Long-term fertilization enhances soil carbon stability by increasing 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 C (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 mechanisms.

Methods We assessed the effect of chemical fertilizer and manure amendment (standard rate and 1.5×standard rate of inorganic fertilizer (NPK) with or without manure (M), with a Control for comparison) at three 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 NPK< NPKM< 1.5NPKM. 1.5NPKM resulting 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.
Conclusions  Long-term fertilization enhanced both AC and PC, but the greater response of PC suggests that fertilization application could enhance the stability of C and thus the potential of cropland for SOC accumulation.

Keywords  Chemical fertilization · Manure · Labile carbon · Non-labile carbon · Response ratio · 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, chemical fertilizer (such as N, P and K) was widely used to increase soil quality and crop yield (Bhattacharyya et al. 2011; Zhang et al. 2017), however with the perennial application of chemical fertilizer, soil quality began to decrease (Sun et al. 2015). Manures are an effective way to improve soil quality, hence more fields use normal or high rates of manure amendment combined with chemical fertilizer to elevate soil quality and crop yield (Duan et al. 2011; He et al. 2015; Maltas et al. 2018). However, to date, the response of SOC stability to fertilization is highly uncertain because the mechanisms controlling the accumulation of SOC are not fully understood. Hence, understanding and characterizing the effect of fertilization on SOC accumulation is important for the management and development of sustainable long-term agriculture.

To better understand the mechanisms by which C is lost or stabilized in soil under long-term fertilization, total SOC can be separated into active (AC) and passive (PC) pools that differ clearly in their residence times (Biswapati et al. 2008; Chan et al. 2001). AC is the fraction of SOC from live microbes and microbial product that has the most rapid turnover rates and decomposes fast (Wu et al. 2012; Zhang et al. 2018), and its oxidation drives the flux of C from soil to the atmosphere. On the other hand, PC is the fraction of SOC from aliphatic, high molecular compounds and humus, which is stabilized against further microbial action due to SOC forming organic-mineral complexes with soil mineral and gets decomposed slowly by microbial activity (Jr et al. 2011). It dominates SOC stores, and is 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 an increase in PC and a decrease in AC. While Huang et al. (2017) through a 6-year fertilization experiment found the opposite was true, and that AC increases were greater than PC. Therefore, changes in AC and PC fractions under long-term fertilization remains unclear. In this paper, we aimed to quantify the effect of different long-term fertilization practices on AC and PC fractions.

Previous studies investigating how fertilization affects SOC have focused mainly on the top 20 cm soil layer in cropland (Sun et al. 2013). However, almost half of SOC is in deeper soil layers below 30 cm (the recommended minimum depth by IPCC) (Balesdent et al. 2018; Jobbágy and Jackson 2000) and sampling to subsoil depths is important for properly evaluating changes in SOC content (Smith et al. 2020). SOC at depth is assumed to be very stable (Jia et al. 2019) and is often associated with minerals, providing both biochemical recalcitrance and physical protection (Chabbi and Rumpel 2009). Some research indicates that fertilization has a positive effect on SOC in deep soil layers (Alavaisha et al. 2019), while other research reveals that fertilization can decrease SOC in deep soil layers (Ali Shah et al. 2021). Despite this, there are remarkably few data on the SOC quantity and its different pools in deep soils as affected by fertilization.

AC and PC fractions are not a fixed component, instead they can be affected strongly by environmental 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)), 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 C dynamics. Thus, there are likely to be different responses of soil
fractions to the fertilizer additions under different climates, soil types and 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 SOC 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 three different soil depths (0–20 cm, 20–40 cm and 40–60 cm depths) were analyzed across four sites in the 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. We hypothesized that all fertilization types could increase the responses of both AC and PC, but with a greater response in PC. In addition, we also predicted that the response ratio of AC and PC to different fertilization may be caused by different factors at different depths, but that soil clay could be the main controlling factor.

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, and sandy clay loam (https://www.nrcs.usda.gov). Information on climate, cropping system and soil types are shown in Table 1.

The field experiments were in a randomized block design, and there was three replications (plots) at ZZ, two replications at QY, but no replication (e.g., single plot) at GZL and CQ due to field availability (He et al. 2015; Wang et al. 2013). Depending on the field availability, a full set of the treatments in a relatively large plot size were designed at each study site and the treatments were considered as being replicated at the study sites (Duan et al. 2011). The plot sizes were 25 m × 16 m at GZL and ZZ, and the plots were 10 m × 12 m at CQ and 10 m × 20 m at QY. To avoid edge effects, each plot was isolated by cement baffle plates. At each site, we focused on four treatments: Control (without fertilization), NPK (chemical fertilization), NPKM (combined chemical fertilizer and manure amendment), 1.5NPKM (greater rate of manure with NPK). The N, P and K fertilizations were urea, calcium superphosphate, and potassium chloride, respectively. The source of amended manure was a mixture of cattle, horse and pig wastes. For the NPKM treatment at GZL and QY sites, 30% of the total N applied was inorganic, as urea, and 70% was as organic manure, while at the ZZ and CQ sites
70% of the total N applied was inorganic, as urea, and 30% was as organic manure. The amount of N applied at the sites ranged from 165~353 kg ha\(^{-1}\) N under chemical fertilizer, and 165~428.5 kg ha\(^{-1}\) N under chemical fertilizer with manure amendment, the amount of N varying according to local cropping system and crop production (Table 2). The rate of fertilization applied for 1.5NPKM was 1.5 times that of NPKM at GZL, ZZ and QY, and 1.5 times that of NPK and of the same M at CQ. We chose 1.5NPKM mainly to look at the yield effect of increased input. It is believed that a 50% increase in manure amendment and chemical fertilizer input could meet the nutrient demand for higher yields. If the nutrient increase ratio is too small, the fertilization effect may not significantly; if the nutrient increase ratio is too high, the environmental risk of nutrient loss is high. At the CQ site, the 1.5 times of chemical fertilizer and same rate of manure amendment could meet the nutrient demand for higher yields, so we choose 1.5NPK and M. 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, P, K fertilizer and manure for the fertilization treatments are presented in Table 2.

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. 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, ground and passed through a 2 mm sieve for pH, SOC, total N (TN), AC and PC analysis.

Soil analysis

The soil pH was measured by a pH electrode (PB-10, Sartorius, Germany) in a 1:2.5 soil:water suspension. 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 of the three depths (0–20, 20–40 and 40–60 cm) were converted to area-based stocks (Mg ha\(^{-1}\)) using the equation of Srinivasarao et al. (2014):

\[
\text{Stock} = \text{SOC concentration} \times \text{Bd} \times D \times 10
\]

where Stock is the SOC stock (Mg ha\(^{-1}\)), SOC concentration is the SOC concentration (g kg\(^{-1}\)), Bd is the bulk density (Mg m\(^{-3}\)), and D is the depth interval (In this study, D was 0.2 m) of the soil layer (m), 10 is the conversion factor.

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.

To better understand SOC accumulation dynamics under long term fertilization, total SOC can be

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Table 2 Annual fertilization rates of N, P and K (kg ha\(^{-1}\)) at the four long-term experimental sites in China

| Site  | GZL Inorganic N-P-K | Organic N | ZZ Inorganic N-P-K | Organic N | CQ Inorganic N-P-K | Organic N | QY Inorganic N-P-K | Organic N |
|-------|---------------------|-----------|-------------------|-----------|-------------------|-----------|-------------------|-----------|
| Control | 0–0-0 | 0 | 0–0-0 | 0 | 0–0-0 | 0 | 0–0-0 | 0 |
| NPK | 165–36-69 | 0 | 353–78-146 | 0 | 300–66-126 | 0 | 300–53-100 | 0 |
| NPKM \(^a\) | 50–36-68 | 115 | 238–78-146 | 116 | 300–66-126 | 128.5 | 90–53-100 | 210 |
| 1.5NPKM | 75–54-103 | 172.5 | 356–117-220 | 173 | 450–99-189 | 128.5 | 135–79-149 | 315 |

\(^a\) The averaged manures input (Mg ha\(^{-1}\) year\(^{-1}\)) since 1990 at GZL, ZZ, CQ, QY were 23.0 12.9, 22.5, 42.0, respectively. All manure amounts at these sites were averaged fresh weight applications during 1990 to 2007. The amounts of P and K in manure and straw were not calculated.
separated into AC and PC pools through a modified Walkley–Black method according to the difference of SOC oxidization, i.e., AC is easily oxidized SOC while PC is less-easily oxidized SOC. (Table S2; Aralappanavar et al. 2022; Chan et al. 2001). Specifically, a 0.50 g sample of finely-ground soil was oxidized with 10 mL K$_2$Cr$_2$O$_7$ solution with 10 mL of concentrate H$_2$SO$_4$ which resulted in acid–aqueous solution ratios of 1:1, that corresponded to 18 N of H$_2$SO$_4$ (Table S2) (Chan et al. 2001; Walkley 1947). The initial amount of SOC, and that remaining following oxidation allowed separation of SOC into the two pools of resistance. AC was defined as the fraction of SOC oxidized from 0 (initial) to 18 N H$_2$SO$_4$ and the PC was defined as the SOC remaining following oxidation at 18 N H$_2$SO$_4$.

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

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

where $X_T$ and $X_C$ represent the mean of the fertilizer treatment and Control 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 Least Significant Difference (LSD) at 5% level of significance by using R ‘agricolae’ package (Mendiburu 2020). Analysis of variance (ANOVA) was also used to evaluate the effects of site, fertilization treatments, soil depth and their interaction on SOC, AC and PC. The treatments and blocking structures used were site * fertilization * depth and site. block / fertilization / depth, respectively. To explore the changes of AC and PC under different fertilization treatments, we calculated the proportional differences and RR of AC and PC to fertilization additions. Correlation analyses were then conducted to examine the relationships between RR and soil texture, environmental variables, soil chemical properties and the variable’s RR by using “corrplot” package (Taiyun and Viliam 2017). Importance analysis 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. 2016) and until all the model predictions fit well with the observed values. The chi-squared ($\chi^2$) 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. 2016; 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 manure application increased SOC stocks by 56.8% and 76.3% under NPKM and 1.5NPKM treatments compared with the Control, respectively (Fig. 1). Fertilizer application increased SOC stocks at GZL, ZZ and CQ sites in the soil profile, but had a lesser effect at depth in ZZ and QY sites. The AC fraction was increased significantly by 53.0% under 1.5NPKM while there was no significant difference under NPK and NPKM treatments (Fig. 2). The PC fraction was increased significantly by 76.7% and 108.5% under NPKM and 1.5NPKM treatments, respectively, but there was no significant difference under NPKM and 1.5NPKM treatments (Fig. 3). The fertilization treatments and soil depths and their interaction had significant effects on SOC stock (Treatment (T), P < 0.001; Depth (D), P < 0.001; D×T, P < 0.001; Table S2), AC (T, P < 0.001; D, P < 0.001; T×D, P < 0.001; Table S2) and PC (T, P < 0.001; D, P < 0.001; T×D, P < 0.001; Table S2). SOC stocks, AC and PC were generally decreased with soil depth under all treatments (0–20 cm > 20–40 cm > 40–60 cm) (Figs. 1, 2, and 3, Fig. S2). In the top layer (0–20 cm), the SOC, AC and PC were significantly increased under NPKM and 1.5NPKM. In the next layer (20–40 cm), the SOC and PC were significantly increased under 1.5NPKM, but there was no significant difference for AC between the treatments. In the deep layer (40–60 cm), the SOC and PC were significantly increased under 1.5NPKM, while there were no significant differences for AC between the treatments.

Fertilization increased the proportion of PC in the total SOC pool from 41.1% to 46.5%, 47.1% and 49.2% across all sites under NPK, NPKM and 1.5NPKM, respectively (Fig. 4). At different sites, there was a similar trend.

Response ratio of SOC, AC and PC

Long-term fertilization significantly increased the RR of SOC, AC and PC at all depths and sites (Fig. S3, Fig. 5). The average RR of SOC was significantly increased by 23.7%, 60.4%, 75.6%, and 13.0%, 44.6%, 57.3% for AC and 40.2%, 84.7% and 119.5% for PC under NPK, NPKM and 1.5NPKM, respectively. In addition, the RR of SOC, AC and PC to different fertilization at all depths and sites were different (P < 0.01).

Modeling drivers of the response ratio of SOC

The RR was correlated with soil chemical properties (SOC, TN, RR_TN, C/N, RR_C/N and pH), environmental variables (MAT and MAP) and soil texture (clay and RR_Bd) (Fig. 6). RR was correlated positively with SOC (r = 0.39), TN (r = 0.32), RR_TN (r = 0.58), C/N (r = 0.29), RR_C/N (r = 0.50), pH (r = 0.28) and RR_Bd (r = 0.20). However, it was correlated negatively with MAT (r = -0.31), MAP (r = -0.24) and clay (r = -0.38).

Considering the RR of SOC had a good relationship with the RR of different variables (TN < RR_TN < RR_C/N < C/N < RR_Bd; Bd < RR_Bd (r = 0.20)), so the RR of the variable was used to construct the model. SEM analysis showed that MAP, MAT, clay and RR_TN had direct effects on RR. Together, these variables predicted 50.0% of variance in RR (Fig. 7a). Taking the indirect and direct effects together, clay was the most important factor influencing the RR in cropland, followed by MAP, RR_TN and MAT (Fig. 7b).

Discussion

Effect of fertilization on SOC, AC and PC

The analysis revealed chemical fertilizer and chemical fertilizer with manure amendment can significantly increase the SOC, AC and PC stocks in the following order 1.5NPKM, NPKM and NPK (Figs. 1, 2, and 3), compared with the Control. The greatest value under 1.5NPKM might be attributed to the greater rate of direct source of C, NPK and other nutrients from manure as well as the indirect effects of root and shoot residues from plants (Fig. S1). The NPK treatment, stimulated the plant to produce more roots or residues to increase the SOC. Previous research has reported that the 1.5NPKM treatment could significantly increase crop yields (Zhao et al. 2009) resulting in greater C from plant roots and shoot residues to soil (Fig. S1; Asiloglu et al. 2021), which has the potential to alter the microbial population size, community structure, and activity of soil- and rhizosphere-associated microorganisms (Jiang et al. 2020;
Fig. 1 Soil organic carbon stock across four sites in different soil depths under chemical fertilizer and organic amendment. See Tables 1 and 2 for experimental treatments in detail. Different lower-case letters over the bars indicate significant differences between different fertilization treatments at $P < 0.05$ level of probability. Error bars in Ave ($n=4$) and GZL, ZZ, CQ, QY ($n=3$) indicates standard error of the mean of treatments for the individual sampling sites with respect to the individual soil depth.
Fig. 2 Active carbon across four sites in different soil depths under chemical fertilizer and organic amendment. See Tables 1 and 2 for experimental treatments in detail. Different lowercase letters over the bars indicate significant differences between different fertilization treatments at $P < 0.05$ level of probability. Error bar in Ave ($n=4$) and GZL, ZZ, CQ, QY ($n=3$) indicates standard error of the mean of treatments for the individual sampling sites with respect to the individual soil depth.
Fig. 3 Passive carbon across four sites in different soil depths under chemical fertilizer and organic amendment. See Tables 1 and 2 for experimental treatments in detail. Different lower-case letters over the bars indicate significant differences between different fertilization treatments at $P < 0.05$ level of probability. Error bar in Ave ($n=4$) and GZL, ZZ, CQ, QY ($n=3$) indicates standard error of the mean of treatments for the individual sampling sites with respect to the individual soil depth.
Du et al. 2020; Poirier et al. 2018). This will in turn increase the amount of SOC (Schimel and Schaeffer 2012). Furthermore, combined manure amendment and chemical fertilizer can affect soil physical and chemical proprieties more than NPK alone and the Control (Table S3). This is because combined manure amendment and chemical fertilizer could increase SOC: SON and decrease bulk density, which may reduce microbial mining of N from soil organic matter (SOM) (Carlesso et al. 2019; Prommer et al. 2020). Similar results have also been reported by Zhang et al. (2017) and Srinivasarao et al. (2014).

The stock of SOC result from the balance between inputs and outputs of C within the belowground environment (Davidson and Janssens. 2006). A number of studies have been done to evaluate how manure amendment and chemical fertilizer affect greenhouse gas (GHG) emissions. They found that more SOC accumulated through increased biomass production and through direct C input from manure and residue retention in soils, however, there was also a significant release of GHGs due to the priming effect (Colman and Schimel 2013). The direct and indirect emissions of CH₄, and N₂O partly offset the benefits.
of increased SOC sequestration. Hence, the overall change in GHG emissions and SOC sequestration should be evaluated when assessing the benefits of any management practice.

We also found the significant increases in PC from fertilizer and manure addition at all depths (Figs. 2 and 3). For the 0–20 cm depth, it might be attributed to biochemically resistant compounds of organic matter and physical protection from minerals (Kallenbach et al. 2016; Rovira et al. 2010; Wu et al. 2021; Dynarski et al. 2020). Belay-Tedla et al. (2009) has reported that farmyard manure application resulted in an increase in lignin and lignin-like products, which are the main components of the resistant C pool. However, with advanced developments in SOC research, a number of research have found that the recalcitrance of C is due to soil physical protection rather than C species (Lehmann and Kleber 2015). In this case, the C is protected by the aggregates which prevents microbial access to the SOC and hence increase recalcitrance. Other factors such as temperature and precipitation may increase the PC in soil surface layers, because it’s the interface between the soil and the atmosphere (Davidson and Janssens 2006; Gabarron-Galeote et al. 2015; Schapel et al. 2018). For soil at deep, this might be related to the physicochemical protection of SOC. Such as binding to soil minerals and existing 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 subsoil layers are more distant from the 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 the PC fractions to long-term fertilization was greater than that of the AC at the four sites (Fig. 5), indicating a greater effect of fertilization on PC. There are many potential reasons for this. For one reason, although both AC and PC increased with fertilization input (Figs. 2 and 3), AC is the chief source of soil nutrients, and may be easily used by the microbes, so reducing AC remains in the soil relative to the PC. In contrast, PC is very stable under the protection of organic–mineral complexes and soil aggregates and is difficult to be accessed (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 compared with the labile C pool might be attributed to 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 found in the recalcitrant C fraction than in the labile C pool in a Mollisol in China. In addition, fertilization might facilitate microbe growth and proliferation (Feyissa et al. 2020), hence leading to the increased input of microbial necromass which is
associated with the mineral-associated organic matter and thus cause accumulation of C in the refractory fraction (Ma et al. 2018). Overall, because AC decomposes rapidly and quickly, it may attain a new equilibrium with inputs matching outputs, reducing the RR. For PC, the low decomposition rates may allow for long-term accumulation and promote the greater RR than AC.

| Driving factors on response ratio |
|----------------------------------|

The SEM and RF models revealed that clay was the major driver of the variation of RR in croplands (Fig. 7, Fig. S4). Soil contains differently sized mineral particles, which are usually classified simply as sand, silt and clay (Ding et al. 2018). In general, clay content is assumed to be positively correlated with
preservation of SOC (Matus 2021). It may be caused by two mechanisms. The formation of complexes between metal ions associated with large clay surface area and substrate accessibility by microbe could explain the reduced decomposition rates with increasing clay content. First, clay and silt particles contain mainly sesquioxide and layer silicates, and provide large specific surface areas and numerous reactive sites at which C can be absorbed by strong ligand exchange and polyvalent cation bridges (Singh et al. 2017). Hence, the response ratio of the soil fraction decreases with the clay content increase.

On the other hand, clay might increase the formation of organic–mineral complexes which can be accessible to soil enzymes to produce AC in soil (Yang et al. 2021). Fertilization is essential for microbial growth and activity (Yang et al. 2018), thus allowing more microbial necro mass to accumulate and impacting the content of PC that returns to the soil directly. Moreover, fertilization can also stimulate the production of compounds in plants aboveground (Chen et al. 2018) and root exudates which will increase the C input to the soil to accumulate more PC.

Methods for AC and PC determination

Based on C turn over time, molecule structure as well as the fraction association with soil particle sizes, identification of the AC (e.g., labile/oxidizable) and PC (e.g., nonlabile/less-easily oxidizable) of SOC has been well developed in the past few years (Table S5). For example, Yu et al. 2015 found fertilizer application mainly increased the levels of recalcitrant organic C components characterized by methoxy/N-alkyl C and alkyl C by using the NMR spectroscopy method (Yu et al. 2015). Yang et al. 2018 found the application of manure combined with mineral NPK (NPKM) resulted in an increase in the passive content in the mineral-associated organic C by using a SOC fraction with the soil particle method. In this study, we found that the fertilizer application had a great effect on PC by using different KMnO₄ oxidation gradient method. This indicates that all these methods
can determination the labile/oxidizable and nonlabile/less-easily oxidizable component of SOC and different methods have a good relationship. Given the oxidation method is simple and easy to operate, it is an effective way to explore SOC stability (Hicks Pries et al. 2013; Jr et al. 2011; Román Dobarco et al. 2020; Qin et al. 2019; Yang et al. 2018).

Conclusion

Long-term fertilization treatments increase SOC stock, AC and PC fractions 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 SOC 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, respectively, which indicated that the response of AC and PC were caused by two different kinds of mechanism which physical and chemical properties of a particular clay mineral and pore size distribution, and fertilization could enhance the stability of carbon.

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Declarations

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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