Long-term Fertilization Enhances Soil Carbon Stability by Increase the Ratio of Passive Carbon: Evidence From Four Typical Croplands

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Abstract

Aims Soil organic carbon (SOC) plays an important role in improving soil quality, however how long-term fertilization influences SOC and contrasting active carbon (AC) and passive C (PC) pools at large scale remains unclear. The aim of this study was to examine the effect of long-term fertilization on SOC, including AC and PC, across four typical croplands in China and to explore the potential relationships and mechanism.

Methods We assessed the effect of different fertilization (standard and $1.5 \times$ standard of inorganic fertilizer (NPK) with or without manure (M), with a control for comparison) at soil depths (0-20 cm, 20-40 cm, 40-60 cm) on SOC, AC and PC.

Results We found that SOC, AC and PC increased in the order Control < NPK < NPKM < 1.5NPKM. 1.5NPKM resulted in a significant increase in SOC, AC and PC, of 76.3%, 53.0% and 108.5% respectively across the soil profile (0-60 cm) compared with Control. The response ratio of PC to long-term fertilization was 2.1 times greater than that of AC across four sites on average. In addition, Clay was identified as the most important factor in explaining the response of AC and PC to different fertilization application, respectively.

Conclusions Long-term fertilization enhanced both AC and PC, but the greater response of PC suggests that fertilization application could enhance the stability of carbon and thus the potential of cropland for soil carbon accumulation.

Introduction

Soil organic carbon (SOC) plays a crucial role in determining soil physical, chemical and biological properties, and is also an important indicator of soil quality (Lehmann and Kleber 2015; Srinivasarao et al. 2014). Numerous studies have shown that crop management practices such as fertilization, tillage, cropping frequency and crop rotation influence SOC dynamics (Fan et al. 2018; Ghosh et al. 2018; Sun et al. 2013). As one of the most widespread management practices in croplands, fertilization can have a great impact on SOC storage and cycling (Bhattacharyya et al. 2011; Zhang et al. 2017). Wang et al. (2017), found that 11 years of chemical and manure fertilization can significantly increase SOC content. Majumder et al. (2008) also observed NPK plus organics increased SOC by 24.3% over the control in a 19-year-old rice (*Oryza sativa* L.)–wheat (*Triticum aestivum* L.) cropping system in subtropical India, while the cropping with only NPK fertilization only maintained SOC content. However, in another study, no clear effects of 25 years of inorganic fertilization on the SOC storage were observed in a monoculture maize (*Zea mays* L.) cropping system in temperate north China because enhanced carbon input were offset by greater decay rates of soil C (Dou et al. 2016). Hence, understanding and characterizing the effect of fertilization on SOC accumulation is important for the development of sustainable long-term agriculture.

To better understand SOC accumulation dynamics under long term fertilization, total SOC can be separated as active (AC) and passive C (PC) pools according to stability (Chan et al. 2001). AC

decomposes fast (Wu et al. 2012) and responds rapidly to changes in C supply before detectable change in total SOC, and is considered to be an early indicator of changes in soil quality (Zhang et al. 2018). PC on the other hand is stabilized against further microbial action due to physical protection and chemical recalcitrance. It dominates SOC stores, and can have a residence time of hundreds or thousands of years (Balesdent et al. 2018; Guo et al. 2017). It is, thus, critical for promoting SOC sequestration (Nath et al. 2018; Xu et al. 2018). Importantly, the influence of organic versus inorganic fertilization on contrasting pools can be very different. Ding et al. (2012) found that 10 years of application of organic manure with chemical fertilization produced a greater size of PC and a decrease in AC, thus increased SOC stability, compared with the application of chemical fertilization alone. Huang et al. (2017), through a 6-year fertilization experiment found the opposite was true, and that AC increases were greater than for PC. Therefore, changes in SOC fractions under long-term fertilization still remain unclear. In this paper, we aimed to quantify the effect of different long-term fertilization practices on active and passive SOC fractions.

Previous studies investigating how fertilization affects SOC have mainly focused on the top 20 cm soil layer in cropland (Sun et al. 2013), which is the depth recommended by the IPCC (Penman et al. 2003). However, almost half of SOC is in deeper soil layers below 30 cm (Balesdent et al. 2018; Jobbágy and Jackson 2000). Deep SOC is assumed to be very stable (Jia et al. 2019) and is often associated with minerals, providing biochemical recalcitrance and protection at greater soil depths (Chabbi and Rumpel 2009). Some research indicates that fertilization has a positive effect on SOC in deep layer soil (Alavaisha et al. 2019), while some research reveals that fertilization can decrease the deep layer SOC (Ali Shah et al. 2021). Despite this, there are remarkably little data on the quantity and SOC pools in deep soils as affected by fertilization.

AC and PC fractions are not a fixed component, instead they can be affected strongly by environment change. A few studies conducted at single sites have recorded some differences in the changes of AC and PC. Climatic factors (e.g., temperature (Wei et al. 2019) and precipitation (Gabarron-Galeote et al. 2015)) as well as soil physical properties (e.g., texture (Gregorich et al. 2006) and bulk density (Toriyama et al. 2015), and nutrient availability (Feyissa et al. 2020) can have substantial effects on SOC dynamics. Thus, there are likely to be different responses of soil fractions to the fertilizer additions under different climates, in different soil types, and at different depths. Improving our understanding of the mechanisms controlling fertilization effects on SOC storage in agroecosystems (Wen and He 2016) is important for identifying options for enhancing soil carbon sequestration and promoting sustainable crop productivity.

In this study, the effects of four different organic and mineral fertilization treatments on SOC, AC and PC storage at different soil depths (0–20 cm, 20–40 cm and 40–60 cm depths) were analyzed across four sites in Chinese Long-term Cropland Fertilization Experiment. The objectives of this study were: i) to compare the responses of AC and PC to the different fertilizations, and, ii) to explore the factors controlling the responses of AC and PC to different fertilization.

Material And Methods

Study sites and experiment design

The study areas were located from northern cool to southern warm regions in China, in Gongzhuling (GZL), Zhengzhou (ZZ), Chongqing (CQ) and Qiyang (QY), based on the National Soil Fertility and Fertilization Effects Long-term Monitoring Network (CNERN, <https://fla.cern.ac.cn/>). Each site represented an important agricultural base with a 20-year long fertilization history. The field experiments at GZL, ZZ and QY were initiated in 1990 and CQ was established in 1991. Soil textures of GZL, ZZ, CQ, QY are respectively Sandy Clay Loam, Silt Loam, Loam, Sandy Clay Loam (<https://www.nrcs.usda.gov>), Information on climate, cropping system and soil types are shown in Table 1.

Table 1
Basic information of the four long-term experimental sites in China

Item	Gongzhuling (GZL)	Zhengzhou (ZZ)	Chongqing (CQ)	Qiyang (QY)
Latitude	43°30'23" N	34°47'25" N	30°26'00" N	26°45'12" N
Longitude	124°48'34" E	113°40'42" E	106°26'00" E	111°52'32" E
Climate region	Mild-Temperate	Warm-Temperate	Sub-Tropical	Sub-Tropical
MAT ^a	7.4	15.0	18.5	18.5
MAP ^a	589	642	1110	1373
Cropping system	Mono-cropping Maize	Double-cropping Wheat–maize	Double-cropping Wheat–rice	Double-cropping Wheat–maize
Soil type ^b	Luvic Phaeozems	Calcaric Cambisol	Chromic Cambiso	Eutric Cambisol
Sand (%) ^c	47.9	27.6	41.0	48.8
Silt (%) ^c	27.4	62.1	38.4	23.3
Clay (%) ^c	24.6	10.3	20.6	27.9
^a Mean annual temperature (MAT) and mean annual precipitation (MAP) are means from 1989 to 2010 from China meteorological sharing service system (https://cdc.cma.gov.cn/).				
^b Soil type is based on FAO classification.				
^c The Sand (2–0.05 mm), silt (0.05–0.002 mm), and clay (< 0.002 mm) were collected from Wu et al. (2013).				

The experimental design comprised plots arranged in a randomized complete block design with three replicate blocks and each plot was isolated by 100 cm cement baffle plates. At GZL and ZZ, the plot sizes were 25 m×16 m, and at CQ and QY the plots were 10 m×12 m and 10 m×20 m, respectively. At each site, we focused on four treatments: Control (without fertilization), NPK (chemical fertilization), NPKM (combination of manure with NPK), 1.5NPKM (greater rate of manure with NPK). The N, P and K fertilizations were urea, calcium superphosphate, and potassium chloride, respectively. The C contained in urea is small compared with the manure inputs and urea in the soil tends to be rapidly mineralized to produce CO₂. The source of amended manure is a mixture of cattle, horse and pig wastes. For the NPKM treatments, 30% of total N applied was inorganic, as urea, and the rest 70% was as organic manure. The rate of fertilization applied for 1.5NPKM was 1.5 times that of the NPKM, except at CQ which was 1.5 times that of the NPK. Manure was applied once a year before seeding of wheat at ZZ and CQ, and before seeding of maize at GZL. The manure was applied twice a year before seeding of wheat and maize at QY (Liang et al. 2019). The annual application rate of chemical N (applied as urea), P (applied as calcium superphosphate), K (applied as potassium chloride) fertilization and manure for various fertilization treatments are presented in Table 2.

Table 2

Application rates of chemical fertilizer (kg ha⁻¹) and manure (Mg ha⁻¹) at the four long-term experimental sites in China

Site	Control	NPK	NPKM	1.5NPKM
Gongzhuling (GZL)	0-0-0-0	165-36-69-0	50-36-69-23	75-54-103-34.5
Zhengzhou (ZZ)	0-0-0-0	188-41-78-0	188-41-78-68	282-62-117-102
Chongqing (CQ)	0-0-0-0	150-33-63-0	150-33-63-22.5	225-49-94-22.5
Qiyang (QY)	0-0-0-0	210-37-70-0	63-37-70-25	95-22-105-40
The application amount of fertilization is N-P-K-M; N, P and K fertilizations were urea, calcium superphosphate, and potassium chloride, respectively; M, is a mixture of cattle, horse and pig wastes.				

Soil sampling and processing

Soil samples were taken at 0–20, 20–40 and 40–60 cm depths after crop harvest in August 2010. Three soil samples were obtained randomly from each replicate plot using a 10-cm diameter auger. Then, three soil cores within each replicated plot were well mixed. Additional triplicate soil samples were taken from

all the plots and depths using the ring method to determine the bulk density (Blake and Hartge 1986). Visible crop residues, root material and stones were removed during sieving. Soil was air-dried and passed through a 2 mm sieve for chemical analysis of soil organic carbon (SOC), total nitrogen (TN), AC and PC.

Soil analysis

SOC and TN were determined by dry combustion using a C/N analyzer (EA-3000, EuroEA Elemental Analyzer). Gravimetric concentrations of SOC (g kg^{-1}) for each depth were converted to area-based stocks (Mg ha^{-1}) using the conventional approach:

$$\text{Stock} = \text{concentration} \times \text{Bd} \times \text{D} \times 10$$

where stocks are in Mg ha^{-1} , SOC concentrations are g kg^{-1} , Bd is bulk density (Mg m^{-3}) and D is the depth interval of the soil layer (m).

The amount of SOC stored in the soil profile (0–60 cm) was estimated as follows:

$$\text{SOC}_{0-60} = \sum_{D=0}^{60} \text{SOC}_D$$

Where SOC_D is the SOC stock at 0–20, 20–40 and 40–60 cm three depths.

The AC and PC were determined through a modified Walkley–Black method (Table S1, Chan et al., 2001; Chandel et al., 2021). A 0.50 g sample of finely-ground soil was oxidized with 10 mL $\text{K}_2\text{Cr}_2\text{O}_7$ solution with 10 mL of concentrated H_2SO_4 which resulted in acid–aqueous solutions ratios of 1:1, that corresponded to 18N of H_2SO_4 (Table S1) (Chan et al. 2001; Walkley 1947). The initial amount of SOC, and that remaining following oxidation allowed separation of SOC into two pools of resistance. AC was defined as the fraction of SOC oxidized from 0 (initial) to 18N H_2SO_4 and PC was defined as the SOC remaining following oxidation at 18N H_2SO_4 .

The effects of fertilization on the variables (X) were quantified by the response ratio (RR) using the following equation:

$$\text{RR} = \frac{X_T - X_C}{X_C}$$

where X_T and X_C represent the mean of the fertilizer treatment and Control groups for variable X, respectively. The results are presented as the ratio of changes in the variables under fertilization. Positive

percentage changes denote an increase due to fertilization application whereas negative values indicate a decrease in the respective variables.

Statistical analysis

To examine differences in the three target variables among the four fertilization treatments, data were analyzed using the Least Significant Difference (LSD) at 5% level of significance by using R “agricolae” package (Mendiburu 2020). Meanwhile, analysis of variance (ANOVA) was also used to evaluate the effects of site, fertilization treatments, soil depth, as well as their interaction on SOC, AC and PC. The treatments and blocking structures used were site * fertilization * depth and site. block / fertilization / depth, respectively. Second, to explore the changes of AC and PC under different fertilization treatments, we calculated the proportion differences and response ratios (RR) of AC and PC to fertilization additions. Third, correlation analyses were then conducted to examine the relationships between RR and soil texture, environmental variables, and soil chemical properties by using “corrplot” package (Taiyun and Viliam 2017). Then, importance analysis (Random Forest) was used to explore the correlation factors controlling the RR of AC and PC by using the “randomForest” package. Finally, we used structural equation modeling (SEM) to explore the direct and indirect factors regulating RR, as well as to evaluate the contributions of these factors by assessing the degree of the standardized total effect (direct effect plus indirect effect) by using “sem” package.

We constructed SEM for the cropland in the consideration of potential differences in the mechanisms underlying RR. To obtain the final SEM, the following two steps involving base model construction and model optimization were specified. First, we established a base model on the basis of empirical knowledge. Specifically, based on the correlation and RF analysis between variables and RR, we included all variables that were significantly correlated with RR in the base model. We then built causal relationships between these variables and RR. In SEM, we assumed that soil chemical properties were expected to play direct roles in RR. Moreover, the RR is also indirectly and directly affected by the soil properties and environmental variables. Second, we optimized the base model on the basis of actual measurements. Specifically, we first examined all indices to ensure that no important paths were left out of the model, and then we removed paths with coefficients that were not significant at $P < 0.05$ (Colman and Schimel 2013; Ding et al. 2016a) and until all the model predictions fit well with the observed values. The chi-squared (χ^2) statistic, degrees of freedom (df), whole-model p -value, root mean square error of approximation ($RMSEA$), comparative fit index (CFI) and standardized root mean square residual ($SRMR$) were used to assess the overall goodness of model fit. $\chi^2/df < 3$, $0.05 \leq P \leq 1.00$, $RMSEA \leq 0.05$, $CFI \geq 0.90$, $SRMR \leq 0.10$ and a high p -value > 0.05 suggest that there is only a small difference between the modeled and observed values (Ding et al. 2016a; Grace 2006).

All data analyses were performed using the R statistical software v. 3.4.3 (R Core Team, 2017, <https://cran.r-project.org>). Statistical significance was set at $P = 0.05$.

Results

SOC stock, AC and PC

Long-term fertilization significantly increased SOC stock by 56.8% and 76.3% under NPKM and 1.5NPKM treatments, respectively (Fig. 1). The AC was significantly increased by 53.0% under 1.5NPKM while there was no significance difference under NPK and NPKM treatments (Fig. 2). The PC was significantly increased by 76.7% and 108.5% under NPKM and 1.5NPKM treatments, but there was no significance difference under NPK (Fig. 3).

The fertilization treatments and soil depths as well as their interaction had significance effects on SOC stock, AC and PC ($P < 0.001$, Table S2). SOC stocks, AC and PC were generally decreased with soil depth at all treatments (0–20 cm > 20–40 cm > 40–60 cm) (Fig. 1, Fig. 2, Fig. 3). In the top layer (0–20 cm), the PC were significantly increased by 36.6%, 77.8%, 117.1% under NPK, NPKM and 1.5NPKM, respectively. While SOC and AC only significantly increased by 56.2%, 80.3% and 40.7%, 53.9% under NPKM and 1.5NPKM, respectively. In the sub layer (20–40 cm), the SOC and PC were significantly increased under 1.5NPKM and NPKM, 1.5NPKM, respectively, but there was no significance difference for AC among all fertilization. In the deep layer (40–60 cm), the SOC was significantly increased by 62.3%, 85.8% under NPKM and 1.5NPKM, respectively. The PC was significantly increased by 113.0% under 1.5NPKM compared with Control, while there were no significance differences for AC among all fertilization.

Fertilization increased the proportion of PC content in the total SOC pool from 44.4–49.5%, 49.8% and 52.2% across all sites under NPK, NPKM and 1.5NPKM, respectively (Fig. 4). At different sites, there were similar trends. At GZL, the proportion of PC in the total SOC pool were from 39.9–45.5%, 45.6 %, 48.4 %, and at ZZ from 46.1% to 51.9 %, 51.4 %, 53.7 %, and at CQ from 46.0–48.5%, 50.1%, 52.0%, and at QY from 45.7–52.1%, 52.4%, 54.7%, respectively. We also found that the 1.5NPKM treatment resulted in PC representing more than 50% of SOC across the sites. Across the three sites, PC was greater than AC under 1.5NPKM treatments, except GZL site.

Response ratio of SOC, AC and PC

The results show that long-term fertilization significantly increased the RR of SOC, AC and PC across all depths and sites (Fig. S2, Fig. 5). The average RR of SOC were significantly increased by 23.7%, 60.4%, 75.6%, and 29.2%, 42.1%, 43.6% for AC while 66.5%, 85.7% and 92.2% for PC under NPK, NPKM and 1.5NPKM, respectively.

The RR of SOC, AC and PC to different fertilization at different depths and sites were differed. For total SOC, the response ratio at all depth were 1.5NPKM > NPKM > NPK. For AC, the response ratio in the top layer (0–20 cm) was 1.5NPKM > NPKM > NPK, and in the sub (20–40 cm) was 1.5NPKM > NPK > NPKM and deep layer (40–60 cm) was NPK > NPKM > 1.5NPKM. For PC, the response ratio in the top layer (0–20 cm) was NPKM > 1.5NPKM > NPK, and in the sub (20–40 cm) was 1.5NPKM > NPK > NPKM and deep layer (40–60 cm) was NPKM > NPK > 1.5NPKM.

In addition, for total SOC, the response ratio at the GZL is 1.5NPKM > NPKM > NPK, at the ZZ is 1.5NPKM = NPKM > NPK, at the CQ is NPKM > NPK = 1.5NPKM, at the QY is NPKM > 1.5NPKM > NPK. For AC, the response ratio at the CQ site was NPKM > NPK > 1.5NPKM, at the GZL and QY sites was NPKM > 1.5NPKM > NPK, and at the ZZ site was 1.5NPKM > NPK > NPKM. For PC, the RR at GZL, CQ and QY sites were 1.5NPKM > NPKM > NPK, whilst at the ZZ site it was 1.5NPKM > NPK > NPKM.

Modeling drivers of response ratio

The RR was correlated with soil chemical properties (SOC, TN, C/N and pH), environmental variables (MAT and MAP) and soil texture (Clay) (Fig. S2). It shows that RR was positively with SOC ($r = 0.32$), TN ($r = 0.31$) and C/N ($r = 0.16$). However, it decreased with MAT ($r = -0.23$) and MAP ($r = -0.14$). In addition, it also shows that RR was negative with the Clay ($r = -0.26$).

SEM analysis showed that SOC, Clay, MAP and fertilization input had direct effects on RR. Together, these variables predicted 52% of variance in RR (Fig. 6). Specifically, soil chemical properties, including SOC had direct positive effects on RR. Environmental variables, including MAT and MAP, had direct and indirect by mediating soil texture and SOC. Soil texture, including Clay, had direct effect on RR. Taking the indirect and direct effects together, Clay was the most important factors influence the RR in cropland, followed by MAP, MAT, fertilization input and SOC (Fig. 7).

Discussion

Effect of fertilization on SOC, AC and PC

The analysis revealed fertilization can significantly increase the SOC, AC and PC in the following order 1.5NPKM, NPKM and NPK (Fig. 1, Fig. 2, Fig. 3), when compared with Control. The greatest value under 1.5NPKM might be attributed to the greater rate of C addition than NPKM, NPK or Control as the 1.5NPKM treatment did not add more M at CQ, and there was no increase in SOC compared with NPKM at this site. In contrast, there were greater C inputs in the 1.5NPKM treatment and SOC increased at the other 3 sites (Fig. 1). Most research has reported that 1.5NPKM treatment could significantly increase crop yields (Zhao et al. 2009) resulting in greater C from plant roots and shoot residues to soil (Asiloglu et al. 2021; Chirinda et al. 2012), which have the potential to alter the microbial population size, community structure, and activity of soil- and rhizosphere-associated microorganisms (Jiang et al. 2020; Du et al., 2020; Poirier et al. 2018). This will in turn increase the amount of SOC (Schimel and Schaeffer 2012). Furthermore, combined manure and chemical fertilization can affect soil physical and chemical properties more so compared with only NPK addition and control (Table S3) as the SOC: SON is significantly greater and bulk density is significantly lower in the NPKM and 1.5 NPKM addition treatments, which may be attributed to reduced need for microbes to mine N from soil organic matter (SOM) resulting in a community shift that favors microbial growth over miners and increases the microbial carbon-use efficiency (Carlesso et al. 2019; Prommer et al. 2020). This may contribute to the higher carbon content under 1.5NPKM. Similar results also have been reported by Zhang et al. (2017) and Srinivasarao et al. (2014).

We also found the significant increases in passive C in fertilizer addition in all depths (Fig. 2, Fig. 3). For the 0–20 cm, it might be attributed to biochemical resistant compounds of organic matter and plant residues input (Kallenbach et al. 2016; Rovira et al. 2010; Wu et al. 2021). Belay-Tedla et al. (2009) has also reported that the farmyard manure application resulted in an increase in lignin and lignin-like products, which are the main components of resistant C pool. Other factors such as temperature and precipitation may increase the PC in soil surface layers (Davidson and Janssens 2006; Gabarron-Galeote et al. 2015; Schapel et al. 2018). For the deep soil, this might be related to physicochemical protection of organic carbon caused by two mechanisms. The organic C can be bound to soil minerals and exists in forms that decomposers cannot access (Fontaine et al. 2007). This, coupled with the SOC decomposition rate at depth being slower, can lead to the C being stable (Ghosh et al. 2018). Microbes in sub and deep soil layers are more distant from rooting zone with little rhizosphere exudation and hence there are less active microbial populations and, therefore, production of enzymes may be lower limiting activity in deeper soil layers (Hao et al. 2021; Korenblum et al. 2020).

Effect of fertilization on response ratio of SOC, AC and PC

In our study, the RR of PC to long-term fertilization was greater than AC at the four sites (Fig. 5), indicating a greater effect of fertilization on PC. There are many potential reasons for this. Although both AC and PC increased with fertilization input (Fig. 2; Fig. 3), however, AC is the chief source of soil nutrients, and may be easily used by the microbes, so reducing AC remaining relative to PC in the soil. In contrast, PC is very stable under the protection of organic–mineral complexes and soil aggregates and is difficult to be used (Shrestha et al. 2008; Zhang et al. 2018), which may increase its relative proportion within the total SOC pool (Biswapati et al. 2008; Chan et al. 2001). The larger increase in the recalcitrant C pool than labile C pool might be attributed to degradation of labile compounds and accumulation of recalcitrant materials over the years (Lopez-Capel et al. 2008). Ding et al. (2012) also observed that the SOC storage due to extraneous C input was more sequestered in recalcitrant C fraction (10–30%) than in labile C pool (6–10%) in a Mollisol in China. In addition, fertilization might facilitate microbe growth and proliferation (Feyissa et al. 2020), hence lead to the increased input of microbial necromass which is associated with the mineral-associated organic matter and thus cause accumulation of C in the recalcitrant fraction (Ma et al. 2018). Moreover, AC is very sensitive to climate change or anthropogenic disturbance and is easily decomposed (Ding et al. 2016b; Zakem et al. 2021). Overall, because AC decomposes rapidly and quickly, it may get into a new equilibrium with inputs matching outputs, reducing the RR. For PC, the low decomposition rates may allow for longer-term accumulation and promote the greater RR than AC.

Driving factors on response ratio

The SEM revealed that Clay was the major driver of the variation of RR in croplands (Fig. 7). Soil contains differently sized mineral particles, which are usually classified simply as sand, silt and clay (Ding et al. 2018). We found clay content has negative relationship with RR of soil fractions. Clay and silt particles contain mainly sesquioxides and layer silicates, and provide large specific surface areas and numerous reactive sites at which carbon can be absorbed by strong ligand exchange and polyvalent cation bridges

(Singh et al. 2017). Hence the response ratio of soil fraction may decrease with the Clay content because the Clay usually contain large amount of carbon, and thus the absolute changes in SOC are being divided by a larger number.

The results of the RF model showed Clay were identified as having the greatest importance as the main attributes to explain the response of AC and PC to fertilization addition, respectively (Fig. S5). Clay might increase the formation of organic–mineral complexes which can be accessible to soil Enzymes to produce active carbon in soil (Yang et al. 2021). Fertilization is essential for microbial growth and activities ((Yang et al., 2018), thus allowing more microbial necromass accumulate and impacting the content of PC that returns to the soil directly. Secondly, fertilization can also stimulate the production of compounds in plants aboveground (Chen et al. 2018) and root exudates which will increase the carbon input to the soil to accumulate more PC.

Conclusion

Long-term fertilization treatments increase SOC stock, AC and PC contents with the greatest values under 1.5NPKM across four typical agriculture sites. Greater applications of manure and chemical fertilization treatment play a key role in crop productively and future global C storage. The response ratio of PC to long-term fertilization was greater than AC at four sites indicating that fertilization increased PC more than AC which could enhance soil C stability. We found fertilization affected PC more than AC at all soil layers. The most important factors for explaining the response of AC and PC are Clay and Fertilization which indicated that the response of AC and PC were caused by two different kinds of mechanism. In short, these findings increase our understanding of how long-term fertilization management affects SOC stock, AC, PC and their response ratio at different depths. And fertilization could enhance the stability of carbon.

Declarations

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Figures

Figure 1

Soil organic carbon stock across four sites in different soil depth under various fertilizations. See Table 1 and Table 2 for experimental treatments in detail. The lower-case letters over the bar indicate significant differences among different fertilization treatments at $P < 0.05$ level of probability. Error bars in Ave ($n = 4$) and GZL, ZZ, CQ, QY ($n = 9$) indicates standard error of the mean of treatments for the individual sampling sites with respect to the individual soil depth.

Figure 2

Active carbon across four sites in different soil depth under various fertilizations. See Table 1 and Table 2 for experimental treatments in detail. The lower-case letters over the bar indicate significant differences among different fertilization treatments at $P < 0.05$ level of probability. Error bar in Ave ($n = 4$) and GZL, ZZ, CQ, QY ($n = 9$) indicates standard error of the mean of treatments for the individual sampling sites with respect to the individual soil depth.

Figure 3

Passive carbon across four sites in different soil depth under various fertilizations. See Table 1 and Table 2 for experimental treatments in detail. The lower-case letters over the bar indicate significant differences among different fertilization treatments at P < 0.05 level of probability. Error bar in Ave (n = 4) and GZL, ZZ, CQ, QY (n = 9) indicates standard error of the mean of treatments for the individual sampling sites with respect to the individual soil depth.

Figure 4

Proportion of active and passive carbon pools of soil organic carbon under different fertilizations. See Table 1 and Table 2 for experimental sites and treatments in detail. Error bar in Ave ($n = 4$) and GZL, ZZ, CQ, QY ($n = 9$) indicates standard error of the mean. Colors correspond to different soil pools: AC (red), PC (blue).

Figure 5

Response ratio (to Control) of active and passive carbon under different fertilization at three depths and four sites as well as an average. See Table 1 and Table 2 for experimental sites in detail. Error bar in Ave ($n = 4$) and GZL, ZZ, CQ, QY ($n = 9$) indicates standard error of the mean. Shapes correspond to different fertilization treatments: NPK (square), NPKM (triangle), 1.5NPKM (circle). Colors correspond to different soil pools: Active carbon (red), Passive carbon (blue).

$\chi^2/df = 1.79$, $P = 0.09$, RMSEA = 0.06, CFI = 0.99, SRMR = 0.02

Figure 6

Structural equation model (SEM) analysis examining the effects of soil properties, soil texture and climate on the response ratio of soil organic carbon (SOC) with fertilization. In the model, square boxes indicate variables, and the abbreviations of the variables are explained in Table 1. The arrows connecting the boxes indicate the direction of causation. The red and blue arrows denote positive and negative relationships, respectively. The arrow widths are proportional to the standardized path coefficients, which reflect the importance of the factors in the model. The proportion of explained variance (R^2) is below each response variable in the model.

Figure 7

Standardized total effects of different factors on response ratios across different croplands. Total effects represent the direct effect plus the indirect effect and were derived from structural equation modeling.

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