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Long-Term Organic Substitution Promotes Carbon and Nitro-Gen Sequestration and Benefit Crop Production in Upland Field

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Abstract: Partial substitution of synthetic nitrogen fertilizer with manure (organic substitution) is highly recommended to minimize environmental risks without compromising crop productivity in intensive agricultural systems. However, our understanding of the effect of organic substitution on soil organic carbon (OC) and total nitrogen (TN) in deep soil and its impact on crop productivity remains limited. However, we investigated OC and TN changes in soil profile down to 100 cm, crop yield, and sustainable yield index under synthetic nitrogen, phosphate, and potassium fertilizers (NPK), NPK plus straw (NPKS), and organic substitution (NPKM) treatment over two decades in four upland fields across different climate zones. Compared with the initial values, two decades of NPKM treatment significantly ($p < 0.05$) increased OC and TN stocks in either topsoil (by 25.6–103.8 and 15.8–89.8%) or deep soil (by 2.9–71.3 and 5.7–36.9%), respectively, across all sites. The increases in OC and TN stocks in 0–100 cm soil receiving NPKM were significantly higher than those receiving NPK at all sites and NPKS at three high-evaporation sites, as well. Compared with NPKS and NPK treatments, crop yield and N uptake were significantly increased under NPKM treatment only at the Qiyang site. Furthermore, OC sequestration in the entire soil profile down to 100 cm and TN accumulation in topsoil exhibited significant positive exponential correlations with crop N uptake, relative crop yield, and sustainable yield index. In conclusion, long-term partial substitution of synthetic N fertilizer with manure facilitates soil OC and TN sequestration in the entire 100 cm profile and thus maintains high crop productivity in upland areas.

Keywords: long-term experiment; soil profile; carbon sequestration; crop productivity; sustainable yield index

1. Introduction

The sequestration of organic carbon (OC) and nitrogen (N) in arable soils is not only essential for crop growth and development [1], but also important in reducing CO_2

and N_2O emissions to the atmosphere, thus significantly contributing to climate change mitigation [2]. This is particularly true for soil organic carbon (SOC) sequestration in soil horizons below 20 cm depth (subsoil), which are not directly subjected to management practices, but have higher potential to sequester OC and N than that above 20 cm depth (topsoil) due to its lower OC and N contents and less exposure to disturbance [3].

Numerous studies have confirmed that long-term single or combined application of synthetic fertilizers and organic manure is the most effective way, among all management practices, to increase soil OC and N sequestration and crop yield [4–6]. Synthetic fertilizers have higher nutrient contents than organic ones but may lead to a series of agro-environmental problems, such as soil acidification, soil degradation, and potential environmental pollution, especially in circumstances of overuse of synthetic N fertilizer [7,8]. Although organic manure is more beneficial for improving soil quality and enhancing SOC, it contains relatively lower nutrients and is too slow in nutrient release to timely meet the needs of crop production as compared with the synthetic fertilizers [9]. To comprehensively take advantage of synthetic N fertilizer and organic manure to either rapidly supply nutrients or improve soil OC, synthetic N fertilizer can be partially replaced with organic manure to maintain the N rate at a recommended level [10]. This practice, also called organic substitution, has increasingly attracted the attention of farmers, stakeholders, and government officials [10]. It has been widely adopted as a judicious management strategy of cropland, where applicable, in China for achieving high crop production while simultaneously reducing the use of synthetic N fertilizer and recycling livestock waste [10,11]. Previous studies have reported the impact of organic substitution on SOC, TN, and crop responses. However, these studies have mainly focused on individual sites or on the topsoil (plough layer soil), which is most directly affected by agricultural practices [12–14]. The sequestration potential of OC and N in subsoil has mostly been neglected, even though it holds ca. 30% to 75% of the total OC and N stocks of the terrestrial surface [15,16]. Moreover, studies have shown that fertilization significantly affects OC and N down to a soil depth of 90 cm [16–19]. For example, Ghosh et al. [17,18] found that synthetic fertilizers combined with manure markedly increased OC and N accumulation at a soil depth of 30–90 cm. In contrast, some studies have also shown that fertilization with synthetic fertilizers and manure causes a decrease in subsoil OC and TN [16,19,20]. However, information on how and to what extent organic substitution may influence organic OC and TN in subsoil, as well as crop yield, is limited. Therefore, it is necessary to further quantify the impact of organic substitution on OC and TN in subsoil, and on crop productivity as well over a longer time scale. Such information will be essential for evaluating the potential of organic substitution in mitigating agro-environmental problems.

Based on short-term experiments or meta-analyses, many studies have reported that OC and TN in plough layer soils profoundly influenced crop production and its sustainability [21–23]. However, subsoil contains more than 30% of the total biomass of crop roots over a 0–100 cm soil depth [15]. Apart from that, it also stores a large amount of plant nutrients, which are reportedly very important in maintaining and improving crop yield in later stages of crop growth [1]. Therefore, crop roots extended to the subsoils may not only affect crop yield through the uptake of nutrients stored there, but also change the subsoil OC and TN pool via the processes of root growth and death, thus possibly eventually influencing the relationship between crop production and the sequestration of OC and TN [15,24]. However, the responses of crop productivity to the subsoil's OC and TN accumulation under long-term fertilization regimes are not well-understood.

Upland fields account for approximately 70% of all land under cultivation in China, and usually have low levels of OC and TN; thus, they may have high potential to sequester C and N [25]. Increasing OC and N sequestration and improving crop yield in upland regions could help to cope with the challenges of food security and environmental sustainability in China [26]. In the present study, we analyzed the archived soil profile samples (0–100 cm) that had been subjected to 23 years of various fertilization treatments at four contrasting upland sites in China. The objectives of this study were to (1) investigate the impact of fertilization regimes, especially partial substitution of synthetic N fertilizer with organic manure, on OC and TN over a soil depth of 0–100 cm as well as on crop productivity, and (2) study the influence of OC and TN accumulation in the soil to a depth of 100 cm on nutrient uptake and crop productivity. We hypothesized that (1) compared with synthetic fertilizer alone and with straw, partial substitution of synthetic N fertilizer with manure is more conducive to OC and TN accumulation and crop growth by potentially introducing a high amount of exogenous C input and reducing mineral N loss and would gain the greatest SOC and TN sequestrations as well as crop productivity; (2) OC and N sequestration across soil profile should facilitate the synergistic improvement of crop yield and its sustainability by promoting nutrient uptake and improving the formation and stability of soil aggregates.

2. Materials and Methods

2.1. Site Descriptions

To comprehensively assess the impact of fertilization on OC and N sequestration and crop productivity in upland areas, we selected four long-term field experiment sites which both belong to the National Network of Soil Fertility and Fertilizer Efficiency Monitoring, namely the Qiyang, Yangling, Zhengzhou, and Gongzhuling sites. Those experiment sites were distributed in different climatic zones along a certain precipitation and temperature gradient (Figure 1). Four upland experiments were established in 1990, with a relatively consistent cropping system and similar fertilization treatments. They represent the typical soil and climate conditions of the main wheat and corn producing areas in Northeast, Central, Northwest, and Southwest areas of China. The information on soil, climate, and cropping system for these long-term experimental sites is presented in Table 1. The Ferralic Cambisol soil at Qiyang, developed from Quaternary red clay, was relatively acidic with a topsoil pH (at a soil/water ratio of 1:5) of 5.7 at the beginning of experiment, and had a clay texture [27]. The Cumulic Anthrosol soil at Yangling and the Calcaric Cambisol soil at Zhengzhou, developed from loess and Yellow River alluvium, respectively, were relatively infertile alkaline soils (pH value at ca. 8.5), with silt loam textures [27]. In contrast, the Luvic Phaeozem soil from Gongzhuling, developed from Quaternary loess-like sediment, was a relatively fertile alkaline soil (pH 7.6) with a clay loam texture [27].

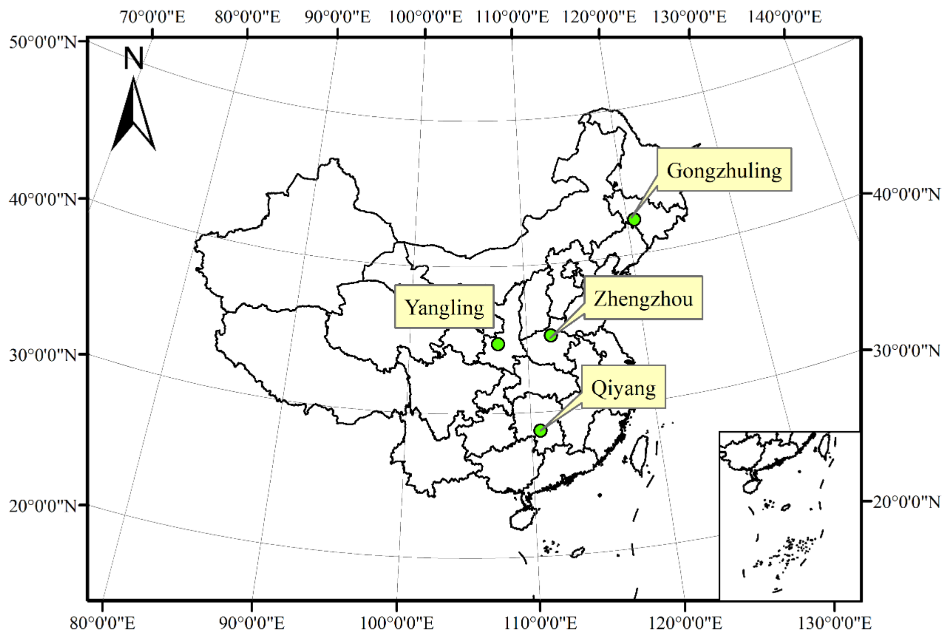

Figure 1. Locations of the long-term field experiments of Qiyang, Yangling, Zhengzhou, and Gongzhuling across China upland.

Table 1. Description of climate, cropping practice, and soil properties at four long-term field experimental sites across China upland.

	Qiyang	Yangling	Zhengzhou	Gongzhuling
Start year	1990	1990	1990	1990
Climate type [a]	ST-H	WT-SH	WT-SH	MT-SH
MAT(°C) [b]	18.0	13.8	14.3	4.5
MAP (mm) [c]	1255	525	632	525
MAE (mm) [d]	1470	993	1450	1400
Cropping [e]	DC-WM	DC-WM	DC-WM	MC-M
Tillage (times, depth)	2, 20 cm	2, 20 cm	2, 20 cm	1, 25 cm
Plot size (m^2)	196	196	43	400
	Initial physical and chemical characteristics, 0–20 cm			
FAO/UNESCO Soil classification	Ferralic Cambisol	Cumulic Anthroso	Calcaric Cambisol	Luvic Phaeozems
Texture (USDA)	light loam	silt loam	silt loam	clay loam
Sand (%)	3.7	31.6	26.5	38.3
Silt (%)	34.9	51.6	60.7	29.9
Clay (%)	61.4	16.8	12.8	31.8
SOC (g kg^{-1}) [f]	8.79	5.68	6.15	13.23
TN (g kg^{-1}) [g]	0.85	0.77	0.60	1.40
BD (g cm^{-3}) [h]	1.19	1.35	1.70	1.19
pH [i]	5.7	8.6	8.3	7.6

Note: [a] ST-H, sub-tropical humid climate; WT-SH, warm-temperate semi-humid climate; MT-SH, mid-latitude temperate semi-humid climate. [b] MAT, mean annual temperature. [c] MAP, mean annual precipitation. [d] MAE, mean annual evaporation. [e] MC-M, monoculture-cropping maize; DC-MW, double-cropping, maize, and wheat. [f] SOC, soil organic carbon. [g] TN, soil total nitrogen. [h] BD, soil bulk density. [i] Soil pH was measured at a soil/water ratio of 1:5 (weight/weight).

2.2. Experimental Design

Four fertilization treatments were applied from 1990 until now at each experimental site: (1) no fertilization (Control, also called CK), (2) synthetic nitrogen (N), phosphorus (P), and potassium (K) (NPK), (3) NPK with 70% of the synthetic N substituted with manure (NPKM), and (4) NPK combined with straw (NPKS). The amount of total N applied in NPK and NPKM treatments was equal (Table 2). The treatment plots were initially randomized with sizes measuring between 43 m^2 and 400 m^2.

Table 2. The annual amount of nitrogen, phosphorus, and potassium applied in synthetic fertilizers and manure/straw at four experimental sites during 1990–2013. The number after the plus sign "+" stands for the quantity of the N, P, or K applied with the incorporation of manure or straw.

Treatments	Qiyang		Yangling		Zhengzhou		Gongzhuling
	Wheat	Maize	Wheat	Maize	Wheat	Maize	Maize
	Nitrogen (synthetic + manure/straw) (kg ha^{-1})						
Control	0	0	0	0	0	0	0
NPK	90	210	165	188	165	188	165
NPKS	90 + 9	210 + 9	165 + 42	188 + 0	123 + 42	188 + 0	112 + 53
NPKM	27 + 63	63 + 147	50 + 115	188 + 0	50 + 115	188 + 0	50 + 115
	Phosphorus (in synthetic + manure/straw) (kg ha^{-1})						
Control	0	0	0	0	0	0	0
NPK	16	37	58	25	36	41	36
NPKS	16 + 1	37 + 1	58 + 4	25 + 0	36 + 8	41 + 0	36 + 6
NPKM	16 + 13	37 + 31	58 + 95	25 + 0	36 + 66	41 + 0	36 + 39
	Potassium (in synthetic + manure/straw) (kg ha^{-1})						
Control	0	0	0	0	0	0	0
NPK	30	70	69	78	68	78	68
NPKS	30 + 17	70 + 17	69 + 57	78 + 0	68 + 86	78 + 0	68 + 58
NPKM	30 + 30	70 + 70	69 + 180	78 + 0	68 + 92	78 + 0	68 + 77

Urea, superphosphate, and potassium chloride/sulfate were used as sources of N, P, and K, respectively. All P and K fertilizers and nearly half of synthetic N fertilizer were applied as basal, and the remaining N fertilizer was applied as top dressing during the growing season at the Zhengzhou and Gongzhuling sites, but all fertilizers were applied as basal at the Qiyang and Yangling sites. Manure was generally incorporated into plough layer soils once a year before wheat sowing at the Yangling and Zhengzhou sites, and before maize sowing at the Gongzhuling site. At the Qiyang site, 30% of it was applied before wheat seeding, the remaining 70% before maize seeding. Organic manure included farmyard manure and excreta from livestock (swine, horse, and dairy manure). For the NPKS treatment, maize straw was incorporated into soil before wheat seeding at Zhengzhou and Yangling and before maize seeding at Gongzhuling. At Qiyang, 50% of the crop residues from the preceding crop (wheat or maize) was incorporated into the soil before the planting of the following crop.

2.3. Cropping and Management Practices

A double cropping system with winter wheat and summer maize, two harvests each year, has been practiced at the Qiyang, Yangling, and Zhengzhou sites, whereas a monoculture cropping system with summer maize has been adopted at the Gongzhuling site. No irrigation was applied at the Qiyang and Gongzhuling sites because of the sufficient precipitation during the growing season of the crops. The plots were irrigated once or twice during winter wheat growing season and twice to 4 times in summer maize growing season when necessary at the Yangling and Zhengzhou sites. The plots were plowed to a depth of 20 cm before winter wheat planting at the Yangling and Zhengzhou sites, and before wheat and maize seeding at the Qiyang site. However, at the Gongzhuling site, the plots were plowed to a depth of 25 cm once every year at maize planting. After crop harvest, straw and grain were air-dried and weighed. Temporal variation of crop yield at those four sites has been reported by Duan et al. [28] and Zhang et al. [29]. Here, we mainly focused on average crop yield and sustainable yield index under the different fertilization treatments.

2.4. Sampling and Analyses

Soil samples were collected down to 100 cm with an increment of 20 cm after maize harvest at four sites using an auger (5 cm inner diameter). Three soil cores were randomly taken in each treatment plot at positions ca. 1.5 m far away from the border. After air-drying and removal of plant residues, soil samples were passed through a 0.25 mm sieve and used for determination of OC and TN with protocols described by Qaswar et al. [30]. Soil bulk density (BD) of the 0–20 and 2040 cm soil layers was measured in situ for each treatment. For BD in each 20 cm layer of soil from 40–100 cm depth under all treatments, the measured value from the protected area at each experimental site was employed to minimize damage to the long-term field experiment. SOC and TN stocks (t ha^{-1}) were determined from soil OC and N concentrations and BD with the method reported by Zhang et al. [31]. The concentrations of N, P, and K in grain and straw were determined with procedures described by Qaswar et al. [30]. Nutrient uptake in above-ground biomass in each plot was estimated from plant nutrient concentration (%), straw biomass (t ha^{-1}), and grain yield (t ha^{-1}).

2.5. Calculations

Relative changes of OC and TN stocks (%) in the soil profile after 23 years of fertilization were calculated with Equations (1) and (2) [32]:

$$\text{Relative change of SOC} = (\text{SOC}_t - \text{SOC}_0)/\text{SOC}_0 \tag{1}$$

$$\text{Relative change of TN} = (\text{TN}_t - \text{TN}_0)/\text{TN}_0 \tag{2}$$

where SOC_0 (TN_0) and SOC_t (TN_t) are SOC (TN) stocks (t ha^{-1}) in 1990 and 2013, respectively.

Carbon sequestration efficiency (*CSE*, %) in the 0–100 cm soil profiles during the study period was calculated with Equations (3) and (4):

$$CSR = (SOC_t - SOC_0)/t \tag{3}$$

$$CSE = CSR/C_{input} \tag{4}$$

where CSR is OC sequestration rate in the 0–100 cm soil profile (t ha^{-1} yr^{-1}), and SOC_0 and SOC_t are OC stocks in the 0–100 cm soil profile (t ha^{-1}) as given above. Here, t is the duration of experiments (yr), and C_{input} is the annual amount of OC input in the 0–100 cm soil profile during the study period (t ha^{-1} yr^{-1}). C_{input} derived from crop residues (roots and stubble) and organic amendments (manure or straw) for a given treatment/plot were estimated with the method described by Zhang et al. [32]. The annual amount of crop root-derived OC input in each 20 cm soil layer was estimated with the distribution proportion of crop root biomass in the soil profile down to 100 cm depth reported by Jobbagy and Jackson [15].

Crop yield in absolute terms could not be directly compared across fertilization treatments and experimental sites because fertilization treatments were not fully inconsistent across the four sites and these four experimental sites were in different regions with varying climates. Relative crop yield (*RCY*) was calculated using Equation (5) to eliminate such influences [33].

$$RCY = Y_t/Y_{max} \tag{5}$$

Here, Y_t is the crop yield of a specific treatment (t ha^{-1}) in a specific year, and Y_{max} is the maximum yield at a particular experimental site (t ha^{-1}) in the same year.

The sustainable yield index (*SYI*), a quantitative measure used to assess the sustainability of agricultural practices [21], is estimated with Equation (6):

$$SYI = (Y_{mean} - \sigma)/Y_{max} \tag{6}$$

where Y_{mean} and Y_{max} are the mean and maximum yields of a specific treatment (t ha^{-1}), respectively, and σ is the standard deviation of crop yield for a particular treatment.

Crop nutrient uptake (kg ha^{-1}) is calculated with Equation (7):

$$\text{Crop nutrient uptake} = \text{Grain}_{yield} \times \text{nutrient}_{grain} + \text{Straw}_{yield} \times \text{nutrient}_{straw} \tag{7}$$

Here, Grain_{yield} and Straw_{yield} are the average yields of grain and straw during 1990–2013 (kg ha^{-1}), respectively, and nutrient_{grain} and nutrient_{straw} are the nutrient concentrations of grain and straw (%), respectively.

2.6. Statistical Analyses

Statistical analysis was conducted using IBM SPSS Statistics 22.0 (IBM Corp, Armonk, NY, USA). Analysis of variance was used to determine the effects of various fertilization treatments, soil layers, sites, and their interaction on all parameters at all four sites. Significant differences ($p < 0.05$) among various fertilization treatments, soil layers, sites were assessed using the least significant difference test for all parameters. Linear regression was used to determine the relationships between OC and TN stocks in different soil layers and between change in OC (or in TN) stocks in topsoil and the entire 100 cm profile. The double linear functions were performed to analysis the relationships between OC and TN stocks in 0–100 cm soil and crop N uptakes, relative crop yield, and SYI.

3. Results

3.1. Soil OC and TN Concentrations and Their Stocks Change in Soil Profile

Soil OC and TN concentrations tended to decrease with increasing soil depth at all sites irrespective of treatment (Figures 2 and 3). For different treatments, the 23 years of fertilization and cropping significantly modified SOC and TN concentrations as compared with the Control treatment (CK) in soils of most horizons of the soil profile, especially upper layers, at all four sites. Among them, soil OC and TN concentrations were significantly higher in the NPKM treatment than the other three treatments in the 0–20 cm layer at all sites and in 20–40 cm layers at the Zhengzhou and Gongzhuling sites. In soils below 0–20 cm at the Qiyang and Yangling sites and below 20–40 cm at the other two sites, both OC and TN largely showed no difference between treatments (Figures 2 and 3).

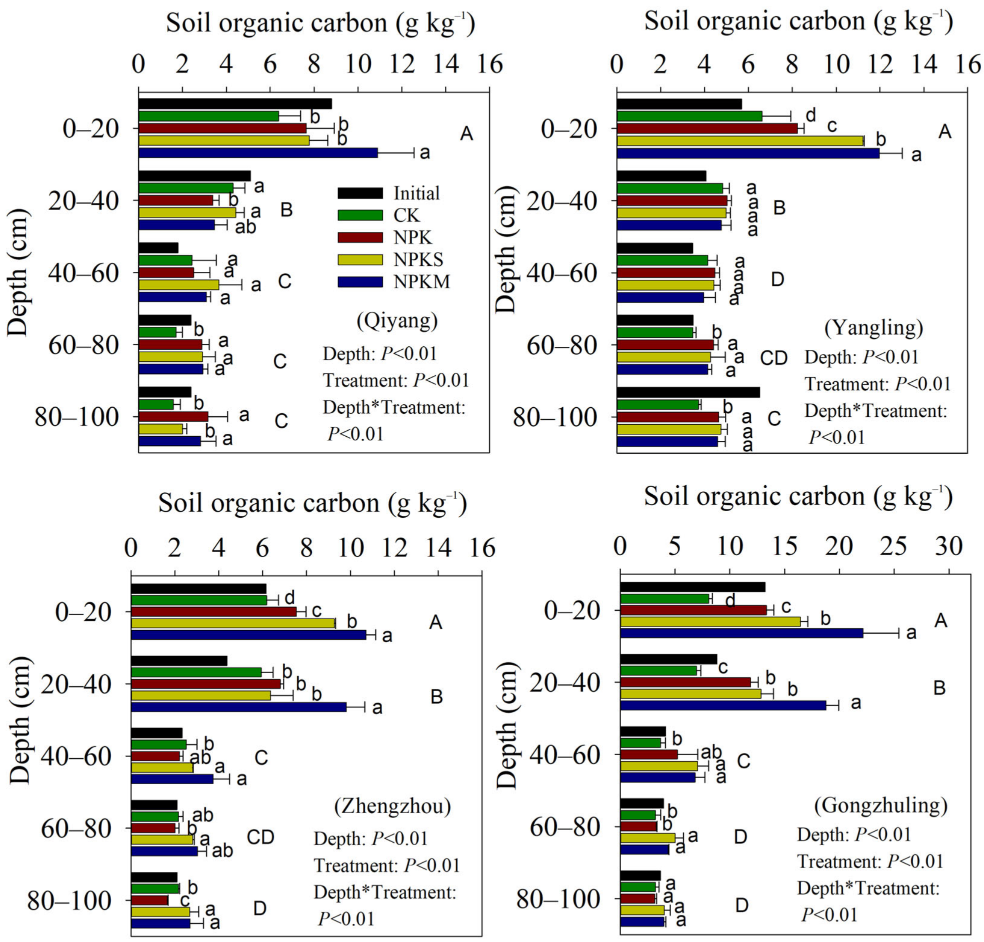

Figure 2. Distributions of soil organic carbon in soil profiles down to 100 cm subjected to fertilization treatments in the Qiyang, Yangling, Zhengzhou, and Gongzhuling experimental sites. Bars are standard errors (n = 3). Different lowercase and uppercase letters indicate significant differences between treatments and soil layer ($p < 0.05$), respectively.

Compared with the initial values, soil OC concentrations of CK treatment decreased in most soil horizons at the Qiyang and Gongzhuling sites and increased in the 0–20 cm and 20–40 cm soil layers at the Yangling and Zhengzhou sites (Figure 2). However, soil TN concentrations under CK treatment increased in 0–80 cm soil layers at the Yangling site and decreased in 40–100 cm soil layers at the Zhengzhou and Gongzhuling sites relative to the corresponding initial values (Figure 3). NPK and NPKS treatments increased soil OC and TN concentrations over initial values to different degrees in the 40–60 and 60–80 cm soil layers at the Qiyang site and in the 0–20 and 20–40 cm layers at the Yangling and

Zhengzhou sites (Figures 2 and 3). However, the NPKM treatment largely increased OC and TN concentrations in most soil layers across all sites compared with the initial values, particularly within the 40–60, 60–80, and 80–100 cm layers at the Qiyang site and within the 0–20, 20–40, and 40–60 cm layers at the other three sites (Figures 2 and 3).

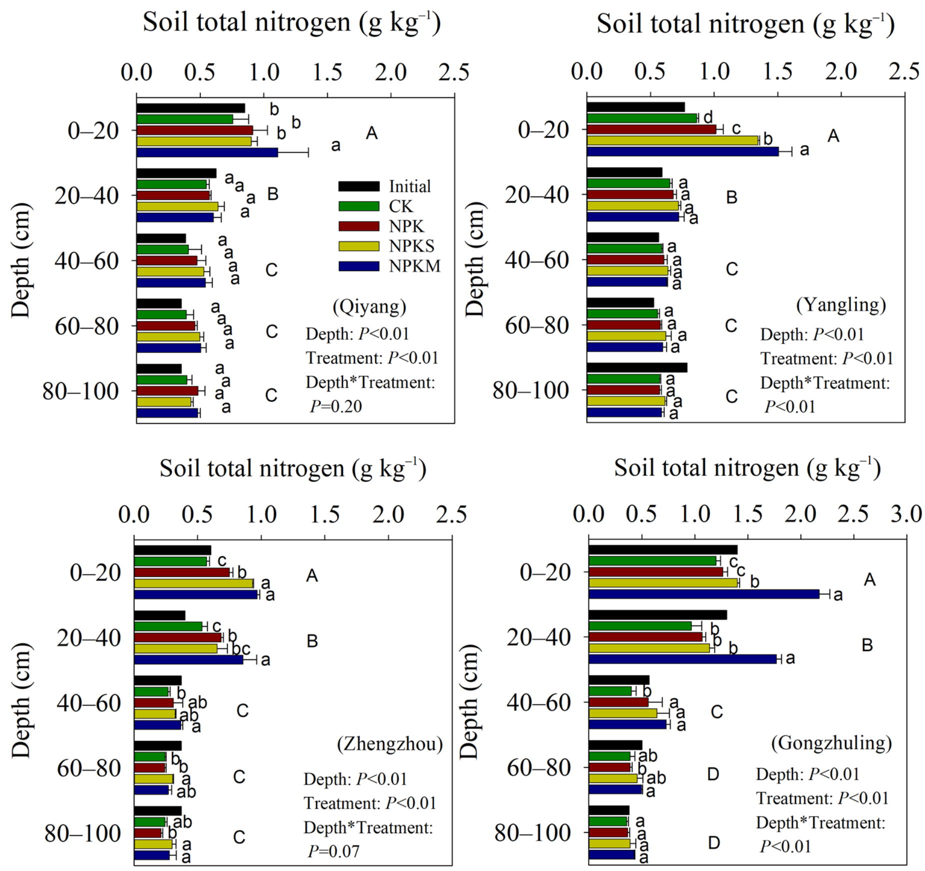

Figure 3. Distributions of soil total nitrogen in soil profiles down to 100 cm subjected to fertilization treatments in the Qiyang, Yangling, Zhengzhou, and Gongzhuling experimental sites. Bars are standard errors (n = 3). Different lowercase and uppercase letters indicate significant differences between treatments and soil layer ($p < 0.05$), respectively.

Meanwhile, changes in OC and TN concentrations inevitably lead to differences in their stocks. Overall, compared to the corresponding initial values, 23 years of fertilization modified OC stocks in topsoil (0–20 cm), subsoil (20–100 cm), and the entire 100 cm soil profile to varying degrees at the four sites (Figure 4a–d). Compared with the initial value, OC stocks in the entire 100 cm soil profile showed a decreasing trend under the CK treatment at all sites (Figure 4), especially at the Qiyang and Gongzhuling sites (decreased by 12.4% and 22.4%, respectively). The addition of NPK enhanced OC stocks in the entire 100 cm soil profile to some extent. The application of synthetic NPK fertilizers in combination with organic supplements (NPKS and MNPK), especially organic manure, further markedly ($p < 0.05$) increased OC stocks in the 0–100 cm soil profile at almost all sites relative to the initial values, of which NPKM increased OC stocks by 25.6–103.8%, 2.9–71.3%, and 26.4–66.8% in topsoil (0–20 cm), subsoil (20–100 cm), and the entire 100 cm soil profile relative to the corresponding initial values, respectively, across all sites. NPKM treatment substantially improved OC stocks of the whole soil profile by 1.7–3.0 and 5.3–12.0 times as much as that of NPKS and NPK treatments, respectively, at all sites except for Yangling (Figure 4a,c,d). Moreover, the increase in OC stock in the 0–100 cm soil profile receiving NPKS was notably greater than that of NPK at all sites except for Qiyang (Figure 4b–d). The increases in SOC stock receiving NPKM in topsoil and subsoil were significantly greater

($p < 0.05$) than those receiving NPK and NPKS in topsoil at all sites and in subsoil at the Zhengzhou and Gongzhuling sites, respectively.

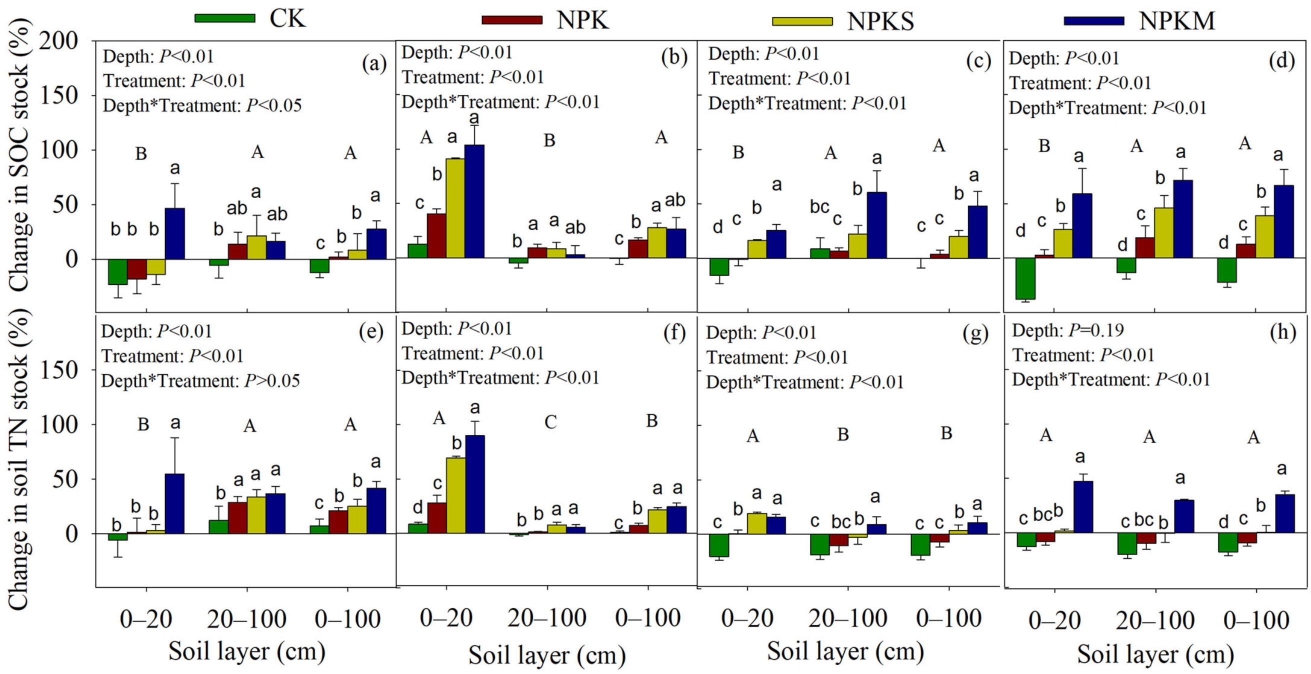

Figure 4. Changes (%) in soil organic carbon (SOC) and total nitrogen stocks (TN) in soils of 0–20 cm, 20–100 cm, and 0–100 cm under different treatments relative to the corresponding initial values at the Qiyang (**a**,**e**), Yangling (**b**,**f**), Zhengzhou (**c**,**g**), and Gongzhuling (**d**,**h**) experimental sites. Bars are standard errors ($n = 3$). Different lowercase and uppercase letters indicate significant differences between treatments and soil layer ($p < 0.05$), respectively.

Under long-term CK and NPK treatment, TN stock in the entire 100 cm profile increased at the Qiyang site, maintained at the Yangling site, but depleted at the Zhengzhou and Gongzhuling sites (Figure 4). Compared to the initial value, NPKS treatment increased TN stocks by 25.3 and 21.7% in 0–100 cm soil, respectively, at the Qiyang and Yangling sites (Figure 4e,f), but kept it at the same level at the Zhengzhou and Gongzhuling sites (Figure 4g,h). Under the NPKM treatment, TN stocks increased by 15.8–89.8, 5.7–36.9, and 10.8–41.9% in topsoil, subsoil, and the entire 100 cm soil profile, respectively, over their respective initial values, across sites. As for treatment effects, the increase in TN stocks in the entire 100 cm soil profile under NPKM was significantly higher ($p < 0.05$) than that of NPK at all sites, and that of the NPKS at sites except for Yangling, where they were at the same level. Moreover, the increase in TN stocks in the 0–100 cm soil profile receiving the NPKS was significantly higher than that receiving NPK at sites except for Qiyang (Figure 4f–h). The increases in TN stock of topsoil and subsoil receiving NPKM were significantly greater ($p < 0.05$) than those of NPK and NPKS in topsoil at sites except for Zhengzhou, and in subsoil at the Zhengzhou and Gongzhuling sites, respectively.

Significant and positive linear correlations ($p < 0.05$) were observed between the change in OC and TN stocks in topsoil, subsoil, and the entire 100 cm soil profile (Supplementary Figure S1), with slopes of 10.0, 4.9, and 7.0, respectively. Further, there were also significant and positive linear correlations ($p < 0.05$) between the change in OC stock in topsoil and that in the entire 100 cm soil profile, and a similar result was also observed for TN, with slope values of 0.39 and 0.37, respectively (Supplementary Figure S2).

3.2. OC Input and SOC Sequestration Efficiency in the Entire 100 cm Soil Profile

Overall, except for the Qiyang site, where there was no significant difference between NPK and NPKS, the OC input into the soils was found in the overall order of NPKS and

NPKM > NPK > CK at four experiment sites (Table 3). At the Qiyang and Yangling sites, OC input for NPKM treatment was 1.6–5.2 and 1.1–3.5 times, respectively, as much as that for treatments receiving NPK and NPKS. However, the NPKS treatment received ca. 2.0–2.3 and 1.2–1.6 times that of OC inputs, compared to that of NPK and NPKM treatments at the Zhengzhou and Gongzhuling sites.

Table 3. Mean annual carbon input (C_{input}) and carbon sequestration efficiency (CSE) in soil profile down to 100 cm during 1990–2013 under different treatments at four experimental sites.

	Treatments	Experimental Sites			
		Qiyang	Yangling	Zhengzhou	Gongzhuling
C_{input} (t ha^{-1} yr^{-1})	Control	0.4 cB	1.2 cA	1.4 dA	1.1 cA
	NPK	1.4 bB	3.8 bA	4.1 cA	2.8 bA
	NPKS	2.1 bC	5.7 aB	9.8 aA	5.7 aB
	NPKM	7.1 aA	6.1 aAB	6.1 bAB	4.7 aB
CSE (%) [a]	Control	−71.1 bB	−1.2 bA	−0.1 bA	−67.6 cB
	NPK	3.9 aB	12.6 aA	2.3 bB	16.7 bA
	NPKS	10.1 aAB	14.0 aAB	5.4 bB	25.4 bA
	NPKM	9.5 aB	12.4 aB	20.0 aB	53.4 aA

Note: [a] For the CSE, the negative value indicates a net loss of organic carbon. Value presented as mean (*n* = 23 and *n* = 3 for C_{input} and CSE, respectively). Different lowercase letters indicate significant differences between treatments at the same experimental site ($p < 0.05$). Different uppercase letters indicate significant differences between experimental sites for the same treatment ($p < 0.05$).

Carbon sequestration efficiency (CSE) for the entire 100 cm soil profile showed a negative value under CK treatment, in sharp contrast to treatments receiving fertilizers, where it was positive at all sites (Table 3). The CSE for NPKM treatment was significantly higher than that of NPK and NPKS treatments at the Zhengzhou and Gongzhuling sites. However, there were no significant differences in CSE between fertilized treatments at the Qiyang and Yangling sites. As for sites, the CSE for CK was significantly higher at the Yangling and Zhengzhou sites than that of the Qiyang and Gongzhuling sites. Moreover, CSE values for NPK and NPKS treatments were considerably greater at the Gongzhuling site than the Zhenzhou site, and the CSE for MNPK was 2.7–5.6 times as much at the Gongzhuling site as that of the other three sites.

3.3. Crop Productivity and Its Relationship with Soil OC and TN Stocks in Soil Profile

Compared with the control, fertilization (NPK, NPKS, and NPKM) significantly increased the average yields of maize and wheat by 1.6–13.0 and 1.4–2.7 times, respectively, at all sites (Table 4). However, there were no significant differences in crop yield between NPK and NPKS treatments at any site. NPKM treatment significantly ($p < 0.05$) enhanced crop yield only at the Qiyang site relative to the NPK and NPKS treatments. Compared with the control, fertilization promoted the SYI of maize by 16–340% across sites, and of wheat by 24–135% at the Yangling and Zhengzhou sites, but decreased the SYI of wheat by 46% at the Qiyang site (Table 4). Fertilization also significantly ($p < 0.05$) increased average N uptake by 2.6–5.6 times at all sites. Crop N uptakes were significantly ($p < 0.05$) greater under NPKM than NPKS treatment at the Qiyang and Gongzhuling sites.

Significant exponential correlations ($p < 0.05$) were observed between OC stocks in topsoil, subsoil, or the entire 100 cm soil profile and N uptake in aboveground biomass (Figure 5 panel a–c), relative crop yield (Figure 5 panel d–f), and SYI (Figure 5 panel g–i). The exponential correlations revealed that the SOC stocks in topsoil, subsoil, and the entire 100 cm soil profile explained 73%, 36%, and 51% of the variation in N uptake (R^2 = 0.73, 0.34, and 0.35, respectively); 87%, 40%, and 65% of the variation in relative crop yield (R^2 = 0.87, 0.40, and 0.65, respectively); and 65%, 35%, and 51% of the variation in SYI (R^2 = 0.65, 0.35, and 0.51, respectively), respectively. However, for the TN stocks in the soil profile, significant exponential correlations ($p < 0.05$) were only observed between topsoil TN stock and N uptake in aboveground biomass (Supplementary Figure S3a), relative crop yield (Supplementary Figure S3d), and SYI (Supplementary Figure S3g). The exponential correlations showed that TN stock in topsoil explained 29%, 46%, and 22% of the variation in N uptake (R^2 = 0.29), relative crop yield (R^2 = 0.46), and SYI (R^2 = 0.22), respectively.

Table 4. The mean crop yield, sustainable yield index (SYI), crop nitrogen (N) uptakes during 1990–2013 under different treatments at four experimental sites. The values are presented as mean± standard error (*n* = 23). Different lowercase letters indicate significant differences between treatments at the same experimental site ($p < 0.05$).

Sites	Treatments	Mean Yield (t ha^{-1})		SYI		Crop N Uptake (kg ha^{-1})
		Maize	Wheat	Maize	Wheat	
Qiyang	Control	0.27 ± 0.21 c	0.36 ± 0.13 c	0.08	0.38	19 ± 4.0 c
	NPK	2.88 ± 1.53 b	1.06 ± 0.61 b	0.24	0.19	95 ± 39 b
	NPKS	3.37 ± 1.67 b	1.19 ± 0.63 b	0.26	0.21	113 ± 44 b
	NPKM	5.11 ± 1.09 a	1.77 ± 0.75 a	0.56	0.22	166 ± 16 a
Yangling	Control	2.25 ± 0.49 b	1.08 ± 0.53 b	0.50	0.19	73 ± 10 b
	NPK	6.11 ± 1.00 a	5.56 ± 1.38 a	0.60	0.48	283 ± 33 a
	NPKS	6.51 ± 1.02 a	5.73 ± 1.44 a	0.62	0.45	303 ± 39 a
	NPKM	6.63 ± 1.15 a	5.82 ± 1.66 a	0.56	0.41	288 ± 38 a
Zhengzhou	Control	1.75 ± 0.42 b	3.02 ± 0.98 b	0.41	0.51	82 ± 11 b
	NPK	6.44 ± 1.02 a	6.96 ± 1.95 a	0.45	0.62	298 ± 34 a
	NPKS	6.33 ± 0.92 a	7.55 ± 1.90 a	0.49	0.64	300 ± 30 a
	NPKM	6.04 ± 0.96 a	7.21 ± 1.86 a	0.49	0.64	283 ± 35 a
Gongzhuling	Control	3.53 ± 1.10 b	-	0.37	-	61 ± 12 c
	NPK	9.13 ± 1.63 a	-	0.63	-	233 ± 36 a
	NPKS	9.15 ± 1.37 a	-	0.65	-	200 ± 25 b
	NPKM	9.23 ± 1.76 a	-	0.63	-	239 ± 39 a

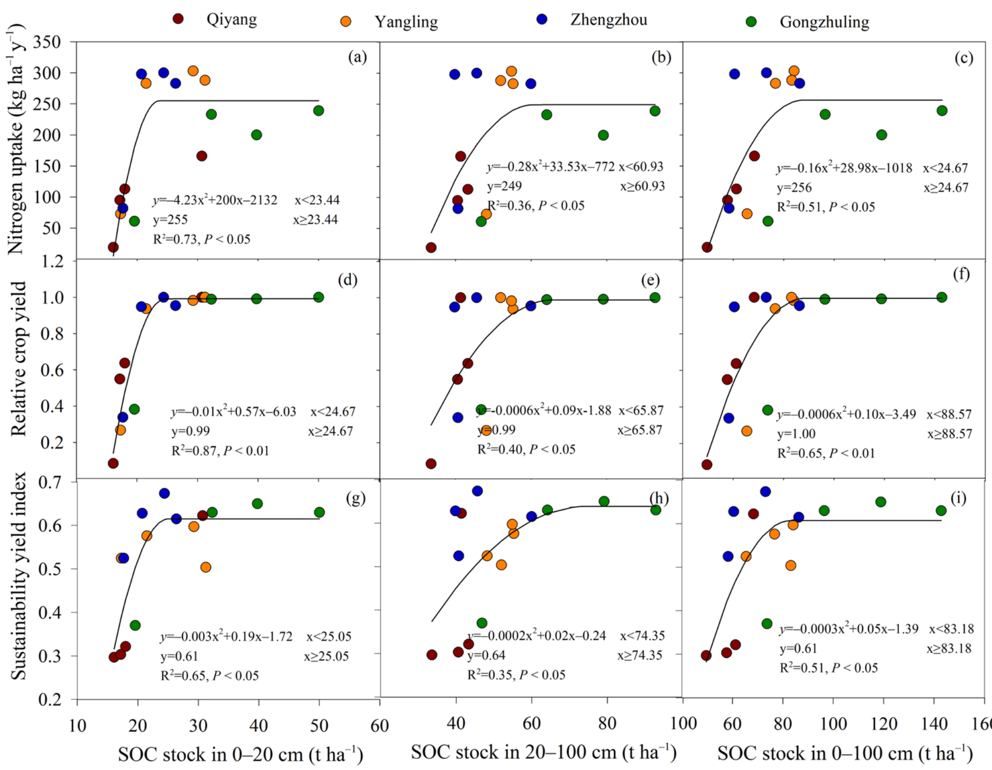

Figure 5. Relationships between soil organic carbon (SOC) stock in 0–20 cm, 20–100 cm, and 0–100 cm soil horizons and crop nitrogen uptake (**a**–**c**), relative crop yield (**d**–**f**), and sustainable yield index (**g**–**i**) during the experiment period of 1990–2013 at four experimental sites (*n* = 16).

4. Discussion

4.1. Impact of Fertilization and Cropping on SOC and TN

Soil OC and N dynamics in arable land reflected the balance between the input of substances containing OC and N, such as crop residues, root exudates, and synthetic fertilizers and organic supplements, and the losses of OC and N, such as soil organic matter (SOM) decomposition and crop harvest [34]. Our results demonstrated that SOC and TN stocks of CK plots has a decreasing trend in the entire 100 cm soil profile at almost all sites except for the Qiyang and Yangling sites, where TN stock slightly increase. This result suggested that soils may act as OC and N sources due to continuous crop harvest without fertilization [29]. Although the decreases in OC and N contents mostly occurred in the topsoil, it also occurred at soil horizons below 60 cm at some sites, i.e., Qiyang, Zhengzhou, and Gongzhuling sites. This implies that the long-term cropping without fertilization not only resulted in a loss of C and N from the topsoil, but also aggravated the depletion of subsoil C and N. This may be because the OC and N inputs from crop residues and root exudates were not sufficient to offset their losses as a result of frequent tillage and crop harvest under such conditions as soil acidification and high primary crop productivity [35]. Our study also found that NPK treatment improved OC stocks in the entire 100 cm profile, which is in line with the findings of previous studies [17,18,20,36]. This is conceivable because balanced application of synthetic N, P, and K fertilizers can considerably increase crop growth and thus OC inputs derived from it [21]. Moreover, fertilizer application slightly increased TN stocks in 0–100 cm at the Qiyang and Yangling sites and decreased TN stock at other two sites. The former might be explained by the relatively low N consumption as a result of soil acidification and lower initial soil fertility at the Qiyang and Yangling sites, respectively. The latter might be due to the high crop production, and thus greater N uptake by aboveground biomass than that applied from exogenous synthetic N at the Zhengzhou and Gongzhuling sites.

The incorporation of NPKM enhanced SOC and TN stocks at all sites could be ascribed to the higher OC inputs, either from organic supplements/manure or crop residues [21,34]. Fertilization can raise the OC and TN contents to soil depths of 40–100 cm at the Qiyang site in the sub-tropical area, and in contrast to that of the 0–60 cm soil layer at the other three sites with a semi-humid climate. On the one hand, this difference might be the result of strong leaching of C and N, in dissolved or particulate forms, caused by heavy rainfall at the Qiyang site [3,37]. On the other hand, in the acidified soil at the Qiyang site, high contents and availability of soil P from manure application could promote the growth and extension of crop roots [38]. The roots residues and exudate have high CSE due to enter the soil directly at the scale of physically protected OC inside aggregates and low SOM decomposition rate in a state of oxygen deficiency, providing a positive effect on subsoil OC and N accumulation [3,39].

In our case, the combination of NPK with organic supplements, specifically organic manure (MNPK), exhibited a greater increase in OC and TN stocks in the 0–100 cm soil profile relative to NPK at sites except for Qiyang. This may be attributable to the direct additional OC and N input and indirect C and N contribution derived from the promoted crop growth [29]. Similar results have been reported in previous studies carried out on topsoil [12,13]. Apart from what is outlined above, amendment of manure could reduce N loss through replacing easily leachable N that is otherwise in synthetic N fertilizer with manure in organic forms, thus improving N use efficiency [28,40]. In addition, amendment of manure could also help to enhance the physical–chemical protection of OC and N from decomposition in soil aggregates [41,42]. The decrease in crop productivity caused by severe soil acidification under NPK and NPKS treatments may be the main reason for the non-significant differences in OC and TN pools at the Qiyang site between these two treatments [27,43]. In the present study, for treatments with organic supplements added, NPKM showed a more significant effect on OC and TN sequestration over NPKS at all sites except for Yangling. A possible reason for this is that manure boosted the OC and N input from crop residues and root exudates by introducing more additional nutrients, which improved crop growth [44]. Further, at the Qiyang site, NPKM may promote crop growth and OC inputs derived from it via alleviating soil acidification [27,43]. However, for the Yangling site with deep soil layers and silt loam textures, no significant difference in OC and N sequestration between NPKS and NPKM may be due to high crop productivity, thus the greater consumption of subsoil OC and nutrients under NPKM treatment.

In the present study, we observed negative CSE values under CK treatments based on the entire 100 cm soil, but positive values under fertilization treatments at all sites. These results also confirm our previous findings that upland soil may serve as a source of OC under long-term cropping without fertilization, but may act as a sink under fertilization. In addition, NPKM treatment also resulted in a significantly higher CSE of 0–100 cm at the Gongzhuling site of mid-latitude temperate relative to the other three sites (Table 3). This may be attributed to the lower temperature there, which reduces the utilization of soil microorganisms to exogenous OC and then releases less CO_2 under an equal amount of exogenous OC input, thereby increasing the amount of exogenous OC converted into SOC [45]. Moreover, the less exposure to disturbance derived from tillage and cropping practices under a monoculture cropping system was also a possible reason for high CSE at the Gongzhuling sites.

4.2. Relationships between Changes in OC and TN Stocks in Different Soil Layers

In cropland soil, the soil OC and N dynamics in the upper soil layers are mainly due to fertilizer application (synthetic fertilizers and manure) and crop biomass return. However, OC and N in soils at deeper horizons may originate from the downward movement of the sources in topsoil in dissolved or particulate forms, directly from crop roots extended to there and the root exudates [3]. Our results showed significant and positive linear correlations ($p < 0.05$) between the change in OC stock in topsoil and that in the entire 100 cm soil profile, and a similar result was also observed for TN, with slope values of 0.39 and 0.37. These results of the change in OC and TN stocks in topsoil only accounted for 39% and 37% of their changes in the entire 100 cm soil profile, respectively. Therefore, OC and TN stocks sequestered in subsoil may be beyond the quantity in topsoil provided that the time span of fertilization is long enough, i.e., 23 years, which highlights the greater potential of subsoil to sequester OC and N in upland fields under long-term fertilization.

Soil N is considered to be a key factor influencing long-term OC sequestration [46], especially before it reaches saturation [41]. In the present study, significant correlations were observed between changes in OC and TN stocks in topsoil, subsoil, and the entire soil profile, implying that SOC dynamics were closely associated with TN dynamics. In upland soils, any increase in soil N may help to reduce N limitation to microorganisms, thereby enhancing OC sequestration [46]. In the present study, soil N accumulation in subsoil was greater than that in topsoil with equal amounts of OC sequestration, which was evidenced by a small slope value of 4.94, against that of 10.11 for topsoil, of the linear equation between changes in OC and TN stocks in subsoil (Supplementary Figure S1). Xiao et al. [47] observed similar results in restored and natural grasslands. The results imply that more attention should be paid to subsoil N accumulation enrichment under long-term fertilization regimes, since it could pose a considerable environmental risk [48].

4.3. Responses of Crop Productivity to OC Accumulation in Soil Profile

The significant effect of NPKM over NPK and SNPK on crop yield and N uptake increase was observed only at the Qiyang site, and there were no significant differences in crop yield among these three treatments at the other sites. This result indicated that the soils receiving 23 years of balanced N, P, and K fertilizers can provide sufficient nutrients and a favorable soil environment for crop growth [21,23]. The further beneficial effects of organic manure at the Qiyang site may be due to the fact that the amendment of organic manure could help to curtail soil acidification via decreasing exchangeable acidity and increasing soil exchangeable base cations, and thereby minimizing the adverse effects of it on crop growth [27,43]. The results mentioned above can also be clarified by SYI. A high SYI suggests a capacity to sustain high crop production over time, whereas a low SYI implies greater susceptibility to biotic and abiotic stress [49]. Our results showed that all treatments receiving NPK, either alone or in combination with organic manure, increased SYI of both maize and wheat at all experimental sites except for Qiyang, where MNPK markedly enhanced only the SYI of maize. This is most likely due to more than two-thirds of organic manure being applied during maize season in the NPKM treatment at the Qiyang site, and the difference of tolerance threshold to the stress of pH for maize and wheat [50].

In our study, both the relative crop yield and SYI exponentially increased with increasing OC stock in topsoil, subsoil, and the entire 100 cm soil profile. Previous studies have also demonstrated that topsoil OC is significantly correlated with crop yield and SYI [21,22]. The increase in SOC substantially improves crop production and SYI may mostly rely on the additional supply of nutrients through its decomposition and its significant effects of enhancing soil water retention and nutrient capacity, improving the biotic and abiotic environments in different soil horizons where crops grow [2,22,42]. Furthermore, our results also showed that the OC accumulation in different soil layers promoted crop N and P uptake (Figure 4 and Supplementary Figure S4). Jiang et al. [51] found that soil fertility improvement, as indicated by OC accumulation, may promote the predation of soil biota (e.g., protists and nematodes) and the activity of soil beneficial microorganism (e.g., arbuscular mycorrhizal fungi), thus enhancing P uptake and transport in crop roots through improving the expression of the P transporter gene (ZMPht1;6). Therefore, the accumulation of OC in the soil profile could facilitate the uptake and utilization of nutrients from both the topsoil and subsoil and eventually promote crop productivity. Our results suggested that the relative crop yield and SYI had a significant exponential correlation with topsoil N accumulation rather than subsoil N enrichment. This can be ascribed to the fact that most of the crop roots were distributed in the topsoil, and on the other hand, sufficient nutrients were supplied from fertilization; therefore, the contribution of the nutrients in subsoils has been masked to a relatively small extent [15,16]. The observed smaller C/N ratio (4.94) in the subsoil in our case may also indicate that subsoil has a relatively abundant N supply due to the long-term excessive N application, which results in an insensitive response of crop productivity to subsoil N accumulation.

4.4. Limitations and Implications of This Study

The substitution rate of synthetic N fertilizer with N from organic supplements in the present study was 70% at all four experimental sites. However, some previous studies have reported that substitution rates could influence the size and direction of the effects of organic substitution practices on OC, N, and crop yield [12–14,52]. Moreover, high rates of organic supplement substitution for synthetic N may result in the accumulation of phosphorus, heavy metals, and antibiotics, which may pose a threat to soil and environmental quality [53]. Therefore, to minimize environmental risk and maximize crop yield benefits, optimal substitution rates of organic supplements for synthetic N fertilizer should be further explored in future studies. In addition, there were slight differences in crop management and the protocol of synthetic fertilizer, manure, and straw application between different sites in this study. Thus, in a few comparisons between different sites, the relative or non-dimensional indicators (relative changes in OC and TN stocks, CSE, RCY) were used to further eliminate the impact of differences in management practices between sites. Meanwhile, further research was needed to determine whether these differences in long-term crop management and fertilization protocol will bias and compromise the results obtained, especially for the relative or non-dimensional indicators.

Despite those inadequacies, our study implied that partial substitution of organic supplements for synthetic N fertilizer could provide a win–win solution for improving soil OC sequestration, thereby achieving desirable crop production, and meanwhile helping to reduce the potential environmental risks caused by the use/overuse of synthetic N fertilizer. At the same time, we should keep in mind that the long-term fertilization may inevitably result in the OC and N enrichment in the subsoil, which accounted for more than 60% of the changes in the entire 100 cm soil profile in our cases. Moreover, it is the OC and N sequestered in both topsoil and subsoil together that improved crop yield and its sustainability. These implications may help to tackle the challenges of food security, climate change, and environmental pollution for intensive cropping systems in upland soils either in China or elsewhere with similar situations. The results also suggested that the subsoil should be taken into consideration when assessing the effects of long-term fertilization on climate change mitigation and food security through C sequestration and N enrichment in upland soils.

5. Conclusions

Our research of the effects of long-term fertilization on soil OC and N demonstrated that compared with synthetic fertilizers alone, synthetic fertilizers combined with crop straw return and 70% substitution of synthetic N with organic N resulted in a higher increase in OC and TN stocks in the entire 100 cm soil profile without compromising crop yield and its sustainability. These results imply that based on reducing the use amount of synthetic N fertilizer in upland areas, adding the appropriate amounts of organic supplements, especially organic manure, can achieve the goal of fertilizing and improving soil while simultaneously solving the resource utilization of agricultural waste and maintaining high crop yield. However, subsoil OC accumulation and N enrichment not only could enhance crop productivity and alleviate climate change, but also may pose a consistent environmental risk, so it is a double-edged sword and should be widely investigated in future studies, especially in upland soils with long-term organic supplement application. In summary, our study provides a basis for N management strategies and sustainable utilization and resource cycle utilization of straw and organic manure under intensive cropping systems in upland soils of China and elsewhere.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agronomy13092381/s1, Figure S1: Relationships between the change in soil organic carbon stock and that of total nitrogen stock in topsoil, subsoil, and the entire soil profile (n = 48); Figure S2: Relationships between the changes in stocks of organic carbon (a), total nitrogen (b) in topsoil and the entire soil profile (n = 48); Figure S3: Relationships between soil total nitrogen (TN) stock in 0–20 cm, 20–100 cm, and 0–100 cm soil horizons, and crop nitrogen uptake (a,d,g), relative crop yield (b,e,h), and sustainable yield index (c,f,i) during the experiment period of 1990–2013 at four experimental sites (n = 16); Figure S4: Relationships between crop phosphorus uptake and soil organic carbon (SOC) stocks in 0–20 cm (a), 20–100 cm (b), and 0–100 cm (c) soil horizons during the experiment period of 1990–2013 at four experimental sites (n = 16).

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