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Long-term manure application enhances organic carbon and nitrogen stocks in Mollisol subsoil

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Abstract

Subsoils contain half of the total soil organic carbon (SOC) that is supposed to be relatively more persistent than that present in the topsoil. Improving SOC and total nitrogen (TN) stocks in croplands is crucial to mitigate climate change and ensuring food security. However, our insight into how the management practices and climatic variables influence stocks of SOC and TN, and crop grain yields in the soil profile is limited. In this study, we assessed the long-term impacts of mineral and manure fertilizers on SOC and TN stocks at soil profile levels (up to 100 cm), and cropping system (wheat–maize–soybean) grain yields. Results indicated that in the top 0–40-cm layers SOC and TN stocks were the highest in manure plus mineral fertilizers (MNPK) compared with control, that is, non-fertilized control (CK). Conversely, compared with NPK, sole application of manure (M) clearly increased SOC stocks by 19%, 40%, and 39% and TN stocks by 51%, 105%, and 116% in 40–60, 60–80, and 80–100 cm, respectively ($p < 0.05$). Moreover, Pearson correlation revealed that climate variables, that is, mean annual temperature (MAT) affected both SOC and TN stocks in 0–40-cm layers only of the soil profile. Our findings implicated that the sole application of manure (M) is vital to augment SOC and TN sequestration, particularly in the subsurface layers. However, trade-offs between SOC and TN sequestration and crop yields should also need to be considered while making recommendations for

SOC and TN stocks maintenance and increasing crop productivity in terms of management strategies.

KEYWORDS

animal manure, climatic variables, grain yield, mineral fertilization, nitrogen stock, SOC sequestration, soil profile

1 | INTRODUCTION

Intensive agricultural practices based on the massive application of mineral fertilizers degrade soil quality and threaten ecosystem health (R. Lal, 2013; Tilman et al., 2002). Therefore, prudent management practices are imperative to achieve higher crop and soil productivity, and soil health, which depends on soil organic carbon (SOC) content (Pan et al., 2019). Soil can store approximately 1500 Pg SOC (R. Lal, 2018), and 140 Pg total nitrogen (TN) in up to 100 cm of soil (Batjes, 2014). The SOC is a key indicator of soil quality, crop production, and overall ecosystem functioning (Waqas, Li, Lal, et al., 2020), while nitrogen (N) is an important element in promoting crop growth and sustaining yields (Wang et al., 2019). Nitrogen also mediates SOC, decomposition of SOC (W. Liu et al., 2018), and soil C and N stocks to attain prolonged sustainability and productivity (Babujia et al., 2010). Subsoils are the soil material below the surface horizons which due to their larger soil volume contain more than 50% of the global SOC stocks (Baumert et al., 2018). They respond to fertilizer management strategies (Dignac et al., 2017; Gregory et al., 2014), and sometimes these SOC levels can also exceed those present in the topsoil (Angst et al., 2018; de Richter & Billings, 2015). In conclusion, subsoils are important (but neglected) to improve SOC storage and can serve as a C sink is provided with an additional organic carbon (OC) input (Rumpel et al., 2012). Small increases in SOC and TN concentrations with improved nutrient management practices could greatly affect national and global C and N cycles and climate change (B. Lal et al., 2019; Luo et al., 2004). Conversely, climate change (e.g., increased temperature and atmospheric carbon) can also affect soil C and N dynamics, as well as microbial activities and ultimately crop yields (Waqas et al., 2021). Therefore, SOC and TN sequestration is widely recognized to improve soil fertility and crop yields and offset climate change (R. Lal, 2004; Macbean & Peylin, 2014; Schmidt et al., 2011).

Cropland soils are considered the main source and sink of atmospheric C which is further governed by management strategies including crop rotation, application of mineral and organic fertilizers, tillage operations, and other agronomic practices (Abrar et al., 2020; Ali Shah et al., 2020; Ghosh et al., 2018; Y. T. He et al., 2015; R. Lal, 2004; Piazza et al., 2020).

Application of mineral fertilizers and manure over a longer period regulates the C and N dynamics (Abrar et al., 2021), and is pivotal for enhancing soil and crop productivity (Cai et al., 2018; Gundale et al., 2014; Yu et al., 2020), and hence critical for improving SOC and TN sequestration in croplands (Cai et al., 2021; Contreras-Cisneros

et al., 2022; Waqas, Li, Lal, et al., 2020). The positive role of manure application alone and in combination with mineral fertilizers in improving soil health dynamics and crop productivity has been described in the past on both regional and national croplands. For example, in Chinese croplands, the average SOC sequestration of 0.43 Mg C ha⁻¹ per year can be achieved through the integrated use of manure and mineral fertilizers, and each increase of 1 Mg C ha⁻¹ leads to an increase in grain yields of rice, wheat, and maize by 143, 255, and 202 kg ha⁻¹, respectively (Waqas, Li, Smith, et al., 2020). Likewise in a long-term study, Kätterer et al. (2011) observed an incremental trend in SOC stock with the application of manure while Zhengchao et al. (2013) found that long-term manure fertilization may result in no change or even a net loss of C and N stocks. In another experiment, Gai et al. (2018) explored that organic manure combined with inorganic fertilizer considerably affected the SOC storage and crop yield more than mineral fertilizer applied alone in a Haplic Luvisol in northern China. The use of mineral fertilizer coupled with manure enhances C storage in rice-wheat cropping systems (Shen et al., 2007), and SOC and TN stocks in rice paddy fields (Cheng et al., 2017). Moreover, other studies by Hobbey et al. (2018), Gauder et al. (2016), M. Liu et al. (2020), and Samson et al. (2021) also reported the SOC increment in subsoil after long-term fertilization. Nonetheless, some studies only investigated that long-term sole addition of inorganic fertilizers reduced SOC stocks (Su et al., 2006), or soil TN stocks (Zhengchao et al., 2013) demonstrating the variable effects of distinct fertilizer management practices on SOC sequestration and their stocks (Blanchet et al., 2016; Sodhi et al., 2009), which were mainly attributed to the differential soil types, agricultural practices, and climatic conditions considerably affecting SOC and TN sequestration (G. Zhang et al., 2021; J. Y. Xie et al., 2015). Considering that conserving SOC and TN under long-term fertilization is potentially important for global climate change mitigation and that a considerable amount of SOC is stored by subsoil (Lorenz et al., 2007; Simo et al., 2019). While previous studies only explored the SOC (W. Zhou et al., 2022), and TN (M. Li et al., 2022) in the topsoil of Mollisols. This study by exploring the SOC and TN storage in the subsoil in the topsoil will essentially enhance our understanding of the role of long-term fertilization to improve SOC and TN storage. Therefore, further investigation is urgently needed for a better understanding of the responses of improving SOC and TN stocks at profile level after long-term mineral and manure fertilization which can improve crop production and reduce greenhouse gas emissions (Bremer et al., 2011; Cai et al., 2016; Mustafa et al., 2020) but is also vital for conserving soil quality and ensuring global food security (Anandakumar et al., 2022;

R. Lal, 2004; Lasanta et al., 2020). Given this, practices to enhance the SOC and TN stocks in croplands have attracted the attention of the scientific community in recent years.

The Northeastern region of China dominated by black soils is covering about 6.9% (ca. 6 million ha) of the global Mollisol area (Osman, 2014) out of which 4.4 million ha is subjected to cultivation (Jian-Bing et al., 2006; Xing et al., 2004). Black soils roughly correspond to the Mollisols order in soil taxonomy (Soil Survey Staff-NRCS/USDA, 2014), and Chernozems and Phaeozems in the World Reference Base for Soil Resources (WRB) (IUSS Working Group WRB, 2015). Black soils/Mollisols are popular for higher SOC concentration than other soils of the Country, and are renowned for maize and soybean production (Ling et al., 2014; Mustafa, Hu, Abrar, et al., 2021), albeit a depreciation in their soil fertility has been noticed over the past decade (Tao et al., 2019; H. Xie et al., 2014), due to intensive cultivation which resulted into serious negative feedbacks for global climate change and food production (Todd-Brown et al., 2014), showing greater prospects for SOC and TN sequestration, food production, and greenhouse gas mitigation (Kopittke et al., 2019; Nicoloso & Rice, 2021; Yang et al., 2016).

Previous cropland studies were mainly focused on evaluating the impacts of long-term fertilization on SOC (Chen et al., 2020; Mustafa et al., 2020; Tong et al., 2014) and TN stocks (Han et al., 2020) in the topsoil layers (0–30 cm), except for one trial conducted by Samson et al. (2021), who quantified long- and short-term influences of mineral fertilizers and organic manure on C and N stocks up to 60-cm depth of a soil profile. Nonetheless, the impacts of long-term manure and mineral fertilizers on SOC and TN stocks in the complete soil profile (0–100 cm) remain neglected in the past, which in the current study demonstrates the uniqueness of this endeavour. Also, the relationships among SOC and TN stocks, cropping system (i.e., wheat–maize–soybean) grain yield, and soil physico-chemical attributes such as bulk density (BD), available nitrogen (AN), total phosphorus (TP), and climatic variables, for example, MAT and MAP are needed to be explored to providing the scientific basis of modelling influence of fertilization on C and N stocks. We hypothesized that the application of mineral fertilizers coupled with manure would enhance both SOC and TN stocks along with a complete profile by improving the soil's physico-chemical properties. The objectives of this study were to: (i) evaluate the long-term effects of manure and mineral fertilizers addition on the SOC and TN stocks and crop grain yields in the cropland soil; (ii) evaluate the relationships between grain yields and SOC

and TN sequestration in the wheat–maize–soybean cropping system and (iii) investigate the influence of climate variables such as mean annual precipitation (MAP) and mean annual temperature (MAT) on SOC and TN stocks distributed in different profile layers.

2 | MATERIALS AND METHODS

2.1 | Site details and experimental design

A long-term soil fertility experiment (126°51'E, 45°50'N) was initiated in the year 1979, in Harbin City, (Heilongjiang Province), Northeast China at an elevation of 151 m. The climate is mid-temperate, with MAP of 533 mm and MAT of 3.5 °C; defined by lengthy cold winter and warm summer seasons. Moreover, the variations in climate variables, that is, (A) MAT and (B) MAP (from 1981 to 2014) can be found in Figure S1 (China Meteorological Administration, <http://cdc.cma.gov.cn/>). According to Xing et al. (2004), the texture of the topsoil layer (~0–20 cm) is sandy clay loam, while the texture of subsoil layers (i.e., 40–100 cm) is clay loam (Xing et al., 2004). The soil of the selected site is a Mollisol, according to the United States Department of Agriculture (USDA) (Soil Survey Staff, 2014), and dominated by montmorillonite and illite. The basic soil physico-chemical properties for the 0–100-cm soil layer in 1979 can be found in Table 1. The aboveground crop residues were removed from the soil surface after harvest.

The experimental layout was designed in a completely randomized design with every treatment repeated three times, and the size of every plot was ~36 m². The cropping system was comprised of wheat–soybean–maize rotation. There are 24 different treatments were devised in total for the whole experimental setup. For this study, we chose five treatments which were comprised of (1) non-fertilized control (CK); (2) incomplete fertilizer application: phosphorus and potassium fertilizers (75 kg ha⁻¹ each) (PK); (3) complete fertilizer application: nitrogen (150 kg ha⁻¹) and PK (NPK); (4) manure (M) (75 kg N ha⁻¹ yr⁻¹) (5) M plus NPK fertilizers (MNPk). Urea, diammonium phosphate (DAP), and sulfate of potash (SOP) were used as the sources of N, P, and K, respectively. The amount of fertilizers N, P₂O₅, and K₂O applied to crops in each season was 150, 75, and 75 kg ha⁻¹, respectively, for wheat and maize; 75, 150, and 75 kg ha⁻¹ for soybean, all the fertilizers applied after crops harvest, except for half amount of nitrogen fertilizer for maize applied during jointing

TABLE 1 Basic physico-chemical properties of initial soil samples collected from black soil long-term experiment in 1979

Depth	SOC (g kg ⁻¹)	TN (ng kg ⁻¹)	TP (pg kg ⁻¹)	TK (g kg ⁻¹)	AN (mg kg ⁻¹)	Olsen-P (mg kg ⁻¹)	AN (mg kg ⁻¹)	pH
0–20	15.66	1.48	1.07	25.3	149.2	51.0	210.0	7.45
20–40	15.31	1.46	1.07	25.0	153.0	51.0	190.0	7.00
40–60	13.86	1.40	1.00	26.3	160.4	48.3	200.4	7.10
60–80	8.17	0.64	0.66	24.1	85.9	8.00	184.0	7.45
80–100	7.89	0.57	0.70	29.1	87.7	21.0	174.0	7.50

Abbreviations: AN, available N; g kg⁻¹, gram per kg; mg kg⁻¹, milligram per kg; ng kg⁻¹, nanogram per kg; Pg kg⁻¹, petagram per kg; SOC, soil organic carbon; TK, total potassium; TN, total nitrogen; TP, total phosphorus.

stage. Organic fertilizer was horse manure and provided as N fertilizer at the rate of 75 kg ha⁻¹ (approximately 18,600 kg manure ha⁻¹) after maize harvesting in each rotation. The contents of OC, N, P, and K in the organic manure were 361, 5.81, 6.49, and 9.07 g kg⁻¹, respectively. The manure was spread in the field before mixing into the soil with plowing up to a 20-cm depth in the autumn. Since this area received an ample quantity of rainfall (MAP = 533 mm) so no irrigation facilities were installed in the field, and the crops were solely dependent on natural rainfall. The aboveground residues of all crops (i.e., wheat–maize–soybean) were manually picked from the experimental plots. The field was ploughed with a moldboard plough to a 20-cm depth.

2.2 | Soil sampling and analysis

Soil samples were acquired from an experimental field located near the city of Harbin in Northeast China in October 2014. Before sampling, plant debris was removed from the field. The soil was sampled at depths of 0–100 cm with 20-cm intervals for five layers. Four soil cores were randomly taken from each plot and each layer, and composited to make one representative sample; resultantly, three samples were acquired per treatment. Then the samples were stored in the sample boxes and transported to the laboratory for further analyses where they were placed on the brown paper for air-drying, then weighed on a weight balance (ME1002E/A, Mettler Toledo, Switzerland), and passed through a sieve (2-mm mesh) in the laboratory. Also, soil BD was measured with the soil core method (cylinders composed of stainless steel with a 5-cm diameter and 100-cm³ volume) in topsoil (0–40-cm depth) in each plot. All the samples were finely ground for further analysis. Fine roots and plant residues were cautiously removed by hand and the samples were then ground with a ball mill for SOC and TN analysis.

2.3 | Carbon and nitrogen analysis

In black soil the inorganic C is usually negligible, therefore, the total C concentration was considered OC. The soil samples for all treatments were tested for total C and TN by dry combustion method using an elemental analyzer (EA-3100, Eurovector, Milan, Italy). All the test samples were run in triplicate.

2.4 | Computation of SOC and TN stocks, and carbon sequestration indicators

The SOC and TN stocks along with different layers of the profile were estimated using the following equations (Yang et al., 2007):

$$\text{Stock}_{\text{SOC}} = \text{SOC}_{\text{conc.}} \times 10 \times \text{Soil}_{\text{BD}} \times D \times 10^{-1} \quad (1)$$

$$\text{Stock}_{\text{TN}} = \text{TN}_{\text{conc.}} \times 10 \times \text{Soil}_{\text{BD}} \times D \times 10^{-1}, \quad (2)$$

Where: Stock_{SOC} and Stock_{TN} are the soil organic C and total N stocks (Mg C ha⁻¹), respectively, and SOC_{conc.} and TN_{conc.} are the SOC and TN concentrations, respectively (g kg⁻¹). In Equations (1) and (2), *D* is the respective layer/depth, and Soil_{BD} is soil bulk density (g cm⁻³) which is one of the key determinants in computing SOC and TN stocks in cropland soils. We used Equation (3) proposed by Xie et al. (2007) to calculate BD for respective depths when BD was not reported:

$$\text{SOC}_{\text{BD}} = -0.1019 \times \text{SOC}_{\text{conc.}} + 1.406 \quad (3)$$

The Soil_{BD} can be easily affected by management practices (Lee et al., 2009), which is crucial for determining the variability in SOC and TN stocks. The change in SOC stock formulated on the equivalent soil mass approach (SOC_{ESM}) is more precise compared with that based on the fixed depth approach (SOC_{FD}) (Lee et al., 2009). Hence, we rectified the SOC and TN stocks following equivalent soil mass using Equation (4) (Poeplau et al., 2011):

$$\text{SOC}_{\text{ESM}} = \frac{\text{Soil}_{\text{BD}} \times \text{SOC}_{\text{FD}}}{\text{Soil}_{\text{BD}}} \quad (4)$$

$$\text{TN}_{\text{ESM}} = \frac{\text{Soil}_{\text{BD}} \times \text{TN}_{\text{FD}}}{\text{Soil}_{\text{BD}}}, \quad (5)$$

Where: Soil_{BD} is the soil bulk density, and SOC_{FD} and TN_{FD} are the SOC and TN stocks, respectively, calculated based on a conventional method depending on a fixed depth (without correction), whereas SOC_{ESM} and TN_{ESM} are the corrected SOC and TN stocks, respectively, with BD.

Since the initial SOC values of the whole profile (0–100 cm) were available, the C sequestration for the selected treatments was estimated using Equations (6)–(8) (adapted from Pathak et al., 2011).

$$\text{Csequestration rate} (\mu\text{g g}^{-1} \text{ soil yr}^{-1}) = \frac{\text{SOC}_{\text{current}} - \text{SOC}_{\text{initial}}}{\text{ED}} \times 1000, \quad (6)$$

Where: SOC_{current} and SOC_{initial} are the SOC of the respective treatment (i.e., CK, PK, NPK, M, and MNPK) and SOC of the initial soil acquired from the sampling plots from different profile (0–100 cm) layers and ED shows the total experiment duration from 1979 to 2014; per year is denoted by yr⁻¹.

$$\text{Csequestered} (\text{g kg}^{-1}) = \text{SOC}_{\text{current}} - \text{SOC}_{\text{initial}} \quad (7)$$

$$\text{Total sequestered C} (\text{Mgha}^{-1}) = \left(\frac{\text{C}_{\text{seq}}}{10} \right) \times 1000 \times \text{BD} \times d, \quad (8)$$

Where: C_{seq} is the sequestered carbon, 10 and 1000 are the conversion factors, BD denotes the bulk density, and *d* is the respective depth of individual soil layer.

2.5 | Statistical analyses

The raw data obtained after experimentation and analyses were pre-processed with Microsoft EXCEL 2016 for Windows. The fertilization effects were evaluated by employing one-way analysis of variance (ANOVA) with IBM SPSS version 23.0 (Chicago, IL, USA) with Tukey's honestly significant difference (HSD) as a post hoc test to evaluate the least significant variations within treatments expressed as the mean \pm standard deviation of three replicates. The normality and homogeneity of the data were analyzed by employing the Shapiro-Wilk and Lavene tests, respectively. The grouped bar graph showing the grain yield of the cropping system was made by using SIGMA-PLOT version 14.0 (Systat Software, Inc., San Jose, CA, USA) for Windows. The stacked bar charts depicting SOC and TN stocks distribution in profile and Pearson's correlation heatmap were performed using ORIGIN PRO 2021, and linear regression model fitting was executed with SIGMAPLOT version 14.0. The Pearson correlation

analysis was performed to analyze associations/relationships among SOC and TN stocks and soil physico-chemical properties such as BD, TP, pH, TK, AN, and climatic variables, that is, MAP and MAT.

3 | RESULTS

3.1 | Distribution of SOC and TN contents, and basic soil parameters along with soil profile

Table 2 shows the distribution of soil BD, contents of SOC and TN, AN, TP, and total potassium (TK) along with different layers (0–100 cm) of a soil profile. In 0–40-cm soil layers, the highest BD was found in the plots receiving no fertilizer or manure (i.e., CK) whereas the lowest BD was observed in manure (M) treated plots. Nevertheless, no significant differences in BD were recorded among CK, PK, and NPK ($p > 0.05$). In the same layers, the highest SOC and TN

TABLE 2 Distribution of soil physical and chemical properties subjected to long-term mineral and manure fertilizers application along with different soil depths of a black soil (Mollisol) profile in 2014

Depth (cm)	Treatments	Elemental contents (g kg ⁻¹)					
		Soil organic carbon (SOC)	Total nitrogen (TN)	BD (g cm ⁻³)	AN (mg kg ⁻¹)	TP (g kg ⁻¹)	TK (g kg ⁻¹)
0–20	CK	16.18 ^d	0.74 ^c	1.39 ^a	156.30 ^c	0.41 ^c	23.59 ^c
	PK	17.10 ^c	0.77 ^c	1.38 ^a	158.72 ^{bc}	0.77 ^b	25.75 ^{ab}
	NPK	17.88 ^b	0.91 ^b	1.38 ^a	161.13 ^{ab}	0.78 ^b	25.66 ^a
	M	17.84 ^b	0.88 ^b	1.29 ^b	157.80 ^{bc}	0.74 ^b	25.72 ^{ab}
	MNPK	18.86 ^a	1.00 ^a	1.31 ^b	164.14 ^a	0.95 ^a	26.89 ^a
20–40	CK	14.25 ^d	0.60 ^c	1.38 ^a	153.5 ^c	0.40 ^c	23.87 ^c
	PK	15.78 ^c	0.67 ^b	1.37 ^a	157.3 ^{bc}	0.75 ^b	24.42 ^{ab}
	NPK	15.85 ^{ab}	0.74 ^{bc}	1.38 ^a	158.7 ^{ab}	0.76 ^b	25.32 ^{ab}
	M	15.57 ^{bc}	0.79 ^{bc}	1.28 ^b	156.9 ^{bc}	0.72 ^b	25.06 ^{ab}
	MNPK	16.60 ^a	0.86 ^a	1.31 ^b	162.8 ^a	0.94 ^a	25.55 ^a
40–60	CK	9.44 ^c	0.38 ^e	1.39 ^a	87.05 ^c	0.27 ^c	25.78 ^c
	PK	11.76 ^b	0.59 ^b	1.38 ^a	89.95 ^b	0.57 ^b	27.37 ^{ab}
	NPK	11.78 ^b	0.47 ^c	1.40 ^a	89.43 ^b	0.58 ^b	27.23 ^{ab}
	M	14.67 ^a	0.76 ^a	1.29 ^b	93.69 ^a	0.66 ^a	27.86 ^{ab}
	MNPK	11.78 ^b	0.54 ^d	1.37 ^a	92.94 ^{ab}	0.70 ^a	28.19 ^a
60–80	CK	7.32 ^c	0.34 ^c	1.39 ^a	82.37 ^b	0.26 ^c	26.11 ^c
	PK	8.30 ^b	0.37 ^b	1.38 ^a	83.33 ^b	0.55 ^b	27.37 ^{abc}
	NPK	7.25 ^c	0.27 ^c	1.41 ^a	81.48 ^b	0.53 ^b	25.90 ^{bc}
	M	12.72 ^a	0.60 ^a	1.30 ^b	89.62 ^a	0.65 ^a	27.86 ^a
	MNPK	8.34 ^b	0.26 ^c	1.38 ^a	83.05 ^b	0.61 ^a	27.53 ^{ab}
80–100	CK	7.14 ^b	0.30 ^b	1.39 ^a	80.51 ^b	0.28 ^d	25.77 ^c
	PK	7.32 ^b	0.29 ^b	1.39 ^a	80.99 ^b	0.52 ^c	27.70 ^{ab}
	NPK	7.07 ^b	0.30 ^b	1.42 ^a	81.62 ^b	0.53 ^{bc}	26.19 ^c
	M	9.33 ^a	0.51 ^a	1.31 ^b	88.49 ^a	0.56 ^b	29.53 ^a
	MNPK	7.49 ^b	0.22 ^b	1.38 ^a	82.36 ^b	0.60 ^a	29.19 ^a

Note: Different lower-case letters depict significant differences between the treatments. The SOC and TN contents data are adopted from Abrar et al. (2020, 2021).

Abbreviations: AN, available nitrogen; BD, bulk density; g cm⁻³, gram per cubic cm; g kg⁻¹, gram per kg; M, manure alone application; mg kg⁻¹, milligram per kg; MNPK, combined application of M and NPK; SOC, soil organic carbon; TK, total potassium; TN, total nitrogen; TP, total phosphorus.

contents were found in MNPK while the lowest values were noticed in CK. Nevertheless, no clear difference in SOC and TN contents was recorded between NPK and M treatments. While in subsoil layers 40–100 cm, manure (M) treatment showed the highest SOC and TN content among all treatments (Table 2). In contrast to other treatments, the maximum AN, TP, and TK were noted in MNPK. Similarly, in 40–100-cm layers, M treated plots had the lowest BD and highest mean values of AN, TP, and TK among all treatments. There were no significant differences in AN, TP, and TK between PK and NPK treatments in the 40–60-cm soil layer. In the 80–100-cm layer, M and MNPK were not significantly different from each other with respect to TK (Table 2).

3.2 | Carbon sequestration at profile level

We observed that all the fertilizer management schemes (PK, NPK, M, and MNPK) significantly improved the C sequestration of the black soil compared to unfertilized CK. In the 0–20-cm layer, among all the fertilizer combinations, the combination of manure (M) and NPK, that

is MNPK maintained the highest C sequestration indices values of 99.71, 9.14, and 0.26 for carbon sequestration rate ($\mu\text{g g}^{-1} \text{ soil yr}^{-1}$), total carbon sequestered, and annual sequestering rate ($\text{Mg ha}^{-1} \text{ yr}^{-1}$), respectively (Table 3). A similar pattern of C sequestration increment among treatments was also observed in the 20–40-cm layer. Contrary to topsoil layers, sole application of manure (M) registered the highest C sequestration indicators compared to other treatments (Table 3). Over long-term period, only manure (M) application sequestered the C throughout the subsoil layers (40–100 cm). Whereas the values of C sequestration indicators maintained by other treatments fall in the negative range (Table 3).

3.3 | SOC and TN stocks, and grain yield of cropping system as a function of long-term mineral and manure fertilization

Overall, the SOC and TN stocks exhibited a diminishing trend with depth increment along with the profile (Figure 1a,b). The interaction between fertilizers treatments and soil layers was also significant

TABLE 3 Total carbon sequestration in 0–100 cm of black soil (Mollisol) of Northeast China subjected to 35 years of mineral and manure fertilizer management strategies

Depth (cm)	Treatments	Carbon sequestration rate ($\mu\text{g g}^{-1} \text{ soil yr}^{-1}$)	Total carbon sequestered (Mg ha^{-1})	Annual sequestering rate ($\text{Mg ha}^{-1} \text{ yr}^{-1}$)
0–20	CK	23.71 ^d	2.31 ^d	0.07 ^d
	PK	48.29 ^c	4.66 ^c	0.13 ^c
	NPK	71.14 ^b	6.87 ^b	0.20 ^b
	M	70.86 ^b	6.40 ^b	0.18 ^b
	MNPK	99.71 ^a	9.14 ^a	0.26 ^a
20–40	CK	17.71 ^d	1.71 ^d	0.05 ^c
	PK	44.57 ^c	4.27 ^c	0.13 ^b
	NPK	53.71 ^b	5.19 ^b	0.15 ^b
	M	52.86 ^b	4.74 ^c	0.14 ^b
	MNPK	76.57 ^a	7.02 ^a	0.20 ^a
40–60	CK	−18.00 ^c	−1.75 ^c	−0.05 ^c
	PK	14.00 ^b	1.35 ^b	0.04 ^b
	NPK	14.57 ^b	1.43 ^b	0.04 ^b
	M	69.14 ^a	6.24 ^a	0.18 ^a
	MNPK	15.14 ^b	1.45 ^b	0.04 ^b
60–80	CK	−54.29 ^c	−5.28 ^c	−0.15 ^c
	PK	−25.71 ^b	−2.48 ^b	−0.07 ^b
	NPK	−55.71 ^c	−5.50 ^c	−0.16 ^c
	M	37.14 ^a	3.38 ^a	0.10 ^a
	MNPK	−25.14 ^b	−2.43 ^b	−0.07 ^b
80–100	CK	−36.29 ^c	−3.53 ^b	−0.10 ^b
	PK	−30.86 ^b	−3.00 ^b	−0.09 ^b
	NPK	−38.86 ^c	−3.86 ^c	−0.11 ^b
	M	25.43 ^a	2.33 ^a	0.07 ^a
	MNPK	−27.14 ^b	−2.62 ^b	−0.07 ^b

Note: Different lowercase letters are significant at $p < 0.05$ following Tukey test.

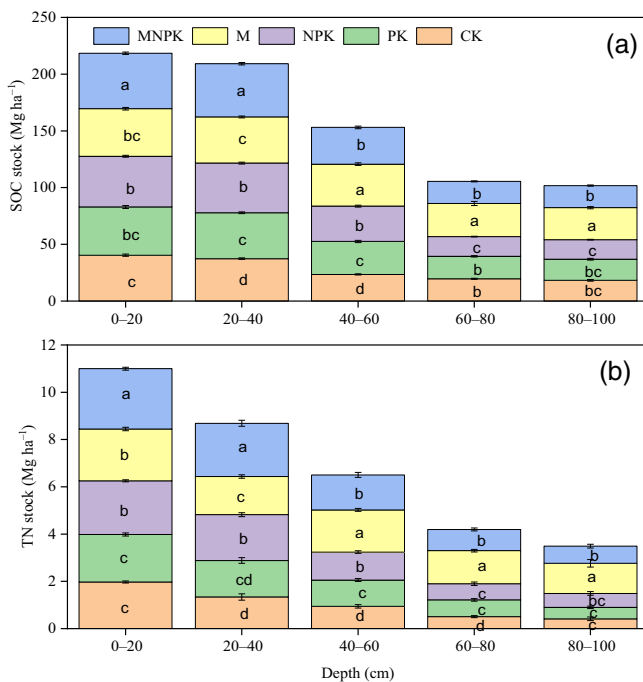


FIGURE 1 Stacked bar chart of distribution of (a) soil organic carbon (SOC) and (b) total nitrogen (TN) stocks (Mg ha^{-1}) in different layers of a Mollisol profile (0–100 cm) subjected to long-term (35 years) application of mineral, manure, and a combination of mineral and manure fertilizers. The different lower-case letters represent significant differences among treatments. Error bars represent the standard deviations (\pm SDs) of SOC and TN stocks for each treatment. Abbreviations used for different treatments: CK, non-fertilized control; PK, combined application of phosphorus and potassium; NPK, combined application of nitrogen and PK; M, manure alone application; MNPK, combined application of M and NPK ($n = 3$). [Colour figure can be viewed at [wileyonlinelibrary.com](https://onlinelibrary.wiley.com)]

(Tables S2 and S3). The MNPK application significantly indicated the maximum SOC and TN stocks in the 0–40-cm layers among all treatments ($p < 0.05$; Figure 1a,b). For example, in the surface layer (up to 20 cm), MNPK increased SOC and TN stocks by 9.41% and 12.09%, respectively, in contrast to NPK while no obvious effects ($p > 0.05$) of NPK and M fertilization on stocks of SOC/TN were found at same depths (Figure 1a,b). The mean SOC stocks in MNPK treated plots ranged $47.99\text{--}50.42 \text{ Mg ha}^{-1}$, $45.54\text{--}48.12 \text{ Mg ha}^{-1}$, and $31.15\text{--}33.85 \text{ Mg ha}^{-1}$ at the soil layers of 0–20, 20–40, and 40–60, respectively (Figure 1a). Likewise, TN stocks followed the same increasing trend in the same soil layers (Figure 1b). The MNPK-treated plots contained 7.07% and 15.88% higher SOC and TN stocks, respectively, in the 20–40-cm layer over those recorded in NPK (Figure 1a,b). Similarly, the TN stocks of the 0–20 and 20–40-cm layers (2.55 Mg ha^{-1} and 2.25 Mg ha^{-1} , respectively) in MNPK were the highest among all treatments (Figure 1a,b; $p < 0.05$).

In the 40–100-cm layers, the application of manure (M) appreciably ($p < 0.05$) enhanced SOC and TN stocks among all treatments. While no clear difference ($p > 0.05$) was noted in the SOC

stocks of the plots treated with NPK and MNPK. Compared with NPK, the addition of M appreciably ($p < 0.05$) improved SOC and TN stocks by 67.54% and 104.83%, respectively, in the 60–80-cm layer whereas no considerable difference was observed among SOC and TN stocks of CK, PK, and MNPK in 60–100-cm layers ($p > 0.05$; Figure 1a,b). The application of MNPK increased TN stock by 29.11% and 67.70% in 0–20 cm and 20–40 cm, respectively. Nevertheless, no meaningful difference was recorded in TN stocks in NPK and manure (M) up to 20-cm depth. Nonetheless, throughout the subsoil, the M application predominantly maintained the SOC and TN stocks in 40–100-cm layers. While no obvious variation was noticed between the SOC and TN stocks of the CK and PK treatments in the 80–100-cm layer (Figure 1a,b). Out of total SOC and TN stocks, the highest proportions of SOC and TN stocks under MNPK were found in the topsoil. In contrast, maximum proportions of SOC and TN stocks under manure (M) treatment were recorded in the subsoil layers (Figure 1a,b).

The application of MNPK also maintained the highest grain yields of rotation (i.e., wheat, maize, and soybean) compared with other treatments ($p < 0.05$; Figure 2a). Compared with CK and NPK, MNPK increased wheat grain yield by 194% and 24.25%, maize yield by 237 and 26.85%, and soybean yield by 62.43% and 19.03%, respectively. While no significant difference in soybean grain yield was recorded among PK, NPK, and M, the CK treatment attained the lowest grain yield of the cropping system (Figure 2a).

3.4 | Association among SOC and TN stocks, depth increment, and cropping system grain yield

In topsoil layers (0–40 cm), a highly significant relationship ($R^2 \geq 0.73$; $p < 0.0001$) between SOC stock and TN stock (Figure 2b; Table 4), with the highest slope value ($b = 0.09$) was observed in 20–40-cm layer (Table 3). Similarly, throughout the subsoil (40–100 cm), a strong correlation was recorded between SOC stock and TN stock (Figure 2b; Table 4). In 40–60 cm, the increment in SOC stock explained 82% variations in TN stock (Table 4). Based on the profile the SOC and TN stocks related exponentially with an increase in soil depth. For instance, SOC stock under CK had a highly strong correlation ($R = 0.961$; $p < 0.01$) with depth increment (Table S1; Figure S2). Also, SOC and TN stocks under other treatments decreased exponentially with increasing depth. Overall, SOC stock and TN stocks maintained by the M application showed the least depreciation (slope values = -0.005 and -0.006 , respectively) with depth increment among all the treatments (Figure S2; Table S1).

Figure 3a–f shows that highly significant associations ($p < 0.0001$) among SOC and TN stocks and rotation grain yields (tonnes ha^{-1}) in 0–40-cm layers of topsoil except for highly significant correlations were also found between SOC stock and wheat grain yield (Figure 3a), and among SOC and TN stocks and soybean grain yield in the first layer (i.e., 40–60 cm) of subsoil (Figure 3e,f; $p < 0.05$). Whereas no significant relationships were recorded among SOC stock

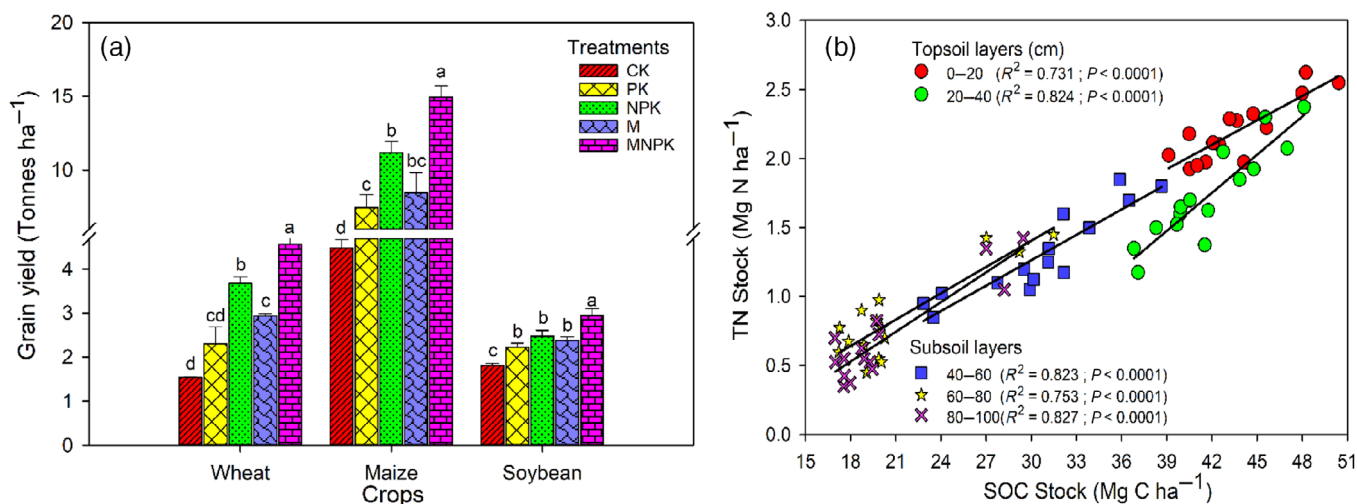


FIGURE 2 Grouped bar chart shows the (a) grain yield (in tonnes ha^{-1}) of the wheat–maize–soybean cropping system at black soil fertility long-term experiment under the influence of long-term mineral and manure fertilization (Panel a). Different lower-case letters (in Panel a) indicate significant differences at $p < 0.05$ followed by Tukey's HSD post hoc test. Panel B depicts the regression between SOC and TN stocks (Mg ha^{-1}) along with soil profile layers ($n = 15$). [Colour figure can be viewed at [wileyonlinelibrary.com](https://onlinelibrary.wiley.com/doi/10.1002/ldr.4498)]

and TN stock and wheat, maize, and soybean grain yield at 60–100-cm layers of the subsoil (Figure 3a–f).

3.5 | Influence of climatic variables, and soil physico-chemical properties on organic C and total N stocks distributed along with soil profile

The correlation analysis (Figure 4a) demonstrates that in the 0–40-cm layers, the climatic variables, that is, MAP and MAT were positively related with SOC and TN stocks and soil physico-chemical properties, that is, TP, pH, and AN at 0–40-cm layers ($p < 0.05$; Figure 4a) except a significant negative relationship was observed between TK and MAP in 40–100-cm layers. Moreover, no significant correlations were found among SOC and TN stocks and MAP and MAT in the 40–100 cm. The BD caused a negative effect on SOC (correlation coefficient $R = -0.55$) and TN stocks ($R = -0.61$), while a positive relationship was recorded among, TP, TK, and SOC stocks. Nonetheless, no significant association was observed between TP and TN stocks, and among AN, SOC, and TN stocks (Figure 4a). The pH has a negative correlation with SOC stock without any significant relationship with TN stock.

In 40–100-cm layers, a close correlation ($R = 0.92$) was observed between SOC stock and TN stock. Similar to 0–40-cm layers, BD recorded negative correlation with SOC stock and TN stock ($R = -0.83$ and -0.74 , respectively) and also with TK ($p < 0.05$; Figure 4b). Moreover, no significant relationships were observed among stocks (both SOC and TN stocks) and all soil physico-chemical attributes except BD. TP and pH are negatively related to each other in subsoil layers (40–100 cm). While in the same layers, TP was associated positively ($R = 0.54$; $p < 0.05$) with TK (Figure 4b).

4 | DISCUSSION

4.1 | Stocks of SOC and TN, carbon sequestration, and grain yields affected by long-term fertilization

In this study, we found an overall depreciation in SOC and TN stocks with depth (Figure 1a,b, respectively), which is in line with the observations of Zhuo et al. (2022) who also found a similar decreasing trend of SOC stocks with depth increment. In 0–40-cm layers, we concluded that the MNPK significantly increased SOC and TN stocks by 14.38% and 48.41%, respectively, over those recorded in CK (Figure 1a,b; $p < 0.05$). These outcomes are in line with those of similar studies (Gai et al., 2018; Gami et al., 2009; Giacometti et al., 2013; Purakayastha et al., 2008), who also observed an identical increasing pattern of SOC and TN stocks in the surface layers after the long-term mixed application of mineral fertilizers and manure that might be ascribed to the higher amount of fertilization annually which enhanced SOC and TN stocks (i) directly by adding larger quantities of OC and N, (ii) and indirectly by increasing plant biomass (He, et al., 2018; Wang et al., 2018). However, throughout the subsoil (40–100 cm), we noticed an appreciable decline in SOC and TN stocks in plots treated with nitrogen-based fertilizers (i.e., MNPK and NPK) that might be associated with the soil compaction and an increase in BD (Table 2) due to the prolonged pertinent addition of NPK and MNPK (Liu et al., 2019; Zhang et al., 2018), other than promoting the decomposition of crop residues and soil C, as we noticed in the subsoil layers (i.e., 40–100 cm) where the NPK applied either alone or combined with manure had improved relatively less SOC and TN stocks than manure alone application, that is, M (Figure 1a,b), that may be linked with the long-term organic matter inputs in addition to the considerable biomass production that is in accord with the identical beneficial

TABLE 4 Relationship between SOC stock and TN stock and wheat–maize–soybean grain yield under long-term mineral and manure fertilization along with different layers of a Mollisol profile

Parameters	Depth (cm)	Regression	Coefficient of correlation (R)	Adjusted regression coefficient (Adj R ²)	Significance
SOC stock versus TN stock	0–20	$y = 0.06x - 0.38$	0.86	0.72	***
	20–40	$y = 0.09x - 2.11$	0.91	0.81	****
	40–60	$y = 0.06x - 0.58$	0.91	0.81	****
	60–80	$y = 0.06x - 0.50$	0.87	0.74	****
	80–100	$y = 0.07x - 0.77$	0.91	0.81	****
SOC stock versus wheat grain yield	0–20	$y = 0.26x - 8.45$	0.82	0.65	***
	20–40	$y = 0.26x - 7.92$	0.87	0.74	****
	40–60	$y = 0.13x - 1.07$	0.60	0.31	*
	60–80	$y = -0.03x + 3.64$	0.02	-0.06	NS
	80–100	$y = -0.00x + 2.96$	0.00	-0.08	NS
TN stock versus wheat grain yield	0–20	$y = 4.02x - 5.87$	0.87	0.74	****
	20–40	$y = 2.66x - 1.65$	0.90	0.80	****
	40–60	$y = 1.55x + 0.95$	0.48	0.17	NS
	60–80	$y = 0.97x + 2.15$	0.30	0.02	NS
	80–100	$y = 0.90x + 2.33$	0.24	-0.02	NS
SOC stock versus maize grain yield	0–20	$y = 1.01x - 34.46$	0.85	0.70	****
	20–40	$y = 1.01x - 32.71$	0.91	0.81	****
	40–60	$y = 0.39x - 2.53$	0.48	0.17	NS
	60–80	$y = -0.22x + 14.11$	0.25	-0.01	NS
	80–100	$y = -0.12x + 11.92$	0.13	-0.06	NS
TN stock versus maize grain yield	0–20	$y = 15.18x - 23.86$	0.88	0.76	****
	20–40	$y = 9.92x - 7.71$	0.91	0.81	****
	40–60	$y = 4.16x + 4.11$	0.34	0.05	NS
	60–80	$y = 2.26x + 7.63$	0.19	0.04	NS
	80–100	$y = 2.14x + 8.03$	0.19	-0.04	NS
SOC stock versus soybean grain yield	0–20	$y = 0.07x - 0.77$	0.75	0.52	***
	20–40	$y = 0.08x - 0.96$	0.78	0.76	****
	40–60	$y = 0.05x + 0.87$	0.72	0.48	**
	60–80	$y = 0.00x + 2.28$	0.02	-0.08	NS
	80–100	$y = 0.01x + 2.07$	0.16	-0.05	NS
TN stock versus maize grain yield	0–20	$y = 1.13x - 0.17$	0.68	0.65	***
	20–40	$y = 0.73x - 1.03$	0.84	0.69	****
	40–60	$y = 0.58x + 1.55$	0.60	0.31	*
	60–80	$y = 0.40x + 1.97$	0.43	0.12	NS
	80–100	$y = 0.33x + 2.07$	0.36	0.06	NS

Note: $n = 15$.

Abbreviation: NS, non-significant relationship.

*Significant when $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$; **** $p \leq 0.0001$.

impacts of manure addition on C and N accumulation investigated also by other studies (He et al., 2015; Xie et al., 2014). Moreover, Wen et al. (2021) concluded that continuous manure fertilization could augment intra-aggregate labile SOC which then assists microbial growth and aggregate formation and stabilization of SOC. Soluble inorganic and organic compounds decomposed from fertilizer can be leached into subsoil (Yan et al., 2018), thus stimulating microbial

nutrient cycling. In accordance with our findings, Fujisaki et al. (2018) explored that the main agent contributing (>5-times) to the more SOC storage in the subsurface layers was the C input via organic fertilization relative to mineral fertilization. In contrast to Hobley et al. (2018), who reported reduced SOC and TN stocks in NPK-treated plots along with soil profile our study results showed higher SOC and TN stocks in NPK- and MNPK-treated plots in the plow layers (0–40 cm),

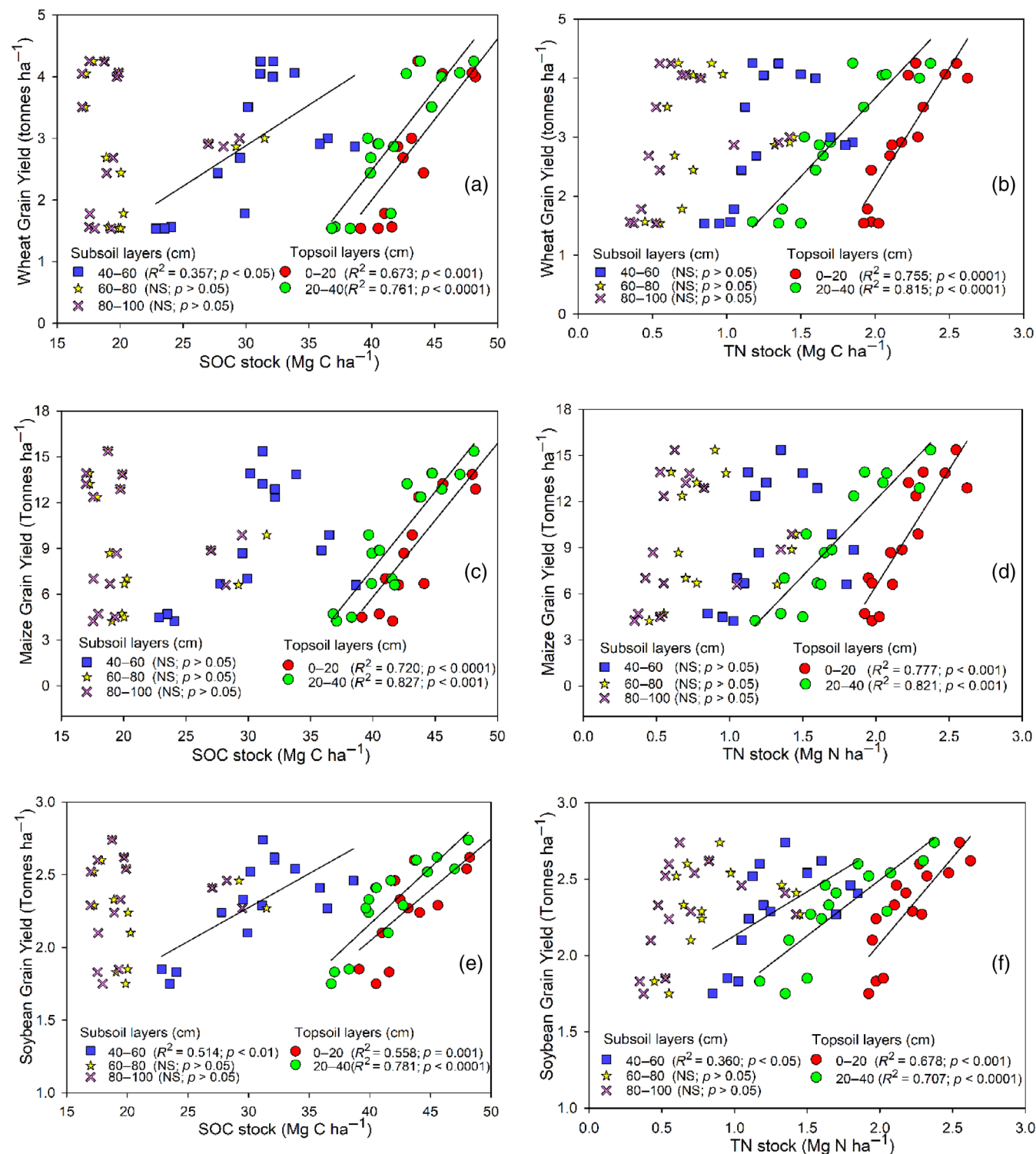


FIGURE 3 Linear regression of (a) soil organic carbon (SOC) stock to wheat grain yield, (b) SOC stock to maize grain yield, (c) SOC stock to soybean grain yield, (d) total nitrogen (TN) stock to wheat grain yield, (e) TN stock to maize grain yield, and (f) TN stock to soybean grain yield ($n = 15$). All regressions are significant ($p \leq 0.05$). [Colour figure can be viewed at [wileyonlinelibrary.com](https://onlinelibrary.wiley.com/doi/10.1002/for.2498)]

validating the positive effects of chemical fertilizers and manure applied with mineral fertilizers (i.e., MNPK application) on SOC and TN stocks only in the plow layers that might be linked with the continuous supply of SOC and TN provided by the mineral and manure fertilization, and partly due to the enhanced immobilization due to the

manure application (Hangs et al., 2021; Li & Han, 2016). Overall, our findings are in line with the speculation that prolonged persistence of OC in subsoil mainly relies on the (1) binding of SOC with the mineral surfaces and less accessibility to the microbes, and (2) additionally the lower oxygen concentration in the subsoil layers could hamper

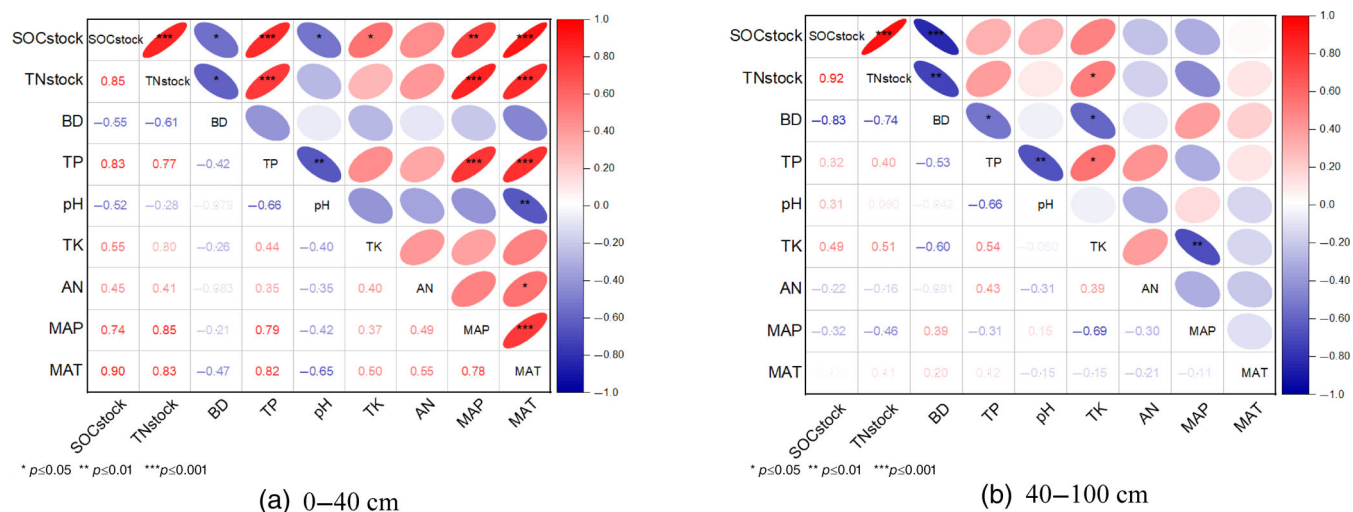


FIGURE 4 Correlation analysis among stocks of carbon (C) and nitrogen (N); climatic variables; and soil attributes in (a) topsoil layers (0–40 cm), and (b) represents subsoil layers (40–100 cm). * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$. AN, available nitrogen; BD, soil bulk density; MAP, mean annual precipitation; MAT, mean annual temperature; SOC stock, soil organic carbon stock; TK, total potassium; TN stock, total nitrogen stock; TP, total phosphorus. The numbers represent the correlation coefficients. Red and blue denote positive and negative correlations, respectively. [Colour figure can be viewed at [wileyonlinelibrary.com](https://onlinelibrary.wiley.com)]

microbes to acquire sufficient energy from the recalcitrant compounds for sustaining microbial activity, thereby greatly decreasing the SOC decomposition (Chabbi et al., 2009; Dungait et al., 2012; Fontaine et al., 2007; Preusser et al., 2017), and hence increased SOC stocks in the deeper layers (Figure 1a). The primary OC inputs in the deep layers are root exudates, and dissolved OC from surface layers (Rumpel & Kögel-Knabner, 2011) transferred by bioturbation (Leuther et al., 2022).

Management practices such as tillage and/or inputs with soluble organic components that navigate fresh C and N inputs to deeper layers could drastically influence organic matter dynamics at deeper soil layers. Moreover, the C and N mineralization was influenced by the DOC, and available N and P (Qiu et al., 2022), which were promptly improved by NPK and MNPK fertilizers (Ashraf et al., 2020; Kumar et al., 2018), that may often depreciate C and N stocks (Xu et al., 2013), implicating that the quantity of recalcitrant OC may increase with depth increment (Jobbágy & Jackson, 2000), while the labile OC proportion decreases with the increasing soil depth which may be associated with relatively slower/insignificant mineralization of the OC (C. Li et al., 2021). Moreover, crop rooting depth and root mass, and microbes associated with roots may also influence the SOC stocks in the subsoil layers (Clemmensen et al., 2013; Fan et al., 2016) as it is well known that OC in the subsoil is considered to be derived from root inputs rather than aboveground biomass, however, C allocation from aboveground biomass also plays a vital role in affecting soil C dynamics (Austin et al., 2017; Kätterer et al., 2011).

Our study results show significant SOC sequestration in the topsoil layers if mineral fertilizer was applied in combination with manure. These findings were also endorsed by previous studies (Gai et al., 2018; Gupta Choudhury et al., 2018; He et al., 2015; Mustafa et al., 2022; Samson et al., 2021; Zhuo et al., 2022). This increase was

mainly attributed to the improved plant biomass, and CO_2 fixation through NPK fertilization and consistent carbon input via manure application (M) (Y. He, Xu, et al., 2018). On average, our findings showed the highest sequestration rate ($0.23 \text{ Mg ha}^{-1} \text{ yr}^{-1}$) under mixed application of NPK and manure, that is, MNPK in 0–40-cm layers. However, in 40–100-cm layers, maximum C sequestration rates were found in the manure-treated plots. Topsoil results of the C sequestration rate corroborate with the knowledge that major C inputs to soils are directly added to the surface layers (Tautges et al., 2019). Moreover, the SOC in the subsoil is protected from decomposition as it is chemically linked with soil minerals (Antony et al., 2022) and due to the lack of fresh C inputs (Fontaine et al., 2007). Also, the role of organo-mineral complexation cannot be neglected as it is significant down the soil profile in terms of storage of sequestered SOC (Torres-Sallan et al., 2017).

Furthermore, we noticed that the MNPK compared with M and NPK enhanced the wheat grain yield by 55.67% and 24.14%, maize grain yield by 76.65% and 33.63%, and soybean grain yield by 23.42% and 18.79%, respectively (Figure 2a). Earlier studies also supported our results and reported that mixed application of manure and mineral fertilization increased grain yields compared with mineral only treatments and implied this yield increment to better nutrient uptake (Gai et al., 2018; Waqas, Li, Smith, et al., 2020; Wei et al., 2016).

4.2 | Relationships among SOC stocks, TN stocks, and cropping system grain yield

Our findings revealed highly significant positive correlations between SOC stock and TN stock ($R > 0.80$), throughout the soil profile (Figure 2b; Table 4), indicating a greater increment in TN stock as a

function of increasing SOC stock, respectively, that might depict a close coupling of C and N cycles (Abrar et al., 2021; Falkowski et al., 2000; Soussana & Lemaire, 2014). It is also considered that in the 0–40-cm layers, the maximum SOC and TN stocks in MNPK compared with CK, M, NPK, and PK were linked with the greater C and N inputs via manure application in MNPK (Gai et al., 2018; Jalali & Ranjbar, 2009; Wankhede et al., 2021), and the higher rhizodeposition (due to greater crop productivity) in the plots treated with MNPK and NPK might be considered the most influencing factors to enhanced soil microbial activity which could increase the mineralization of SOC and TN (Cheng et al., 2016; Malhi & Lemke, 2007). Also, nutrient stoichiometry (C:N ratio) might play a crucial role in nutrient turnover/mineralization (Abrar et al., 2021; Ashraf et al., 2020). Whereas M-only application might limit the microbial activity by improving more macro aggregation (Mustafa, Hu, Shah, et al., 2021) in those plots and might also hamper the O₂ diffusion essential for microbial activity (Gupta & Germida, 2015). These outcomes may also corroborate our results in the subsoil layers (60–100 cm) where we found that M treated plots had significantly higher SOC and TN stocks compared with MNPK and NPK ($p < 0.05$). Whereas, in the same layers, the decreased SOC and TN stocks in NPK treated plots might be due to the priming effect of the applied nutrients on fresh SOC since all the nutrients promote microbial activity thus helping more SOC decomposition (Di Lonardo et al., 2017; Fang et al., 2018b).

In this study, we further noticed an increase in cropping system grain yield with an increment in SOC and TN stocks in 0–40 cm topsoil layers (Figure 3a–f). This is highly expected and mainly attributed to the improved soil properties (such as soil aggregation, BD, aeration, etc.), and the acquisition of plant nutrients through the addition of manure (Dai et al., 2019; Zhang et al., 2018), and higher SOC enhances grain yields by providing plant nutrients and ameliorating soil health and quality (R. Lal, 2016; Oldfield et al., 2018). Also, Han et al. (2018) have associated improved crop production with an increase in SOC stock. In agreement with these outcomes, we also noticed greater wheat, maize, and soybean grain yield in plots treated with NPK alone or mixed with manure, that is, MNPK (Figure 2a). Consistent with the other long-term studies (Bhattacharyya et al., 2011; Q. Li et al., 2017) who also reported similar findings since N is one of the most important nutrients for enhancing crop productivity and microbial activity along with mediating the soil C cycle (Bichel et al., 2016; Schmidt et al., 2011). Higher amounts of N present in both MNPK and NPK significantly increased cropping system grain yield in contrast to other treatments lacking N (Figure 2a). Yield generally correlates well with the availability of nutrients in the soil, especially nitrogen and P in the topsoil (Lemaire et al., 2021), because plant roots grow mainly on the surface and their density decreases with depth (Vanhees et al., 2021). Due to lower microbial activity, TOC and TN are less mineralized in the subsoil than in the topsoil (Ma et al., 2022). Second, the BD of the subsoil was higher than that of the topsoil (Ren et al., 2022), while the AN was about half that of the topsoil (Ma et al., 2022). Therefore, AN correlated strongly with yield, while subsoil nitrogen content did not correlate significantly with grain yield. Subsoil TOC content and TN are more important for

sequestering soil carbon and reducing nitrogen losses (Hobley et al., 2018). Lower SOC mineralization and concentrations of available nutrients explain little or no correlation between SOC in the subsoil and grain yield of the cropping system.

4.3 | Relationship of SOC and TN stocks with climatic variables and soil properties varying down the profile

In addition to the management practices (such as mineral and manure fertilization, crop residue incorporation, crop yield, etc.), the SOC and TN stocks of arable land were also influenced by climatic variables, that is, MAP and MAT (Ladha et al., 2011; Zhou et al., 2019; Zhuo et al., 2022). The MAT may augment SOC decomposition, while the effect of precipitation on SOC is generally perceived to be positive because it is believed that SOC increases with increasing precipitation (Fang et al., 2018a; Mishra et al., 2021). However, Kirschbaum (2000) argued that temperature is on one hand anticipated to depreciate SOC by increasing decomposition rates while concurrently enhancing SOC through improved net primary production (NPP). In this study, consistent with Y. Zhou et al. (2019), we noticed a positive influence of both MAP and MAT on SOC and TN stocks in surface layers only (Figure 4a). Whereas, from 40 to 100-cm soil layers, no significant correlations were detected among MAP and MAT and SOC and TN stocks (Figure 4b). However, these influences of MAP and MAT are often less pronounced in the deeper layers possibly due to soil buffering capacity than surface layers (Mishra et al., 2021). Additionally, the fresh C inputs from surface layers predominantly altered SOC accrual in the subsurface layers (Abrar et al., 2020; Kan et al., 2022). Here, we noticed that soil properties such as BD and porosity are considered to be the main factors controlling the SOC and TN stocks both in topsoil and subsoil layers in addition to the influences posed by MAP and MAT (Figure 4a,b). Consistent with Alhassan et al. (2018), we noticed negative correlations of SOC and TN stocks with BD, respectively, and BD is an indicator of soil compaction which influence SOC and TN turnover/decomposition by altering microbial activity (Yin et al., 2019). Furthermore, compacted soil inhibits root penetration and minimized subsurface biomass (Colombi & Keller, 2019), leading to minimal C input into the subsoil. The pH has negative correlations with SOC and TN stocks in agreement with the other study by M. Li et al. (2019). This negative relationship could be ascribed to the acquisition of greater organic matter, through oxidation, and provided the soil solution with organic acids leading to reduce soil pH (Ali et al., 2017).

5 | CONCLUSIONS

Long-term use of mineral fertilizers and manure improves Northeastern China's SOC and TN stocks and cropping system productivity. Among fertilizers, the manure added with mineral fertilizers resulted in the highest increases in grain yields and SOC and TN stocks in the

surface layers, implying that SOC and TN reserves still have the potential for C and N sequestration. However, manure application was a more effective practice for enhancing SOC and TN subsoil stocks. This improvement may be linked to more quantity of dissolved organic matter translocated to the deep layers, improved aggregation, and reduced microbial activity. The close relationship between the stocks of SOC and TN makes it imperative to increase C inputs to stabilize and/or increase TN stocks across soil profiles. Higher SOC and TN sequestration rates in deep layers when manure is applied can offset the negative effects of greenhouse gas emissions and improve soil health under existing climatic conditions. At this point, we should also consider that the carbon sequestered in the subsurface profile has a longer mean residence time due to very low decomposition rates. However, manure application alone could also compromise the system productivity, so based on our results, we recommend using a greater amount of organic manure along with mineral fertilizer to ensure the highest crop productivity and carbon and nitrogen sequestration.

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CONFLICT OF INTEREST

We declare that all the authors have no conflicts of interest to this work. We also declare that we do not have any commercial or associative interest that represents a conflict of interest in connection with the work submitted.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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