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Soil organic carbon sequestration and its synergetic effect on crop yield in cropland under different fertilizations

Hu Xu

Promoteurs : Prof. Gilles Colinet
Prof. Ming-Gang Xu (CAAS, China)

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Résumé

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Résumé:

Sous la double pression de la croissance de la demande alimentaire mondiale et de l'aggravation des changements climatiques, il devient crucial d'identifier des pratiques agricoles qui concilient séquestration du carbone organique du sol (COS) et amélioration de la production végétale en vue d'assurer la sécurité alimentaire d'une part mais aussi pour mettre en œuvre concrètement l'« Initiative quatre pour mille » et la « Neutralité carbone ». La fertilisation, en particulier l'ajout d'amendements organiques, est la pratique la plus efficace pour augmenter le COS dans les systèmes de culture intensive. En effet, ces dernières années, les pratiques traditionnelles (par exemple, incorporation de paille et substitution d'engrais chimiques par du fumier (substitution organique)) et nouvelles (par exemple, application de biochar) ont montré un grand potentiel pour améliorer le rendement des cultures et le stockage de C. Cependant, notre compréhension des impacts de la substitution organique et de l'incorporation de paille sur le COS du sous-sol et le rendement des cultures reste limitée. De plus, les résultats de l'application de biochar sur le COS et le rendement des cultures ont montré une grande variation et une grande incertitude dans la littérature. Par conséquent, il est essentiel d'améliorer notre compréhension des mécanismes de séquestration du COS et du rendement des cultures en réponse à ces pratiques de fertilisation.

Dans la première partie de cette thèse, une expérience de terrain avec une culture de maïs double nous a permis d'étudier le fractionnement du COS en réponse à une fertilisation à long terme et de révéler le mécanisme sous-jacent de la séquestration du COS dans les systèmes de culture intensifs. Ensuite, quatre expériences à long terme de substitution organique et d'incorporation de pailles, en Chine, ont été valorisées pour quantifier les effets de ces pratiques sur le COS à différentes profondeurs et sur le rendement des cultures. Enfin, nous avons estimé l'effet de l'application de biochar sur le COS et le rendement des cultures par une méta-analyse globale et en avons également identifié les facteurs clés à l'aide d'une analyse par *boosted regression tree*.

L'expérience de fertilisation à long terme a montré qu'une fertilisation de 24 ans, en particulier avec le fumier, augmentait considérablement la concentration totale de COS, celle dans les agrégats et celle associée aux argiles, par rapport à l'absence de fertilisation.

Les essais de substitution organique ont montré qu'après 23 ans, les stocks de COS ont augmenté significativement dans la couche arable (0 à 20 cm) et le sous-sol (20 à 100 cm). En outre, les modalités avec substitution organique ont également augmenté ou maintenu de manière significative le rendement des cultures et donc leur durabilité. Accumulation de C et rendement semblent bien compatibles.

L'expérience d'incorporation de pailles a par contre montré que le stock de COS avait augmenté dans la couche arable, mais également considérablement diminué dans la tranche 40-100 cm. L'analyse de redondance a montré que l'apport de carbone et d’éléments nutritifs expliquaient conjointement jusqu'à 90 % de la variation des stocks de CO dans la couche arable, mais seulement 32 % de celle du sous-sol.

D’après la méta-analyse mondiale, l'application de biochar a augmenté le rendement des cultures et le carbone organique du sol de 33% et 22%, respectivement. Cependant, si la combinaison de biochar avec des fertilisants permet d’augmenter les rendements, il n’y a pas réellement d’effet sur le stockage de C. Les propriétés du sol et du biochar expliquaient conjointement 70 à 79 % et 90 à 93 % des variances du rendement des cultures et du COS, respectivement. Le rapport C: N du biochar et le pH du sol étaient les facteurs les plus importants.

En conclusion, l’incorporation d’un amendement organique dans la fertilisation, a amélioré la séquestration du COS principalement en améliorant la protection physique et chimique des agrégats et de la fraction argileuse. Les résultats des expériences sur le terrain ont confirmé que la substitution organique était le mode optimal d'utilisation du fumier et de la paille pour augmenter la séquestration du COS dans le profil du sol et le rendement des cultures. De plus, nos résultats ont mis en évidence qu'ignorer le sous-sol ne permettait pas d’apprécier la capacité potentielle du sol pour la séquestration du C, et pouvait même conduire à de conclusions erronées. Le biochar, combiné avec des engrais, est une solution d'amélioration des stratégies de fertilisation pour augmenter le COS et le rendement des cultures, qui dépend des propriétés du biochar et du pH du sol. Nos résultats fournissent un nouvel aperçu et une base pour l'optimisation des stratégies de fertilisation et l'utilisation durable des terres cultivées dans les systèmes intensifs.

Mots-clés: expérimentation à long terme, système de culture très intensif, substitution biologique; incorporation de paille; application de biochar; séquestration du carbone organique du sol; particules de sol, formation d'agrégats de sol; le rendement des cultures.

Abstract

Hu Xu. (2021). “Soil organic carbon sequestration and its synergetic effect on crop yield in cropland under different fertilizations” (PhD Dissertation in English). Gembloux, Belgique, Gembloux Agro-Bio Tech, University of Liège, 154 pages, 14 tables, 27 figures.

Abstract: Under the dual pressure of food demand growth and climate change intensification, how to promote soil organic carbon (SOC) sequestration and synergistically improve crop production via agricultural practice is not only a major strategic demand to ensure food security but also a key way to implement the "Four Per Mille Initiative" and "Carbon Neutrality". Fertilization, especially adding organic amendment, is the most effective practice to enhance SOC, but limited information is available on the underlying mechanism of SOC sequestration in intensive cropping systems. Moreover, in recent years, the application practices of both traditional (e.g., straw incorporation and organic substitution (substitution of chemical fertilizers by manure)) and emerging (e.g., biochar application) organic amendment have shown a great potential in enhancing crop yield and SOC. However, an understanding of how and to what extent organic substitution and straw incorporation impact SOC at different depths and crop yield remains limited. Moreover, previous results regarding the effects of biochar application on SOC and crop yield showed wide variation and high uncertainty. Therefore, it is essential to enhance the understanding of SOC sequestration mechanisms and crop yield response to those practices.

In this thesis, firstly, we used a field experiment with double maize cropping to study the responses of OC in soil fractions to long-term fertilization and revealed the underlying mechanism of SOC sequestration in intensive cropping systems. Secondly, four organic substitution and one straw incorporation field experiments were conducted in China to quantify the effects of these practices on SOC at different depths and crop yield as well as their relationship. Finally, the effects of biochar application, as a new perspective of fertilization strategy evolution, on SOC and crop yield were evaluated via a global meta-analysis, and their key factors were determined using a boosted regression tree model. The main results are as follows:

The field experiment with double maize cropping showed that long-term fertilization, especially in the presence of manure, increased significantly ($p < 0.05$) the concentration and proportion of OC in aggregates, clay particles, and bulk soil compared with no fertilization. Moreover, OC in aggregates and clay particles showed linear and non-linear responses to OC accumulation in bulk soil, respectively. Meanwhile, OC in clay particles contributed approximately 47% to soil OC. Moreover, the mass proportion of aggregates and the mass ratio of aggregates to silt particles significantly increased with OC enriched in fine silt and clay fractions.

Organic substitution field experiments showed that, compared with the initial values, SOC stock significantly increased by 25.6~103.8% and 2.9~71.3% in topsoil (0-20 cm) and subsoil (20-100 cm), respectively, under long-term 70% substitution of mineral nitrogen fertilizer by manure (70%NPK+M). Moreover, a significant linear correlation was observed between SOC change in topsoil and the entire 100 cm profile, with a slope of 0.39. Compared with chemical fertilizers (NPK) and NPK plus straw (NPK+S), 70%NPK+M resulted in the significantly higher increment of SOC stock in the entire 100 cm profile, and maintained or even increased significantly crop yield and its sustainability. SOC accumulation in soil profile exhibited significant exponential correlations with crop yield, sustainable yield index, and nutrients uptakes.

Straw incorporation field experiment suggested that, compared with straw removal (SR), SOC stock greatly increased in topsoil but substantially decreased in 40-100 cm under long-term straw incorporation. The highest crop yield in the most years and the greatest increase in topsoil OC stock was observed in CM (cattle manure produced by straw) treatment. For the entire 100 cm profile, compared with SR, SOC stock greatly increased in CM treatment, but reduced in other straw incorporation modes. Redundancy analysis showed that OC input and soil nutrients jointly explained up to 90.1% of the variance of OC change in topsoil, but only 31.8% of that in subsoil.

Global meta-analysis indicated that biochar application substantially promoted SOC and crop yield by 33% and 22%, respectively. However, there were significant differences in improving crop yield (15.1% and 48.4%, respectively), but almost no differences in increasing SOC between biochar alone (B) and biochar plus chemical fertilizers (BF). The effect sizes of B and BF on crop yield declined with biochar C:N ratio and soil pH increasing. The wetness index, soil properties, and biochar properties jointly explained 70-79% and 90-93% of the variances of the biochar's effect on crop yield and SOC, respectively. Biochar C:N ratio and soil pH were the most important factors that determine the effects of B and BF on crop yield and SOC.

In conclusion, fertilization, especially in the presence of organic amendment, improved SOC sequestration mainly by enhancing OC's physicochemical protection from aggregates and clay fractions. In intensive cropping systems, clay particles play important roles in OC sequestration and soil aggregation via enriching OC. Field experiment results confirmed that organic substitution and straw incorporation after livestock digestion were the optimal modes of manure and straw utilization to increase crop yield and SOC sequestration in soil profile. However, ignoring subsoil OC change could not actually recognize the soil's potential capability for C sequestration, and may even lead to false conclusions regarding the impact of organic amendment application on SOC sequestration in agroecosystem. Moreover, global meta-analysis implied that biochar application was a valid fertilization strategy to increased SOC and crop yield, which depended on biochar properties and soil pH. The results provided novel insight and basis for fertilization strategy optimization and cropland sustainable utilization in intensive cropping systems.

Keywords: long-term experiment, intensive cropping system, organic substitution; straw incorporation; biochar application; soil organic carbon sequestration; soil particles, soil aggregates formation; crop yield.

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List of Abbreviations

CK	No fertilization
NPK	Chemical nitrogen, phosphorus, and potassium fertilizers
2NPK	Double application rate of the NPK
NPK+S	Chemical NPK fertilizers combined with straw
NPK+M	Chemical NPK fertilizers combined with manure
70%NPK+M	70% substitution of mineral nitrogen fertilizers by manure
M	Manure application alone
SR	Straw removal
SM	Direct straw mulching
SC	Straw incorporation after crushing
CM	Cattle manure produced by an equal amount of straw
B	Biochar application alone
BF	Biochar combined with chemical fertilizers
C	Carbon
OC	Organic carbon
SOC	Soil organic carbon
OM	Organic matter
N	Nitrogen
TN	Total nitrogen
C: N	Ratio of carbon to nitrogen
P	Phosphorus
AP	Available phosphorus
TP	Total phosphorus
K	Potassium
AK	Available potassium
TK	Total potassium
BD	Soil bulk density
SYI	Sustainable yield index
RCY	Relative crop yield
CSE	Carbon sequestration efficiency
CCI	Cumulative carbon input

MAT	Mean annual temperature
MAP	Mean annual precipitation
Topsoil	Top 20 cm soil
Subsoil	Soil below 20 cm
LSD	Least significant difference
BRT	Boosted regression tree

Chapter I

General Introduction

Abstract

Under the rapid growth of global population, how to promote SOC accumulation and synergistically improve crop production through optimizing fertilization practices is very important for ensuring food security and mitigating climate change. Fertilization, especially when combined with organic amendment (e.g., manure, straw, biochar, etc.), is regarded as the most effective practice for SOC improvement. In cropland, a series of physical-chemical protection occurs in the processes of OC accumulation and sequestration under organic amendment applications, but the underlying mechanisms of SOC sequestration and stabilization are still limitedly understood in intense cropping systems. Moreover, to gain high crop production, overused chemical fertilizers have caused a series of environmental and soil quality degradation problems in the past. Therefore, improving crop yield and SOC via organic amendment addition has become an effective strategy to reduce chemical fertilizer input and increase fertilizer use efficiency in intensive cropping systems in China. Numerous studies showed that traditional (organic substitution and straw incorporation) and emerging (biochar application) organic amendment applications have shown great potential for improving crop yield and SOC, which has received extensive attention and research recently. However, the understanding of SOC (especially in subsoil) and crop yield as affected by those practices remains limited. Therefore, it is essential to enhance the understanding of how and to what extent those practices influence SOC and crop yield. This chapter introduced a general review regarding the mechanisms of SOC sequestration and stabilization under fertilization as well as the impacts of manure, straw, and biochar application on SOC and crop yield. Based on the above review, we put forward the knowledge gap in the related study, and then presented the research objectives and strategies of this thesis.

Keywords: organic substitution; straw incorporation; biochar application; soil organic carbon sequestration; crop yield; soil particle fractions;

1. Background

Sustaining crop production to feed the growing population with the limited arable soil is a great challenge for agriculture and environment. In cropland, soil OC, as a driver of soil structure, nutrient cycling, water dynamics, microbial activity, and biodiversity, plays an important role in enhancing food security (Hijbeek et al., 2017; Lal, 2004; Tautges et al., 2019; Xu et al., 2011). Moreover, cropland OC pool, as the most active C pool, accounts for 8%-10% of the total OC pool in the terrestrial ecosystem (Flach et al., 2019), which has a profound impact on climate change (Zhang et al., 2013). Therefore, in agricultural production, increasing SOC sequestration and improving crop yield synergistically via effective practices are important for agricultural and environmental sustainability (Lal, 2009a; Lal et al., 2007).

Soil OC accumulation is a long-term and slow evolution process (Xie et al., 2018). Soil OC content depends on net balances between OC input and loss (Cai et al., 2020). On the one hand, increasing OC input via organic amendment (e.g., manure, straw, biochar, etc.) addition is the most direct way to enhance SOC (Six et al., 2002). However, with the increase of OC input, SOC content does not indefinitely increase and will approach a maximum value (Stewart et al., 2007; West and Six, 2007). At different OC levels, the response of soil OC content to OC input shows the following trends: (1) at low soil OC level, soil OC content sharply increases with OC input, showing OC accumulation for a period of time; (2) SOC accumulation rate then slows down, and SOC content tends to a saturation level with OC input; (3) finally, SOC content approaches saturation value, then will increase again and reach a new saturation state only when management practices (e.g., increasing OC input) and soil properties were changed (Stewart et al., 2007; West and Six, 2007). Thus, SOC saturation level is mainly limited by internal factors, such as clay and silt content, and mineral composition (Harter and Stotzky, 1971; Anderson et al., 1981). On the other hand, exogenous OC derived from organic amendment is chemically protected by the formation of organic-mineral complexes (Six et al., 2002, 2000, 1998), and/or physically protected by the formation and stability of soil aggregates, both of which are key for SOC sequestration in agroecosystem (Han et al., 2016; Lal et al., 2015). Hence, studying the response of OC in soil particle fractions to fertilization is crucial for a better understanding of OC sequestration and stabilization mechanisms.

Moreover, fertilization, especially when in combination with organic amendments, is considered to be the most valid solution for improving SOC and crop yield (Wang et al., 2018; Zhu et al., 2013). As the most common and rich organic amendments, livestock manure and crop straw contain plenty of OC and nutrients (Ji, 2015; Li et al., 2020), their outputs in 2015 about 2.7×10^9 and 9.3×10^8 t in China, respectively (Lal, 2005; Shi et al., 2021; Song et al., 2018). Meanwhile, manure and crop straw incorporations into the field not only reduce environmental problems caused by improper application (Gao et al., 2019; Shi et al., 2014), but also increase OC and nutrients input, thus are conducive to crop growth and SOC sequestration (Liu et al., 2014; Xu et al., 2016, 2015). In addition, biochar, as an emerging organic amendment, is usually made from crop straw and manure by low-temperature carbonization and pyrolysis technology (Clough et al., 2013; Sohi et al., 2010). Biochar application could be considered as an effective solution to increase SOC and crop growth by improving OC input, nutrient use efficiency, and soil microenvironment (Biederman and Stanley Harpole, 2013; Novak et al., 2009). Thus, manure, straw, and biochar appropriately incorporated into the field have shown great potential for increasing SOC and crop yield, which has gradually received extensive attention and research in recent years (Sutherland et al., 2010; Wang et al., 2018; Yan et al., 2018). Therefore, it is essential to enhance the understanding of how and to what extent manure, straw, biochar application influence SOC and crop for ensuring food security and enhancing environmental sustainability by optimizing management practices.

2. Literature review

2.1. The mechanism of soil organic carbon sequestration and stabilization under fertilization in cropland

In recent years, the intensive utilization of cropland has led to a considerable loss of OC pool in agroecosystem (Li et al., 2018; Sanderman et al., 2017). Enhancing SOC in intensive cropping farmland has a profound effect on the sustainable intensification in agricultural systems (Lal et al., 2015, 2007). Organic amendment addition, as the most direct and effective practice to regulate SOC, could just SOC mineralization rate, then may accelerate SOC mineralization to cause a positive priming effect, or slow down SOC mineralization to generate a negative priming effect (Blagodatsky et al., 2010; Kuzyakov et al., 2000). However, the magnitude and direction of priming effect are not only impacted by the quantity and quality of exogenous OC but also influenced by SOC stability (Kuzyakov et al., 2000). Therefore, in addition to exogenous OC input, SOC stability is also a key for SOC accumulation under organic amendment application (Gunina and Kuzyakov, 2014; Hobley et al., 2014). The stabilization mechanism of SOC generally includes three aspects: the physical protection of aggregates, the chemical protection of soil minerals, and the biochemical protection of complex structure of OM, which are all shown in Figure 1-1 (Six et al., 2002; Xie et al., 2018). Thus, in agroecosystem, the physicochemical protection of soil particles to OC is key for SOC stability and sequestration (Han et al., 2016; Lal et al., 2015).

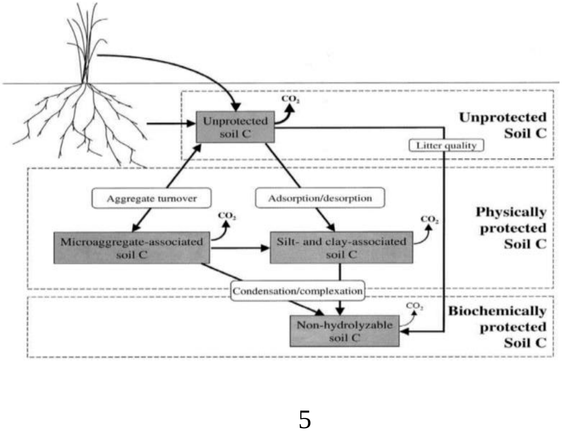

Figure 1- 1 Stabilization mechanisms of soil organic carbon (Six et al., 2002).

In general, most OC associates with soil particles (Six et al., 2002). Coarse particles associated with OC (> 53 µm, aggregate) is usually more active, and is more sensitive to management practices (Xu et al., 2011). In contrast, fine soil particles (< 53 µm, clay and silt) have a large specific surface area, which enables the adsorption and stabilization of SOC through ligand exchange, hydrogen bonding, and hydrophobic interaction (Leinweber and Reuter, 1992; Schulten and Leinweber, 1991). Thus, the phenomenon of OC saturation is firstly observed in fine soil particles, especially clay fractions, with the gradual increase of exogenous OC input and/or SOC accumulation (Hassink 1997; Six et al. 2002). To sum up, the responses of OC in different particle-sized fractions to management practices may be different. Previous studies argued that long-term manure alone or with chemical fertilizers promotes significantly OC in all soil particles (Liang et al., 2014; Zhang et al., 2015). However, Tong et al. (2009) found that the concentration and distribution of OC increase significantly in coarse particles (> 53 µm), but decrease in silt and clay fractions under fertilization. Moreover, Jiang et al. (2017) reported that chemical fertilizers increase significantly OC in silt and clay fractions (< 53µm) and decrease OC in aggregates (> 53 µm), but manure increases OC in all soil particles. Thus, although numerous studies have investigated the effect of long-term fertilization on soil particles associated with OC, their results are still controversial.

Moreover, SOC is closely related to the mass proportion of soil fractions (Six et al., 2002). For example, Ou et al. (2016) and Benbi et al. (2016) reported that SOC increases with the increase of aggregates' mass proportion. Meanwhile, some studies have argued that clay' mass proportion exhibits significant correlations with OC in clay fractions and bulk soil, implying that clay content is important for SOC accumulation, and determines the distribution and saturation levels of soil OC (Six et al., 2002; Vogel et al., 2015). Therefore, SOC could be controlled by the mass proportion of aggregates and clay. However, with SOC content and/or OC input increasing, due to the differences in the physical and chemical properties among different particle-sized fractions, OC enriched in soil fractions may affect the mass proportion of soil fractions by the destruction of coarse soil particles or the agglomeration of fine soil particles (Tisdall and Oades, 1982; Six et al. 2000; Six et al., 2002). However, knowledge about the mass proportion of soil fractions response to OC in soil fractions is still limited under long-term fertilization, and is needed to enhance our understanding of SOC sequestration mechanism in cropland.

2.2. Effect of manure application on soil organic carbon sequestration and crop yield in cropland

Manure, as the most common and rich biological resource, could continuously provide all essential nutrients for crop growth due to the slow release of nutrients, while chemical fertilizers could quickly and effectively provide mineral nutrients (Chen, 2006; Zhu et al., 2013). Chemical fertilizers combined with manure is a valuable practice, which has received widespread attention in the world from past to today, from crop production to soil fertility improvement (Ghosh et al., 2012; Wang et al., 2018; Zhu et al., 2013). Numerous studies have shown that this practice could increase SOC (depth up to 90 cm) and crop yield (Ghosh et al., 2012; Shahid et al., 2017; Zhang et al., 2016). Menichetti et al. (2015) found that 53-years chemical fertilizers combined with manure greatly improves SOC in 0-35 cm soil layer, and has little impact on SOC below 35 cm soil. However, Ghosh et al. (2018) argued that SOC accumulation and sequestration in this practice over control plots is 0.74 and 0.22 t ha^{-1} y^{-1}, respectively, in 0-90 cm soil layer with >50% of the accumulated OC in 30-90 cm. Meanwhile, a study conducted 17 long-term experiments in China reported that this practice improves crop yield and its stability (Zhang et al., 2016). However, in most previous studies, manure is additionally applied without reducing chemical N fertilizer rate (Xu et al., 2019; Duan et al., 2011; Ghosh et al., 2012; Zhang et al., 2016), then increasing the total amount of N fertilizer in the field (Wang et al., 2018). Overused chemical N fertilizer increases crop yield and meets food demand (Yan et al., 2018; Zhu et al., 2013), but causes negative effects on agroecosystem, such as low N utilization efficiency, soil acidification, SOC decline (Gu et al., 2013; Guo et al., 2010; Ju et al., 2003, 2009; Roelcke et al., 2004).

Partial substitution of chemical fertilizers by manure (organic substitution), as a widely recommended fertilization strategy in China today, recycles organic wastes and minimizes adverse environmental effects caused by chemical fertilizers overapplication (Li et al., 2017; Wang et al., 2018). Moreover, previous studies reported that organic substitution may increase, maintain, or even reduce crop yield compared with chemical fertilizers alone, thus there is no general agreement on those results (Dai et al., 2021; Greenberg et al., 2019; Ji et al., 2020; Lv et al., 2020; Wang et al., 2015). Meanwhile, although numerous studies showed that organic substitution improves OC, they limited their focus mainly on individual experimental sites and topsoil (Dai et al., 2021; Ning et al., 2017; Yue et al., 2016). Moreover, subsoil holds more than 50% of total soil C stocks in the entire 100 cm profile, having greater volume and potential to sequester C (Batjes, 2014; Jobbagy and Jackson, 2000). However, there is remarkably little information on how and to what extent organic substitution influences subsoil OC. Therefore, in various climatic zones and long-term timescales, it is essential to further study the effect of organic substitution on SOC in soil profile and crop production.

In addition, soil OC level usually limits crop productivity (D'Hose et al., 2014; Zhang et al., 2016). Numerous field experiments have reported a positive correlation between topsoil OC pool and crop yield, which implied that there is a synergistic effect of topsoil OC on crop yield under fertilization (Oldfield et al., 2019; Wei et al., 2020; Zhang et al., 2016). With the postponement of crop growth period, the proportion of crop roots in subsoil increases and will account for more than 30% of that in 0-100 cm soil (Jobbagy and Jackson, 2000; Yang and Zhang, 2011). Moreover, subsoil also stores plenty of nutrients, which are important for maintaining and improving crop yield especially in the later stages of crop growth (Hartmann et al.,2008; Ma et al., 2009). Thus, under long-term fertilization, the downward extension of crop roots could influence crop yield by uptaking subsoil nutrients, and might change OC pool in subsoil by increasing OC input derived from crop roots, which finally would affect crop production and SOC as well as their relationships (Fontaine et al., 2007; Jobbagy and Jackson, 2000). However, how crop productivity responds to SOC accumulation in soil profile remains unclear, and needs further study.

2.3 Effect of straw incorporation on soil organic carbon sequestration and crop yield in cropland

Straw, likes manure, is the most common and abundant organic amendment. Straw returned into the field not only reduces greenhouse gas emission caused by in-situ straw burning (Jacinthe et al., 2002; Shi et al., 2014), but also provides rich nutrients and OC sources and stimulates soil microbial and enzyme activities (Li et al., 2020), then enhancing SOC and food production (Zhao et al., 2020). Thus, straw return, as the most economical and sustainable way of straw resource utilization, is a feasible practice to improve soil fertility and crop yield (Yang et al., 2020). For example, it is estimated that about 0.6-1.2 Pg of C could be sequestered into soil by straw incorporation in the world every year (Lal, 2009b; Zhao et al., 2020, 2015). Moreover, via a global meta-analysis comprising 176 studies, Liu et al. (2014) argued that straw incorporation increases SOC content by 12.8%. However, straw return could easily render soil microorganisms in a state of nutrient limitation, and accelerate SOC mineralization, therefore resulting in a strong priming effect (Kuzyakov et al., 2000; Shahbaz et al., 2018). Thus, some studies found that SOC sequestration could not promote under long-term straw incorporation (Zhang et al., 2019). Moreover, numerous studies have shown that straw return increases significantly crop yield (Pathak et al., 2006; Berhane et al., 2020). However, straw return may enhance the temporary biological contention and fixation of N, which could inhibit the growth of crop population at the initial stage of growth, resulting in no increase or even decrease of crop yield (Liu et al., 2007; Zheng et al., 2019). In summary, although numerous studies have reported the impacts of straw incorporation on SOC and crop yield, the results are controversial and deserve further study.

Straw decomposition and transformation are crucial links in the C cycle and nutrient turnover (Parton et al., 2007). However, those processes are not only affected by straw properties (Liu et al., 2014), but also controlled by straw return mode (Fan et al., 2018; Zhang et al., 2017). Straw incorporation in different modes could lead to the differences in size, nature, and returning depth of straw, which, in turn, may influence soil microbial community diversity and straw decomposition rate (Coppens et al., 2006), finally resulting in inconsistent impacts on SOC and crop yield. Fan et al. (2018) found that, compared with straw mulching, straw return after crushing increases significantly SOC content in Northeast China. Moreover, Liu et al. (2014) argued that topsoil OC increase by 28.7% and 12.4% under straw return after crushing and livestock digestion in black soil, respectively. Moreover, Xu et al. (2018) suggested that straw covering the field and straw return with deeply plowing increase crop yield by 15.1% and 5.1% compared with straw removal. To sum up, previous studies mainly focus on topsoil or individual incorporation mode (Coppens et al., 2006; Fan et al., 2018; Powlson et al., 2008; Zhao et al., 2015), but the information on subsoil OC and crop yield as affected by different straw incorporation modes remains limited.

Moreover, since straw C: N is much higher than microbial C: N and then usually is negatively correlated with SOC mineralization rate (Yanni et al., 2011), straw addition will bring about a microbial nutrient limitation in the initial stage, finally leading to a low priming effect and the lag of straw effect in time (Lyu et al., 2019). Therefore, the effect of straw duration on SOC and crop yield could be controlled by straw return duration. For example, some studies found that SOC content changes insignificantly under short-term straw return, but will significantly increase and even reach saturation with time (Li et al., 2018; Lu et al., 2009). Moreover, Yang et al. (2020) reported that crop yield improvement may become more obvious with time. Thus, SOC (especially subsoil OC) and soil fertility evolution are a slow long-term process (Carvalhais et al., 2014; Luo et al., 2019). The long-term experiment provides unique opportunities to study the responses of agroecosystem and cropland soils to various fertilization practices over a long-term timescale (Wang et al., 2008; Xu et al., 2015; Zhao et al., 2010). It has the characteristics of being long in time, repeatable in climate, and consistent in field management practices, which makes experiments data more reliable and accurate, and can answer scientific questions that cannot be solved by short-term experiments (Wang et al., 2008; Xu et al., 2015; Zhao et al., 2010). To sum up, how and to what extent straw incorporation influences SOC in soil profile and crop yield, needs more in-depth and comprehensive study via long-term experiment, especially considering different return modes.

2.4 Effect of biochar application on soil organic carbon sequestration and crop yield in cropland

Biochar, as an emerging organic amendment, is a carbon-rich byproduct produced by the pyrolysis of agricultural waste (e.g., crop straw, manure, etc.) at high temperatures (400-700 °C) and anoxic conditions (Smith, 2016; Sohi et al., 2010). Biochar usually has high C content, rich nutrients, strong adsorption capacity, and oxygen-rich functional groups (Smith, 2016; Sohi et al., 2010). Except for being a stable C source, biochar application affects soil OC mainly via the following ways: (1) the easily decomposed fractions in biochar are preferentially mineralized, then could enhance soil microbial activity and lead to microbial co-metabolism, finally resulting in a positive priming effect (Prayogo et al., 2014; Lu et al., 2014); (2) in the biochar's external surface and internal pore structure, the strong adsorption and encapsulation to OC could promote the formation and stabilization of organic-inorganic complex and inhibit soil microbial and enzyme activities, finally causing a negative priming effect (Brodowski et al., 2005; Fang et al., 2014). Meanwhile, biochar has a rich pore structure and strong adsorption capacity, which could effectively intercept soil nutrients and improve nutrient use efficiency, consequently improving crop growth (Lehmann et al., 2003; van Zwieten et al., 2010; Zhang et al., 2017). Thus, biochar application is also an effective practice to regulate SOC and crop yield in cropland (Biederman and Stanley Harpole, 2013; He et al., 2017; Liu et al., 2013), which has received far-reaching attention and research (Sutherland et al., 2010).

However, several study results regarding the effects of biochar application on crop yield and SOC have great variation and high uncertainty (Liu et al., 2016, 2013). For example, previous results from global meta-analyses proved that biochar application increases crop yield and SOC by 5.0-51.0% and 28.0-60.0%, respectively (Biederman and Stanley Harpole, 2013; Cayuela et al., 2014; He et al., 2017; Liu et al., 2013). The great variation and high uncertainty are mainly due to human factors, especially chemical fertilizers addition (Al-Wabel et al., 2018; Lehmann and Joseph, 2009). Compared with biochar alone (B), biochar combined with chemical fertilizers (BF) not only directly provides mineral nutrients needed for crop growth but also increases crop return due to chemical fertilizers addition (Al-Wabel et al., 2018; DeLuca et al., 2009). Therefore, an enhanced understanding of the biochar's effect on SOC and crop yield by distinguishing the effects of B and BF is needed for accurate estimation.

Moreover, the great variation and uncertainty of biochar's effect on crop yield and SOC are dependent on climate factors (i.e., climate zone, temperature, precipitation, wetness index), management practices (i.e., crop types, and biochar properties), and soil properties (i.e., soil nutrients, soil texture) (He et al., 2017; Jeffery et al., 2017, 2011; Liu et al., 2016). Due to interaction of these factors, it will cause a considerable uncertainty to predict the biochar's effect on crop yield and SOC based on individual factors (He et al., 2017; Jeffery et al., 2017, 2011; Liu et al., 2016, 2019). However, previous meta-analysis studies only report the biochar's effect on crop yield and SOC among different factors, but the importance of these factors still remains unclear. Therefore, the relative contribution of these factors needs further study to accurately improve the potential benefits of biochar.

In summary, although numerous studies have shown that fertilization, especially the one with organic amendment application, helps to alleviate the decline of SOC caused by long-term intensive cropping, the understanding of SOC sequestration mechanism still remains limited in high-intensity intensive cropping systems. Meanwhile, numerous studies have investigated the responses of SOC and crop yield to manure, straw, and biochar applications, but how and to what extent organic substitution, straw incorporation modes, biochar alone and combined with chemical fertilizers influence SOC and crop yield are limitedly understood.

3. Objectives

The general objectives of this work were to reveal the underlying mechanism of SOC sequestration in intensive cropping systems, and to study the effects of organic substitution, straw return, and biochar application, which were highly concerned and recommended in recent years, on SOC and crop yield using field experiments and meta-analysis. The technology roadmap of our research was shown in Figure 1-2. Firstly, via a field experiment (red dashed box), the responses of OC in soil particles to long-term fertilization were studied to reveal the underlying mechanism of OC sequestration under intensive cropping systems (purple dashed box, Chapter II). Secondly, through long-term fertilization field experiments (red dashed box), the impacts of organic substitution and straw incorporation on SOC in soil profile, crop yield, and their relationship (green dashed box, including Chapter III and Chapter IV) were investigated to provide novel insight for optimizing manure and straw applications. Finally, the effects of biochar (an emerging organic amendment) on topsoil OC and crop yield as well as the relative contribution of various factors were quantified (green dashed box, Chapter V) via a global meta-analysis (blue dashed box) to provide a new perspective of fertilization strategy improvement.

To expound on the above main objectives, the specific aims are as follows:

(1) To evaluate the responses of OC in soil fractions to long-term fertilization;

(2) To reveal the underlying mechanisms of SOC sequestration and stabilization in double maize cropping systems;

(3) To investigate the effect of organic substitution on SOC in soil profile and crop yield;

(4) To investigate the effect of straw incorporation modes on SOC in soil profile and crop yield;

(5) To investigate the responses of topsoil and subsoil OC to long-term fertilization as well as to their key factors;

(6) To explore the synergistic effects of SOC accumulation in soil profile on crop yield under long-term fertilization;

(7) To quantify the effect of biochar application on topsoil OC and crop yield by distinguishing the effects of B and BF as well as to identify their key factors;

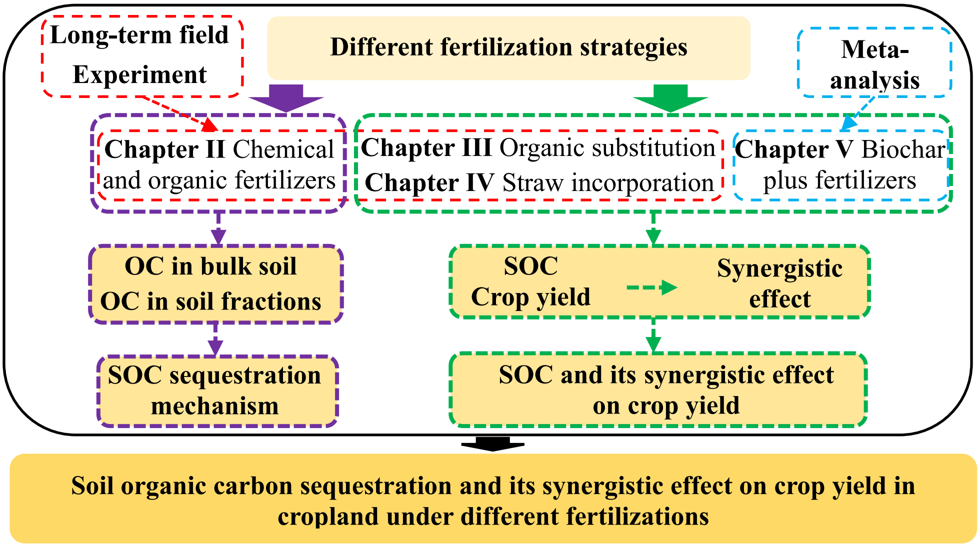

Figure 1- 2 The technology roadmap of this thesis.

4. Overview of the chapters

This thesis was structured into the following 6 chapters:

Chapter I General introduction.

In this chapter, the general information of research progress and objectives was described. We presented a general review regarding the mechanisms of SOC stabilization and sequestration under long-term fertilization as well as the effects of manure, straw, and biochar application on crop yield and SOC, especially in China, and then put forward the knowledge gap in related research. Finally, the objectives and structure of this thesis were summarized in this chapter.

Chapter II Long-term fertilization and intensive cropping enhance carbon and nitrogen accumulated in soil clay-sized particles of red soil in South China.

The objective of this chapter was to evaluate the responses of soil particle fractions and their association with OC and N to long-term fertilization for revealing the underlying mechanisms of topsoil OC and N sequestration in intensive cropping systems. Here we used a long-term field experiment established in 1986 with double maize cropping. By using soil fractionation and 13C-isotope analyses, we analysed OC, TN, and $\delta^{13}C$ in bulk soil, aggregates (53-2000 μm), coarse silt fraction (5-53 μm), fine silt fraction (2-5 μm), and clay fraction (<2 μm) under different fertilizations, including CK, NPK, 2NPK, M, and NPK+M.

Chapter III Partial substitution of chemical nitrogen fertilizer by manure promotes carbon and nitrogen accumulation in deep soils

The objectives of this chapter were to investigate the impacts of different fertilization regimes, especially partial substitution of chemical N fertilizer with manure, on SOC and TN contents and stocks in the soil profile of 0–100 cm and crop yield, as well as to explore the responses of crop productivity to SOC accumulation in 0-100 cm soil profile. Four typical upland experimental sites of Qiyang, Yangling, Zhengzhou, and Gongzhuling across various climate zones involved in this study were all established in 1990. Soil samples were collected in September 2013 after maize harvest at four sites. Sampling depth was 0–100 cm, which was divided into five layers (each measuring 20 cm). Soil OC, TN, and bulk density were determined under different fertilization regimes, including CK, NPK, NPK+S, and 70%NPK+M. The N, P, K contents and weight of crop straw and grain in maize and wheat were measured in each treatment at four experiment sites after crop harvest every year.

Chapter IV Long-term straw incorporation significantly reduced subsoil organic carbon stock in cinnamon soil

The objective of this chapter was to study the effects of straw return with an equal amount in different modes on SOC in the soil profile of 0–100 cm and crop yield in cinnamon soil. The long-term field experiment of straw incorporation was conducted at Shouyang County of Shanxi Province in 1992. A split-plot design was used with main plots under the application seasons (spring and autumn) of chemical fertilizers, four straw incorporation modes (SR, SM, SC, and CM) as sub-plots. Soil samples were collected in September 2013 after harvest. Sampling depth was 0–100 cm, which was divided into five layers (each measuring 20 cm). Soil bulk density, crop yield as well as soil OC, TN, TP, TK, AP, and AK contents were measured for all treatments.

Chapter V Effects of biochar application on crop productivity and soil carbon sequestration controlled by biochar C: N ratio and soil pH: A global meta-analysis

The objectives of this chapter were to quantitatively examine the effects size of B and BF on crop yield and topsoil OC under different external conditions using a global meta-analysis, and to identify their key factors via a BRT model. In this study, 1080 paired crop yields under biochar application from 143 published literatures were collected, including 648 paired under B, 430 paired under BF, and 333 paired with SOC determinations. In addition to crop yield, information including site location (country, latitude, and longitude), climate variables (MAT, MAP), crop type, biochar properties (biochar type, application rate, pH, C: N, C and N contents), soil properties (SOC, soil TN, soil pH, and soil texture) were also extracted from the targeted literature to explain the variations of crop yield and SOC with biochar application.

Chapter VI General discussion, conclusions, and perspective

In this chapter, the meaning, importance, and relevance of general results in the four main chapters were introduced. We stated the answers to the main questions of this study, made future prospects for this topic and demonstrated what new knowledge we have contributed.

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Chapter II

Long-term fertilization and intensive cropping enhance carbon and nitrogen accumulated in soil clay-sized particles of red soil in South China

From Xu, H., Liu, K. L., Zhang, W. J., Rui, Y. C., Zhang, J. Y., Wu, L., Colinet, G., Huang, Q. H., Chen, X. N., Xu, M. G., 2020. Long-term fertilization and intensive cropping enhance carbon and nitrogen accumulated in soil clay-sized particles of red soil in South China. *Journal of Soils and Sediments* 20, 1824-1833.

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Abstract

Understanding the underlying mechanism of soil carbon (C) and nitrogen (N) accumulation is of great significance for soil C sequestration and climate change mitigation, as well as soil fertility improvement. The objective of this study was to evaluate the response of C and N accumulation in aggregates and fine soil particles to long-term chemical fertilizers and manure application. Five treatments from a long-term experiment with double maize cropping were examined in this study: (1) no fertilizer (CK); (2) chemical nitrogen, phosphorus and potassium application (NPK); (3) doubled application rate of the NPK (2NPK); (4) pig manure alone (M); and (5) chemical NPK fertilizers and manure combination (NPK+M). By using physical particle-sized fractionation, we analysed soil organic carbon (OC) and total nitrogen (N), and $\delta^{13}C$ of OC in bulk soil and aggregates (53-2000 µm) and, coarse silt-sized fraction (5-53 µm), fine silt-sized fraction (2-5 µm), and clay-sized fraction (<2 µm) under those five treatments. 24 years' fertilizers application, particularly M and NPK+M treatments, significantly increased the concentration and proportion of OC and total N associated with aggregates and clay-sized fraction as compared with CK treatment. Manure application significantly increased the proportion of OC by 6.6-7.8 points in aggregates, whereas it was by 22.6-25.0 points in clay-sized fraction. Clay-sized fraction associated C and N showed a non-linear response to C and N accumulation in bulk soil, contributing approximately 47.0% and 69.0% to soil OC and total N, respectively. Moreover, the mass proportion of aggregates and the mass ratio of aggregates to fine soil particles increased significantly with C accumulation in fine silt-sized and clay-sized fraction. Organic carbon and total nitrogen accumulation in soil clay-sized particles play important role in soil C and N sequestration in red soil. Our results also suggested that C accumulation in fine soil particles might benefit soil aggregation in intensive cropping systems of South China.

Keywords: Long-term fertilization, organic carbon, total nitrogen, physical particle-sized fractionation, foil aggregation

1 Introduction

Soil organic carbon (SOC) and nitrogen (N) in agro-ecosystem have attracted much attention over the past two decades, due to their crucial roles in soil fertility, crop productivity, and climate change mitigation (Ghimire et al. 2017; Paustian et al. 2016). Higher SOC and N levels are usually associated with higher crop productivity contributed by the enhanced nutrient cycling, and physicochemical and biological properties (Bell et al. 2014). On the other hand, greater crop yield can also contribute to a greater amount of crop residues incorporated into soils, thereby enhancing C and N accumulation (Zhang et al. 2010). Therefore, appropriate agricultural management practices, such as crop rotation, optimal fertilization and organic amendments, are highly recommended to enhance soil C sequestration by increasing crop residues return and C and N input (Fan et al. 2019; Wen et al. 2019).

SOC and N are mainly stabilized with soil minerals. Numerous studies have confirmed that most OC and N are bound in silt-sized and clay-sized fractions, highlighting the predominant role of fine mineral particles in C and N stabilization (Antil et al. 2005; Poeplau et al. 2017). Some studies show that C and N within particle-sized fractions display contrasting decomposition characteristics (Chan et al. 2002; Koiter et al. 2017). Generally, organic matter in aggregates (>53 μm) is active and sensitive to management practices changed. In contrast, fine soil particles (<53 μm) have a larger specific surface area, and can adsorb and stabilize C and N through ligand exchange, hydrogen bonding and hydrophobic effects (Leinweber and Reuter 1992; Schulten and Leinweber 1991). Accordingly, OC and N in fine soil particles usually have a long turnover time and contribute greatly to C and N stock (Balesdent et al. 2000; Denef et al. 2007). It is believed that long-term manure application can significantly enhance C and N accumulation in all particle-sized fractions in arable soils (Liang et al. 2014; Zhang et al. 2015a). Some studies show that chemical fertilizers application has little influence on, or even decreases C and N concentration in different particle-sized fractions (Aoyama et al. 1999; Ling et al. 2014). Some other studies also suggest that long-term fertilization can increase C distribution in aggregates and silt-sized fractions, but not affected in clay-sized fraction of topsoil (Dong et al. 2017). Therefore, more evidence is needed to unravel the impact of chemical fertilizers and manure application on C and N stabilization in soil particles.

Red soil accounts for about 30.0% of the total area in China’s arable land. This highly weathered soil is widely distributed in tropical and subtropical regions of Southern China with abundant precipitation and thermal resource. However, over a long period of intensive cultivation and farming, soil fertility and productivity in these areas have seriously degraded due to the high risk of soil erosion and acidification resulted from intensive cropping and excess use of chemical nitrogen fertilizer (Guo et al. 2010). Moreover, developed from the Quaternary red clay, the red soil usually is poorly structured (Xu et al. 2015). A study from this area indicates that long-term organic manure increases C accumulation in the macro-aggregates and micro-aggregates (Huang et al. 2010). Due to the intensive cropping in this area, we hypothesized that organic C derived from crops or applied manure would be bond to fine soil particles (<53 μm) first, resulted in C enrichment in the fine particles, then acted as binding agents to benefit the formation of soil aggregates. To test this hypothesis, we select a more than 20-year field experiment in upland red soil to explore the response of OC and total N in soil fractions to long-term fertilizers application.

2 Materials and Methods

2.1 Experiment site description

This long-term experiment site (28°37'N, 116°26'E) was located in Jinxian County, Jiangxi province. The red soil was classified as Ferralic Cambisol (FAO 1988) in this study. The MAT and MAP were 17.70 °C and 1727 mm, respectively. The experiment started in 1986. The initial topsoil (0-20 cm) physiochemical properties were as follows: SOC: 8.93 g kg^{-1}, total N: 0.98 g kg^{-1}, available N: 60.30 mg kg^{-1}, pH value: 6.00, bulk density: 1.19 g cm^{-3}.

2.2 Cropping practice

Prior to the long-term experiment, the field was cultivated with peanuts (C_3 plant, Arachis hypogaea) and/or soybean (C_3 plant, Glycine max (L.) for several years. Since 1986, double maize (C_4 plant, Zea mays L.) plantation had been conducted. Spring and summer maize were sown in mid-April and late July, and harvested in early July and early November, respectively. Herbicides and pesticides were applied during the growth periods when necessary. Crops were harvested by cutting manually. The above-ground biomass was removed from the field after the harvest, leaving about 5-10 cm height stalks in situ. Grains and straws yield for each crop were recorded yearly after air-dried.

Table 2-1 Annual amount of nitrogen (N), phosphorus (P), potassium (K) fertilizer, and manure applied for fertilization treatments in double maize system from 1986-2010 year

Treatments	Chemical fertilizer$^{\$}$			Organic fertilizer$^{\#}$
	N (kg ha^{-1})	P_2O_5 (kg ha^{-1})	K_2O (kg ha^{-1})	Fresh pig manure (kg ha^{-1})
CK	0	0	0	0
NPK	120	30	60	0
2NPK	240	60	120	0
M	0	0	0	30000
NPK+M	120	30	60	30000

Note: $^{\$}$ Chemical nitrogen fertilizer was as urea, chemical phosphorus fertilizer was as calcium-magnesia phosphate, and chemical potassium fertilizer was as potassium chloride;

$^{\#}$The water content of fresh pig manure was 72%. The organic carbon, nitrogen, phosphorus, and potassium content of pig manure was 376 g kg^{-1}, 33.14 g kg^{-1}, 23.77 g kg^{-1}, and 15.09 g kg^{-1}, respectively.

The application rate of chemical fertilizer and organic fertilizer was half and half for each maize season. Fresh pig manure, potassium chloride and calcium-magnesia phosphate were applied as basal fertilizers. 70% of nitrogen in urea was applied as top dressing one week after seeding. Then the rest of nitrogen fertilizer was applied two weeks after seeding.

2.3 Experiment design

The experiment was conducted based on a completely randomized design with three replicates. Five treatments were included in this study: (1) no fertilizer (CK); (2) chemical nitrogen, phosphorus, and potassium application (NPK); (3) doubled application rate of the NPK (2NPK); (4) pig manure alone (M); and (5) chemical NPK fertilizers and pig manure combination (NPK+M). Each plot was randomly designed with an area of 22.20 m^2, and was isolated by 100 cm cement baffle plates.

Chemical N, P and K fertilizers were applied in urea, calcium-magnesia phosphate, and potassium chloride, respectively (Table 2-1). For NPK and NPK+M treatments, chemical N, P and K fertilizers were applied at rates of 60 kg N ha^{-1} $season^{-1}$, 15 kg P ha^{-1} $season^{-1}$ and 30 kg K ha^{-1} $season^{-1}$, respectively. Manure was applied at 15 t ha^{-1} (fresh basis weight) under M and NPK+M treatments for each maize season. All chemical P, K fertilizers and organic manure were used as a base fertilizer. 70% of chemical N fertilizer was applied as topdressing, and the rest N fertilizer was applied two weeks after seeding. The contents of carbon, nitrogen and water in pig manure were 376 g kg^{-1}, 33.14 g $kg^{-1}$and 716 g kg^{-1}, respectively.

2.4 Sampling and analysis

Soil samples (0-20 cm) were collected after summer maize harvest in 2010. Each plot was randomly sampled for 5-10 cores in 5 cm diam. These soil cores from each plot were thoroughly mixed and air-dried, and then passed through 2.0 mm and 0.25 mm sieve for chemical analyses.

Air-dried soil samples (<2000 μm) were fractionated into aggregates (53-2000 μm), coarse silt-sized fraction (5-53 μm), fine silt-sized fraction (2-5 μm), and clay-sized (<2 μm) fraction with a modified method (Wu et al. 2005). In this method, aggregates (53-2000 μm) were selected by wet-sieving, and mineral-associated fractions (<53 μm) by centrifugation of the suspension after wet-sieving according to Stockes' law (Amelung et al. 1998; Wu et al. 2005). Before wet sieving, the air-dried samples were pre-treated as follows: air-dried soil was capillary rewetted to field capacity plus 5% (kg kg^{-1}) and equilibrated at 4 °C over night before immersion in water (rewetted treatment). Coarse silt-sized (5-53 μm), fine silt-sized (2-5 μm), and clay-sized (<2 μm) fractions were isolated by different centrifugation speed and time. Suspension with clay-sized fraction was flocculated with 0.20 mol/L $CaCl_2$, and then collected by centrifugation. Aggregates (53-2000 μm) were retained on the sieve, and the centrifuged fractions were washed to aluminium boxes, first being evaporated in water bath, and then being put in an oven at 40 °C to a consistent weight (48-72 h) and finally being grounded by hand to pass through a 0.15 mm sieve for C, N, and $\delta^{13}C$ analysis.

The concentration of OC and total N in bulk soil and soil fractions were determined by CN Analysis using a Euro EA3000 (*Eurovector Milan, Italy*). The δ^{13}C value of OC in bulk soil and soil fractions were analysed by an Isoprime MAT Delta Plus XL (*Bremen, Germany*). Since aggregates (>53 μm) differed in content from other aggregate-sized class, SOC content was corrected on the sand free basis (Six et al. 1998). The recoveries of soil mass (97.0%-101.0%), SOC (88.0%-106.0%), and total nitrogen (94.0%-110.0%) after particle-sized fractionation averaged 99.0%, 102.0%, and 93.0%, respectively.

2.5 Data calculation and statistical analysis

Soil OC and total N stocks (t ha^{-1}) in bulk soil and soil fractions were calculated as:

$$OC(total\ N)_{stock} = (C \times A_i \times BD \times d)$$

In the above equation, C was the concentration of OC (total N) in bulk soil and soil fractions (g kg^{-1}), A_i was the mass proportion of the i fractions content to the bulk soil (%). BD was the bulk density (g m^{-3}), and d was the soil depth (0.20 m).

One-way ANOVA was conducted to determine the effect of various fertilization treatments on the concentration and distribution of OC and total N in bulk soil and soil fractions using SPSS 22.0. Significant differences among various fertilization treatments were assessed by the LSD test at 5% levels for all the parameters. The linear and non-linear regression was performed to check relationships between OC concentration (and total N) in soil fractions and OC concentration (and total N) in bulk soil, and between the mass proportion of soil particle fractions and OC concentration in soil particle fractions under various fertilization.

3 Results

3.1 Soil OC and total N in bulk soil

Long-term fertilization significantly changed SOC and total N concentration in double maize cropping systems (Figure 2-1). In bulk soil, SOC concentration was the highest in NPK+M treatment (9.89 g kg^{-1}), followed by M treatment (Figure 2-1). Compared with the starting year, SOC concentration significantly increased by 11.0% and 7.0% in NPK+M and M treatments, respectively, while decreased by 18% in CK treatment. Total N concentration was the highest (1.14 g kg^{-1}) in NPK+M treatment (Figure 2-1b), followed by M treatment (1.09 g kg^{-1}). As compared with the starting year, total N concentration significantly increased by 11.0% and 16.0% in NPK+M and M treatments, respectively, while decreased by 18.0% in CK treatment. Moreover, Long-term fertilizers application did not significantly change soil C: N ratios, except in NPK treatment (Figure 5-1).

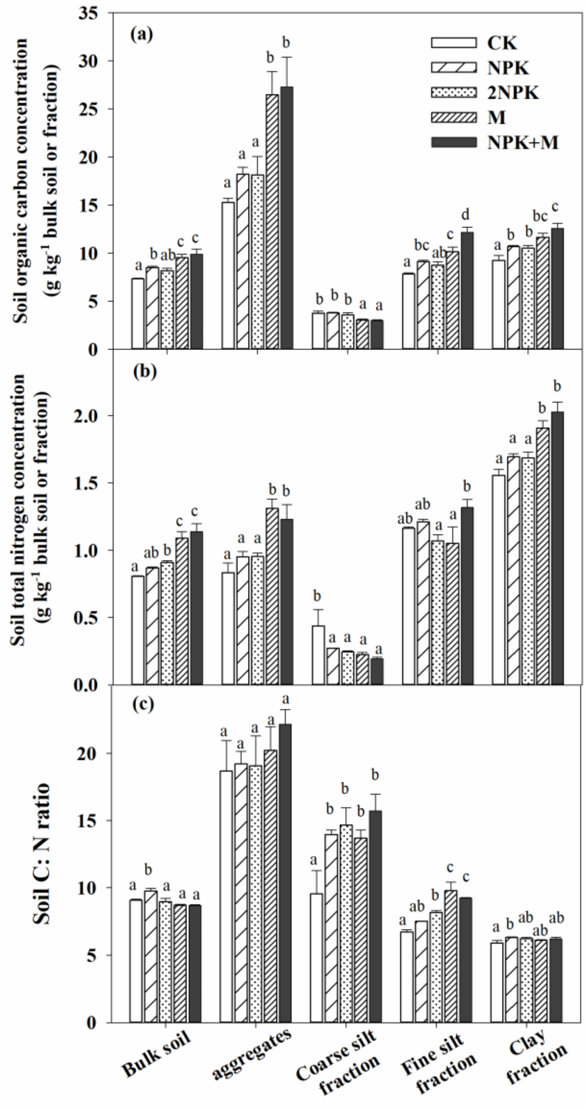

Figure 2-1 Organic carbon (a), total nitrogen (b), and C: N ratio (c) in bulk soil and soil fractions after two decades of fertilization (1986-2010) in a double maize system. Aggregates (53-2000 μm), coarse silt-sized fraction (5-53 μm), fine silt-sized fraction (2-5 μm), and clay-sized fraction (<2 μm). Bars indicate SE (n=3). Different letters above the bars indicate significant differences in organic carbon (a), total nitrogen (b) and C/N ratio (c) of bulk soil and soil fractions among different treatments at the 5% level.

3.2 Soil particle-sized fraction and their association with OC and total N

Fractionation analysis showed that the mass proportion of coarse silt-sized fraction was the highest (with an average of 42.0%), but the mass proportion of aggregates was the lowest (with an average of 6.0%) (Table 2-2). Compared with CK treatment, fertilizers application increased the mass proportion of aggregates and clay-sized fraction, particularly in manure application (M & NPK+M), the mass proportion increased by an average of 2.30 points and 15.80 points, respectively.

Results showed that OC concentration was the highest in aggregates (with the average of 21.07 g kg^{-1} fraction), but the lowest in coarse silt-sized fraction (with the average of 3.43 g kg^{-1} fraction) among all treatments (Figure 2-1a). Compared to CK treatment, manure application (M & NPK+M) increased OC concentration in aggregates, fine silt-sized fraction and clay-sized fraction by an average of 76.0%, 42.0%, and 31.0%, respectively. However, manure application (M & NPK+M) significantly decreased OC concentration by an average of 17.0% in coarse silt-sized fraction. As for total N concentration, it was the highest in clay-sized fraction (1.77 g kg^{-1} fraction), and the lowest in coarse silt-sized fraction (0.27 g kg^{-1} fraction). And it increased by an average of 52% and 26% in aggregates and clay-sized fractions under M and NPK+M treatments, respectively, as compared to that under CK treatment. Accordingly, soil C: N ratio was the highest in aggregates (19.87), and the lowest in clay-sized fraction (6.16), showing a decreasing trend with the decrease of soil particle size (Figure 2-1c). Compared with CK treatment, chemical fertilizers application (NPK & 2NPK) significantly increased C: N ratio ($p < 0.05$) by 4.75 units in coarse silt-sized fraction, whereas manure application (M & NPK+M) increased C: N ratio by 5.13 and 2.78 units ($p < 0.05$) in coarse silt-sized and fine silt-sized fractions, respectively.

The distribution proportions of OC and total N were the highest in clay-sized fraction (47.0% and 69.0%, respectively), while the lowest in aggregates (14.0% and 6.0%, respectively) for all treatments (Table 2-2). Compared with CK treatment, manure application (M & NPK+M) increased the distribution proportions of OC by an average of 7.20 points and 23.80 points, and total N by an average of 2.40 points and 17.30 points in aggregates and clay-sized fraction, respectively. Meanwhile, as for coarse silt-sized and fine silt-sized fractions, manure application decreased the distribution proportions of OC by an average of 17.10 points and 3.70 points, and total N by an average of 9.60 points and 10.00 points as compared to CK treatment, respectively.

3.3 Relationship between soil particle fractions and OC and total N accumulation

The OC stock of aggregates was significantly and positively correlated with OC in bulk soil ($p < 0.05$, Figure 2-2a). There was a significant exponential relationship between SOC stock in clay-sized fraction and in bulk soil (Figure 2-2d), but a significantly negative relationship between SOC stock in coarse silt-sized fraction and in bulk soil (Figure 2-2b). A significant exponential relationship between total N stocks in clay-sized fraction and in bulk soil was observed (Figure 2-3d), whereas there was a significant linear correlation appeared in aggregates (Figure 2-3a).

Table 2-2 Mass proportion of soil fractions to bulk soil ($M_{\%}$, %), the proportion of soil fractions associated OC ($C_{\%}$, %) and total N ($N_{\%}$, %) to soil OC and total N under various fertilizations in double maize system, respectively.

Treatments	Aggregates (53-2000 μm)			Coarse silt-sized fraction (5-53 μm)			Fine silt-sized fraction (2-5 μm)			Clay-sized fraction (<2 μm)		
	$M_{\%}^{\#}$	$C_{\%}$*	$N_{\%}$*	$M_{\%}$	$C_{\%}$	$N_{\%}$	$M_{\%}$	$C_{\%}$	$N_{\%}$	$M_{\%}$	$C_{\%}$	$N_{\%}$
CK	4.29a	10.01a	4.58a	44.81b	30.94c	14.61c	13.38ab	19.29 b	24.18 c	23.58a	29.51 a	56.63 a
NPK	6.11b	13.72b	6.25b	40.83a	19.53b	6.25 b	15.50 b	17.90 b	20.54 c	36.10b	48.85 b	66.97 b
2NPK	5.77b	13.38b	6.16b	41.28a	19.12b	6.31 b	13.57ab	15.43 a	16.18 b	37.92b	52.06 c	71.35 c
M	6.02b	16.59c	6.89b	40.44a	14.48a	4.56 a	12.80 a	14.68 a	12.84 a	39.97b	54.56cd	75.71 d
NPK+M	7.17b	17.79c	7.07b	40.67a	13.13a	5.38ab	12.82 a	16.62ab	15.45ab	38.76b	52.10 c	72.09cd

Note: Data are means (n=3). Different letters in the column indicate significant differences among different treatments at the 5% levels. $^{\#}M_{\%}$ represent the proportion of each soil fractions mass to bulk soil. $^{*}C_{\%}$ ($N_{\%}$) represent the proportion of soil fractions associated OC (total N) to soil OC (total N) in bulk soil.

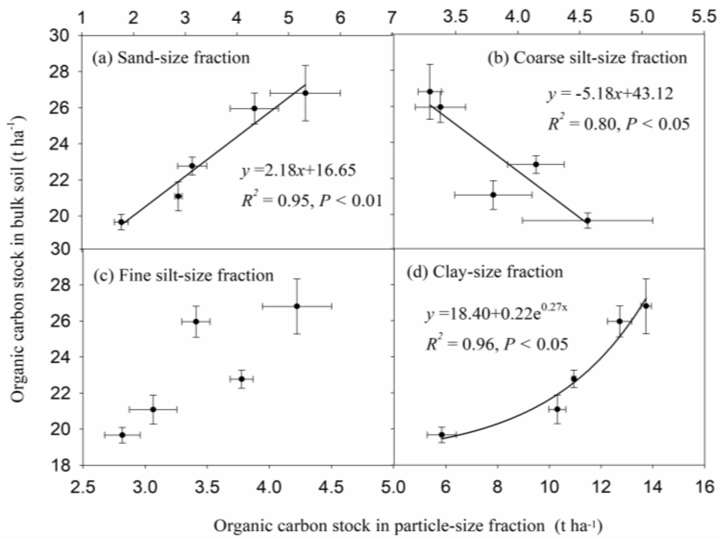

Figure 2-2 Relationship between organic carbon stock in bulk soil and organic carbon stock in aggregates (a, 53-2000 µm), coarse silt-sized fraction (b, 5-53 µm), fine silt-sized fraction (c, 2-5 µm), and clay-sized fraction (d, <2 µm) fractions after two decades of fertilizations (1986-2010) in a double maize system. Error bars represents the standard errors of organic carbon in bulk soil and soil fractions (n=3). R^2 represents the determination coefficient of the linear equation.

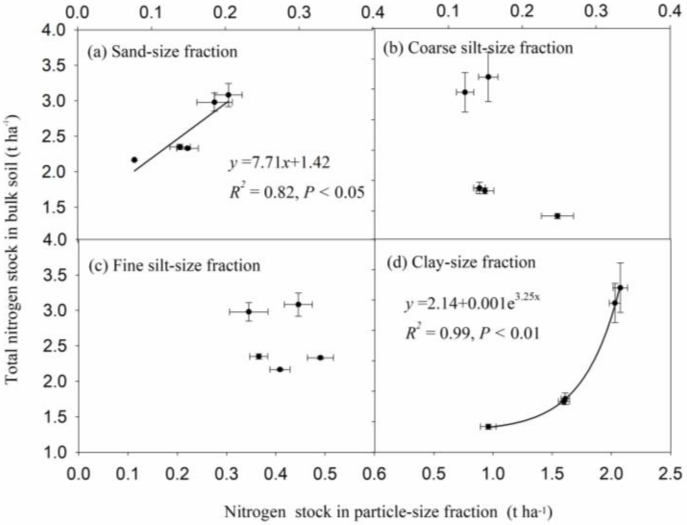

Figure 2- 3 Relationship between total nitrogen in bulk soil and total nitrogen stock in aggregates (a, 53-2000 µm), coarse silt-sized fraction (b, 5-53 µm), fine silt-sized fraction (c, 2-5 µm), and clay-sized fraction (d, <2 µm) fractions (d) fractions after two decades of fertilizations (1986-2010) in double maize system. Error bars represents the standard errors of organic carbon in bulk soil and soil fractions (n=3). R^2 represents the determination coefficient of the linear equation.

The mass proportion of aggregates to bulk soil showed a significant ($p < 0.05$) exponential-growth with the increase in the concentration of fine silt-sized and clay-sized fraction associated OC (Figure 2-4a and b). However, a significant ($p < 0.05$) logarithmic-decay trend was observed between the mass proportion of coarse silt-sized fraction and the concentration of fine silt-sized fraction associated OC (Figure 2-4c) and clay-sized fraction associated OC (Figure 2-4d). Furthermore, the mass ratio of aggregates to coarse silt-sized fraction and fine silt-sized fraction also suggested a significantly positive and linear correlation with the concentration of fine silt-sized fraction associated OC (Figure 2-4e and g) and clay-sized fraction associated OC (Figure 2-4f and h).

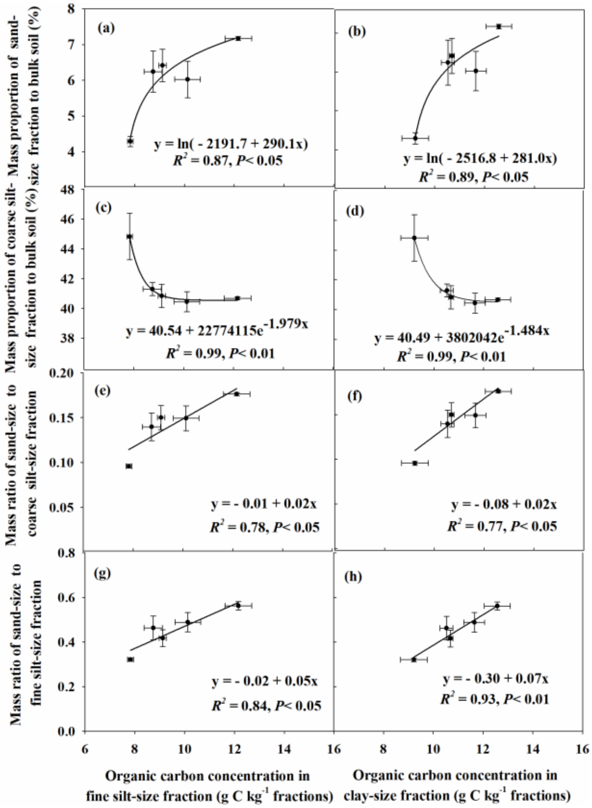

Figure 2-4 Relationship between the mass proportion of aggregates (53-2000 μm) and the concentration of silt-sized (a, 2-5 μm) and clay-sized (b, <2 μm)fraction associated OC; between the mass proportion of coarse silt-sized fraction and the concentration of silt-sized (c) and clay-sized (d) fraction associated OC; between the mass ratio of aggregates to coarse silt-sized fraction (5-53 μm) and the concentration of silt-sized (e) and clay-sized (f) fraction associated OC; between the mass ratio of aggregates to fine silt-sized fraction and the concentration of silt-sized (g) and clay-sized (h) fraction associated OC after two decades of fertilizations (1986-2010) in double maize system. R^2 represents the determination coefficient of the linear equation. Error bars represents the standard errors of organic carbon in bulk soil and soil fractions (n=3).

4 Discussion

Our study confirmed that manure application facilitated OC and total N accumulation in bulk soil. Fertilization, especially organic fertilizers application, mainly enhanced crop yields, consequently increasing the return amounts of stubble and root exudates in the previous studies of this area (Zhang et al., 2015b), introducing plenty of exogenous C and N into soil. Furthermore, the enhanced C and N accumulation might benefit the physical and chemical protection of OC and N by soil aggregate formations (Campbell et al. 2001). Our findings showed the highest OC concentration in aggregates and highest total N in clay-sized fraction, which was in accordance with those reported by He et al. (2009) in northern China and Gelaw et al. (2015) in northern Ethiopia. The possible reason was that the refractory and incompletely decomposed plant residues and stubble (with high C: N ratios) were mainly accumulated in aggregates. Organic matter in clay-sized fraction is highly decayed by microbes with N enrichment and lower C: N ratio. OC and N in clay-sized fraction were likely adsorbed and stabilized as organo-mineral complexes (Marx et al. 2005). Furthermore, our results indicated that annual additions of organic manure significantly decreased OC and total N concentration in coarse silt-sized fraction. The main reason might be that, other than the physical protection from aggregates and the chemical bonding from clay, coarse silt-sized fraction associated with OM was relatively easy to be decomposed by soil microbes (Vogel et al. 2015). This likely indicated that coarse silt-sized fraction associated C and N were in an intermediate state of decay, and might have a relatively fast turnover rate which could facilitate the stabilization of microbial products in clay-sized fraction.

According to the theory of aggregates turnover proposed by Six et al. (2002), plant residues which are not completely degraded, are physically protected in coarse particle-sized fraction (Kirchmann et al. 2004). In this study, the C: N ratio showed a decreasing trend with the decrease of particle sizes from 19.87 in aggregates to 6.16 in clay-sized fraction. Soil C: N ratio usually decreases with the increase of residues decomposition. As the soil particle size decreased, the decomposition degree of soil particle fractions associated with OM increased gradually (Yan et al., 2012; Liang et al., 2014). Thus, clay-sized fraction shows a high degree of microbial decomposed organic matter (Gerzabek et al. 2006), and it is also particularly enriched by microbial debris and degradation products (Vogel et al. 2015). The C: N ratio of 6.6 in clay-sized fraction in this study also suggests a possible source from fungi (C: N ratios of 15:1-5:1) and bacteria (C: N ratio of 5:1- 3:1) (Amelung et al. 1998).

Our study found that about 47.0% of OC and 69.0% of total N were stored in the clay-sized fraction, indicating that clay fraction played an important role in C and N sequestration in red soil. It might be due to the high concentrations of clay-sized fraction associated C and N, and the larger mass proportion of clay-sized fraction (35%) in this soil. These results were in agreement with Christensen (2001) and Long et al. (2015) that more C and N were stored in clay-sized fraction than in other particle-sized fractions of soil. This result also highlighted the capacity of OM accumulation via organo-mineral interactions and adsorption, and its nonsusceptibility to microbial decomposition due to the strong physical-chemical protection (Diekow et al. 2005; Vogel et al. 2015). It is likely that clay-associated C and N are mainly semi-decomposed, and are relatively stable and hard to be decomposed due to the encapsulation of inorganic-organic compounds (Fernandez-Ugalde et al. 2016).

As the stubble and root exudates were incorporated into soil, the $\delta^{13}C$ value of SOC gradually approached the $\delta^{13}C$ value of the above-ground growth plant (Bai et al., 2012). For the treatments without manure (CK and NPK of which C was mainly derived from maize), the $\delta^{13}C$ of soil particles associated OC showed an increasing trend as a decrease in particle size (Table 2-3). The increment of the $\delta^{13}C$ of OC in NPK treatment was relatively high than that in CK treatment, especially in clay-sized fraction (Table 2-3). Its possible reason was those chemical fertilizers application enhanced maize yield and biomass, and correspondingly increased carbon input from maize in the previous studies of this area (Zhang et al. 2015b). In addition, the averaged value of $\delta^{13}C$ in fine silt-sized and clay-sized fraction showed a relatively high abundance ($\delta^{13}C$ increased by 2.40 and 1.09 points, respectively) after 18 years for CK and NPK treatments compared with the initial value (Table 2-3), whereas the increment was 0.78 points in the coarse silt-sized fractions. These results indicated that maize-derived C from residues and roots was mainly enriched in fine silt-sized and clay-sized fractions. Besides fractionation and stabilization of ^{13}C with SOM decomposition (Bird et al. 2003, Roscoe et al. 2001), more microbial decomposition products and maize-derived C might also be associated with these soil fine minerals, leading to the increase in abundance of ^{13}C of fine soil particles. It might reveal the gradual increase in the proportion of C derived from maize residues and roots in SOC. Furthermore, the relatively high increase in $\delta^{13}C$ of fine soil particles associated OC suggested that fine soil particles might be the main sequestration site of maize-derived C.

Table 2-3 The $\delta^{13}C$ value of organic carbon in bulk soil and different soil fractions of red soil under long-term fertilization

Year	Treatments	$\delta^{13}C$ value of organic carbon (‰)				
		Bulk soil	Aggregates	Coarse silt fraction	Fine silt fraction	Clay fraction
1992	CK	-21.42	-23.43	-22.15	-21.54	-20.13
2010	CK	-19.66	-22.37	-21.59	-19.17	-19.48
2010	NPK	-19.57	-22.43	-21.16	-19.10	-18.60
2010	M	-20.33	-22.20	-22.12	-20.33	-19.56
2010	NPK+M	-20.49	-22.68	-22.51	-20.63	-19.33

Note: aggregates (53-2000 μm), coarse silt-sized fraction (5-53 μm), fine silt-sized fraction (2-5 μm), and clay-sized fraction (<2 μm) fractions

In our study, SOC and TN in bulk soil increased exponentially with the increase of clay-sized particle associated OC and total N. This indicated that clay-sized fraction was an important part of C and N sequestration in soil. According to Hassink (1997) and Castellano (2015), however, a maximum holding capacity (defined as the maximum amount of clay-sized fraction associated C and N under the current management practices) of clay associated-OM did exist. In our study, clay-sized fraction associated OC and total N would probably reach the estimated maximum value (14.21 g C kg^{-1} and 2.28 g N kg^{-1}, respectively) with the increase of soil OC and TN in this red soil. This also further confirmed that the maximum capacity of clay-sized fraction associated OC and total N was limited by a saturation phenomenon (Hassink 1997; Six et al. 2002), since all adsorption and interaction sites on the surfaces of clay-sized fraction were occupied (Du et al. 2014; Gulde et al. 2008). Our maximum estimation of clay-sized fraction associated OC was lower than that of 32.50 g C kg^{-1} in a Dark Red Latosol Soil reported by Roscoe et al. (2001). It might be due to the high clay content (> 80.0%) for a Dark Red Latosol Soil (Roscoe et al. 2001), which was more than twice of the clay content (35.0%) for red soil in our study. In addition, the intensive disturbing of double maize cropping in our study, might also contribute to the low capacity of the maximum estimation in the clay-sized fraction associated OC, which was different from the cultivated pasture under natural conditions from Roscoe et al. (2001). The linear response of aggregates associated OC and total N to soil OC and total N also might indicate that soil OC and total N stock would be further increased through aggregates associated OC and total N after clay-sized fraction associated OC and total N became saturated gradually (Carter 2002).

The significantly positive correlations revealed that the absolute content of aggregates (the mass proportion of aggregates) and relative content of aggregates (the mass ratio of the aggregates to coarse silt-sized fraction and fine silt-sized fraction) increased with the enrichment of OC in the fine soil minerals. However, the significantly negative correlations indicated that the amount of coarse silt-sized fraction gradually decreased with the increase of OC accumulation in fine soil particles. According to the theory of aggregate formation proposed by Tisdall and Oades. (1982), soil aggregates form as OC in particle fractions associate with fine soil particles or microbial mucus, and then fine soil particles agglomerate and form aggregates through organic binding agents. As organic matter can act as binding agents for soil aggregates formation (Six et al. 2002), we speculated that soil aggregation was facilitated by the binding of the enriched OC in fine soil particles (the silt-sized and clay-sized fractions) with the fine particles into aggregates, resulting in a decrease in the relative proportion of coarse silt-sized fraction. Those results also confirmed the theory that the soil aggregates hierarchically and continuously formed from small to large through organic binding agents (Tisdall and Oades.1982; Six et al. 2000; Six et al. 2002).

5 Conclusion

Manure application substantially enhanced C and N sequestration in bulk soil and fine soil particles, as well as aggregates in upland red soil. Fine soil particles, especially clay-sized fraction, were of great importance for C sequestration and N accumulation with a limited capacity. Soil OC and total N levels might further increase through its association with aggregates after clay-sized associated C and N gradually reaches the upper limitation. Moreover, C accumulation in soil clay-sized particles benefited the formation of aggregates. In conclusion, organic amendments with fertilization can effectively enhance soil C and N sequestration through C and N accumulated in fine soil particles, as well as aggregates under intensive cropping in South China.

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Chapter III

Partial substitution of chemical nitrogen fertilizer with manure promotes carbon and nitrogen accumulation in deep soils

From Xu, H., Cai, A. D., Zhang, W. J., Yang, X. Y., Huang, S. M., Wang, B. R., Zhu, P., Colinet, C., Xu, M. G., 2021. Partial substitution of chemical nitrogen fertilizer by manure promotes carbon and nitrogen accumulation in deep soils. Journal of Integrative Agriculture (Major Revision).

Abstract

Partial substitution of mineral nitrogen (N) fertilizer with manure is highly recommended to minimize environmental risks without reducing crop productivity in intensive agricultural systems. However, our understanding of the effect of organic substitution on soil organic carbon (SOC) and total N (TN) below topsoil (0–20 cm) remains limited. This present study investigated the SOC and TN changes in 0-100 cm soil profile and crop productivity under mineral fertilizers (NPK), mineral fertilizers combined with straw (NPKS), and 70% mineral N fertilizer substituted with manure (NPKM) over two decades in four upland fields across various climate zones. Compared with the initial values, two decades of NPKM treatment significantly ($p < 0.05$) increased SOC and TN stocks in both topsoil (by 25.6%–103.8% and 15.8%–89.8%) and subsoil (by 2.9%–71.3% and 5.7%–36.9%) at all sites, respectively. The increments of SOC and TN stocks in 0–100-cm soil receiving NPKM treatment were significantly higher than those of NPK treatment at all sites and NPKS treatment at three high-evaporation sites. Carbon sequestration efficiency in NPKM treatment (53.4%) was the highest at Gongzhuling site, which was 2.7–5.6-fold of those at the rest sites. Compared with NPKS and NPK treatments, crop yield and N and phosphorus (P) uptakes significantly increased at Qiyang site but insignificantly changed at the rest sites under NPKM treatment. Furthermore, SOC accumulation along soil profile exhibited a significant exponential correlation with N and P uptakes, relative crop yield, and sustainable yield index. In conclusion, long-term partial substitution of mineral N fertilizer with manure facilitates SOC and TN sequestrations and maintains high crop productivity in upland, which could mitigate climate change and benefit food security.

Keywords: long-term experiment, soil profile, Carbon sequestration, crop productivity, Sustainable yield index.

1. Introduction

In croplands, soil organic carbon (SOC) and nitrogen (N) accumulations are not only essential to crop growth and development (Ma et al., 2009), but also can alleviate CO_2 and N_2O concentrations in the atmosphere, significantly contributing to climate change mitigation (Amelung et al., 2020). This is particularly true because horizons below 20 cm (subsoil), which are not directly subjected to management practices, have greater SOC and N sequestrations potential due to the relatively less soil disturbance and the relatively lower SOC and N contents (Rumpel and Kögel-Knabner, 2011).

Numerous studies have confirmed that long-term mineral fertilizers combined with manure is the most effective practice to substantially increase SOC and TN sequestration as well as crop yield (Ghosh et al., 2012; Xu et al., 2020, Wei et al 2020). However, in most previous studies, manure was applied additionally without reducing mineral N fertilizers application rate, therefore increasing the total amount of mineral N fertilizers into field (Dai et al., 2021). Moreover, long-term overuse of mineral N fertilizers gained high crop production and met food demand, while generated a series of agro-environmental problems, such as soil degradation, soil acidification, and environmental pollution (Guo et al., 2010a; Ju and Zhang, 2017). To address the above-mentioned issues without reducing food production, partially replacing mineral N fertilizer with manure keeps the total application rate of N fertilizer unchanged (Dai et al., 2021; Zhang et al., 2021). This practice, also named organic substitution, has increasingly attracted attention of stakeholders and has been recommended in cropland by the Chinese government as an effective fertilization strategy for achieving high crop production, simultaneously reducing mineral N fertilizer use and recycling livestock manure utilization (Guo et al., 2020a; Zhang et al., 2021). Although some previous studies have reported the impacts of organic substitution on SOC and TN as well as crop yield, these studies have been mainly based on individual site or focused on the top 20 cm soil (topsoil) which is directly affected the most by agricultural management practices (Li et al., 2017; Ji et al., 2020; Dai et al., 2021). However, these studies ignored the subsoil's potential to sequester C and N, as subsoil holds 30.0%-75.0% of total soil C and N stocks (Jobbagy & Jackson, 2000; Tautges et al., 2019). Moreover, lots of researches showed that fertilization significantly affects C and N up to a depth of 90 cm in subsoil (Ghosh et al., 2018a; 2018b; Rodrigues et al., 2021; Tautges et al., 2019). For example, Ghosh et al (2018a; b) found that chemical fertilizers combined with manure significantly increase OC and N accumulation in 30-90 cm, with the increments of OC and N in 30-90 cm even accounting for more than 50.0% of those of 0-90 cm. In contrast, some studies also showed that fertilization (e.g., mineral fertilizers, manure, etc.) decreases subsoil OC and TN significantly (Rodrigues et al., 2021; Tautges et al., 2019; Wang et al., 2020). Despite many of these studies concern a lot about the effect of fertilization on crop yield and deep soil C and N, the information on how and to what extent organic substitution will influence subsoil C and N as well as crop yield is remarkably limited. Therefore, on long-time scales, it is essential to quantify the impact of organic substitution on SOC and TN in subsoil and crop yield for mitigating climate change and understanding C and N cycle in cropland.

Typically, SOC significantly influences crop production and its sustainability (Oldfield et al., 2018). Previous studies have reported significant positive correlations between SOC and nutrient uptake and crop production, but these studies have been based on short-term experiments or meta-analyses, with a primary focus on topsoil (Manna et al., 2005; Oldfield et al., 2019; Wei et al., 2020). However, crop roots in subsoil account for more than 30.0% of total biomass over a 0–100-cm depth (Jobbagy & Jackson, 2000). Moreover, subsoil stores plenty of nutrients, which are important for maintaining and improving crop yield in later stages of crop growth (Ma et al., 2009). Therefore, under long-term fertilization, the downward extension of crop roots not only could affect crop yield by uptaking nutrients stored in subsoil, but also may change SOC pool by increasing root biomass, which would finally influence the relationship between crop production and SOC accumulation (Jobbagy & Jackson, 2000; Fontaine et al., 2007). However, the responses of nutrient uptake, crop yield and its sustainability to SOC accumulation along soil profile are not well understood under long-term fertilization.

Upland fields, accounting for approximately 70.0% of all land under cultivation in China, usually have low C and N levels but high potential to sequester C and N (Xie et al., 2007). Consequently, increasing SOC and TN sequestration especially in subsoil and realizing the synergetic improvement of crop yield in upland regions could help to solve the challenges of food security and environmental sustainability in China (Lal, 2004). In this present study, soil samples of 0-100 cm were collected from four sites subjected to more than 20-year upland field experiments and similar fertilization practices in China. The objectives of this study were to (1) investigate the impact of fertilizer regimes, especially partial substitution of mineral N fertilizer with manure, on SOC and TN over a depth of 0–100 cm as well as crop yield, and (2) study the responses of nutrient uptake and crop productivity to SOC accumulation in 0–100 cm. It is hypothesized that (1) compare with mineral fertilizers alone and plus straw, organic substitution practice should have higher input of OC and essential nutrients, which is more conducive to soil carbon and nitrogen accumulation and crop growth, and will gain the greatest SOC and TN sequestrations as well as crop yield; (2) across the entire 100 cm profile, subsoil which is not subjected to frequent tillage, has larger volume and lower OC and TN contents, thus fertilization may lead to greater changes of subsoil OC and TN stocks under long timescale; (3) SOC sequestration across the entire soil profile should facilitate the synergistic improvement of crop yield and its sustainability by promoting nutrients uptakes.

2. Materials and Methods

2.1 Site descriptions

Four typical upland experimental sites of Qiyang, Yangling, Zhengzhou, and Gongzhuling across various climate zones involved in this study were all established in 1990 (Fig 3-1). Basic information on these four long-term experiments sites was presented in Table 3-1. The Ferralic Cambisol soil at Qiyang site, which developed from Quaternary red clay, was relatively acidic (topsoil pH = 5.7 at the beginning of experiment) with a clay texture. The Cumulic Anthrosol soil at Yangling site and the Calcaric Cambisol soil at Zhengzhou site developed from loess soil and Yellow River alluvium, respectively, were relatively infertile alkaline soils (pH = 8.5), with silt loam textures. In contrast, the Luvic Phaeozems soil from Gongzhuling site developed from Quaternary loess-like sediment, was relatively fertile alkaline soils (pH = 7.6) with a clay loam texture.

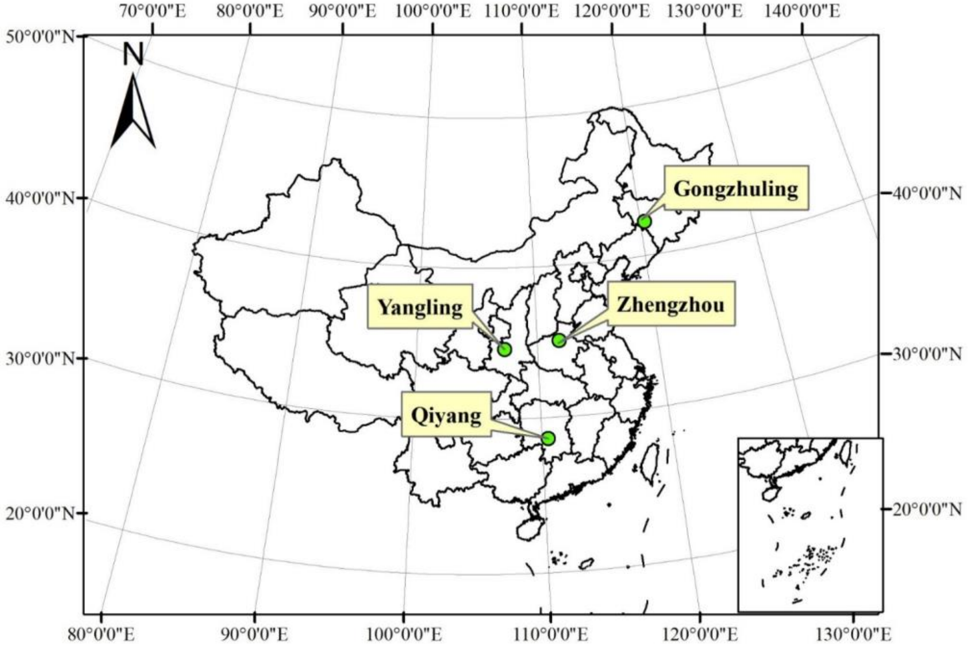

Figure 3-1 Locations of long-term field experiment of Qiyang, Yangling, Zhengzhou, and Gongzhuling across China in upland.

2.2 Experimental design

Four fertilization treatments were applied at each experimental site: no fertilization (CK), mineral nitrogen, phosphorus, and potassium (NPK), 70% mineral N substituted with manure (NPKM), and mineral NPK combined with straw (NPKS). The amounts of total N applied between NPK and NPKM treatments were identical (Table 3-2). In NPKM treatment, 30% of total N was derived from mineral fertilizer, and the remaining 70% was derived from manure. The treatment plots were initially randomized and isolated using 100-cm cement baffle plates in plot sizes measuring 43–400 m^2.

Table 3-1 Description of climatic condition, cropping, and soil properties at four long-term field experiment sites across China upland.

Sites	Qiyang	Yangling	Zhengzhou	Gongzhuling
Start year	1990	1990	1990	1990
Climate type [a]	ST-H	WT-SH	WT-SH	MT-SH
MAT [b](°C)	18.0	13.8	14.3	4.5
MAP [c] (mm)	1255	525	632	525
MAE [d] (mm)	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 soil properties, 0–20 cm		
FAO/UNESCO Soil classification	Ferralic Cambisol	Cumulic Anthrosol	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
pH	5.7	8.6	8.3	7.6

Note: [a] MT, mid-latitude temperate; WT, warm-temperate; ST, sub-tropical; SH, semi-humid; H, humid. [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 annually.

Urea, calcium superphosphate, and potassium chloride were used as sources of N, P, and K fertilizers, respectively. All P and K fertilizers and nearly half of mineral N fertilizer were applied as basal fertilizers, and the remaining mineral N fertilizer was applied as top dressing during the growing season. Manure was generally applied once a year before wheat sowing at Yangling and Zhengzhou sites, but before maize sowing at Gongzhuling site. At Qiyang site, 30% of manure was applied before wheat seeding, and 70% of manure was applied before maize seeding. Organic manure included farmyard manure and excreta from livestock (e.g., as pigs, horses, and cattle). For NPKS treatment, maize straw was incorporated into soil before wheat seeding at Zhengzhou and Yangling sites but before maize seeding at Gongzhuling site. However, after maize and wheat harvests, 50% of crop straw was incorporated into soil before the next crop seeding at Qiyang site.

Table 3- 2 The annual amounts of nitrogen, phosphorus, and potassium applied in mineral fertilizers and manure/straw at four experiments sites during 1990-2013.

Treatment	Qiyang [a]		Yangling [b]		Zhengzhou [c]		Gongzhuling [d]
	Wheat	Maize	Wheat	Maize	Wheat	Maize	Maize
	Nitrogen (kg ha^{-1}) (in chemical fertilizers + manure/straw)						
NPK	90+0	210+0	165+0	188+0	165+0	188+0	165+0
NPK+S	90+9	210+9	165+42	188+0	123+42	188+0	112+53
70%NPK+M	27+63	63+147	50+115	188+0	50+115	188+0	50+115
	Phosphorus (kg ha^{-1}) (in chemical fertilizers + manure/straw)						
NPK	16	37	58	25	36	41	36
NPK+S	16+1	37+1	58+4	25	36+8	41	36+6
70%NPK+M	16+13	37+31	58+95	25	36+66	41	36+39
	Potassium (kg ha^{-1}) (in chemical fertilizers + manure/straw)						
NPK	30	70	69	78	68	78	68
NPK+S	30+17	70+17	69+57	78	68+86	78	68+58
70%NPK+M	30+30	70+70	69+180	78	68+92	78	68+77

Note: CK treatment, no fertilizer applied. [a] 40.0 t ha^{-1} pig manure (30% before wheat, 70% to maize) for 70%NPK+M, 50% wheat straw, 50% maize straw for NPK+S. [b] 26.2 t ha^{-1} cattle manure for 70%NPK+M, 4.5 t ha^{-1} maize straw for NPK+S. [c] 22.5 t ha^{-1} horse manure for 70%NPK+M, 4.1 t ha^{-1} maize straw for NPK+S. [d] 23.2 t ha^{-1} manure for 70%NPK+M, 7.5 t ha^{-1} maize straw for NPK+S.

2.3 Cropping and management practices

A double cropping system with winter wheat and summer maize rotation was implemented at Qiyang, Yangling, and Zhengzhou sites, whereas a monoculture cropping system with summer maize was implemented at Gongzhuling site. Because sufficient precipitation occurred during the growing season, irrigation was not carried out at Qiyang and Gongzhuling sites. However, in Yangling and Zhengzhou sites, irrigation was carried out two or three times during the wheat growing season as well as one or two times during the maize growing season. The plots subjected to each treatment were plowed to a depth of 20 cm twice annually after wheat and maize harvests at Yangling and Zhengzhou sites, and before wheat and maize seeding at Qiyang site. However, at Gongzhuling site, the plots subjected to each treatment were plowed to a depth of 25 cm every year. After crop harvest, straw and grain were air-dried and weighed. Temporal variation of crop yield at these four long-term experiments sites has been reported by Duan et al. (2011) and Zhang et al. (2009) in previous studies. Here, we mainly focused on the average crop yield and sustainable yield index under different fertilizations.

2.4 Sampling and analyses

Soil samples were collected in September 2013 after maize harvest at four sites using a 5 cm diameter auger. The sampling depth was 0–100 cm, which was divided into five layers, each measuring 20 cm. Twelve to fifteen cores from each plot were collected randomly (S-shaped sampling) and the soil from a random combination of four or five cores was to form three samples for each plot. After air-drying and plant residues removal, soil samples were passed through 0.25 mm sieve to determine SOC and TN contents by procedures described by Qaswar et al. (2020). At all long-term experiment sites, the initial contents of SOC and TN were both determined in 1990 by a method consistent with these in 2013. Soil bulk density (BD) in the 0–20 cm and 20–40 cm soil layers were measured in situ for each treatment. For BD in each layer of 40–100 cm soil under all treatments, the measured value from the protected area in each experiment site was employed to minimize damage to long-term field experiment. OC and TN stocks were corrected to t ha^{-1} using a method reported by Xu et al. (2020).

After crop harvest, stubbles and roots remained in the field, and crop straw and grain were air-dried and weighted. Samples of air-dried grain and straw were oven-dried for 30 min at 105 °C, then heated at 70 °C to a constant weight for determining dry matter and nutrient contents. The contents of N, P, and K in grain and straw were measured by procedures described by Qaswar et al. (2020). Nutrient uptake by crop in the above-ground biomass at each plot was estimated based on plant nutrient content (g kg^{-1}), crop biomass (t ha^{-1}), and grain yield (t ha^{-1}).

2.5 Calculations

Relative changes of SOC and TN stocks along soil profile (%) after two decades of fertilizations were calculated using Equations (1) and (2) to eliminate the effect of different sites (Zhang et al., 2012):

$$Relative\ change\ of\ SOC = (SOC_t - SOC_0)/SOC_0 \tag{1}$$

$$Relative\ change\ of\ TN = (TN_t - TN_0)/TN_0 \tag{2}$$

Here, SOC_0 (TN_0) and SOC_t (TN_t) are the SOC (TN) stock (t ha^{-1}) in the initial year (1990) and 2013, respectively.

Carbon sequestration efficiency (CSE) along 0-100-cm soil profile during study period (t ha^{-1} $year^{-1}$) was calculated using Equations (3) and (4):

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

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

where CSR is the SOC sequestration rate along 0-100 cm soil profile (t ha^{-1} $year^{-1}$), SOC_0 and SOC_t are the SOC stock along 0–100 cm soil profile (t ha^{-1}) in 1990 and 2013, respectively. Here, t is the duration of fertilizer application (years), and C_{input} is the annual input of OC in 0-100 cm soil profile during the study period (t ha^{-1} $year^{-1}$). C_{input} derived from crop residue (roots and stubble) and organic amendments (manure or straw) in different treatments were estimated using the method of Zhang et al. (2012). According to the distribution proportion of crop roots biomass in soil profile of 0-100 cm reported by Jobbagy and Jackson. (2000), OC input derived from crop roots in each layer of soil profile was estimated.

Crop yield could not be directly compared across treatments because these four experiments were carried out in different regions with different climates. Relative crop yields (RCY) were calculated using Equation (5) to eliminate such influences (Bai et al., 2013).

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

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

The sustainable yield index (SYI) is a quantitative measure used to assess the sustainability of agricultural practices (Manna et al., 2005), and is estimated using 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. σ is the standard deviation of crop yield for a particular treatment.

2.6 Statistical analyses

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

3. Results

3.1 SOC and TN contents in 0-100 cm soil profile

Compared with the initial value, SOC content decreased in most layers at Qiyang and Gongzhuling sites (Figure 3-2a; h), and TN contents decreased in most layers at Zhengzhou and Gongzhuling sites (Figure 3-2f; g) under long-term CK treatment. Compared with the initial value, NPK and NPKS treatments increased SOC and TN contents at different degrees in each layer of the 40–80 cm at Qiyang site and the 0–40 cm at Yangling and Zhengzhou sites (Figure 3-2c, e, and h). However, NPKM treatment largely increased SOC and TN contents in most soil layers across all sites compared with the initial value, particularly within 0–60 cm at three northern sites and 40–100 cm at Qiyang site. Moreover, NPKM treatment resulted in significantly higher SOC and TN contents than those in NPKS and NPK treatments in 0–20cm at Qiyang and Yangling sites, but in 0–40 cm at Zhengzhou and Gongzhuling sites.

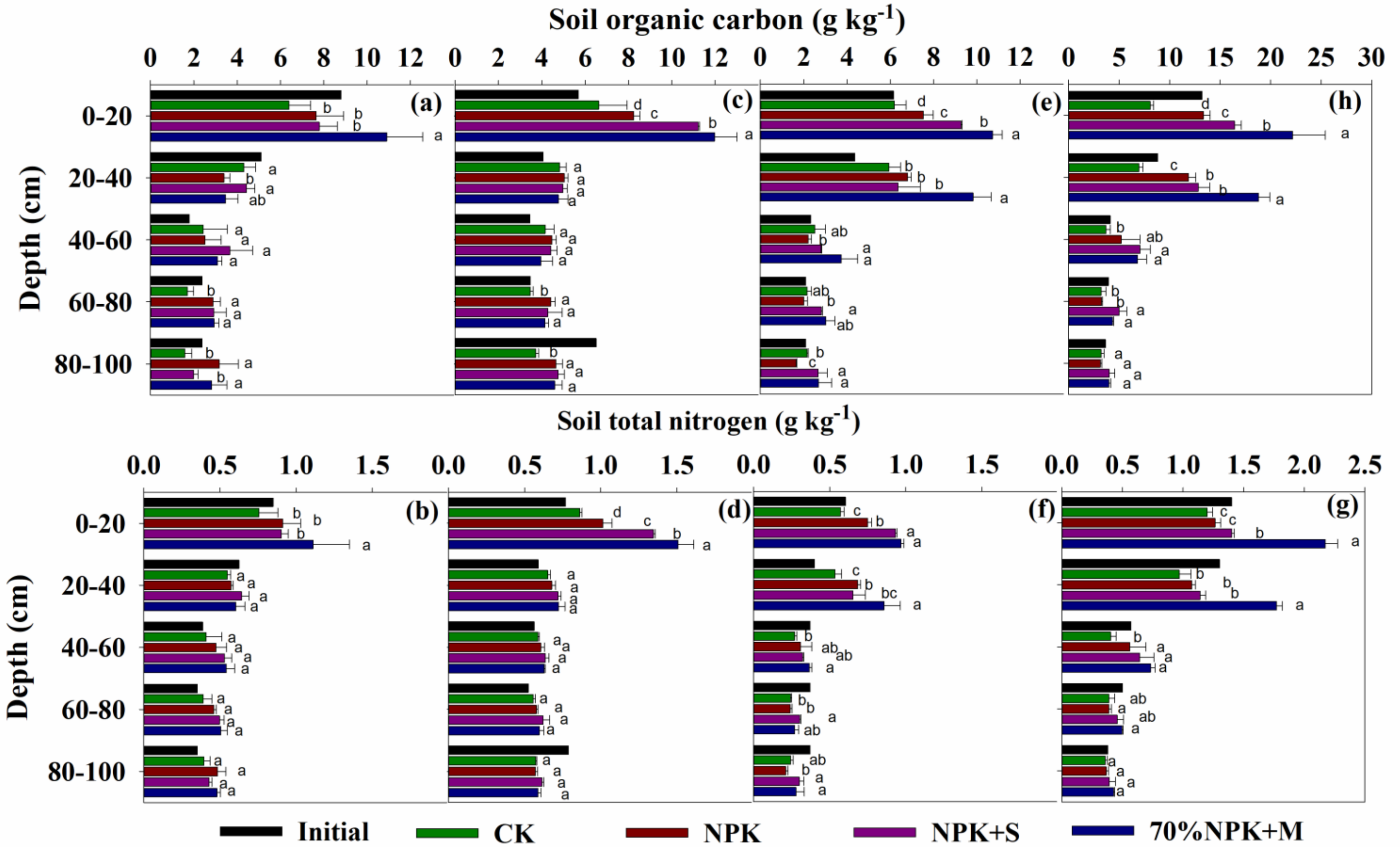

Figure 3-2 Soil organic carbon (SOC) and total nitrogen (TN) contents at 0-100 cm soil profiles under different fertilization from Qiyang (a, b), Yangling (c, d), Zhengzhou (e, f) and Gongzhuling (h, g) sites. Value indicates mean ± standard errors (n = 3).Different lowercase letters indicates significant differences among treatments at same soil layer ($p < 0.05$).

3.2 SOC and TN stocks change in topsoil, subsoil, and the entire 100 cm profile.

Compared with the initial value, overall, SOC stock in 0-100 cm decreased under CK treatment (Figure 3-3), especially at Qiyang and Gongzhuling sites (decreased by 12.4% and 22.4%, respectively), but NPK treatment alleviated the decrease of SOC stocks to a certain extent at all sites. However, two decades of NPKS and NPKM treatments both significantly ($p < 0.05$) increased SOC stock in 0-100 cm soil profile relative to the initial value, particularly in NPKM treatment. The two decades of NPKM treatment increased SOC stock 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 profile, respectively, at all sites. Significantly greater increment ($p < 0.05$) of SOC stock in 0-100 cm soil profile was observed in NPKM treatment, which was 1.7–3.0 and 5.3–12.0 times of those in NPKS and NPK treatment, respectively, at Qiyang, Zhengzhou, and Gongzhuling sites (Figure 3-3). Meanwhile, the increment of SOC stock in 0-100 cm receiving NPKS treatment was significantly higher than that of NPK treatment at three northern sites (Figure 3-3c, e, and g). Furthermore, the increment of SOC stock following NPKM treatment was significantly higher ($p < 0.05$) than that of NPK and NPKS treatments in topsoil at all sites but in subsoil at Zhengzhou and Gongzhuling sites (Figure 3-3 e; g).

In the entire 100 cm profile, TN stock changed insignificantly at Yangling site, but slightly increased at Qiyang site, and decreased at Zhengzhou and Gongzhuling sites under two decades of CK treatment (Figure 3-3). TN stock following NPKS treatment increased by 25.3% and 21.7% in 0–100 cm soil at Qiyang and Yangling sites, respectively, (Figure 3-3b; d), and the increment mainly occurred in subsoil at Qiyang site and in topsoil at Yangling site, but showed no obvious change at Zhengzhou and Gongzhuling sites. Under two decades of NPKM treatment, TN stock increased by 15.8%–89.8%, 5.7%–36.9%, and 10.8%-41.9% in topsoil, subsoil, and the entire 100 cm profile compared with the initial values, respectively, at all sites. The increment of TN stock in the entire 100 cm profile following NPKM treatment was significantly higher ($p < 0.05$) than that of NPK treatment at all sites and NPKS treatment at Qiyang, Zhengzhou, and Gongzhuling sites (Figure 3-3b, f, and h). Meanwhile, the increment of TN stock in the 0-100 cm receiving NPKS treatment was significantly higher than that of NPK treatment at three northern sites (Figure 3-3d, f, and g).

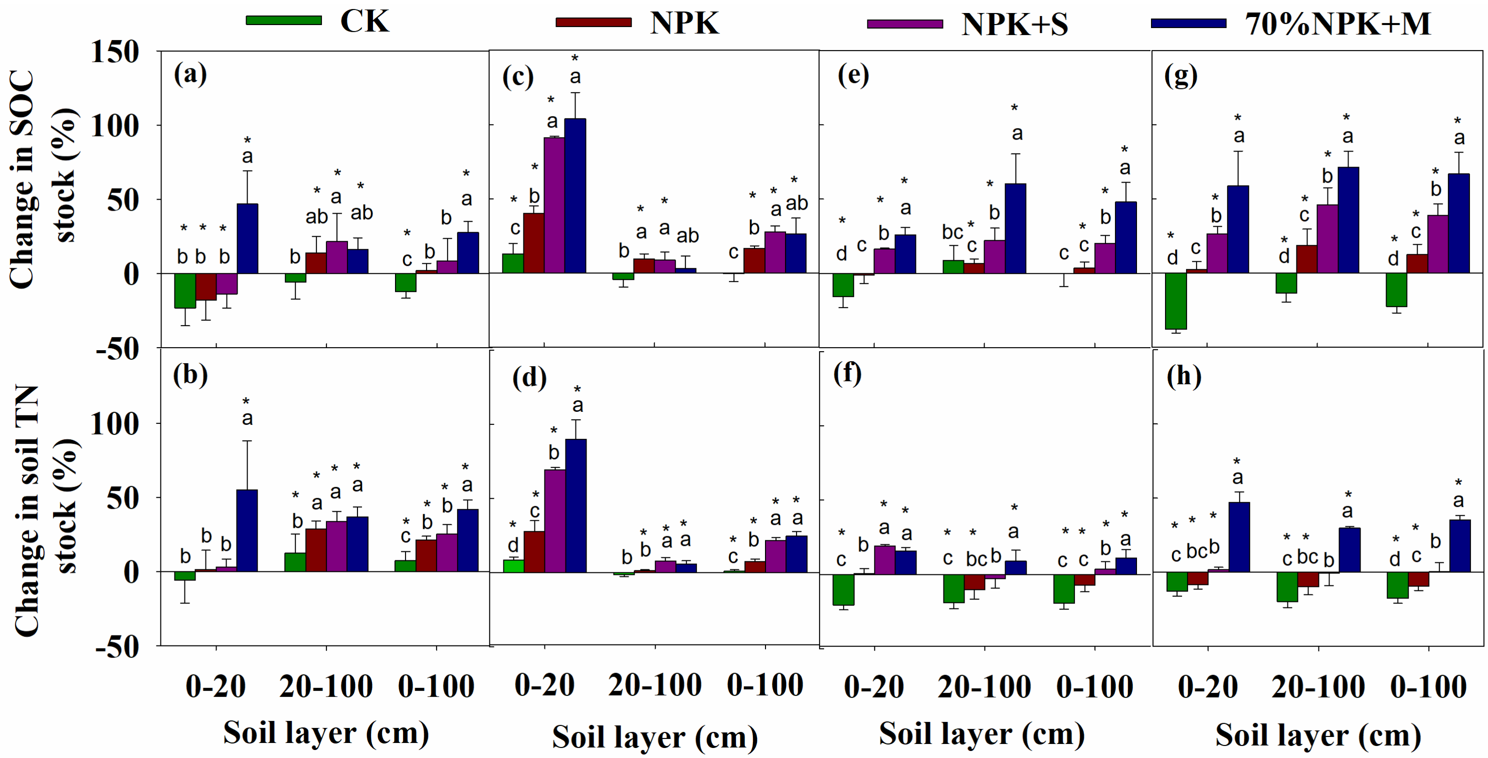

Figure 3-3 Changes (%) of soil organic carbon (SOC) and total nitrogen (TN) stocks in 0-20 cm, 20-100 cm, and 0-100 cm under different treatments compared with the initial values at Qiyang (a, b), Yangling (c, d), Zhengzhou (e, f) and Gongzhuling (h, g) experiment sites. Value indicates the mean ± standard errors (n = 3). Different lowercase letters indicates the significant differences among treatments at same soil layer ($p < 0.05$). * denotes change in soil OC or TN is significantly ($p < 0.05$) different from zero.

Table 3-3 Averaged annual carbon input (C_{input}, t ha^{-1} y^{-1}) and carbon sequestration efficiency (CSE, %) in 0-100 cm during 1990-2013 under different treatments at four experiment sites.

Index	Site	CK	NPK	NPK+S	70%NPK+M
Averaged annual carbon input (t ha^{-1} y^{-1})	Qiyang	0.4	1.4	2.1	7.1
	Yangling	1.2	3.8	5.7	6.1
	Zhengzhou	1.4	4.1	9.8	6.1
	Gongzhuling	1.1	2.8	5.7	4.7
CSE (%)	Qiyang	/	3.9aB	10.1aAB	9.5aB
	Yangling	/	12.6aAB	14.0aAB	12.4aB
	Zhengzhou	/	2.3bB	5.4bB	20.0aB
	Gongzhuling	/	16.7bA	25.4bA	53.4aA

Note: Different lowercase letters indicates significant differences among treatments at same experiment site ($p < 0.05$). Different capital letters indicate significant differences among experiment sites for same treatments ($p < 0.05$). "/" represented a net loss of carbon stock in 0-100 cm.

In Qiyang and Yangling sites, the average input of organic carbon in the entire 100 cm profile receiving NPKM treatment was the highest, which was 1.6–5.2 and 1.1–3.5 fold that of NPK and NPKS treatments, respectively (Table 3-3). For Zhengzhou and Gongzhuling sites, however, the average input of organic carbon in the entire 100 cm profile receiving NPKS treatment was the highest, which was 2.0–2.3 and 1.2–1.6 fold that of NPK and NPKM treatments, respectively. Carbon sequestration efficiency (CSE) in the entire 100 cm profile receiving NPKM treatment was the highest at Gongzhuling site, which was 2.7–5.6 fold that of the rest sites (Table 3-3). Moreover, CSE in the entire 100 cm profile receiving NPKM treatment was significantly higher than that of NPK and NPKS treatments at Zhengzhou and Gongzhuling sites.

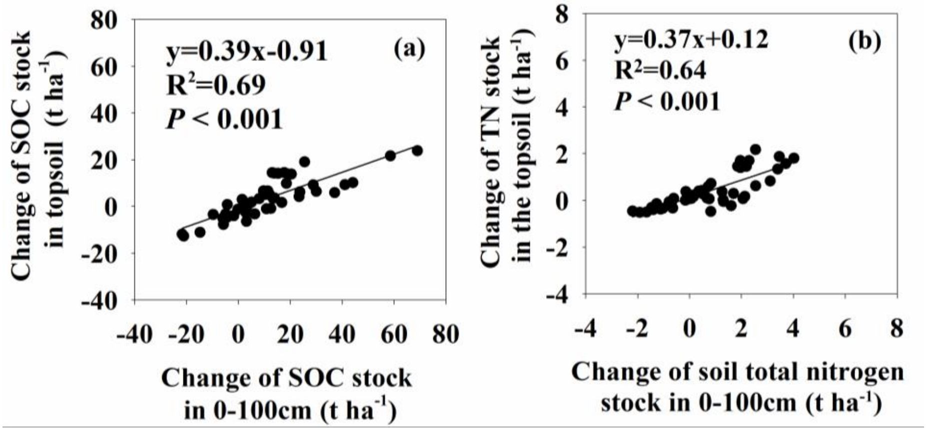

Figure 3-4 Relationships between soil organic carbon and total nitrogen stocks change in topsoil (a, 0-20 cm), subsoil (b, 20-100 cm), the entire 100 cm profile (c, 0-100 cm) (n=48).

Significant linear correlations ($p < 0.05$) were observed between change of SOC and TN stocks in topsoil (Figure 3-4a), subsoil (Figure 3-4b), and the entire 100 cm profile (Figure 3-4c), with the slope values of 10.00, 4.90, and 7.00, respectively. Furthermore, significant linear correlations ($p < 0.05$) between SOC stock change (Figure 3-5a) in topsoil and the entire 100 cm profile as well as between TN stock change (Figure 3-5b) in topsoil and the entire 100 cm profile were observed, with the slope values of 0.39 and 0.37, respectively.

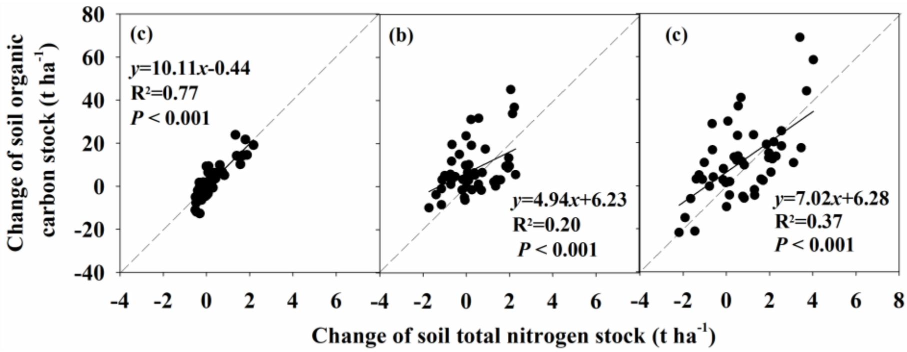

Figure 3-5 Relationships between soil organic carbon (SOC, a) or total nitrogen (TN, b) stock change in topsoil (0-20 cm) and the entire 100 cm profile (0-100 cm) (n=48).

3.3 Relationships between SOC stock in soil profile and crop productivity

Compared with CK treatment, fertilizations (NPK, NPKS, and NPKM) significantly increased average (1990–2013) maize and wheat yield by 1.6–13.0 and 1.4–2.7 times, respectively, at all sites (Table 3-4). Compared with NPK and NPKS treatments, NPKM treatment significantly ($p < 0.05$) increased crop yield only at Qiyang site. However, there were no significant differences in crop yield between NPK and NPKS treatments at all sites. Moreover, compared with CK treatment, fertilization also promoted maize SYI by 16.0%–340.0% at all sites and wheat SYI by 24.0%–135.0% at Yangling and Zhengzhou sites, but decreased wheat SYI by 46% at Qiyang site. Meanwhile, fertilizations significantly ($p < 0.05$) increased average N and P uptakes both (1990–2013) by 2.6–5.6 and 1.9–6.3 times relative to CK treatment, respectively, at all sites. Compared with NPKS treatment, NPKM also significantly ($p < 0.05$) increased N and P uptakes by crop at Qiyang and Gongzhuling sites.

Significant exponential correlations ($p < 0.05$) were observed between SOC stock in the entire 100 cm profile and average N (Figure 3-6a) and P uptakes (Figure 3-6b) by crop, and relative crop yield (Figure 3-6c) and SYI (Figure 3-6d). According to the results of exponential correlation, SOC stock in the entire 100 cm profile explained 35.0%, 64.0%, 51.0%, and 38.0% of variations of N uptake ($R^2 = 0.35$), P uptake ($R^2 = 0.64$), relative crop yield ($R^2 = 0.51$), and SYI ($R^2 = 0.38$), respectively.

Table 3-4 The averaged crop yield (t ha^{-1}), sustainable yield index (SYI), average nitrogen and phosphorus uptakes (kg ha^{-1} y^{-1}) during 1990-2013 under different treatments at four experiment sites.

Site	Treatment	Crop productivity				Nutrient uptake	
		Average yield		SYI			
		Maize	Wheat	Maize	Wheat	N	P
Qiyang	CK	0.27c	0.36c	0.08	0.38	19c	3.7d
	NPK	2.88b	1.06b	0.24	0.19	95b	16c
	NPK+S	3.37b	1.19b	0.26	0.21	113b	29b
	70%NPK+M	5.11a	1.77a	0.56	0.22	166a	37a
Yangling	CK	2.25b	1.08b	0.50	0.19	73b	11c
	NPK	6.11a	5.56a	0.60	0.48	283a	48b
	NPK+S	6.51a	5.73a	0.62	0.45	303a	46b
	70%NPK+M	6.63a	5.82a	0.56	0.41	288a	53a
Zhengzhou	CK	1.75b	3.02b	0.41	0.51	82b	14c
	NPK	6.44a	6.96a	0.45	0.62	298a	38b
	NPK+S	6.33a	7.55a	0.49	0.64	300a	42a
	70%NPK+M	6.04a	7.21a	0.49	0.64	283a	43a
Gongzhuling	CK	3.53b	/	0.37		61c	16d
	NPK	9.13a	/	0.63		233a	44c
	NPK+S	9.15a	/	0.65		200b	49b
	70%NPK+M	9.23a	/	0.63		239a	58a

Note: The different lowercase letters indicates significant differences among treatments at the same experiment sites ($p < 0.05$).

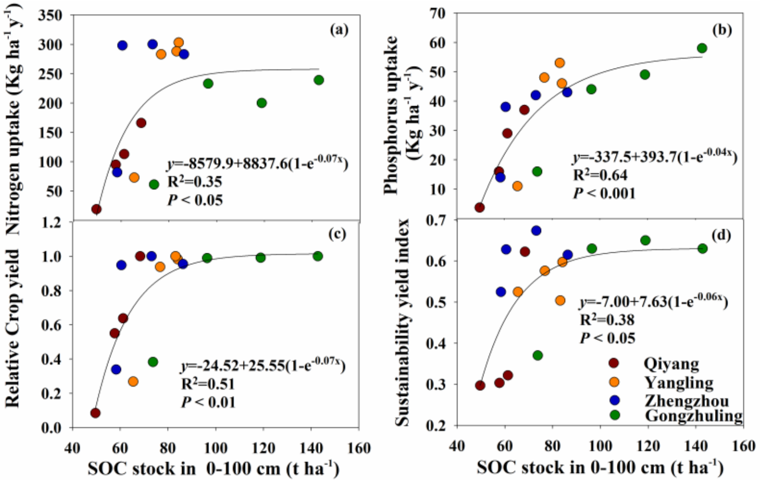

Figure 3-6 Relationships between soil organic carbon (SOC) stock in 100 cm soil profile, and averaged nitrogen (a) and phosphorus (b) uptakes by crop, relative crop yield (c), and sustainable yield index (d) during 1990-2013 at four sites (n=16).

4. Discussion

4.1 Impact of fertilization and cropping on SOC and TN

In cropland soils, SOC and TN dynamics reflect the balance between OC and N inputs from crop biomass (e.g., residue and roots) and fertilization (e.g., mineral fertilizers, manure, and straw return) and OC and N losses caused by SOM decomposition and crop harvest (Zhang et al., 2010). Our results showed that long-term CK treatment decreased SOC and TN stocks in the entire 100 cm profile at all sites, which implied that upland soil may act as OC and N sources under long-term continuous cropping without fertilization. It was probably because frequent tillage and crop harvest lead to greater OC and N losses than OC and N inputs derived from crop biomass (Zhang et al., 2009). Furthermore, the decrease of SOC and TN contents mainly occurred in topsoil as well as at depths greater than 60 cm at Qiyang and Gongzhuling sites. The results implied that long-term CK treatment not only resulted in SOC and TN losses in top 20 cm soil layer mainly due to frequent cropland practices, but also aggravated C and N depletions below 60 cm soil probably caused by enhancing priming effect under long-term fresh carbon input derived from crop roots (Fontaine et al., 2007). Our study also found that NPK treatment improved SOC stock in the entire 100 cm profile, which is similar to the findings of previous studies (Nave et al., 2009). The possible reason is that mineral fertilizers improving OC input derived from crop residue and roots by increasing crop yield (Manna et al., 2005).

Moreover, NPKM largely increased SOC and TN stocks in the entire 100 cm profile at all sites due to higher inputs of organic matter and higher crop biomass restitution (Manna et al., 2005). However, the increase of SOC and TN contents mainly occurred in the 0–60 cm soil layer at three northern sites of semi-humid areas and the 40–100 cm soil layer at Qiyang site of sub-tropical area under NPKM treatment. Fertilizers application (mineral fertilizers and manure) and crop biomass return mainly occurred in top 20 cm soil, which resulted in the increase of SOC and TN contents in the upper layer of soil profile at three northern sites. Meanwhile, we speculated that strong leaching caused by heavy rainfall accelerated the downward movement of dissolved C and N, then could help SOC and TN accumulation in subsoil at Qiyang site (Rumpel and Kögel-Knabner, 2011; Hess et al., 2020). Meanwhile, Guo et al (2010b) also found that long-term application of chemical fertilizers and manure leads to the nitrate nitrogen accumulation in the soil profile of 0-300 cm, and its tendency of downward movement. Therefore, dissolved OC and N accumulated in deep soil, could help to supplement soil OC and N pool, but may produce underutilization nutrients to a certain extent and pose a potential environmental risk (Guo et al., 2010b). Moreover, in the acidified soil at Qiyang site, high contents and availability of soil P could promote the growth and extension of crop roots due to manure application (Suriyagoda et al., 2014). This could impose a positive effect on subsoil OC accumulation via increasing OC inputs derived from root biomass, which have high CSE due to oxygen lack (Rumpel and Kögel-Knabner, 2011; Menichetti et al., 2015).

Meanwhile, our results showed that the increment of SOC and TN stocks in the entire 100 cm profile receiving NPKS was significantly higher than those of NPK at three northern sites. This is likely because straw addition brings in plenty of exogenous C and nutrients, which not only is conducive to SOC and TN accumulation, but also increases crop biomass return by improving crop growth (Zhang et al., 2009). Therefore, on the basis of mineral fertilizers application alone, crop straw incorporation was an effective way to improve SOC and TN stocks. Our results also showed that NPKM resulted in significantly higher SOC and TN stocks in the entire 100 cm profile relative to NPK. Similar results have been reported in previous studies carried out on topsoil (Ji et al., 2020; Dai et al., 2021). Compared with mineral fertilizers, manure application potentially introduces a high amount of exogenous C input, and reduces mineral N loss due to the prevention of the concentrated release of mineral N, and, in turn, improves SOC accumulation and N-use efficiency (Gutser et al., 2005; Duan et al., 2011). Meanwhile, manure addition could enhance the physical-chemical protection of SOC and TN by aggregates and clay fractions, which may benefit SOC and TN accumulation (Six et al., 2002; Xu et al., 2020). Furthermore, in this present study, NPKM had a better effect on SOC and TN accumulation relative to NPKS at Qiyang, Zhengzhou, and Gongzhuling sites. Possible reasons for this phenomenon are as follows: (i) manure increases crop residue and roots return by introducing richer and diverse nutrients and also has a high CSE due to high N availability (Silver & Miya, 2001); (ii) at Qiyang site, NPKM treatment could increase OC input derived from crop residue by considerably alleviating soil acidification and then increasing crop yield, which is proved by higher crop yield in our study and higher pH in previous studies under NPKM (topsoil pH=6.1 in 2013) rather than NPKS (topsoil pH=4.75 in 2013) treatment (Cai et al., 2015, Xu et al., 2015).

4.2 Relationships between change in SOC and TN stocks at different soil layers

Furthermore, we found that the changes of SOC and TN stocks in topsoil explained 69% and 64% of their variation in the entire 100 cm profile, respectively. Therefore, SOC and TN dynamics in topsoil could reflect SOC and TN trends in the entire 100 cm profile to a certain extent. This phenomenon was observed because fertilization practices are directly applied to topsoil, but the major OC and N sources for subsoil are dissolved organic matter from topsoil, followed by crop root litters and exudates (Rumpel and Kögel-Knabner, 2011). The slopes of linear equation for SOC and TN were 0.39 and 0.37, respectively, thus the change amount of SOC and TN stocks in topsoil only accounted for 39% and 37% of those in the entire 100 cm profile, respectively. The results implied that SOC and TN sequestered in subsoil may be more than those of topsoil after two decades of fertilization, which highlighted the greater potential of subsoil to sequester SOC and TN in the upland fields under long-term fertilization.

Soil N is considered a key factor influencing long-term SOC sequestration (Luo et al., 2004), and typically limits SOC accumulation before OC saturation (Six et al., 2002). In this present study, significant correlations were observed between changes of SOC and TN stocks in topsoil, subsoil, and the entire 100 cm profile, implying that

SOC dynamics were closely associated with TN dynamics in soil profile. In uplands, an increase of soil N reduces N limitation, which enhances SOC sequestration (Luo et al., 2004). Meanwhile, soil N accumulation in subsoil was greater than that of topsoil under an equal amount of SOC sequestration, as evidenced by a small slope in subsoil (4.94). Xiao et al. (2017) observed similar results in restored and natural grasslands. The results implied that more N accumulation in subsoil should be paid more attention to under long-term fertilization since it could pose a considerable environmental risk (Guo et al., 2010b).

4.3 Responses of crop productivity to SOC stock accumulation in soil profile

Compared with NPK and NPKS treatments, NPKM significantly increased crop yield as well as N and P uptakes by crop only at Qiyang site. This phenomenon was observed because manure application minimized soil acidification, as well as the adverse effects of acidification on crop growth in Qiyang site, such as aluminum and manganese toxicity (Duan et al., 2011; Cai et al., 2015). Xu et al. (2015) and Cai et al. (2015) found that, compared to NPK and NPKS treatments, two decades of NPKM increases topsoil pH by 1.4 units due to higher alkalinity and base content as well as organic matter in manure and then decreases exchangeable acidity and increases soil exchangeable base cations, which also indirectly proves our inference. Meanwhile, our result also showed that there were no significant differences in crop yield among NPKS, NPK, and NPKM treatments at three northern sites. These results implied that NPKS and NPKM both could maintain high crop yields compared with NPK. A high SYI suggests a capacity to sustain high crop production over time, whereas a low YSI implies greater susceptibility to biotic and abiotic stress (Waqas et al., 2019). Our results showed that fertilization increased maize and wheat SYI in most experimental sites because of a stable and adequate nutrient supply and soil environment improvement for crop growth (Manna et al., 2005; Wei et al., 2020). However, in Qiyang site with acidified soil, fertilization increased maize SYI, but slightly decreased wheat SYI. The possible reason is the differences in responses of maize and wheat growth to soil pH, which has previously been demonstrated by varying pH thresholds (5.1 and 5.5, respectively) for acid damage in maize and wheat (Zeng et al., 2017). Moreover, in this present study, the obvious differences in average yield between wheat and maize also indirectly proved it.

Relative crop yield and SYI exponentially increased with an increase of SOC stock in the entire 100 cm profile. Previous studies have demonstrated that topsoil OC is significantly correlated with crop yield and SYI (Manna et al., 2005; Oldfield et al., 2019). An increase of SOC in soil profile significantly improves crop production and SYI because of the suppling nutrient through SOM decomposition and the improving soil biotic and abiotic environment in different soil layers (Oldfield et al., 2018, 2019; Amelung et al., 2020). Conversely, an increase of SOC in soil profile, along with the enhanced retention capacity of soil water and nutrient through improving the formation and stability of soil aggregates, has contributed to a high and stable crop production (Xu et al., 2020). Furthermore, SOC accumulation improves soil fertility that may promote soil biota predation (e.g., protists and nematodes) and the activity of beneficial microorganisms (e.g., arbuscular mycorrhizal fungi) (Jiang et al., 2020). It could further enhance the uptake and transport of P in crop roots through improving the expression of P transporter gene (*ZMPht1;6*), and, in turn, promote crop yield (Jiang et al., 2020). Therefore, we speculated that SOC accumulation in soil profile could potentially facilitate the uptake and utilization of nutrients both from topsoil and subsoil by improving soil microenvironment and the genes expression of nutrient transporter, and, in turn, promotes crop yield and its sustainability.

4.4. Limitation and implication of this study

Due to the limitations of fertilization treatments design at the beginning of experiments, substitution rates of mineral N fertilizer with organic N fertilizers in this present study were 70% at all long-term experiment sites. However, some studies have reported that substitution rates could influence the effects size of organic substitution practice on SOC and TN as well as crop yield (Li et al., 2017; Ning et al., 2017; Ji et al., 2020; Dai et al., 2021). Moreover, more manure addition could result in the accumulation of phosphorus, heavy metals, and antibiotics that may threaten soil and environmental quality under high substitution rates (Tang et al., 2020). Therefore, to minimize environmental risk and maximize crop yield benefits, researchers should explore the optimal substitution rates of mineral N fertilizer with organic N fertilizer under organic substitution regimes in future studies.

Despite these drawbacks, two important implications arose from our study for sustainable agricultural management in upland soil of China and other countries under intensive cropping systems, which will help tackle the challenges of food security and climate change. Firstly, 70% mineral N fertilizer substituted with manure can improve SOC and TN sequestrations along soil profile and maintain high and stable crop yield. Therefore, partial substitution of mineral N fertilizer with manure could provide a win-win solution for improving SOC sequestration, reducing the environmental risk caused by the overuse of mineral N fertilizer, and achieving high crop yields. Secondly, subsoil OC and N changes accounted for more than 60% of those in the entire 100 cm profile under long-term fertilization. Moreover, SOC accumulation in soil profile improved crop yield and its sustainability. Thus, subsoil should be considered when assessing the effects of fertilization on climate change mitigation and food security through C sequestration and N enrichment in upland soil.

5. Conclusion

Our research demonstrated that the change of SOC and TN stocks in topsoil accounted for less than 40% of those of the entire 100 cm profile. It implied that only considering topsoil may underestimate the impact of long-term feralization on carbon and nitrogen sequestration in upland soil. Moreover, compared with mineral fertilizers alone and mineral fertilizers combined with straw, 70% substitution of mineral N with organic N resulted in a higher increment of SOC and TN stocks in the entire 100 cm profile without reducing crop yield and its stability. Overall, more than 20-year field experiment confirmed that 70% substitution of mineral N with manure was an effective fertilizer strategy for improving SOC and TN and maintaining high and stable crop yields while helping to simultaneously achieve the "Fertilizer Use Zero-Growth Action Plan" and recycling livestock manure utilization in upland soils areas. Our study provides a novel insight and a basis for nitrogen management strategies and sustainable utilization of upland soils under intensive cropping systems in China and other countries. Furthermore, the potential transformation mechanisms of organic N derived from manure especially in subsoil need further research to maximize soil OC sequestration and N fertilizer use efficiency for sustainable crop production.

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Chapter IV

Long-term straw incorporation significantly reduced subsoil organic carbon stock in cinnamon soil

From Xu, H., Cai, A. D., Zhou, H., Colinet, C., Zhang, W. J., Xu, M. G., 2021. Long-term straw incorporation significantly reduced subsoil organic carbon stock in cinnamon soil. Journal of Plant Nutrition and Fertilizers, 27, 768-776.
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Abstract

Straw incorporation, as an effective fertilization way, has a significant effect on topsoil OC sequestration, but its effect on subsoil OC still remains unclear. We studied the change of SOC stock in 0-100 cm soil profile in cinnamon soil under different straw incorporation modes to provide a scientific basis for optimizing straw incorporation practices. The long-term straw incorporation field experiment was started in 1992. A split-plot design was used, with main plots under chemical fertilizers application seasons (spring and autumn), while subplots were subjected to four straw incorporation modes (SR, straw removal; SM, direct straw mulching; SC, straw incorporation after crushing; CM, cattle manure produced by an equal amount of straw). After maize harvest in 2013, samples of 0-100 cm soil profile were collected to study SOC and nutrient changes under different straw incorporation modes. Compared with SR treatment, regardless of chemical fertilizers applications seasons, SOC content significantly increased in topsoil under SM, SC, and CM treatments, but significantly decreased in 40-60 cm and 80-100 cm soil layers under SM and SC treatments. There were significant differences in SOC stock change of different soil layers among straw incorporation modes. Compared with that under SR treatment, SOC stock receiving SM, SC, and CM treatments increased by 2.32, 5.41, and 12.61 t ha^{-1} in topsoil, respectively, but decreased by 3.98, 6.99, and 3.76 t ha^{-1} in 40-100 cm soil layer, respectively. The increment of topsoil OC stock under CM treatment was significantly higher than that of SM and SC treatments. Compared with SR treatment, SOC stock in 0-100 cm increased by 9.62 t ha^{-1} under CM treatment, but decreased by 1.81 and 5.36 t ha^{-1} under SM and SC treatments, respectively. Redundancy analysis showed that accumulative carbon input and soil nutrient jointly explained 90.1% and 31.8% of the variation of topsoil and subsoil OC changes, respectively. The main factors affecting topsoil and subsoil OC changes were AP (80.1%) and TN (25.3%), respectively. Long-term straw incorporation facilitated topsoil OC accumulation by increasing carbon input and soil nutrients, but led to subsoil OC depletion due to insufficient N supply. Cattle manure was the optimal mode of straw incorporation to improve fertility and crop yield in cinnamon soil.

Keywords: Cinnamon soil; long-term experiment; straw incorporation; subsoil; soil organic carbon depletion;

1. Introduction

Soil organic carbon, as a key indicator of soil fertility in cropland, is a basis for high and stable crop yield (Schmidt et al., 2011). Soil carbon (C) pool is the largest C pool in terrestrial ecosystem, and a tiny change of soil C pool may cause a huge impact on climate change (Zhang et al., 2013). Therefore, SOC not only has a direct impact on soil fertility and crop productivity but also plays a very important role in climate change mitigation (Schmidt et al., 2011; Zhang et al., 2013). In China, crop straw is rich in output that in 2015 was about 9.31×10^8 t (Ji et al, 2015). Crop straw contains plenty of organic carbon (OC) and nutrients. Straw incorporation into field could not only reduce greenhouse gas generated by straw burning in situ (Song et al., 2018), but also increase OC input and crop residue return, which, in turn, effectively maintains or even improves OC stock in cropland (You et al., 2020; Liu et al., 2014; Yuan et al., 2017). Therefore, straw return has great potential for SOC sequestration, consequently improving soil fertility and mitigating climate change (Cai et al., 2020). Yang et al. (2018) found that SOC in 0-30 cm soil layer increases by 4.5%-9.0% after 5-years straw incorporation. Liu et al. (2014) reported that SOC in 0-20 cm increases by 28.7% and 12.4% under straw incorporation after crushing and livestock digestion in black soil, respectively. At present, those studies regarding the effect of straw incorporation on SOC mostly focus on topsoil (Liu et al., 2014; You et al., 2020; Yang et al., 2018). However, SOC pool stored in subsoil was 2-3 times of that in topsoil (Jobbagy and Jackson, 2000). Therefore, exploring the response of subsoil OC to straw return is important for climate change mitigation and understanding C cycle (Jobbagy and Jackson, 2000). Moreover, SOC improvement was a slow and long-term process. Therefore, more reliable results could be obtained based on a long-timescale study (Xu et al., 2015). Ma et al. (2011) found that SOC increases in 0-45 cm in paddy red soil under 17-years straw incorporation. Xie et al. (2012) showed that SOC significantly increases by 41% in 20-40 cm in black soil after two decades of straw combined with chemical fertilizers. However, Xu et al. (2016) in upland red soil and Gai et al. (2019) in fluvo-aquic soil reported that SOC significantly increases below 40 cm soil under long-term straw incorporation. Cinnamon soil is one of the main soil types in China with a total area of about 2516×10^4 ha (Xu et al., 2015), which as the area for the main production of wheat and maize in China, has plenty of crop straw resources (Xu et al., 2015). Therefore, studying the response of SOC in soil profile to straw incorporation is crucial for improving crop straw utilization efficiency in this area, as well as solving the problems of food security and environmental sustainability. In addition, cinnamon soil has loose textures (Xu et al., 2015), and the response of subsoil OC to straw incorporation especially in different modes remains unclear. Therefore, via two decades of straw incorporation field experiment in Shouyang County of Shanxi Province, we studied OC and soil nutrients in 0-100 cm soil profile and crop yield in cinnamon soil under different straw incorporation modes. On this basis, the characteristics SOC change in soil profile and their key factor were clarified, which provided a scientific basis for optimizing straw incorporation practices, improving soil fertility and C sequestration in this area.

2 Material and methods

2.1 Overview of the experimental site

The long-term straw incorporation experiment was conducted in Shouyang County, Shanxi Province (113°06' E, 37°58' N), located in warm-temperate semi-humid area. The MAT and MAP were 7.4°C and 501 mm, respectively. The precipitation was mainly spanned from June to September each year. The experiment soils were cinnamon soil, and its parent material was Malan loess. The initial (1992) topsoil properties were as follows: SOC 15.71 g kg^{-1}, TN 1.07 g kg^{-1}, TP 0.76 g kg^{-1}, TK 23.7 g kg^{-1}, AP 4.84 mg kg^{-1}, AK 95.0 mg kg^{-1}, and pH 8.30.

2.2 Experiment design and field management

The experiment was started in 1992, and implemented a monoculture cropping system with spring maize. During maize growth (seeding from April 15 to 25, harvesting from September 20 to October 10), the employed field management practices were the preventions of weeding and insect pests. A split-plot design was used with main plots under chemical fertilizers applications in spring and autumn, four straw return modes as sub-plots (straw removal, SR; direct straw mulching, SM; straw incorporation after crushing, SC; cattle manure produced by an equal amount of straw, CM). The plot area was 54 m^2, without duplication. The urea (N 46.0%) and superphosphate (P_2O_5 14.0%) were used as sources of N and P fertilizers, respectively, in the experiment. The contents of OC, TN, TP (P_2O_5), and TK (K_2O) in maize straw (air-dried) were 443.0, 7.39, 0.44, and 27.50 g kg^{-1}, respectively. The contents of OC, TN, TP (P_2O_5), and TK (K_2O) in cattle manure (air-dried) were 52.50, 3.93, 1.37, and 14.10 g kg^{-1}, respectively (Xu et al., 2015).

Table 4-1 Annual input of chemical fertilizers and straw incorporation in each treatment.

Treatment	Chemical fertilizer rate (kg ha^{-1} y^{-1})		Straw incorporation rate (t ha^{-1} y^{-1})	
	N	P_2O_5	Straw (Air dried)	Manure (Fresh)
SR	150	84	/	/
SM	150	84	6	/
SC	150	84	6	/
CM	150	84	/	45

Chemical fertilizers were shallowly applied (4-7 cm) in spring, combining with spring sowing, and were deeply applied in autumn, combining with deep tillage and plowing (10-30 cm). No topdressing was conducted during the growth period. In SM treatment, crop straw from previous maize was evenly covered on ground at the end of May each year, then the undecomposed straw was incorporated into field via deep tillage and plowing after maize harvest in current season. In SC treatment, maize straw was directly chopped (15 cm), and then incorporated into field via deep tillage and plowing after maize harvest in current season. In CM treatment, crop straw (an equal amount of straw with SM and SC treatments) from previous maize was fed to cattle.

Cattle manure produced by feeding cattle was decomposed, evenly spread, and then incorporated into field via deep tillage and plowing in current season. The amounts of chemical fertilizers and straw in each treatment were shown in Table 4-1.

2.3 Sampling and analysis

Soil samples were collected in September 2013 after maize harvest at four sites using a 5 cm diameter auger. The sampling depth was 0–100 cm, which was divided into five layers, each measuring 20 cm. Twelve to fifteen cores from each plot were collected randomly (S-shaped sampling) and the soil from a random combination of four or five cores was to form three samples for each plot. After air-drying and plant residues removal, soil samples were grounded through a 2 mm sieve to determine AP and AK contents, and grounded through a 0.25 mm sieve to determine SOC, TN, TP, and TK contents. The contents of SOC and TN were determined by the potassium dichromate volumetric and semi-micro Kjeldahl method, respectively. The contents of TP and TK were determined by the sodium hydroxide fusion method. The contents of AP and AK were determined by the Olsen and ammonium acetate extraction-flame photometry method, respectively. Soil bulk density (BD) was determined by the cutting ring method. Moreover, the weights of maize grain and straw for each plot were measured after harvest every year.

2.4 Data calculation and statistical analysis

Carbon input mainly includes crop roots, crop stubble, and organic amendment (straw or manure) in cropland. According to the method reported by Cai et al. (2020), Carbon inputs derived from crop roots, crop stubble, organic amendment (straw or manure) were estimated. According to the distribution proportion of crop roots biomass in soil profile of 0-100 cm reported by Jobbagy and Jackson (2000), Carbon inputs derived from crop roots in each layer of soil profile were estimated.

The calculation formula of SOC stock was as follow:

$$\mathrm{SOC_{stock}} = (C \times BD \times d)/10$$

Here, SOC_{stock} is the SOC stock (t ha^{-1}); C is the SOC content (g kg^{-1}); BD is the soil bulk density (g cm^{-3}); d is the soil depth (cm).

All data were plotted using SigmaPlot 12.5 software. Canoco 5 software was used for redundancy analysis. Using SPSS 22.0 software, the LSD method was used to test the significance of differences among treatments ($p < 0.05$).

3 Results

3.1 Crop yield and cumulative carbon input

Under chemical fertilizers applications in spring (Figure 4-1a) and autumn (Figure 4-1b), maize yield in all treatments showed a similar upward trend with planting time increasing. Moreover, SR treatment had the lowest maize yield in most years and large inter-annual fluctuations (2.21-10.91 t ha^{-1}). Moreover, compared with SR treatment, the median value (1992-2013) of maize yield increased by 7.7%-14.7%, 17.1%, and 25.8% in SC, SM, and CM treatments, respectively.

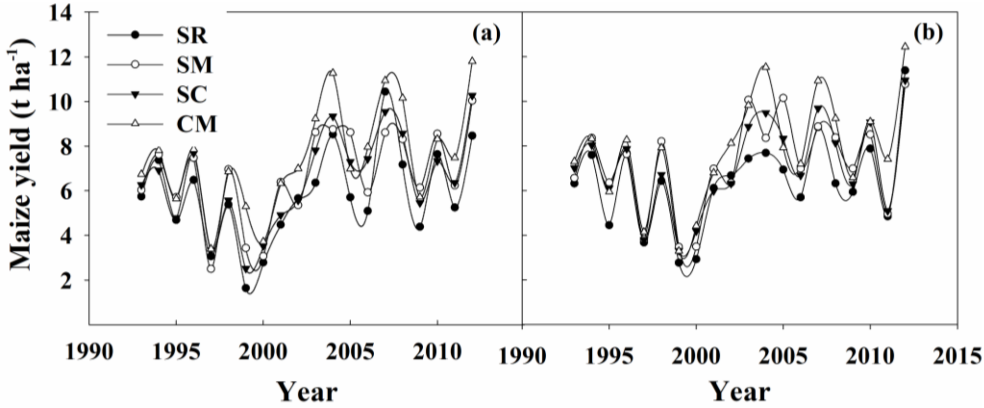

Figure 4-1 Maize yield under different straw incorporation modes and chemical fertilizations in spring (a) and autumn (b) (1993-2012).

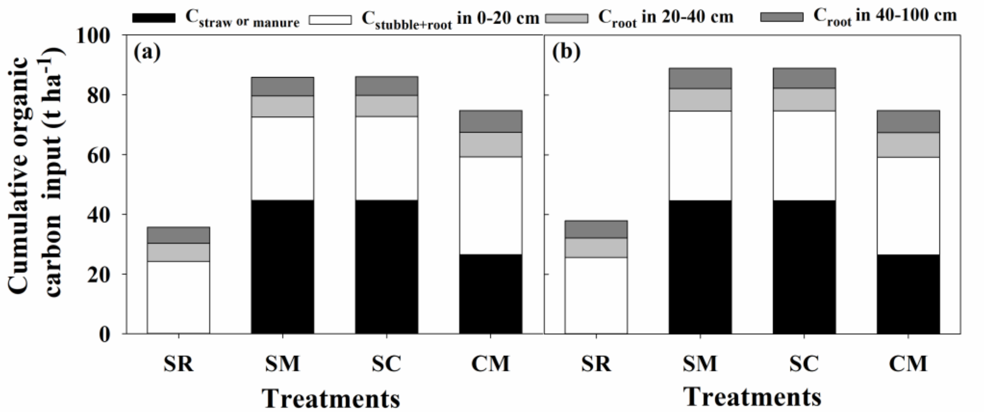

Figure 4-2 Cumulative carbon input under different straw incorporation modes and chemical fertilizations in spring (a) and autumn (b) (1993-2012). $C_{straw\ or\ manure}$—Carbon input derived from straw or manure; $C_{stubble+root}$—Carbon input derived from crop stubble and root; C_{root}—Carbon input derived from crop roots.

Straw incorporation modes also affected OC input by influencing crop yield (Figure 4-2). OC input derived from crop straw in SM and SC treatments are 3.7 times that of cattle manure in CM treatment. Under fertilizations in spring (Figure 4-2a) and autumn (Figure 4-2b), compared with SR treatment, cumulative OC input derived from crop stubble and root in the entire 100 cm profile increased by 16.1%,16.5%, and 31.5%, respectively, and total cumulative OC input increased by 1.4, 1.4, and 1.0 times in SM, SC and CM treatments, respectively.

3.2 SOC content and stock in different layers

Fertilization seasons and straw incorporation modes significantly affected SOC content (Table 4-2). Compared with chemical fertilizers application in spring, SOC content in CM treatment significantly increased in 0-40 cm soil layer, but SOC content in SM and SC treatments significantly decreased in 40-80 cm soil layer under chemical fertilizers application in autumn. Regardless of chemical fertilizers application in spring or autumn, compared with SR treatment, SOC content significantly increased in topsoil under CM, SM, and SC treatments, but significantly decreased in 40-60 cm and 80-100 cm soil layers under SM and SC treatments.

Table 4-2 Soil organic carbon (SOC) content in 0-100 cm soil profile with different straw incorporation modes under chemical fertilizations in spring and autumn.

Fertilization seasons	Treatments	SOC content in soil profile (g kg^{-1})				
		0-20 cm	20-40 cm	40-60 cm	60-80 cm	80-100 cm
Spring	SR	21.37cA	18.20aA	10.13aA	5.22bA	3.67aA
	SM	22.98bA	17.74aA	8.09bA	6.43aA	2.93bB
	SC	23.77bA	16.78aA	8.52bA	5.13bA	2.38cB
	CM	26.67aB	18.57aB	10.05aA	4.45bA	3.08bB
Autumn	SR	21.36cA	18.19bA	7.71aB	4.01aA	3.46aA
	SM	23.50bA	18.52bA	6.25bB	4.38aB	3.23bA
	SC	24.05bA	16.80cA	5.78bB	4.00aB	3.15bA
	CM	28.21aA	20.71aA	6.27bB	4.05aA	3.45aA

Note: Values followed by different small letters in same column mean significant differences among straw incorporation modes in same soil layer and chemical fertilization season ($p < 0.05$); Values followed by different capital letters in same column mean significant differences among chemical fertilization season in same soil layer and straw incorporation modes.

There were significant differences in SOC stock change at different depth among straw incorporation modes, but no significant difference among fertilization seasons (Figure 4-3). Compared with SR treatment, topsoil OC stock increased by 2.32, 5.42, and 12.60 t ha^{-1} under SM, SC, and CM treatments, respectively. The increment of topsoil OC stock in CM was significantly higher than that of SM and SC treatments. For 20-40 cm soil layer, compared with SR treatment, SOC stock slightly increased by 0.56-0.99 t ha^{-1} under CM treatment, and did not obviously change under SM treatment, but decreased 2.98-4.62 t ha^{-1} under SC treatment. Compared with SR treatment, SOC stock in 40-100 cm decreased by 3.62-4.34, 6.09-7.89, and 3.67-3.85 t ha^{-1} under SM, SC, and CM treatments, respectively. In the entire 100 cm profile, compared with SR treatment, SOC stock decreased by 1.18-2.44 and 5.92-4.80 t ha^{-1} under SM and SC treatments, respectively, but increased by 9.62 t ha^{-1} under CM treatment. However, the increment of SOC in the entire 100 cm profile was less than that of topsoil under CM treatment.

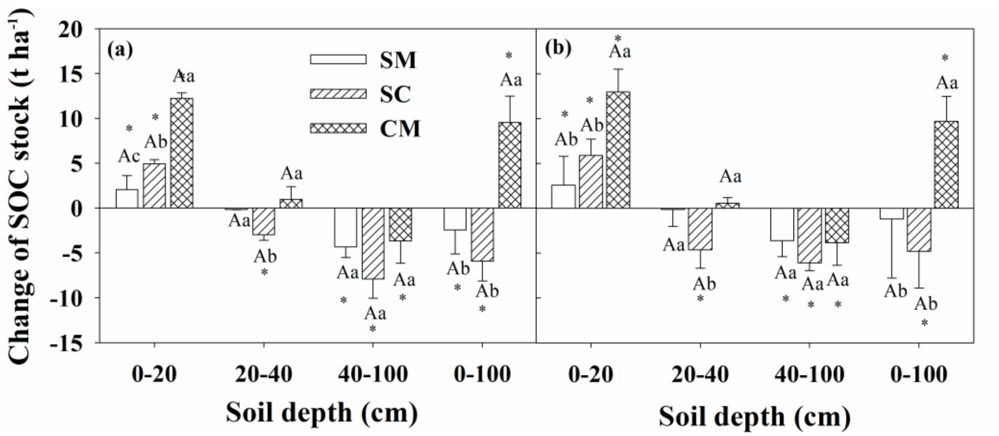

Figure 4-3 Change of soil organic carbon (SOC) stock relative to straw removal in different soil layers under straw incorporation modes and chemical fertilizations in spring (a) and autumn (b). Different small letters above the bars represent significant differences among straw incorporation modes in same soil layer and fertilization season. Different capital letters represent significant differences between fertilization seasons in same soil layer and straw incorporation mode ($p < 0.05$). * denotes the change of soil OC is significantly ($p < 0.05$) different from zero.

3.3 Nutrient content in soil profile

Straw incorporation modes also significantly affected nutrient contents in 0-100 cm soil profile (Table 4-3). Compared with SR treatment, topsoil TN, AP, and AK contents in SM, SC, and CM treatments increased at varying degrees under chemical fertilizers applications in spring and autumn. Meanwhile, topsoil TN, AP, and AK contents in CM treatment were significantly higher than these of SM and SC treatments. For 20-40 cm soil layer, TP and AP contents receiving CM treatment were significantly higher than these of SM treatment under chemical fertilizers applications in spring, but AP content in CM treatment was significantly higher than that of other treatments under chemical fertilizers applications in autumn. For 40-100 cm soil layer, TN and TK contents in CM treatment were significantly higher than these of SC treatment under chemical fertilizers applications in spring, and TK and AP contents in SM treatment were significantly higher than these of SR treatment under chemical fertilizers applications in autumn.

Table 4- 3 Soil nutrient contents with different straw incorporation modes under chemical fertilizations in spring and autumn

Depths (cm)	Treatments	Chemical fertilizers applications in spring					Chemical fertilizers applications in autumn				
		TN (g kg^{-1})	TP (g kg^{-1})	TK (g kg^{-1})	AP (mg kg^{-1})	AK (mg kg^{-1})	TN (g kg^{-1})	TP (g kg^{-1})	TK (g kg^{-1})	AP (mg kg^{-1})	AK (mg kg^{-1})
0-20	SR	1.17c	0.90ab	17.43a	12.63b	88.67c	1.06d	1.05b	15.31b	16.43c	94.17c
	SM	1.25bc	0.71b	16.59ab	14.50b	101.00b	1.31c	1.18ab	17.58a	18.93c	110.00b
	SC	1.32b	1.00ab	15.29b	11.30b	95.33bc	1.37b	1.05b	16.31ab	28.23b	105.5b
	CM	1.63a	1.08a	17.04ab	68.00a	156.00a	1.66a	1.37a	15.96ab	77.65a	182.5a
20-40	SR	0.94a	0.66ab	17.87a	5.52a	67.33a	0.85a	0.83a	15.27b	2.20b	75.83a
	SM	1.01a	0.58b	16.86a	3.25b	69.67a	0.94a	0.82a	17.63a	4.17b	80.50a
	SC	0.95a	0.80a	15.49a	2.80b	71.22a	0.95a	0.84a	16.46a	4.27b	82.83a
	CM	1.08a	0.79a	17.24a	6.55a	75.00a	1.09a	0.91a	15.97ab	10.75a	89.50a
40-100	SR	0.50ab	0.65a	17.81a	1.52a	68.22a	0.44a	0.60a	16.56b	1.74b	77.17a
	SM	0.48ab	0.36b	16.16b	1.40a	64.78a	0.46a	0.65a	17.24a	2.31a	71.72a
	SC	0.46b	0.58a	16.71b	1.56a	64.33a	0.44a	0.62a	17.15a	1.91ab	76.72a
	CM	0.52a	0.60a	18.29a	1.63a	65.33a	0.47a	0.59a	16.55b	1.90ab	75.95a

Note: Values followed by different small letters represent significant differences among different straw incorporation modes in same soil layers ($p < 0.05$). TN, total nitrogen; TP, total phosphorus; TK, total potassium; AP, available phosphorus; AK, available potassium.

3.4 The factors influencing SOC stock changes in topsoil and subsoil

Cumulative OC input and soil nutrient contents (TN, TP, TK, AP, and AK) were used as factors influencing SOC stock change. Redundant analysis was carried out to identify the importance of those factors. Results showed that cumulative OC input and soil nutrient contents jointly explained 90.1% and 31.8% of the variation of topsoil and subsoil OC stocks changes, respectively. The main factors affecting OC stock change were AP, TN, and carbon input in topsoil (with explanation rates of 80.1%, 4.4%, and 2.8%, respectively) (Figure 4-4a), but were TN, carbon input, and TP in subsoil (with explanation rates of 25.3%, 3.7%, and 4.2%, respectively) (Figure 4-4b).

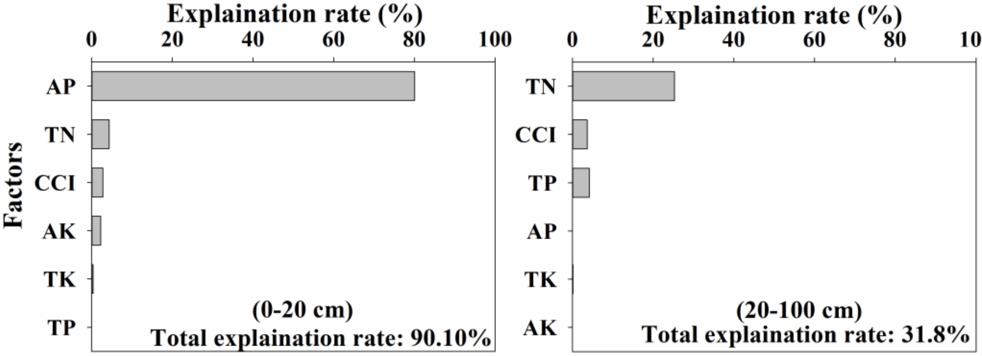

Figure 4-4 The explanation rate of cumulative organic carbon input and soil nutrient contents to soil organic carbon stock change in 0-20 cm (a, topsoil) and 20-100 cm soil layers (b, subsoil). CCI, Cumulative carbon input; TN, total nitrogen; TP, total phosphorus; TK, total potassium; AP, available phosphorus; AK, available potassium.

4 Discussion

4.1 The effects of different straw incorporation modes on crop yield and soil nutrients contents

Our results showed that, compared with SR treatment, topsoil TN, AP, and AK contents significantly increased under straw incorporation, and the largest increase was observed in CM treatment. This is mainly because straw and fresh cattle manure were applied into topsoil and then were gradually decomposed and utilized to release nutrients. Moreover, cattle manure contains more carbon and richer nutrients, leading to higher nutrients content than straw (Hadas et al., 2004). Overall, maize yield showed an upward trend with planting time increasing in all treatments, indirectly indicating that chemical fertilizers alone or combined with straw application could both improve soil fertility and crop growth environment (Tian et al., 2010). In addition, maize yield receiving SR treatment was the lowest in most years and fluctuated greatly. This may be because chemical fertilizers application alone reduces the stability of soil system. Meanwhile, crop yield in CM treatment was higher than that of SM and SC treatments, which implied that CM treatment has a better effect on soil fertility and crop production improvement. Those results were also proved by higher topsoil OC and nutrient contents in CM treatment rather than SM and SC treatments. However, a higher crop yield was observed in SM treatment rather than SC treatment. This was because mulching straw regulates soil temperature and reduces surface runoff and water evaporation, which has a better effect on soil water and nutrients retention (Yang and Tian, 2004).

4.2 SOC stock changes in different soil layers and their influencing factors

In cropland, SOC content depends on a net balance between OC input (crop stubble and root, straw, and manure) and OC loss caused by OM decomposition (Cai et al., 2020). It was found that, compared with straw removal, straw incorporation significantly increased topsoil OC stock, which is consistent with previous results (Cai et al., 2015; Xu et al., 2016). This is because the accumulative OC input in topsoil under straw return treatments was 2.0-3.0 times that of straw removal treatment. In general, SOC generally increases with OC input increasing before SOC saturation (Six et al., 2002; Xu et al., 2020). In addition, the increment of topsoil OC stock in CM treatment was significantly higher than that of SM and SC treatments. This is mainly due to that cattle manure is generally in a semi-decomposed state of OM, which brings more available N into field, resulting in higher C conversion efficiency than straw (Silver and Miya, 2001). Moreover, cattle manure has a better effect on improving the formation and stability of aggregates than straw, which benefits to SOC accumulation (Xu et al., 2020). Meanwhile, the increment of topsoil OC stock in SC treatment was significantly higher than that of SM treatment, implying that SC treatment was more conducive to topsoil OC accumulation under an equal amount of OC input. Fan et al. (2018) also found that, compared with straw mulching, SOC content increases in a cultivated layer under straw incorporation after crushing. The possible reasons for this phenomenon are as follows: (1) under SC treatment, crushed crop straws are in full contact with soil and are more prone to be decomposed and transformed, which is conducive to produce more metabolites (e.g., polysaccharides) and promote soil aggregates formation, thereby increasing SOC adsorption and fixation (Dou et al., 2011); (2) however, whole straw is covered on ground which has frequent dry-wet alternation and high microbial activity under SM treatment, causing more C derived from straw to be decomposed, thereby releasing into atmosphere in the form of CO_2 (Abiven et al., 2009).

Furthermore, we found that, compared with straw removal, SOC stock significantly reduced in 40-100 cm soil layer under straw return, which is inconsistent with the results of Xu et al. (2016) and Gai et al. (2019). It implied that an amount of OC input could not compensate for OC loss caused by OM mineralization in subsoil (Yang et al., 2018). We speculated that subsoil OC depletion had the following reasons: (1) crop roots extend downwards under insufficient water conditions in our study site which belongs to a drought area. Therefore, to provide nitrogen for crop growth, microbial mining for N enhances soil OM decomposition and then causes OC loss, which would particularly occur when N supply is insufficient due to low N content in subsoil (Rumpel and Kögel-Knabner, 2011). Moreover, the explanation rate of TN (25.3%) to subsoil OC change was the highest in our study, indirectly proving this inference. (2) straw incorporation brings in plenty of dissolved OC and nutrients, then increases fresh C source in subsoil (such as dissolved OM and root exudates) via leaching and improving root growth, finally leading to OC loss due to priming effect (Fontaine et al., 2007). (3) fertilization and deep tillage could promote roots growth and break plow pan, respectively, which benefits to improve soil aeration and the downward extension of crop roots, finally accelerating OM decomposition by increasing soil disturbance (Han et al., 2020). Moreover, we found that SOC stock in the entire 100 cm profile reduced under SM and SC treatments, but significantly increased under CM treatment. Meanwhile, the increment of SOC stock in the entire 100 cm profile was less than that of topsoil under CM treatment. Those result highlighted that only considering topsoil may overestimate the effect of straw incorporation on SOC improvement benefits. Therefore, subsoil OC stock change should be paid more attention to in cinnamon soil under straw incorporation into field.

Redundant analysis showed that the total explanation rate of cumulative OC input and soil nutrient content to topsoil OC change was 90.1%, which implied that topsoil OC change was mainly controlled by OC input and soil nutrients. This is because straw and fresh cattle manure contain plenty of OC and nutrients (Song et al., 2018). On the one hand, straw and cattle manure mainly applies on topsoil, which greatly increases OC input and directly increases topsoil OC. On the other hand, straw and cattle manure bring in plenty of nutrients, which reduces microbial nutrient limitation in the conversion process of straw and cattle manure, finally benefiting topsoil OC accumulation (de Oliveira Ferreira et al., 2018; Murugan and Kumar, 2013; Xu et al., 2016). However, our results also showed that the total explanation rate of OC input and soil nutrients to subsoil OC change was only 31.8%. It may be due to the low amounts and small changes of OC input and soil nutrients in subsoil. In addition, some studies also found that subsoil OC may be more prominently affected by clay content, Ca^{2+}, cation exchange capacity, soil porosity, iron and aluminum oxides (dos Reis et al., 2014; Han et al., 2018; Rumpel and Kögel-Knabner, 2011). In summary, due to the differences in source, content, and stability between topsoil and subsoil OC under straw incorporation, topsoil OC change was mainly controlled by OC input and nutrient contents, but subsoil OC change may be more affected by other properties rather than OC input and nutrient contents (de Oliveira Ferreira et al., 2018; Rumpel and Kögel-Knabner, 2011). Therefore, we should further study the driving factors of subsoil OC change to provide a basis for SOC sequestration and model prediction in cinnamon soils.

5 Conclusion

Regardless of fertilization in spring or autumn, straw incorporation significantly increased topsoil OC, TN, AP, and AK contents and crop yield in cinnamon soil, and the greatest increment was observed in CM treatment. Compared with straw removal, SOC stock was largely increased in topsoil and greatly reduced in 40-100 cm under straw incorporation. For the entire 100 cm profile, SOC stock reduced under SC and SM treatments, but increased under CM treatment. However, the increment of SOC stock in the entire 100 cm was less than that of topsoil under CM treatment. Therefore, when evaluating the impact of straw incorporation on SOC in cinnamon soil, only considering topsoil may overestimate the effect on SOC improvement. In summary, our results highlighted that CM was an important practice of straw utilization for improving soil fertility and C sequestration in cinnamon soil. However, subsoil OC change should also be paid attention to under straw incorporation.

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Chapter V

Effects of biochar application on crop productivity and soil carbon sequestration controlled by biochar C:N ratio and soil pH: A global meta-analysis

From Xu, H., Cai, A. D., Wu, D., Liang, G. P., Xiao, J., Xu, M. G., Colinet, C., Zhang, W. J., 2021. Effects of biochar application on crop productivity, soil carbon sequestration, and global warming potential controlled by biochar C: N ratio and soil pH: A global meta-analysis. *Soil &Tillage Research* 213, 105125.
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Abstract

Biochar application has been widely recommended as a potential solution to tackle the challenges of food security and climate change in agroecosystems, but the effect sizes of biochar application on crop productivity and soil carbon sequestration shows great uncertainties. To explore the effect variation of biochar application alone (B) and biochar plus chemical fertilizers (BF) on crop yield and soil organic carbon (SOC), this study reviewed updated datasets with 455 and 131 independent experiments globally to identify the key factors influencing the responses of crop yield and SOC to B and BF, respectively. Overall, the effect sizes were different between B and BF in both improving crop yield (15.1% and 48.4%, respectively) whereas there was almost no difference in terms of increasing SOC (32.9% and 34.8%, respectively). In addition, the effect sizes of B and BF on crop yield were coupled with these on SOC. Increased biochar carbon to nitrogen ratio (C:N ratio) and soil pH decreased the impact of B and BF on crop yield, while increased SOC promoted the impact of BF on crop yield. The wetness index, soil properties (SOC, pH, and clay), and biochar properties (type, pH, C:N ratio, and application rate) jointly explained 70%-79% and 90%-93% of the effect variations in crop yield and SOC, respectively. The biochar C:N ratio and soil pH were the most important factors determining the effect size of biochar application on crop yield and SOC. Taken together, biochar application is a win-win solution for improving crop yield and increasing SOC mainly depending on biochar properties and soil pH.

Keywords: Biochar properties; Fertilizer; Crop yield; Soil carbon

1. Introduction

Sustaining crop production to feed the growing population with limited arable soil is a great challenge for agriculture (Valin et al., 2013). In addition to its significant role in crop production, agricultural soil is also expected to contribute to mitigating climate warming by soil carbon (C) sequestration through effective agricultural management practice (Lal, 2004; Wiesmeier et al., 2014). Biochar is a carbon-rich byproduct produced by the pyrolysis of agricultural waste (e.g., crop straw, wood, and manure) at high temperatures (400-700 °C) and anoxic conditions (Smith, 2016, Sohi et al., 2010). It has a large specific surface area, stable physical and chemical properties, abundant pore structure and surface functional groups, and has been proposed as an emerging agricultural soil amendment material (Smith, 2016, Sohi et al., 2010). Biochar application has been regarded as an effective agricultural management practice to mitigate climate change and ensure global food security (Crane-Droesch et al., 2013; He et al., 2017; Smith, 2016).

Results on the effect of biochar on crop yield and soil organic carbon (SOC) have shown wide variation and high uncertainty (Liu et al., 2016a; Liu et al., 2013). For example, previous results from global meta-analyses proved that biochar application could improve crop yield by 5%-51% (Biederman and Harpole, 2013; Borchard et al., 2019; Cayuela et al., 2014; He et al., 2017; Liu et al., 2013). The large variation and uncertainty of the effect of biochar could be attributed to anthropogenic factors, especially for biochar applied alone (B) and combined plus chemical fertilizers (BF). Compared with B, BF application could not only directly provide mineral nutrients needed for crop growth but also increase crop return due to the addition of chemical fertilizers (Al-Wabel et al., 2018; DeLuca et al., 2009). Therefore, to obtain a more accurate estimate of the effect of biochar, it is imperative to focus on distinguishing the effects of B and BF on crop yield and SOC.

The large effect variation of B and BF on crop yield and SOC is also dependent on different influencing variables, which can be divided into three categories: climatic factors, management practices, and soil properties (He et al., 2017; Jeffery et al., 2011; Liu et al., 2016a). Among them, climate is generally regarded as the dominant variable that regulates crop yield by directly providing temperature and precipitation for crop productivity (Crane-Droesch et al., 2013; Jeffery et al., 2017). However, the increase in crop productivity or biomass could result in a higher rate of root deposits and crop residue retention in fields and thus eventually enriching SOC (Cai et al., 2019). With a high proportion of recalcitrant carbon, biochar has been regarded as a potential medium for soil C sequestration (Schmidt et al., 2002). Besides carbon substrate input, the application of biochar with a high C:N ratio results in microbial nitrogen immobilization in soil (Kirkby et al., 2014). Decreased soil microbial activities as a consequence of biochar with a high C:N ratio would lead to an increase in SOC (Cleveland and Liptzin, 2007; Kirkby et al., 2014). Biochar with moderate alkalinity would benefit lower SOC mineralization, while biochar with extremely high or low pH values would incur macronutrient deficiencies and finally generate low crop yield (Chan and Xu, 2012). Climate and biochar properties also influence the potential effect of biochar by regulating soil properties (Biederman and Harpole, 2013; Smith, 2016). Biochar application in acid soils greatly enhances its consumption by microorganisms for balanced soil pH conditions, which may trigger a more vigorous priming effect on native SOC mineralization (Foereid et al., 2011; Jones et al., 2011). Instead, biochar amendment to neutral or alkaline soils would incur an inhibition of soil carbon mineralization with enhanced soil pH (Liu et al., 2016a). Owing to the interactions among different variables (e.g., climate, management practices, and soil properties), the practice of predicting the effect of biochar on crop yield and SOC based on individual factors (e.g., temperature, soil texture, biochar application rate, etc.) would produce considerable uncertainty (He et al., 2017; Jeffery et al., 2017; Jeffery et al., 2011; Liu et al., 2016a; Liu et al., 2019).

Therefore, an enhanced understanding of the relative importance of different variables influencing the effect size of biochar application on crop yield and SOC, particularly B and BF application, is urgently needed to accurately predict and improve the potential benefit of biochar. In this study, 95 individual experiments derived globally from 143 published literatures were synthesized to examine the responses of crop yield and SOC to B and BF. The specific objectives of this study were to (1) quantitatively examine the effect size of B and BF on crop yield and SOC under different external conditions, and (2) identify the key factors influencing the effect size of B and BF application on crop yield and SOC to a better understanding of the implications of biochar application in sustainable agricultural ecosystems.

2. Materials and methods

2.1. Database collection

To establish a comprehensive database of the effect biochar on crop yield in the global agricultural ecosystem, strict criteria (described below) were set up to obtain closely relevant data from the Web of Science (http://apps.webofknowledge.com), Google Scholar (https://scholar.google.com), and China Knowledge Resource Integrated Database (http://www.cnki.net/) with combined keywords ("biochar", "crop productivity", "crop yield", and "grain yield"). All the selected published literature and experimental data adhered to the following requirements: (a) Manipulative experiments were conducted in the field (outdoor environment), while laboratory and incubation experiments were excluded to avoid the disturbance of the environmental variables of crops growth from humans. Targeted experiments included control (with no biochar addition) and treatment groups (with biochar addition), and each group had no less than three plots as replicates; (b) No biochar was applied before or during the targeted experiments in the control group. For treatment groups, a study was obliged to specify whether it was the application of B or BF; (c) Studies meeting the above two criteria and having data of SOC under B and BF were as well recorded; (d) Experimental data could be obtained directly from the figure, table, or text. For these data in figures, the GetData Graph Digitizer 2.24 was used. Relevant meta-analysis literature of previously published syntheses and the subsequent experimental data until December 2019 were included in this database.

A total of 1080 paired crop yield data under biochar addition experiments from 143 published literature were collected, including 648 paired under B and 430 paired under BF treatments, and 333 paired with SOC determinations (Table 5-1). The spatial distributions of targeted sites were shown in Figure 5-1. In addition to crop yield, information including the first author, publication year, site location (country, latitude, and longitude), climate variables (MAT and MAP), crop type, biochar properties (biochar type, biochar application rate, biochar pH, biochar carbon content and biochar nitrogen content), soil physicochemical properties (SOC, soil total nitrogen, soil pH, and soil texture) were as well extracted from the targeted literature to explain the variation of crop yield.

Finally, another database (489 paired crop yield data) was established to explore the interaction of biochar and fertilizers on crop yield because there were 41 related publications relevant to single control, two-factor treatments (biochar and fertilizer), and combinations of factors (biochar plus fertilizers).

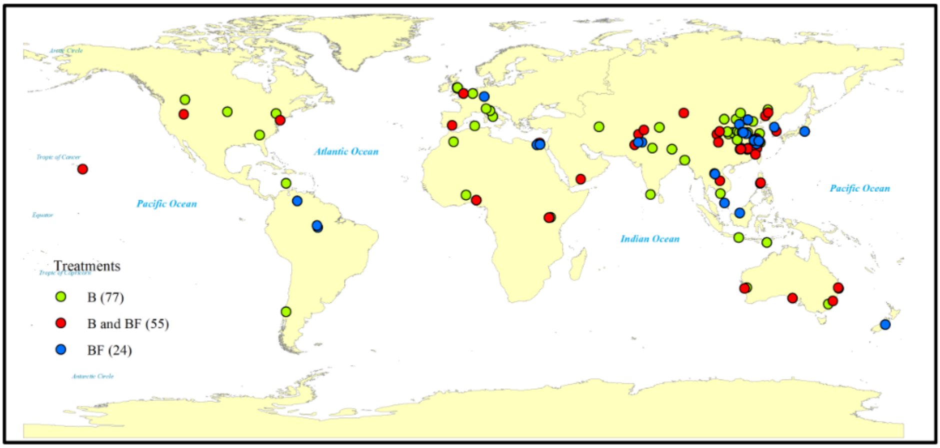

Figure 5- 1 Global distribution of 455 independent experiments from 156 study sites selected. Letters B (278 independent experiments) and BF (177 independent experiments) represent biochar applied alone and biochar plus fertilizers, respectively.

2.2. Data preparation

In our study, more than half of the environmental variables data (e.g., MAT (67%), MAP (75%), biochar properties (87%), and soil properties (62%)) were directly extracted from the targeted literatures (Table 5-S1, supplementary table at the end of this chapter). The non-reported data (e.g., MAT, MAP, and soil texture) in the targeted literature were obtained from relevant websites. Nonetheless, such data were not included to ensure the accuracy of the database. Overall, experimental sites were spanned from -43.7° to 53.5° in latitude and from -155.7° to 172.5° in longitude, with MAT varying from 4.0 °C to 29.0 °C, and MAP ranging from 45 mm to 2870 mm. Detailed biochar and soil properties were shown in Table 5-S1. The wetness index was a composite indicator of climate. The wetness index of each experimental site was calculated using MAT and MAP as De Martonne (1926) equation:

$$Wetness\ index = MAP/(MAT + 10) \tag{2}$$

For the comparison of the effect of biochar on crop yield and SOC among regions, experimental sites were divided into two ways. First, experimental sites were grouped according to absolute latitude into tropic (23.5 °S to 23.5 °N), subtropic (23.5-35 °S and °N), temperate (35-50 °S and °N), and (sub)arctic (> 50 °S and °N) zones. Second, experimental sites were separated into four groups based on their wetness index: < 20.0, 20.0-40.0, 40.0-60.0, and > 60.0. The type of crop and biochar were divided into five (wheat, maize, rice, vegetable, and other) and four categories (straw, wood, manure, and other), respectively. The application rates (Mg ha^{-1}) of biochar were divided into three categories: < 10.0, 10.0-40.0, and > 40.0 Mg ha^{-1}. The pH and C:N ratio of biochar were categorized into four (< 8.0, 8.0-9.0, 9.0-10.0, and > 10.0) and three categories (< 50.0, 50.0-100.0, and > 100.0), respectively. For soil properties, soil organic carbon was grouped into three categories: < 10.0, 10-20.0, and > 20.0 g kg^{-1}. If soil pH was measured by the method of $CaCl_2$ solution, the following equation was used to obtain the value of soil pH (H_2O): pH (H_2O) = 1.65+0.86 pH ($CaCl_2$) (Guo and Xin, 2009). Soil pH were divided into acidic soils (pH < 5.5), weak acidic soils (pH 5.5-6.5), neutral soils (pH 6.5-7.5), and alkaline soils (pH > 7.5). Soil texture was classified into sandy soil, loamy soil, and clayey soil based on the U.S. Department of Agriculture soil classification system.

2.3. Non-independence of sampling

In one experiment, the same control treatment was shared by several experimental treatments, such as different biochar application rates. These experimental treatments are treated as non-independent treatments. Previous research results have shown that non-independent treatment plays an important role in the difference of effect size for meta-analyses, which could significantly affect the result (Hungate et al., 2009; Verhoeven et al., 2017). In this study, the application rates of biochar were considered as non-independent factors because different biochar application rates were compared with the same control. Finally, the mean and standard deviation of treatments under different application rates were weighted by the amount of the biochar application, the weight (W_B) of which could be estimated as the following equation:

$$W_B = Q/\sum_{i=1}^{N} Q_i \tag{3}$$

where Q is the amount of the biochar application during the experiment (Mg ha^{-1}); N is the number of treatments with the same control treatment.

With the consideration of non-independence, 455 independent experiments of crop yield and 131 independent experiments of SOC were eventually obtained (Table 5-1). A meta-analysis was then performed based on these independent experiments.

Table 5-1 Effect size of biochar applied alone (B) and biochar plus fertilizers (BF) on crop yield and soil organic carbon (SOC).

Index	Treatments	Effect size (Lower *CI*, Upper *CI* (%))	No. of independent experiments	Sampling sizes
Crop yield	Total	22.2 (18.7, 25.7)	455	1078
	B	15.1 (12.1, 18.3)	278	648
	BF	48.4 (41.8, 55.3)	177	430
SOC	Total	33.5 (23.2, 45.2)	131	333
	B	32.9 (21.7, 44.8)	86	230
	BF	34.8 (24.2, 46.5)	45	103

2.4. Meta-analysis

This study focused on the effect size of biochar application on crop yield and SOC at the global scale by weighting the natural log-transformed response ratio (Ln (*RR*)) with the inverse variance. The Ln (*RR*) was calculated as follows:

$$Ln(RR) = ln(X_B/X_C) \tag{4}$$

where X_B and X_C are the mean values under the biochar application and control, respectively.

If there was no a heterogeneity among treatments ($P > 0.1$ and $I^2 < 50\%$), the fixed-effect model (FEM) would be used for analysis. Otherwise, the random effect model (REM) would be used. For that purpose, data of mean, standard deviations (SD), and sample sizes (N) were needed. If standard error (SE) rather than SD was obtained, SD was transformed using the following equation:

$$SD = SE\sqrt{N} \tag{5}$$

In this database, only 19% of the data did not report *SD* or *SE*, where part of these SDs was obtained from the corresponding author, and the missing SDs were derived from the mean by the variance coefficient of all datasets (Hou et al., 2020).

The variance (V) of X was calculated as follows:

$$V = {SD^2}_B \Big/ N_B {X^2}_B + {SD^2}_C \Big/ N_C {X^2}_C \tag{6}$$

where SD_B and SD_C are the standard deviation under biochar and control, respectively. N_B and N_C are the sample number under biochar and control, respectively.

The weighting factor (W_{ij}) and standard error of S (Ln (*RR*)) were calculated as using the following equations:

$$W_{ij} = 1 \big/ V_{ij} \tag{7}$$

$$S(Ln(RR)) = \sqrt{1 \Big/ \sum_{i=1}^{m} \sum_{j=1}^{ki} W_{ij}} \tag{8}$$

95% confidence interval (95% *CI*) was calculated as the following equation:

$$95\%CI = Ln(RR) \pm 1.96S\big(Ln(RR)\big) \tag{9}$$

If 95% *CI* overlapped with zero, biochar application would not affect crop yield and

SOC.

The effect size was transformed by using the equation (10):

$$RR = (e^{Ln(RR)} - 1) \times 100\% \tag{10}$$

For the effect size of interaction between biochar and fertilizer on crop yield (the interaction effect size), Ln (RR) and variance were calculated as using the following equations:

$$Ln(RR) = Ln({}^{X_{AB}}/_{X_B}) - Ln({}^{X_A}/_{X_C}) \tag{11}$$

$$var(RR) = {}^{SD_{AB}^2}/_{X_{AB}N_{AB}} + {}^{SD_B^2}/_{X_B N_B} + {}^{SD_A^2}/_{X_A N_A} + {}^{SD_C^2}/_{X_C N_C} \tag{12}$$

where X_{AB}, SD_{AB}, and N_{AB}; X_B, SD_B, and N_B; X_A, SD_A, and N_A; X_C, SD_C, and N_C represent the mean, SD, and sample size of the group of BF, B, fertilizer alone, and control, respectively. In the BF treatment, the effect of biochar and chemical fertilizer on crop yield is to promote, inhibit or have no interaction from each other, respectively, when their interaction effect size is greater, less than or overlaps with zero.

2.5 Effect sizes

The effect size is often used the measures of the effect magnitude in the Meta-analysis, which provides a statistical mean for summarizing the results of independent experiments (Hedges et al., 1999). The effect size provides comprehensive information about how much the subjects (e.g., crop yield, SOC, etc.) have been changed under the treatment group (e.g., biochar application rate, interaction between biochar and chemical fertilizer, etc.) relative to the control group across all studies. Meanwhile, the optimal application conditions of the treatment can be obtained by comparing the effect size under different variables (e.g., temperature, precipitation, biochar type, soil texture, etc.). Meanwhile, the optimal treatment can be obtained by comparing the effect size under different treatments (e.g., biochar, biochar plus fertilizers). Furthermore, the relative contributions of different variables to the effect size could be quantified by some other statistic methods, such as a BRT analysis.

2.6. Statistical analysis

Before conducting the meta-analysis, two ways were performed to check the quality of database, of which one was the leave-one-out meta-analysis by the "meta" packages (Figure 5-S1, supplementary figure at the end of this chapter) and the other was the frequency distribution by the Gaussian distribution function based on effect sizes:

$$y = A\, exp^{{}^{(x-\mu)^2}/_{2\sigma^2}} \tag{13}$$

where y is the sample size of effect sizes; x is the mean of effect sizes; μ and σ^2 are the mean and variance across all effect sizes, respectively; A is a coefficient.

A BRT analysis was performed to quantify the relative contributions of climate, crop type, biochar properties, and soil properties to the effect size of crop yield and SOC under B and BF treatments. Before the BRT analyses, some variables were not included to avoid correlation based on theoretical knowledge. Specifically, (1) wetness index, rather than MAT and MAP, is adopted to represent climate because it is a composite indicator. (2) SOC, rather than soil total nitrogen, is adopted as an indicator of soil fertility because of their high correlation ($R^2 = 0.81$, $p < 0.01$, n = 326). (3) Soil clay, rather than soil sand and silt, is adopted as an indicator of soil texture. (4) Only the biochar C:N ratio is included because it is a composite indicator of high correlation with biochar carbon content and biochar nitrogen content. For each effect size, the BRT analyses were performed following the recommended parameters from previous studies (learning rate of small value (0.01-0.001), bag fraction from 0.50 to 0.75, cross-validation of 10, and tree-complexity (2-5)) to ensure the number of regression trees exceeding the sample size (Cai et al., 2020; Elith et al., 2008). The Gaussian distribution was used in the BRT analysis because of the continuous numerical variables of effect sizes. The predicted effect sizes of selected factors through the BRT analyses were compared with the observed effect sizes to check their reliability of selected factors. The package of generalized boosted regression models from Elith et al. (2008) was used in the R version 3.3.3.

3. Results

3.1. Global effect sizes of B and BF on crop yield and SOC

This synthesis showed that the effect of biochar on crop yield and SOC was globally distributed, which varied greatly among experimental sites and displayed normal/Gaussian distributions (Table 5-1 and Figure 5-S1 to 5-S3). Biochar had a decreased effect size with absolute latitude ($R^2 = 0.22$, $p < 0.01$, n = 85) on crop yield, but without the same occurrence on SOC (Figure 5-S3). Overall, biochar with/without fertilizers significantly increased crop yield and SOC by 22.1% (18.7% to 25.7% CI, same as below) and 33.5% (23.2% to 45.2%), respectively compared with the control. The effect sizes on crop yield were substantially changed under B (15.1%) and BF (48.4%). Moreover, the effect size s of B (Fig. 5-2a) and BF (Fig. 5-2b) on crop yield was significantly and linearly correlated with that on SOC (Figure 5-2).

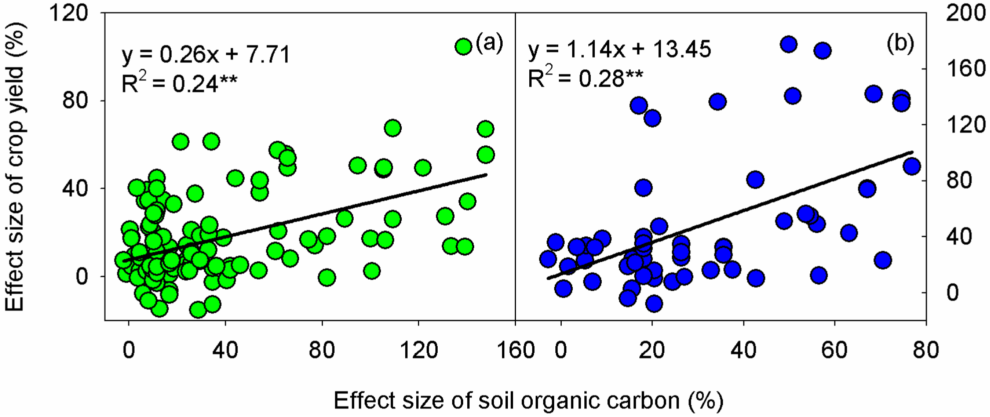

Figure 5-2 Relationships between the effect size (%) of crop yield and the effect size (%) of soil organic carbon under biochar applied alone (a) and biochar plus fertilizers (b).

3.2. Effects sizes of B and BF on crop yield and SOC

Crop yield significantly increased in tropic, subtropic, and temperate zones under B and BF (Figure 5-3). The highest effect size of B and BF on crop yield was observed in the tropic zone and > 60 wetness index compared with other climatic zones and < 60 wetness index. The effect size of B and BF on crop yield tended to decrease with the biochar C:N ratio and soil pH increase, while an opposite trend appeared with SOC under BF. These results further revealed that the interaction between biochar and chemical fertilizers on crop yield was rare under various climates, crop types, soil properties, and biochar properties (Figure 5-S4), which even revealed a negative interaction in the subtropic zone and for the crop of maize. The application of B and BF significantly increased SOC compared with the control under various climates, crop types, soil properties, and biochar properties (Figure 5-4). However, there were no significant differences in the effect size on SOC between B and BF.

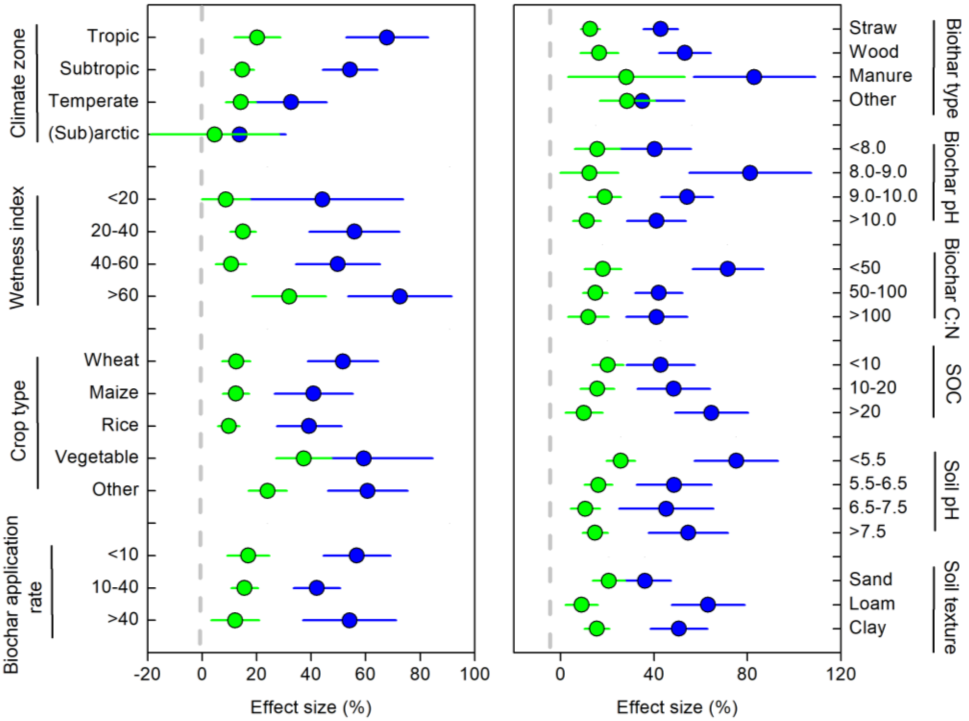

Figure 5-3 Effect sizes (%) of crop yield at various climate zones, wetness index, crop types, biochar properties and application rates, and soil properties under biochar applied alone (green) and biochar plus fertilizers (blue). Values represent the effect sizes ± 95% confidence intervals (see Table 5-S2). The dashed lines indicate the non-significant effect size. C: N is the ratio of carbon to nitrogen. SOC is the soil organic carbon. Only groups with a total sample size ≥5 are shown.

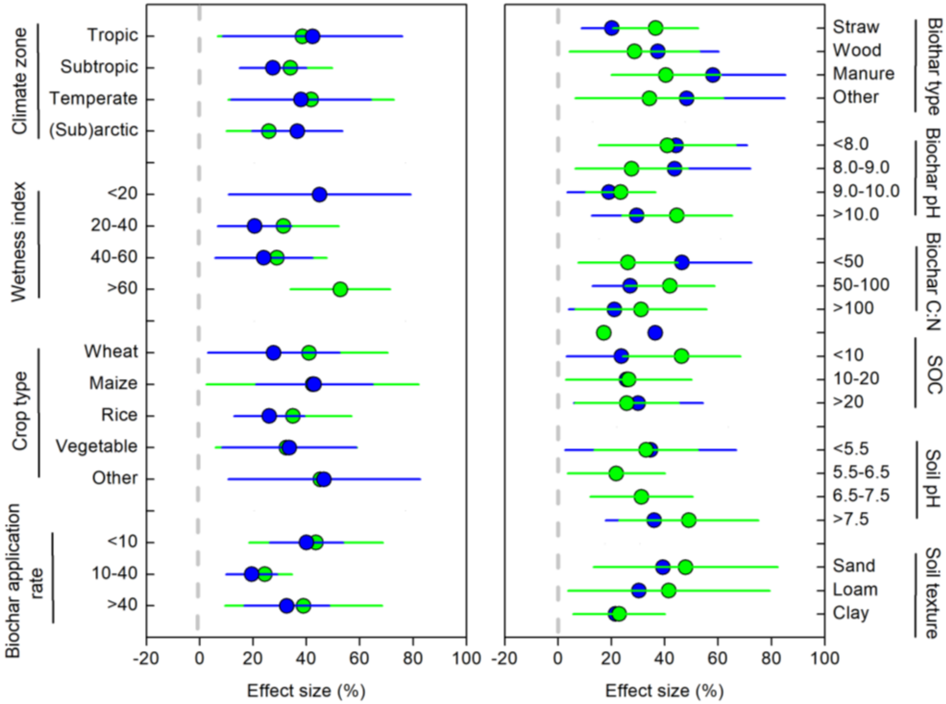

Figure 5-4 Effect sizes (%) of SOC at various climate zones, wetness index, crop types, biochar properties and application rates, and soil properties under biochar applied alone (green) and biochar plus fertilizers (blue). Values represent effect sizes ± 95% confidence intervals (see Table 5-S3). The dashed lines indicate the non-significant effect size. C: N is the ratio of carbon to nitrogen. SOC is soil organic carbon. Only groups with a total sample size ≥5 are shown.

3.3. Predicted effects sizes of B and BF on crop yield and SOC

The responses of crop yield and SOC to B and BF were driven by multiple environmental variables rather than a single factor (Figure 5-3 to 5-5). Biochar C:N ratio, SOC, and soil pH were the three most influential variables on the effect size of crop yield under B (relative influence: 14.2%, 15.4%, and 15.6%, respectively) and BF (26.0%, 12.4%, and 22.8%, respectively) among the selected 9 variables. The overall influence of biochar properties to the effect size on SOC was larger than that of climate, crop type, and soil properties, with their relative influence of 55.3% and 64.9% under B and BF, respectively. However, some variables with the relative influence of <3% were deleted from the BRT analysis. Overall, the BRT model driven by the 9 variables could explain 70%-79% and 90%-93% of the variance for the effect size on crop yield and SOC, respectively.

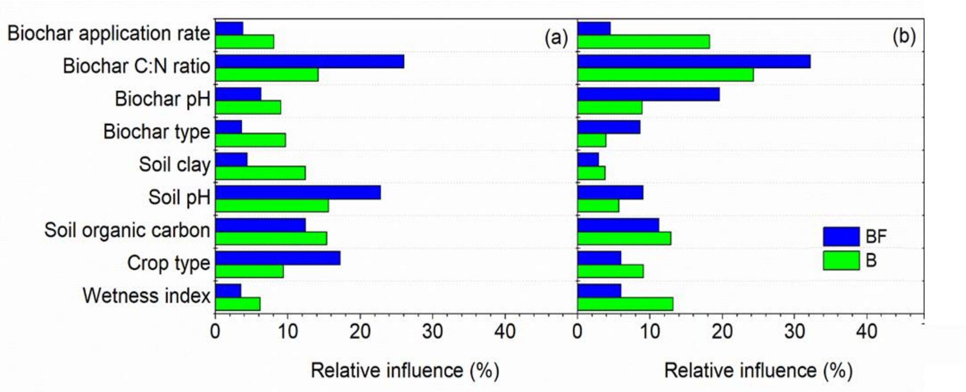

Figure 5-5 The relative influence (%) of climate, crop type, edaphic factors, and biochar properties, soil properties, crop type, and wetness index to the effect sizes of biochar applied alone (B, green) and biochar plus fertilizers (BF, blue) on crop yield (a) and soil organic carbon (b, SOC) estimated by the BRT model. Notes: Biochar properties include biochar type, biochar pH, biochar carbon to nitrogen (C: N) ratio, and biochar application rate. Soil properties include SOC, soil pH, and soil clay.

4. Discussion

4.1. The magnitude of biochar's effect size

According to 455 independent experiments (1078 samples) on crop yield, biochar application significantly increased crop yield by 22.2% (Table 5-1), higher than an increase of 4.8% increased crop yield from 86 samples (Jeffery et al., 2011), 8.5% increase from 228 samples (Liu et al., 2013), 11.0% increase from 81 samples (Liu et al., 2019), and 17.1% increase from 30 independent experiments (Biederman and Harpole, 2013). Two reasons could be used to explain these discrepancies: (1) compared with our results, previous results mainly based on small scale samples, which might lead to a relatively greater(Sutton et al., 2007); (2) the application of BF has been widely used to substantially increase crop yield due to the abundance of nutrients provided for crop growth recently (Liu et al., 2016b; Oladele et al., 2019), but while almost all previous studies did not clearly distinguish the effect size on crop yield under B and BF (rarely involving BF). Our study verified that the effect size of BF on crop yield was higher than that of B (Table 5-1). Compared with B, BF could further promote crop growth by enhancing nutrient supply and increase nutrient use efficiency (Agegnehu et al., 2016; Horák et al., 2017). For instance, Biederman and Harpole (2013) reported that BF could increase crop yield by 35.0% (19.0% to 51.0% CI) compared with the no-biochar application based on 30 samples. However, according to 177 independent experiments (430 samples), the average effect size of BF on crop yields (48.4%) was not only higher than the Biederman and Harpole's (2013) result (35.0%), but also with a lower variability (from 19.0-51.0% CI to 41.8-55.3% CI).

Soil organic carbon sequestration by biochar application has been proposed as an effective way to increase crop yields and mitigate global warming (Smith et al., 2008). Our result was consistent with the conclusion of Liu et al. (2016a) that biochar application has a positive effect on SOC by global meta-analysis. However, the positive effect (33.5%) revealed in this study was less than that (52.2%) of Liu et al. (2016a). An accurate biochar's effect could be obtained by supplementing the experimental sample size and considering the experimental non-independence (Hungate et al., 2009; Verhoeven et al., 2017). Besides, the formation of SOC is a long-term process of soil retention of exogenous organic materials, which is affected by climate, management practices, and soil properties (Cai et al., 2016). Compared with B, BF could further promote crop growth and secrete more organic materials by crop roots, which contribute to SOC formation (Chew et al., 2020; Prendergast-Miller et al., 2014). The experimental time in targeted literature was relatively short, with an average of only 1.5 years, consequently resulting in no difference between the effect size of B and BF (32.9% and 34.8%, respectively) (Table 5-1).

4.2. Mechanisms of biochar's effect

The applications of B and BF were effective management practices for increasing crop yield. However, the effects of B and BF on crop yield depended on various climatic conditions, management practices, soil properties, and biochar properties. High effect size on crop yield under B and BF occurred in the tropic climate zone with > 60 wetness index (Figure 5-3), which may provide evidence for the statement that crop yield derived from nutrients effect (Haider et al., 2017; Tilman et al., 2002). Compared with the relatively fertile temperate climate zone, the addition of nutrients by B and BF was likely to have an impact on crop yield in the tropical climate zone. This may be because soil nutrients are a major limiting factor for crop growth in the tropical climate zone (Tilman et al., 2002). Some studies reported that biochar addition does not significantly increase crop yield in the temperate zone (Haider et al., 2017; Jeffery et al., 2017). This study further quantified the relationship that the effect size on crop yield under B and BF decreased with absolute latitude (Figure 5-S3). In addition, the effect size of B and BF on crop yield was declined with the increase of soil pH, which was consistent with the previous research (Jeffery et al., 2017). Soil pH was likely to promote crop yield, especially in acidic soil (Cai et al., 2019). The positive effect of B and BF on crop yield offset the negative effect from low soil pH by the high pH and C:N ratio of the added biochar, leading to the immobilization of some nutrient elements. Interestingly, the effect size on crop yield tended to be decreased under B but increased under BF with the increase of SOC. It suggested that biochar must be combined with fertilizer to further increase crop yield in fertile soils (Agegnehu et al., 2016). That's because biochar application alone affects crop yield by mainly improving soil physical properties and then creating an optimum environment for crop growth in high-fertility soil (Biederman and Harpole, 2013). Overall, biochar C:N ratio, SOC, and soil pH were the three most influential variables on the crop yield among the selected nine variables (Figure 5-5).

Biochar has a high performance in chemical and microbiological stability in soil due to its high carbon content, complex aromatization structure, and inherent chemical inertness (Lehmann et al., 2006; Sohi et al., 2010). Therefore, biochar may change the composition of soil organic matter to increase the total SOC content. By providing an abundant nutrient supply from fertilizers, the BF could significantly improve crop growth and correspondingly increase the carbon input from crops compared with B application (Chew et al., 2020). However, the response of SOC to B did not significantly differ from that BF under various influencing variables (Figure 5-4), mainly because of the too-short experiment times (1-2 years). Although more organic matter through crop roots was input into the soil under BF, the formation of SOC was still a complex and time-consuming process (Carvalhais et al., 2014; Luo et al., 2019). Meanwhile, this meta-analysis showed that the biochar C:N ratio was the most influential variable for the effect size of B and BF on SOC among all influencing variables. Biochar with a high C:N ratio may result in microbial nitrogen immobilization in soil, which is conducive to the formation of SOC (Cayuela et al., 2010). Biochar with a low C:N ratio is generally available in microbial processes such as SOC mineralization that decreases SOC content (Singh and Cowie, 2014). The effect size of B and BF on crop yield was additionally found to increase linearly with that on SOC (Figure 5-2). It directly quantified how B and BF could affect crop yield by regulating SOC and demonstrated the importance of soil fertility in crop yield (Cai et al., 2019). In a summary, more soil property indicators should be taken into account to further explore the network of biochar and soil properties in determining crop yield.

Biochar has high stability and will remain in the soil for a long time due to its slow decomposition rate (Smith, 2016). Our study further showed that the effect size of biochar application on crop yield and SOC could not be increased with the experimental time due to low nutrients content and high passive carbon content in biochar (Figure 5-S6). Biochar could act as a long-term soil conditioner with benefits for crop yield improvement and SOC sequestration (Woolf and Lehmann, 2012). In summary, distinguishing the effect of biochar alone and biochar plus fertilizers could reduce the variation of biochar's effect on crop yield and SOC compared with the previous studies. Our results could improve the accuracy of estimating biochar's effect. By quantifying the relative importance of environmental variables in controlling the effect of biochar, this present study provides novel insights into the optimization of biochar application in the agroecosystem.

5. Conclusion

Evidence presented in this study showed that both B and BF applications could significantly increase SOC sequestration and crop yield. Compared with B, however, BF could increase the effect size on crop yield by three times, while no obvious changes in the effect size on SOC. A significant coupling relationship was found between the effect size on crop yield and that on SOC under both B and BF application. The biochar C:N ratio and soil pH are the two most important variables controlling the effect size of B and BF application on crop yield. However, the overall influence of biochar properties on the effect size to SOC is larger than that to climate, crop types, and soil properties. Our results highlight that more economic and environmental benefits could be achieved by optimizing the properties (C:N ratio) of biochar that was applied in soil, especially in the acidic soil.

6. Data accessibility

All data related to this manuscript are available from the Dryad Digital Repository: https://figshare.com/articles/Biochar_by_a_global_meta-analysis/12368930.

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8. Supplementary Figures and Tables

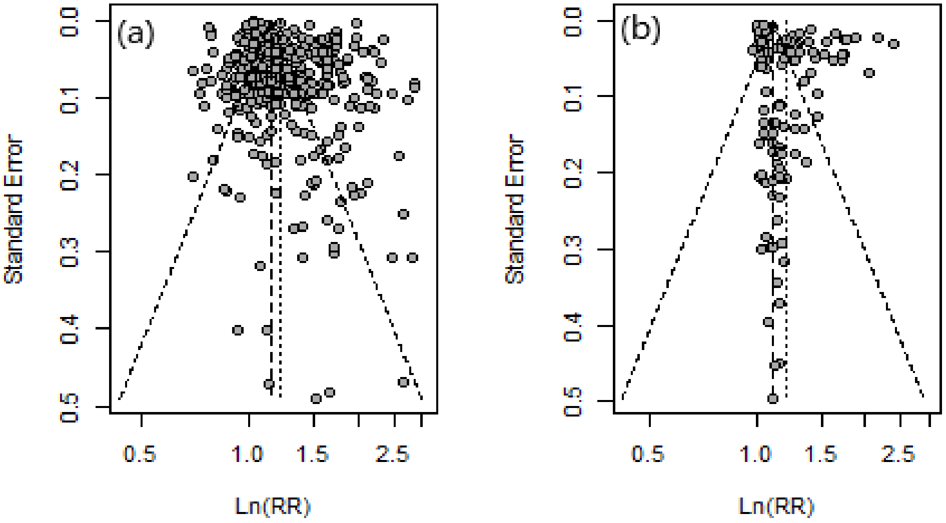

Figure 5-S1 Funnel plots for the effect of biochar application on crop yield (a) and soil organic carbon (b, SOC). Asymmetry test showed a symmetric distribution for crop yield (t = 2.14, $p < 0.05$) and SOC (t = 3.30, $p < 0.05$).

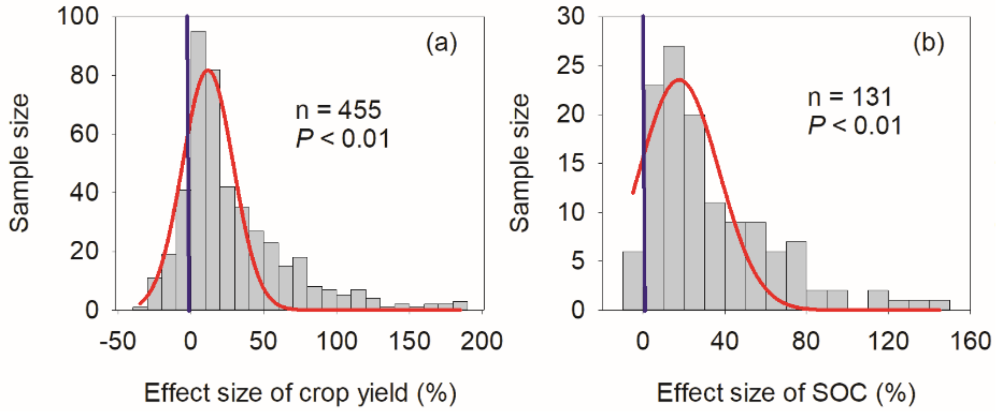

Figure 5-S2 Distribution of the effect size of biochar applications on crop yield (a) and soil organic carbon (b, SOC). The red curve is a Gaussian distribution fitted to frequency data. The blue dashed line is at effect size = 0.

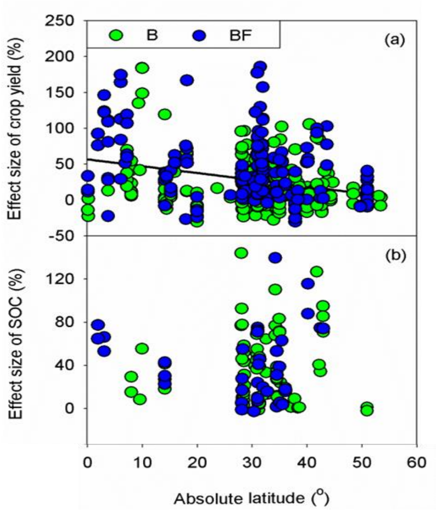

Figure 5-S3 Relationships between the effect sizes of biochar applicated alone (B, green dots) and biochar plus fertilizers (BF, blue dots) on crop yield (a) and soil organic carbon (b, SOC) and absolute latitude at a global scale. (a) The effect sizes of B and BF on crop yield decrease with absolute latitude (meta-regression, $R^2 = 0.22$, $p < 0.01$).

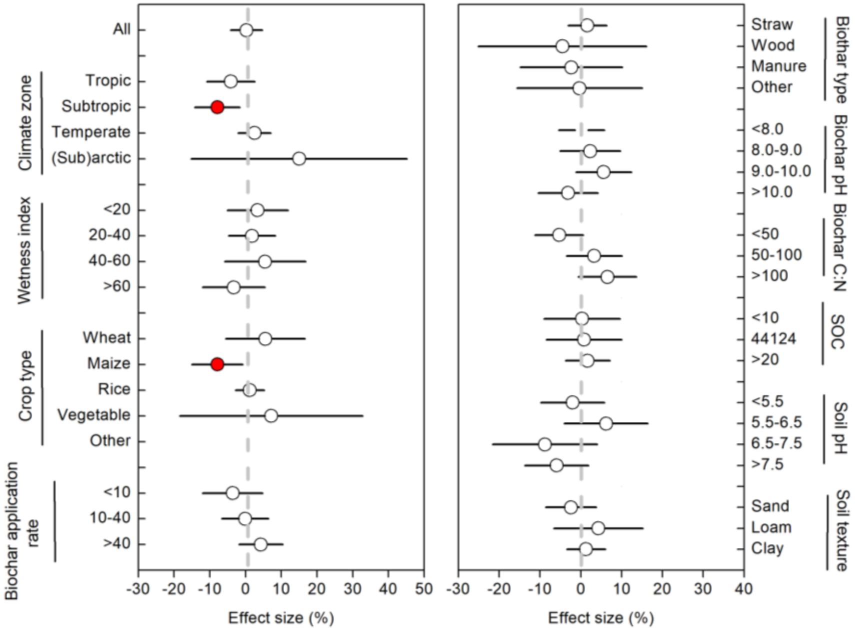

Figure 5-S4 Interaction effect size (%) of crop yield at various climate zones, wetness index, crop types, biochar properties and application rates, and soil properties under biochar applied alone (green) and biochar plus fertilizers (blue). Values represent effect sizes ± 95% confidence intervals (see Table5-S2). The dashed lines indicate the non-significant effect. C:N is the ratio of carbon to nitrogen. SOC is the soil organic carbon. Only groups with a total sample size ≥5 are shown.

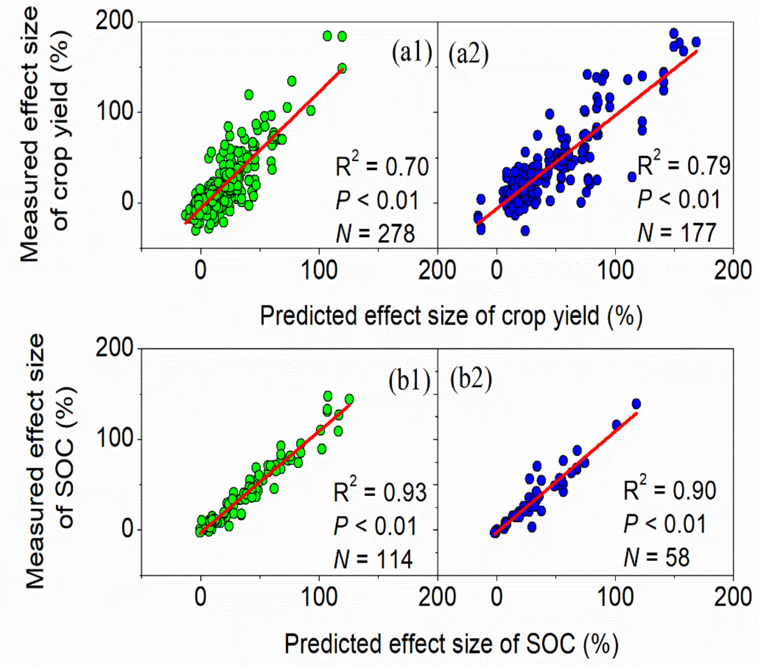

Figure 5-S5 Relationships between the predicted effect size from the boosted regression tree model and the measured effect size of crop yield and soil organic carbon under B (a1 and b1) and BF (a2 and b2). In all figures, the red line is the fitted function between predicted effect size and measured effect size.

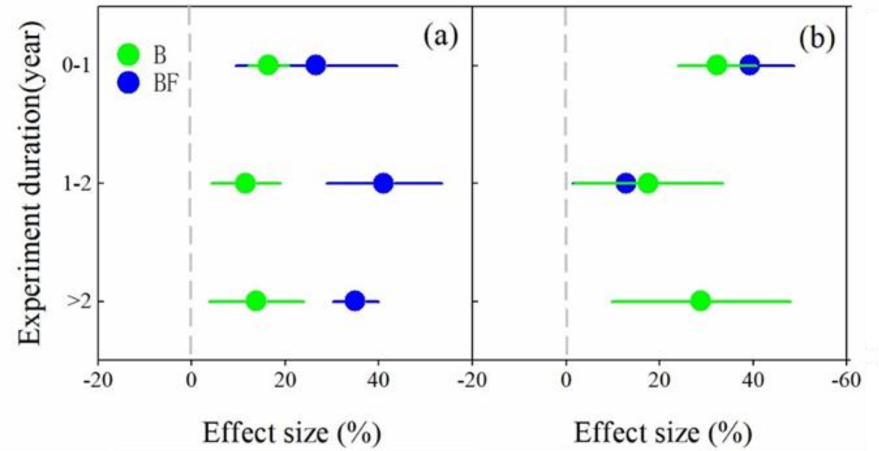

Figure 5-S6 Effect size of biochar applied alone (B, green dots) and biochar plus fertilizers (BF, blue dots) on crop yield (a) and soil organic carbon (b, SOC) under experiment durations. The dashed lines indicate the non-significant effect size. Only groups with a total sample size ≥5 are shown.

Table 5-S1 Summary of site climate, soil and biochar properties in biochar applied alone and biochar plus fertilizers treatments. SD indicates the standard deviation. *N* represents the number of independent experiments. MAT, Mean annual temperature. MAP Mean annual precipitation. WI, Wetness index. BAR, Biochar application rate. B-pH, Biochar pH. B-C, Biochar content. B-N, biochar nitrogen content. B-C:N, Biochar-C:N ratio. B-P, Biochar phosphorus content. S-C, soil organic carbon. S-N, soil total nitrogen. S-pH, soil pH. S-sand, Soil sand content. S-silt, Soil silt content. S-clay, Soil clay content.

Indexes	Unit	Biochar applied alone			Biochar plus fertilizers			Interaction of biochar and fertilizer		
		N	Mean SD)	Range	*N*	Mean (SD)	Range	*N*	Mean (SD)	Range
Longitude	°	278	76.3 (72.4)	-155.7-153.4	177	70.6 (71.1)	-155.7-172.5	83	82.8 (54.8)	-114.4-153.2
Latitude	°	278	27.0 (18.0)	-38.6-53.5	177	24.8 (20.3)	-43.7-51.0	83	15.7 (22.5)	-32.8-43.0
MAT	℃	199	15.8 (4.9)	4.0-28.0	104	16.6 (5.6)	7.0-29.0	59	15.4 (4.3)	4.0-27.5
MAP	mm	215	1118 (541)	45-2870	116	1149 (615)	82-2530	71	1080 (491)	48-2027
WI		199	40.8 (15.6)	1.6-79.7	103	39.4 (16.5)	1.6-70.7	71	59.3 (52.5)	1.5-202.7
BAR	Mg ha^{-1}	278	24 (19)	1.4-128.5	177	24 (18)	1-93	83	21 (13)	2.5-2.9
B-pH		261	9.4 (1.2)	5.7-13.0	166	9.2 (1.3)	5.7-11.0	74	9.4 (1.2)	6.0-10.7
B-C	g kg^{-1}	268	533 (180)	33-82	170	544 (171)	34-187	73	488 (149)	30.2-868.0
B-N	g kg^{-1}	263	8.2 (4.7)	0.8-33.0	172	8.6 (5.0)	1.5-28.0	73	8.3 (5.7)	1.5-33.0
B-C:N		261	87 (73)	12-522	170	80 (49)	11-300	75	76 (52)	6-300
B-P	g kg^{-1}	149	8.9 (17.6)	0.0-76.6	66	9.4 (15.2)	0.1-64.0	32	13.3 (17.6)	0.1-55.6
S-C	g kg^{-1}	223	13.9 (7.9)	1.3-48.0	132	15.1 (7.8)	3.3-36.4	70	15.6 (7.9)	3.3-48.0
S-N	g kg^{-1}	210	1.5 (1.6)	0.2-21.0	120	2.0 (3.2)	0.3-21.0	65	2.1 (2.6)	0.3-21.0
S-pH		246	6.4 (1.3)	3.9-8.8	146	6.3 (1.3)	3.9-8.8	69	6.2 (1.3)	4.1-8.8
S-sand	%	122	39.3 (23.0)	2.4-98.9	76	38.6 (28.1)	3.5-97.3	46	36.1 (24.7)	5.2-77.0
S-silt	%	122	35.6 (16.5)	1.1-73.0	76	35.5 (19.9)	2.8-73.0	46	37.0 (17.9)	12.0-73.0
S-clay	%	124	24.6 (14.8)	0.1-71.6	78	25.3 (16.6)	0.0-71.6	47	26.9 (14.4)	5.0-71.6

Table 5-S2 Effects sizes (%) of crop yield at various climate zones, wetness index, crop types, biochar properties and application rates, and soil properties under biochar applied alone, biochar plus fertilizers, and interaction between biochar and fertilizers. Only groups with a total sample size ≥5 are shown. N represents the number of independent experiments.

Treatments		Biochar				Biochar plus fertilizers				Interaction of biochar and fertilizers			
		N	Effect size (%)	Lower CI (%)	Upper CI (%)	*N*	Effect size (%)	Lower CI (%)	Upper CI (%)	*N*	Effect Size (%)	Lower CI (%)	Upper CI (%)
All		278	15.1	3.1	3.2	177	48.4	6.6	6.9	83	0.2	4.4	5.2
Climate zone	Tropical	45	20.2	8.4	9.1	46	67.8	15.1	16.5	19	-4.1	6.7	8.8
	Subtropic	143	14.7	4.2	4.4	79	54.3	10.1	10.8	38	-7.9	6.3	6.5
	Temperate	84	14.2	5.5	5.8	36	32.7	12.1	14.5	17	2.5	4.6	5.2
	(Sub)arctic	6	4.7	23.5	30.4	16	13.8	16.8	19.8	9	15.0	30.2	32.0
Wetness index	<20	23	8.7	8.9	9.7	11	44.2	29.6	37.2	8	3.4	8.5	8.7
	20-40	96	15.0	4.7	4.9	39	55.9	16.5	18.5	26	1.8	6.5	7.0
	40-60	63	10.6	5.6	5.9	40	49.8	15.4	17.2	23	5.5	11.3	12.2
	>60	17	31.9	13.5	15.1	13	72.6	19.0	18.3	14	-3.3	8.7	9.9
Crop type	Wheat	56	12.5	5.3	5.5	54	51.7	12.9	14.2	11	5.6	11.1	11.4
	Maize	70	12.4	4.8	5.1	40	40.9	14.4	16.0	19	-7.9	7.1	7.4
	Rice	87	9.8	4.0	4.2	53	39.3	11.9	13.0	40	1.2	4.0	4.2
	Vegetable	25	37.3	10.2	11.0	17	59.3	25.3	30.0	11	7.2	25.6	27.6
	Others	40	24.0	7.1	7.5	13	60.7	14.7	22.3	2			
Biochar application rate (Mg ha^{-1})	<10	70	16.9	7.7	8.3	56	56.8	12.3	13.3	17	-3.6	8.4	8.9
	10-40	160	15.6	4.9	5.2	94	42.1	8.6	9.1	46	-0.1	6.5	6.9
	>40	48	12.2	8.8	9.6	27	54.1	17.1	19.2	20	4.3	6.1	6.5

Biochar type	Straw	180	12.7	4.1	4.3	97	42.9	7.5	7.9	64	1.6	4.7	5.4
	Wood	52	16.5	8.1	8.7	51	53.3	11.1	12.0	3			
	Manure	12	28.2	25.0	31.0	12	83.0	26.1	28.9	8	-2.3	12.5	13.1
	Others	32	28.5	11.8	13.0	17	35.0	18.1	20.9	6	-0.3	15.3	24.8
Biochar pH	<8.0	43	15.7	9.7	10.6	32	40.4	15.6	17.5	9	0.2	5.6	6.9
	8.0-9.0	25	12.3	12.5	14.1	18	81.2	26.0	30.4	38	2.3	7.4	8.4
	9.0-10.0	87	19.0	6.9	7.3	69	54.2	11.2	12.1	24	5.6	6.8	7.4
	>10.0	105	11.3	6.0	6.3	47	41.0	12.6	13.8	12	-3.1	7.3	8.2
Biochar C:N ratio	<50	66	18.1	7.9	8.5	48	71.7	15.2	16.7	21	-5.3	5.9	6.7
	50-100	145	14.9	5.2	5.5	77	42.0	10.2	10.9	42	3.3	6.8	7.2
	>100	50	11.9	8.7	9.5	45	41.2	13.1	14.4	12	6.5	7.1	8.2
SOC (g kg^{-1})	<10	100	20.2	6.7	7.1	48	42.9	14.6	16.3	21	0.3	9.3	9.8
	10-20	75	15.7	7.3	7.8	45	48.4	15.6	17.4	29	0.8	9.2	10.2
	>20	58	10.0	8.0	8.6	54	64.6	15.6	17.3	20	1.7	5.4	6.1
Soil pH	<5.5	68	25.8	6.1	6.4	45	75.2	17.9	19.9	23	-2.0	7.8	8.3
	5.5-6.5	59	16.2	6.0	6.3	39	48.6	16.1	18.0	24	6.2	10.2	11.4
	6.5-7.5	53	10.6	6.3	6.7	24	45.3	20.2	23.5	10	-8.8	12.8	14.3
	>7.5	66	14.8	5.4	5.7	36	54.7	17.0	19.1	12	-5.9	7.8	8.3
Soil texture	Sand	68	20.7	7.0	7.4	60	36.2	11.1	12.0	21	-2.4	6.2	6.9
	Loam	52	9.0	6.9	7.4	43	63.2	15.7	17.3	25	4.3	10.9	11.8
	Clay	109	15.6	5.3	5.6	60	50.7	12.2	13.3	30	1.3	4.7	5.7

Table 5-S3 Effects sizes (%) of soil organic carbon (SOC) at various climate zones, wetness index, crop types, biochar properties and application rates, and soil properties under biochar applied alone and biochar plus fertilizers. Only groups with a total sample size ≥5 are shown. N represents the number of independent experiments.

Treatments		Biochar applied alone				Biochar plus fertilizers			
		N	Effect size (%)	Lower CI (%)	Upper CI (%)	*N*	Effect size (%)	Lower CI (%)	Upper CI (%)
Total		86	32.9	11.3	11.9	45	34.8	10.6	11.7
Climate zone	Tropic	7	38.6	31.9	41.2	7	42.3	33.7	45.8
	Subtropical	56	34.0	15.7	18.0	28	27.5	12.5	14.3
	Temperate	17	41.8	31.1	41.2	10	38.0	26.2	33.4
	(Sub)arctic	6	26.0	15.9	25.5	8	36.6	17.0	20.7
Wetness index	<20	4				6	45.0	34.1	46.3
	20-40	28	31.4	20.7	25.2	19	20.6	13.8	15.9
	40-60	33	29.0	18.7	22.3	9	24.0	18.3	22.8
	>60	5	52.7	18.7	21.3	3			
Crop type	Wheat	21	40.9	29.6	38.7	8	27.7	24.7	31.6
	Maize	12	42.3	39.8	57.9	12	43.0	21.9	26.4
	Rice	35	34.9	22.0	26.9	17	26.0	13.2	15.4
	Vegetable	10	32.4	26.5	32.4	7	33.5	25.2	23.5
	Others	8	45.2	33.6	40.3	6	46.6	36.0	49.5
Biochar application Rate (Mg ha^{-1})	<10	14	43.6	25.1	33.3	14	40.0	13.8	15.5
	10-40	61	24.5	10.2	11.2	19	19.5	9.6	10.6
	>40	10	39.0	29.5	38.7	10	32.7	16.0	18.5

Biochar type	Straw	64	36.6	16.0	18.4	30	20.2	11.4	12.8
	Wood	13	28.6	24.3	37.6	11	37.4	22.8	28.1
	Manure	6	40.5	20.5	32.0	7	58.0	27.2	30.2
	Others	8	34.2	27.8	29.2	5	48.2	36.8	26.3
Biochar pH	<8.0	13	40.9	25.7	32.2	9	44.2	26.7	33.6
	8.0-9.0	11	27.5	21.2	27.2	8	43.7	28.5	36.5
	9.0-10.0	35	23.5	13.1	14.8	11	19.1	15.7	19.2
	>10.0	20	44.6	20.7	24.6	16	29.6	16.9	19.8
Biochar C:N ratio	<50	15	26.2	18.7	19.0	10	46.5	26.0	32.4
	50-100	52	41.9	16.7	19.2	22	27.1	14.2	16.3
	>100	17	31.1	24.6	31.9	12	21.2	17.2	20.9
Soil organic Carbon (g kg^{-1})	<10	37	46.3	22.0	26.5	12	23.8	20.7	25.5
	10-20	23	26.5	23.6	30.0	21	25.7	15.5	18.1
	>20	17	25.8	19.5	34.2	10	30.1	24.2	30.7
Soil pH	<5.5	26	33.0	19.4	23.2	7	34.7	32.1	43.9
	5.5-6.5	22	21.8	18.2	22.2	12	21.9	17.2	21.3
	6.5-7.5	8	31.3	19.2	27.1	4			
	>7.5	24	49.0	26.1	31.5	19	36.1	18.3	21.5
Soil texture	Sand	17	47.8	34.6	46.8	12	39.4	23.7	29.3
	Loam	13	41.6	37.8	53.9	11	30.4	21.0	25.7
	Clay	46	22.9	17.2	20.5	18	21.5	14.2	16.6

Chapter VI

General discussion, conclusions, and perspective

The research in this thesis demonstrated that clay fraction, as the major site of soil OC accumulation and exogenous OC sequestration, plays important roles in SOC sequestration and soil aggregation by enriching OC under chemical fertilizers and organic amendment application in intensive cropping systems. Moreover, organic substitution and straw incorporation after livestock digestion were the optimal modes of manure and straw applications to improve SOC sequestration and crop yield. However, subsoil should be paid more attention to when assessing the effects of long-term manure and straw application on climate change and food security through C sequestration in agroecosystem. Finally, the application of biochar, as an emerging organic amendment, is a win-win solution for improving crop yield and SOC sequestration mainly depending on biochar properties and soil pH. Different sections of this thesis aimed at studying (i) the underlying mechanism of SOC sequestration in highly intense cropping soil under long-term fertilization, (ii) the effects of organic substitution and straw incorporation on SOC in soil profile and crop yield, (iii) the responses of topsoil and subsoil OC to long-term fertilization and their key factors, (iv) the synergistic effects of SOC accumulation in soil profile on crop yield, and (v) the effects of biochar application on SOC and crop yield and their driving factors.

1. General discussion

1.1 Response of OC in soil particles to long-term fertilization in intensification cropland

Over the past three decades, intensive cropping plays a very important role in meeting the rising need for food, but leads to a constant change of OC content, especially in cropland of China (Lal et al., 2015; Paustian et al., 2016; Sanderman et al., 2017). Therefore, the effect of fertilization on SOC balance has always been a research hotspot in intensive cropping systems. Exploring the impact of long-term fertilization on OC in soil fractions helps to better understand SOC sequestration and stabilization mechanisms, which has a profound impact on SOC improvement in intensification cropland (Lal, 2004; Lal et al., 2015). Field experiment showed that two decades of fertilization, especially in the presence of manure, increased significantly the concentration and proportion of OC in aggregates and clay particles, and then significantly improving SOC (Figure 2-1). Fertilization mainly introduces plenty of exogenous OC into soil, and also indirectly increases OC input derived from crop stubble and root exudates by improving crop yield (Zhang et al., 2015), finally promoting OC in bulk soil and soil particles. Thus, fertilization, especially that in combination with organic amendment, might benefit SOC sequestration mainly by enhancing the physicochemical protection of aggregates and clay fractions to OC.

This present study showed that the largest increment and highest concentration of OC were observed in aggregates (Figure 2-1), which was in accordance with those reported by He et al. (2009) in northern China and Gelaw et al.(2015) in northern Ethiopia. The possible reason was that the refractory and incompletely decomposed plant residues and stubble (with high C: N ratios) were mainly accumulated in aggregates. However, the highest distribution proportion of OC was observed in clay fractions (47%) (Table 2-2), implying that clay particles were the most important site of OC sequestration in red soil. It might be due to the high OC concentration and mass proportion (35%) in clay fractions. These results were in agreement with Christensen (2001) and Long et al. (2015) that OC stored in clay fractions is more than that of other soil fractions. This phenomenon was likely because that clay associated with OM is mainly semi-decomposed, and is relatively stable and nonsusceptible to microbial decomposition due to strong physicochemical protection from the encapsulation and adsorption of inorganic-organic compounds (Fernández-Ugalde et al., 2016). This result also highlighted clay particles' capacity to improve OC stability via organo-mineral interaction and adsorption (Diekow et al., 2005; Vogel et al., 2015). Isotopic analysis indicated that $\delta^{13}C$ in soil particles showed an increasing trend as particle-sized decreasing under treatments without manure (CK and NPK treatments of which C input was mainly derived from maize) (Table 2-3). It could be due to the reason that more maize-derived OC and microbial metabolite might be associated with fine minerals, resulting in an increase of $\delta^{13}C$ in fine soil particles. Thus, fine soil particles are the main sites of maize-derived OC accumulation and sequestration.

1.2 The underlying mechanism of SOC sequestration after exogenous OC input in intensification cropland

In cropland, OC content usually depends on the dynamic balance among three processes of OC input, transformation, and output under exogenous OC addition, which are time-consuming and slow evolution processes (Xie et al., 2018). Physicochemical protection plays an important role in the transformation and output of exogenous OC, which mainly improves SOC accumulation by directly combining exogenous OC with soil minerals (Six et al., 2002, 1998), or promoting the formation and stability of soil aggregates (Gunina and Kuzyakov, 2014; Han et al., 2016; Six et al., 2002). Therefore, exploring the relationships between OC in soil fractions and bulk soil as well as soil fraction' mass proportion is conducive to better understand the mechanism of SOC accumulation and sequestration. We found that OC in bulk soil exponentially increased with the increase of OC in clay particles (Figure 2-2). It implied that clay fractions are an important part of soil C sequestration, and have a maximum holding capacity for OC. In this present study, clay fractions associated with OC would probably reach a saturation level (14.2 g C kg^{-1}) with soil OC accumulation. In addition, when comparing OC in clay particles under different treatments, we found that OC stock in clay particles receiving NPKM treatment was 13.7g C kg^{-1}, approaching the saturation level. Thus, the maximum capacity of clay fractions associated OC was limited by saturation phenomenon (Hassink, 1997; Six et al., 2002), since all adsorption and interaction sites on the surfaces of clay fraction were occupied (Du et al., 2014; Gulde et al., 2008). The saturation value of OC in clay fractions in this study was lower than that of 32.5 g C kg^{-1} in a Dark Red Latosol Soil reported by Roscoe et al. (2001). It might be due to a relatively low clay content (35%) and an intensive disturbance in double maize cropping systems in this present study, then contributing to a low saturation value of OC in clay fractions. The linear response of OC in aggregates to soil OC might imply that OC in aggregates does not display saturation trend under current management practices (Figure 2-2), then soil OC stock would be further increased through OC in aggregates after OC in clay fractions saturate gradually (Carter, 2002).

Significantly positive correlations were observed between OC in fine soil particles (fine silt and clay particles) and the mass proportion of aggregates as well as the mass ratio of aggregates to silt fraction (Figure 2-3). It indicated that the absolute and relative values of aggregates mass both significantly increased with OC enriched in fine soil particles. According to the theory of aggregate formation proposed by Tisdall and Oades. (1982), soil aggregates form as OC in particle fractions associate with fine soil particles or microbial mucus, and then fine soil particles agglomerate and form aggregates through organic binding agents. Since OM may act as binding agents for the formation of soil aggregates (Six et al., 2002), we speculated that soil aggregation was facilitated by the binding of OC enriched in fine soil particles with fine particles into aggregates. Those results also confirmed the theory that soil aggregates hierarchically and continuously formed from small to large through organic binding agents (Tisdall and Oades.1982; Six et al. 2000; Six et al. 2002). To sum up, with OC enriched in fine soil particles, OC in clay fraction tends gradually to saturation and aggregates' mass proportion increases, finally further increasing soil OC stock mainly via OC in aggregates.

1.3 Effect of organic substitution on SOC in soil profile and crop yield in cropland

This present study has confirmed that chemical fertilizers combined with organic amendment can significantly improve SOC (Figure 2-1). Partial substitution of chemical N fertilizer by manure, as one of those practices, is a widely recommended fertilization strategy in China today (Wang et al., 2018), due to simultaneously reduce chemical fertilizers application rate and recycle livestock manure utilization without reducing food production (Li et al., 2017; Ning et al., 2017). However, there are remarkably little data on subsoil OC as affected by partial substitution of chemical N fertilizer by manure. Compared with the initial value, the higher increments of SOC stock both in topsoil (25.6%–103.8%) and subsoil (2.9%–71.3%) were observed in 70%NPK+M treatment rather than in NPK and NPK+S treatments (Figure 3-3), exceeding the benchmark of 4‰ increase in soil C per year targeted by the 4 per 1,000 initiative (Minasny et al., 2017). The possible reason was that, compared with chemical fertilizers or straw, manure could bring in plenty of exogenous OC and available nutrients, which may directly increase SOC or indirectly improve SOC accumulation by reducing microbial nutrient limitation in the transformation process of exogenous OC (Duan et al., 2011; Gutser et al., 2005; Ning et al., 2020). Moreover, manure has a better effect on improving aggregates formation and stability, then enhances OC's physicochemical protection, eventually benefiting OC sequestration and adsorption (Six et al., 2002; Xu et al., 2020). In this present study, an increase of

SOC mainly occurred in the top 60 cm soil layer at three northern sites of semi-humid areas but below 40 cm soil layer at Qiyang site of sub-tropical area under 70%NPK+M treatment (Figure 3-2). This phenomenon may be due to the following reasons: (1) fertilizers application and crop residue return mainly occur in topsoil, resulting in an increase of SOC in the upper layer of soil profile; (2) leaching caused by heavy precipitation accelerates the downward movement of dissolved OC, supplementing OC pool in deep soil at Qiyang site (Hess et al., 2020; Rumpel and Kögel-Knabner, 2011); (3) in acidified soil of Qiyang site, the high content and availability of soil phosphorus under manure application could improve the growth and extension of crop roots (Suriyagoda et al., 2014). This imposed a positive effect on subsoil OC accumulation through increasing crop roots biomass and litter, which have a high C sequestration efficiency due to oxygen lack (Menichetti et al., 2015). These results also further confirmed that OC input derived from root litter and exudates plays an important role in enhancing subsoil OC sequestration (Jobbagy and Jackson, 2000; Richter et al., 2015).

Fertilization not only influences SOC by increasing exogenous OC input, but also affects crop yield by providing essentials nutrients for crop growth. This present study results showed that fertilization, especially the one with organic amendments, increased significantly crop yield and nutrient uptakes during 1990-2013 in all sites compared with CK treatment (Table 3-4). Similar results were reported by Cai et al. (2019) and Duan et al. (2011). Fertilization improves crop growth by bringing in plenty of nutrients, then promoting crop yield. However, compared with NPK and NPK+S treatments, 70%NPK+M treatment increased significantly crop yield only in Qiyang site but changed insignificantly crop yield in the rest sites (Table 3-4). This may be because manure application minimizes soil acidification, as well as the adverse effects of acidification on crop growth in Qiyang site, such as aluminum and manganese toxicity (Duan et al., 2011; Cai et al., 2021). A high SYI suggests the capability of sustaining high crop production over time, but a low SYI implies more susceptibility to biotic and abiotic stresses for crop growth (Waqas et al., 2019). This present study found that fertilization also increased SYI for maize and wheat in most sites (Table 3-4). Possible reasons are as follows: (1) fertilization provides stable nutrient supplies and improves soil environment for healthy growth of crops, which makes crop yield reach a relatively stable level (Manna et al., 2005; Wei et al., 2020); (2) fertilization, particularly phosphorus addition, could promote the growth and extension of crop roots (Suriyagoda et al., 2014), and improve nutrient uptakes from subsoil (Hartmann et al., 2008; Ma et al., 2009), thereby maintaining nutrient requirements for high and stable crop yield. However, we found that compared with CK, fertilization increased maize SYI, but slightly decreased wheat SYI at Qiyang site (Table 3-4). The possible reason was that maize and wheat growth have different sensitivity to soil pH, as revealed by different pH thresholds (5.1 and 5.5, respectively) of acid damage (Zeng et al., 2017).

1.4 Effect of straw incorporation modes on SOC in soil profile and crop yield in cropland

Straw, like manure, is the most common and abundant biological resource (Shi et al., 2014). Straw incorporation not only brings plenty of OC input and nutrients, but also improves soil properties, which is an important practice to improve soil fertility and SOC in the world (Li et al., 2020). We observed a higher crop yield in CM treatment rather than in SM and SC treatments (Figure 4-1), implying that CM has better effects on improving soil fertility due to introduce more nutrients. Those results were further proved by the higher topsoil OC and nutrient in CM. However, we also found that crop yield in SM treatment was higher than in SC treatment (Figure 4-1). This is likely because straw mulching could adjust soil temperature and reduce surface runoff and water evaporation, which has better effects on soil water and nutrient retentions, especially in arid and semi-arid areas, thereby benefiting crop growth (Yang and Tian, 2004).

In this study, compared with straw removal, straw return increased significantly topsoil OC stock (Figure 4-3), which was consistent with previous study results (Cai et al., 2015; Xu et al., 2016). This is because straw return treatment increased OC input by 1.8-3.0 times that of straw removal treatment. SOC generally increases with OC input increasing before saturation (Cai et al., 2020; Xu et al., 2020). In addition, the increment of topsoil OC stock in CM treatment was significantly higher than in SM and SC treatments (Figure 4-3). Possible reasons for this phenomenon are as follows: (1) fresh cattle manure is mainly in a semi-decomposed state, which brings more available N into field, resulting in higher C conversion efficiency than straw (Silver and Miya, 2001); (2) fresh cattle has better effects on improving aggregates formation and stability than straw, which does benefit to SOC accumulation due to enhance OC's physical and chemical protection (Xu et al., 2020). Meanwhile, we found a significantly higher increment of topsoil OC in SC rather than in SM treatment (Figure 4-3), which was similar to the results reported by Fan et al. (2018). Thus, SC treatment was more conducive to OC accumulation under an equal amount of OC input. This is because crushed straws are in full contact with soil particles and are more prone to be decomposed and transformed under SC treatment, which could benefit to produce more metabolites and promote soil aggregates formation, thereby increasing SOC adsorption and fixation (Dou et al., 2011). However, under SM treatment, whole straw is covered on the ground that has frequent dry-wet alternation and high microbial activity, which caused more straw-derived C to be decomposed, then releasing it into the atmosphere in the form of CO_2 (Abiven et al., 2009).

Furthermore, this present study found that, compared with straw removal, straw return reduced significantly subsoil OC stock (Figure 4-3), which was inconsistent with the results reported by Xu et al. (2016) and Gai et al. (2019). It implied that OC input could not compensate for OC loss caused by OM decomposition in subsoil under long-term straw incorporation (Cai et al., 2020). We speculated that subsoil OC depletion may be due to the following reasons: (1) Because the experimental area belongs to a drought area, crop roots extend downwards under insufficient water conditions. Therefore, to provide N for crop growth, microbial mining for N enhances soil OM decomposition and then causes C loss, which would particularly occur when N supply is insufficient due to low N content in subsoil (Boilard et al., 2019; Dijkstra et al., 2013; Rumpel and Kögel-Knabner, 2011). Moreover, the explanation rate of TN (25.3%) to subsoil OC change was the highest in this present study (Figure 4-4), which also indirectly proved this inference. (2) Straw incorporation brings in plenty of dissolved OC and nutrients, and consequently increases fresh C source (e.g., dissolved OM, root exudates, and organic acids, etc) in subsoil via leaching and roots growth, causing OC loss due to enhance the priming effect (Fontaine et al., 2007; Keiluweit et al., 2015). (3) fertilization and deep tillage could promote roots growth and break plow pan, respectively, which may benefit to improve soil aeration and the downward extension of crop roots, consequently accelerating OM decomposition due to increased soil disturbance (Han et al., 2020; Six et al., 2000). Moreover, for the entire 100 cm profile, SOC stock reduced under SM and SC treatments, but significantly increased under CM treatment (Figure 4-3). Meanwhile, the increment of SOC stock in the entire 100 cm profile was less than in topsoil under CM treatment (Figure 4-3). This result highlighted that only considering topsoil may grossly overestimate the effect of straw incorporation on SOC improvement, or even lead to a false conclusion.

1.5 The responses of topsoil and subsoil OC to long-term fertilization and their key factors

The results showed that organic substitution and straw return both increased significantly topsoil OC, but had opposite effects on subsoil OC (Figure 3-3 and Figure 4-3). In cropland ecosystems, a common recognition is that OC in top 20 cm soil would be most affected by fertilization, crop roots, tillage, etc (Minasny et al., 2017). Fertilization directly increases exogenous OC input, or indirectly increases crop biomass return by improving crop yield, thereby promoting OC accumulation in topsoil. However, due to being indirectly subject to management practices, subsoil OC input is mainly sourced from plant roots and root exudates, dissolved OM from topsoil, and bioturbation (Rumpel and Kogel-Knabner, 2011). Fertilization increases topsoil OC input via direct or indirect ways, which correspondingly increases subsoil OC input to a large extent (Manna et al., 2005; Rumpel and Kogel-Knabner, 2011). Thus, increasing subsoil OC inputs (e.g., dissolved organic matter, roots litters, and root exudates) could supplement OC pool and help OC accumulation in subsoil (Rumpel and Kogel-Knabner, 2011; Dijkstra et al., 2021). In contrast, subsoil C loss may be attributed to the priming effect mediated by the following aspects: (1) the soil OM degradation from the priming of resource-limited deep microbial communities (Dignac et al.,2017); (2) the enhancing priming effect due to fresh C source input via leaching and roots growth (Fontaine et al. 2007); (3) the mechanical disruption (Blankinship and Schimel, 2018). Therefore, OC input, especially root exudates and dissolved OM, impacts on OC in subsoil may be a double-edged sword, which is controlled by the direction and magnitude of priming effect mediated by soil microbial and enzymatic activities (Dijkstra et al., 2021). Moreover, numerous studies have highlighted the importance of clay content, extractable Ca, Fe, and Al in stabilizing SOC in subsoils (McSherry and Ritchie, 2013). Thus, the direction and size of long-term fertilization effect on SOC in subsoils may depend on climate parameters, soil inherent properties, nutrients availability, and fertilization practices.

Moreover, under long-term fertilization, there was a significant linear correlation ($p < 0.05$) between SOC change in the topsoil and the entire 100 cm profile (Figure 3-4). It indicated that topsoil OC dynamics could reflect SOC pools change in the entire 100 cm profile to a certain extent. This was mainly because agricultural management practices were directly and mainly applied in topsoil, and the main C sources in subsoil are dissolved OM from topsoil, followed by crop roots litter and exudate (Fontaine et al., 2007; Menichetti et al., 2015; Rumpel and Kögel-Knabner, 2011). Statistical analysis implied that the slope of linear equation was 0.39 and significantly < 1 (Figure 3-4), which suggested that SOC change in topsoil only accounted for 39% of that in the entire 100 cm profile. Thus, subsoil OC accumulation may account for more than 60% of that in the entire 100 cm profile. This was probably because subsoil may not yet be saturated in OC and has a greater potential to sequester OC due to low C concentration. These results also highlighted the importance and potential of subsoil to sequester C in cropland. Thus, subsoil should be considered when assessing the effect of long-term fertilization on mitigating climate change and ensuring food security via C sequestration.

Meanwhile, using a redundancy analysis, we explored the key factors controlling topsoil and subsoil OC changes under long-term fertilization from the perspectives of OC input and soil nutrients. This present study showed that the total explanation rate of OC input and soil nutrients to topsoil OC change was up to 90.1% (Figure 4-4), implying that topsoil OC change mainly depended on OC input and soil nutrients. This is because chemical fertilizers or/and organic amendment contains plenty of OC and soil nutrients (Song et al., 2018). On the one hand, fertilization mainly applies on topsoil, then greatly increases OC input, finally directly improving SOC in topsoil (Cai et al., 2020; Zhang et al., 2010). On the other hand, chemical fertilizers or/and organic amendment brings in rich nutrients, which reduces microbial nutrient limitation, benefiting topsoil OC accumulation (de Oliveira Ferreira et al., 2018; Murugan and Kumar, 2013; Xu et al., 2016). However, the total explanation rate of OC input and soil nutrients to subsoil OC change was only 31.8% (Figure 4-4), which may be due to the low amounts and small changes of OC input and soil nutrients. In addition, some studies implied that subsoil OC may be prominently affected by clay content, Ca^{2+}, cation exchange capacity, soil porosity, Fe and Al oxides (dos Reis et al., 2014; Han et al., 2018; Rumpel and Kögel-Knabner, 2011). In summary, topsoil OC changes are mainly controlled by OC input and soil nutrients, but subsoil OC changes may be more influenced by its own properties rather than OC input and soil nutrients (de Oliveira Ferreira et al., 2018; Rumpel and Kögel-Knabner, 2011).

1.6 Synergistic effects of C accumulation in the entire 100 cm profile on crop yield under long-term fertilization

Soil OC, as a core indicator of soil fertility and cropland use sustainability, is a basis of high and stable crop yield (Lal, 2009). Numerous studies suggested that enhancing SOC sequestration via organic amendment addition is considered to be an effective way to improve crop yield (Agegnehu et al., 2016; Al-Wabel et al., 2018). Previous studies have also demonstrated that SOC content in 0–20 cm significantly correlated with crop yield and its stability (Manna et al., 2005; Oldfield et al., 2019; Zhang et al., 2016). Moreover, based on a global meta-analysis, we also found that the effect size of biochar application on crop yield linearly increased with that on SOC (Figure 5-2). Thus, there is a synergistic effect between enhancing OC sequestration in the cultivation layer and improving crop yield under long-term fertilization. On the one hand, SOC affects cropland productivity by altering soil physical and chemical properties as well as biological functions (Murphy, 2015). For example, SOC affects soil structure, soil porosity, and water infiltration, and improves soil chemical buffering capacity, biological activity, and nutrient cycling (Johnston et al., 2009; Murphy, 2015; Oelofse et al., 2015). On the other hand, fertilization, especially mineral fertilizers application, improves crop yield, then increases OC input derived from crop residue and roots biomass, which is also conducive to enhance SOC (Manna et al., 2005; Zhang et al., 2009).

Four long-term field experiments showed that crop yield and its stability as well as nutrient uptakes exponentially increased with the increase of SOC stocks in 0–100 cm soil profile (Figure 3-6). This phenomenon may be due to the following reasons: (1) increasing SOC in the entire 100 cm profile significantly promotes crop production and its sustainability by improving soil biotic and abiotic environment in both topsoil and subsoil (Amelung et al., 2020; Oldfield et al., 2019, 2018). Previous study results showed that OM decomposition plays key roles in nutrient supplies for crop yield, especially in subsoil at later stage of crop growth (Hartmann et al., 2008; Jobbagy and Jackson, 2000; Yang and Zhang, 2011); (2) conversely, increasing SOC in soil profile, along with enhancing the retention capacity of soil water and nutrient in the entire 100 cm profile by potentially improving the formation and stability of soil aggregates, has contributed to a high and stable crop yield (Xu et al., 2021); (3) SOC accumulation may promote the predation of soil biota (e.g., protists and nematodes) and the activities of beneficial microorganisms (e.g., arbuscular mycorrhizal fungi) due to improving soil fertility (Jiang et al., 2020), which could further enhance P uptake and transport in crop roots via the expression of P transporter gene (*ZMPht1;6*), and, in turn, promoting high crop yield (Jiang et al., 2020). To sum up, SOC accumulation in soil profile potentially facilitates the uptake and utilization of nutrients from both topsoil and subsoil by improving soil microenvironment and enhancing the genes expression of nutrients transporter, thereby promoting crop yield and its stability. Thus, these results indirectly clarified how fertilization affected crop yield by regulating SOC, and demonstrated the synergistic effects of OC accumulation in the entire 100 cm profile on crop productivity (Cai et al., 2019).

1.7 Effect of biochar application on crop yield and SOC and their driving factors

As an emerging organic amendment, biochar is usually produced by agricultural waste (e.g., crop straw, manure, and wood, etc) at high temperatures (400-700 °C) and anoxic conditions (Smith, 2016, Sohi et al., 2010). Biochar application, as an improvement strategyof traditional fertilization practice, has shown great potential to improve SOC and crop yield, which has been widely recommended as an effective solution to tackle the challenges of food security and climate change in agroecosystems (Agegnehu et al., 2016; Al-Wabel et al., 2018). The results in this present study were consistent with the results of Liu et al. (2016) that biochar application has a positive effect on SOC via a global meta-analysis (Table 5-1). However, the positive effect (33.5%, Table 5-1) revealed in this present study was less than that (52.2%) of Liu et al. (2016). An accurate effect of biochar could be obtained by supplementing the experimental sample size and considering the experimental non-independence (Hungate et al., 2009; Verhoeven et al., 2017). In addition, this present study showed that chemical fertilizers addition insignificantly increased OC based on biochar application. SOC formation is a long-term process of soil retention of exogenous C materials, which is affected by climate, management practices, and soil properties (Cai et al., 2016). Compared with B, BF application could further promote crop growth and secrete more organic materials by crop roots, which may contribute to SOC formation (Chew et al., 2020; Prendergast-Miller et al., 2014). Although more organic materials through crop roots and stubble are incorporated into soil under BF, the formation of SOC is still a complex and time-consuming process (Carvalhais et al., 2014; Luo et al., 2019). The experimental time in targeted literature was relatively short (an average of only 1.5 years), consequently resulting in no differences of SOC between the effect sizes of B and BF (32.9% and 34.8%, respectively). Meanwhile, this meta-analysis showed that biochar C:N ratio was the most influential variable for the effect sizes of B and BF on SOC among all influencing variables (Figure 5-5). Biochar with a high C:N ratio might result in microbial N immobilization in soil, which is conducive to SOC formation (Cayuela et al., 2014). Biochar with a low C:N ratio is generally available in microbial processes such as SOC mineralization that decreases SOC content (Singh and Cowie, 2014).

Biochar, with a rich pore structure and strong adsorption capacity, could effectively intercept soil nutrients and improve nutrient use efficiency, consequently helping to improve crop growth (Lehmann et al., 2003; van Zwieten et al., 2010; Zhang et al., 2017). In this study, biochar application significantly increased crop yield by 22.2% (Table 5-1), which was higher than that of 4.8~17.1% reported by the previous studies (Biederman and Stanley Harpole, 2013; Jeffery et al., 2011; Liu et al., 2019, 2013). This was largely due to two reasons: (1) previous results mainly based on small samples, which might lead to a relatively greater deviation (Sutton et al., 2007); (2) almost all previous studies did not clearly distinguish the effect sizes on crop yield under B and BF (a few involved BF). This present study confirmed that chemical fertilizers addition significantly improved crop yield benefits based on biochar application. Compared with B, BF could further promote crop growth by enhancing nutrient supply and increasing nutrient use efficiency (Agegnehu et al., 2016; Horák et al., 2017). For instance, Biederman and Stanley Harpole (2013) reported that BF could increase crop yield by 35.0% (19.0%~51.0% CI) compared with the no-biochar application. However, the average effect size of BF on crop yields (48.4%) in this present study was not only higher than the Biederman and Stanley Harpole's (2013) result (35.0%), but also with a lower variability (from 19.0~51.0% CI to 41.8~55.3% CI), which implied that distinguishing the effects of B and BF could reduce the variation of biochar's effect on crop yield compared with previous studies.

Biochar has high stability and would remain in soil for a long time due to its slow decomposition rate (Smith, 2016). This present study showed that biochar’s effect sizes on crop yield and SOC could not promote with experiment duration increasing (Figure 5-S6) due to low nutrient and high passive C contents in biochar. Therefore, biochar could act as a long-term soil conditioner, benefiting to improve crop yield and SOC sequestration (Woolf and Lehmann, 2012). In summary, the results in this present study may reduce the variations and improve the accuracy of estimating biochar's effect on crop yield and SOC by distinguishing the effects of B and BF as well as quantifying the importance of variables controlling biochar's effect. Thus, those results provided novel insights for the optimization of biochar application in agroecosystem.

2. General conclusions

This present study showed that OC in bulk soil, soil aggregate, and soil particles were significantly affected by long-term chemical fertilizers and organic amendment applications. To reveal the underlying mechanism of SOC sequestration in intensive cropping systems, OC and δ^{13}C in bulk soil and soil fractions were measured, then the responses of OC in soil fractions to chemical fertilizers and organic amendment applications were assessed in this present study. Furthermore, to provide a scientific basis for the optimization of straw and manure applications, long-term organic substitution and straw incorporation field experiments were conducted to study the effects of those practices on SOC in soil profile and crop yield as well as their synergistic effects. Finally, from a new perspective for fertilization strategy evolution, the effects of biochar alone and plus fertilizer application on crop yield and SOC were estimated via a global meta-analysis and their key factors were identified via a BRT model.

Using physical particle-sized fractionation, this present study showed that fertilization, especially in the presence of organic amendment, promoted significantly SOC by increasing OC in aggregates and fine soil particles. Manure increased significantly the proportion of OC physiochemically protected by aggregates and clay fractions. The highest value and increment of δ^{13}C (‰) as well as the highest distribution proportion (up to 47%) of OC were observed in clay fractions. Therefore, clay fractions were the most important sites of exogenous OC sequestration and soil OC accumulation. Moreover, OC in clay fractions showed a nonlinear response to soil

OC, with a saturation trend, but OC in aggregates showed a linear response to soil OC. Regression analysis implied that the absolute and relative values of aggregates mass increased with OC enriched in fine soil particles, indicating that fine soil particles, especially clay fractions, play important roles in soil aggregation by enriching OC. Therefore, those results implied that organic C derived from organic amendment would be bond to fine soil particles (<53μm) first, resulting in C enrichment in the fine particle and then brought benefit to soil aggregates formation, finally further increased soil OC sequestration via aggregates after OC saturation in clay fractions. To sum up, these results enhanced understanding of the underlying mechanism of soil OC sequestration under organic amendment application in intensive cropping systems.

Field experiments showed that, under long-term intensive cropping, the unfertilized soil of 0-100 cm showed SOC depletion, and may act as a C source. However, long-term NPK and NPK+S treatments could slow down SOC depletion in 0-100 cm to a certain extent, which depended on soil properties (e.g., soil acidification and initial fertility) and climatic conditions. Compared with NPK and NPK+S treatments, 70%NPK+M treatment increased significantly SOC stock in the entire 100 cm profile and maintained a high and stable crop yield. Overall, the long-term field experiment confirmed that 70%NPK+M was the most effective fertilization strategy for improving SOC sequestration and being helpful to sustainable crop production while achieving the “Fertilizer Use Zero-Growth Action Plan” in upland areas. Meanwhile, SOC accumulated in the entire 100 cm profile improved significantly nutrient uptakes, relative crop yield, and sustainable yield index. However, SOC change in topsoil only accounted for less than 40% of that in the entire 100 cm profile. Those results implied that subsoil should be considered when assessing the effects of long-term fertilization on climate change and food security via C sequestration in upland soil.

Long-term straw incorporation experiment showed that the greatest increments of crop yield and topsoil nutrients contents were observed in CM treatment. Moreover, compared with SR, SOC stock increased greatly in topsoil but substantially in subsoil under straw return. For the entire 100 cm profile, SM and SC treatments reduced SOC stock, but CM treatment increased SOC stock. In addition, the increment of SOC stock in the entire 100 cm profile was less than in topsoil under CM treatment. Those results implied that only considering topsoil OC change may grossly underestimate the impact of straw return on SOC sequestration benefits, or even lead to false conclusions. Redundancy analysis suggested that topsoil OC stock change was mostly controlled by nutrient management and carbon input, but subsoil OC depletion mainly was caused by insufficient nitrogen supply. Those results highlighted that CM was the best mode of straw incorporation to improve SOC sequestration and crop yield. However, OC stock change in subsoil should also be paid attention to under straw return.

Global meta-analysis showed that biochar application increased significantly crop yield and SOC by 22.1% and 33.5% compared with the no-biochar application. However, compared with B, the effect size of BF on crop yield increased by three times, but that on SOC insignificantly increased. Thus, chemical fertilizers addition significantly improved crop yield benefits but insignificantly influenced SOC sequestration based on biochar application. Regression analysis indicated that SOC accumulation had a synergistic effect on crop yield improvement under biochar application. Moreover, regardless of B or BF, a greater effect size on crop yield was found under N-rich rather than N-deficient biochar application, in acid rather than neutral soil due to the liming effects. The BRT model suggested that biochar C: N ratio and soil pH were the two most important variables controlling the effects size of B and BF on crop yield. However, the main factors that controlled the biochar's effect on SOC were biochar properties and application rate, with the total explanation rates of 55.3% and 64.9% under B and BF, respectively. Consequently, optimizing biochar properties (C:N ratio) and selecting appropriate application conditions are valid solutions to maximize the economic and environmental benefits of biochar application.

In conclusion, fertilization, especially the one with organic amendment, increased SOC mainly by enhancing OC's physical and chemical protection from aggregates and clay fractions. Fine soil particles, especially clay fractions, play important roles in OC sequestration and soil aggregation via enriching OC in intensive cropping systems. Organic substitution and straw incorporation after livestock digestion were the optimal modes of manure and straw applications to increase SOC sequestration in the entire 100 cm profile and crop yield. However, this present study highlighted that ignoring subsoil OC change could not actually recognize the soil's potential capability to sequester C and may even lead to false conclusions, when evaluated the effect of organic amendment application on SOC sequestration. Moreover, biochar application, as a new perspective for fertilization strategy evolution, was a win-win solution for improving SOC sequestration and crop yield, which depended on biochar properties and soil pH. Those results provide novel insight and basis for cropland sustainable utilization and optimizing fertilization strategies under intensive cropping systems in China and other countries.

3. Innovations

This thesis revealed the underlying mechanism of SOC sequestration under long-term fertilization in intensive cropping systems by soil particles fractionation and 13C-isotope analyses. Moreover, in this present thesis, the effects of long-term organic substitution and straw incorporation on SOC in subsoil and crop yield, as well as their synergetic effects were investigated based on more than 20-year field experiments. Finally, the accuracy of estimating biochar's effect sizes was improved by distinguishing the effects of biochar alone and biochar plus fertilizer and quantifying the relative contribution of various factors controlling the biochar's effect size via a global meta-analysis and BRT model.

4. Perspectives

Based on the results of this thesis, the following recommendations are proposed to better understand the mechanism of SOC sequestration as well as the potential benefits of organic substitution, straw incorporation, and biochar application to improve OC sequestration and crop yield in intensive cropping systems:

(1) Further reveal the transformation process of exogenous and native OC in soil particles under chemical fertilizers and manure application using 13C-isotope technology. Isotope technology provides a very important means to study C transformation and sequestration processes. Deeply understanding those processes (e.g., priming effect, C mineralization, and C balance) in intensive cropping systems is very important for enhancing OC, cropland sustainable utilization, and climate change mitigation.

(2) Explore the optimal substitution rate that simultaneously achieves the maximizing of SOC sequestration and crop production benefits and the minimizing of possible environmental risk. Limited by treatment design in these four long-term experiments, the substitution rate of chemical N fertilizer with manure in this study is 70%. However, previous studies showed that organic substitution rate could not only change the effect size of organic substitution practice on SOC and crop yield, but also influence the accumulation of available phosphorus and harmful substances (e.g., heavy metals, antibiotics) derived from manure in the soil. Therefore, it is essential to optimize organic substitution practice via exploring the best substitution rate;

(3) Comprehensively evaluate the benefits of different straw return modes from economic and environmental perspectives (e.g., crop yield improvement, SOC sequestration, labour input, energy consumption, greenhouse gas emissions, implementation difficulty, etc.), which helps to obtain the optimal straw return mode for maximizing economic benefits and minimizing environmental pollution;

(4) Study the mechanisms of how organic amendment application (manure and straw) influences SOC and crop yield. In this thesis, only the effects of those practices on SOC and crop yield were quantified, but the mechanism remains unclear. Via multivariate analysis (BRT and SEM), the driving factors of SOC and crop yield under those practices are identified from the perspectives of management practices (e.g., OC and nutrients input, tillage, cropping system), climate factors (e.g., precipitation, temperature, and aridity index), and soil properties (e.g., pH, soil nutrients, Ca^{2+}, soil silt and clay contents, Fe and Al oxide). Soil organisms (e.g., bacteria, AFM, earthworm, etc.) play an important role in crop yield and SOC sequestration response to those practices by influencing OC and nutrients transformation processes. By the sophisticated techniques (e.g., PLFA and isotope analysis), the relationships between those practices and crop yield and SOC sequestration should be established by measuring earthworm density, soil microbial community composition, and enzymes (e.g., soil β-glucosidase, β-N-acetyl glucosidase, leucine aminopeptidase, and alkaline phosphatase, etc) from the perspective of OC and nutrient conversion and utilization;

(5) Carry out more extensive and long-timescale field experiments of biochar application. Biochar, as an emerging organic amendment, could be used as a long-term soil conditioner to improve soil structure and soil water status, which may be beneficial to crop yield and SOC. In the current studies, the limited duration of biochar application (an average of 1.5 years) does not allow us to wholly determine the long-term economic and environmental benefits of biochar application via a meta-analysis.

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Appendix - Publications

1. Publications

(1) **Xu, H.**, Liu, K., Zhang, W., Rui, Y., Zhang, J., Wu, L., Colinet, G., Huang, Q., Chen, X., Xu, M. Long-term fertilization and intensive cropping enhance carbon and nitrogen accumulated in soil clay-sized particles of red soil in South China. *Journal of Soils and Sediments*, 2020, 20: 1824-1833.

(2) Wu, L., **Xu, H**., Xiao, Q., Huang, Y., Suleman, M.M., Zhu, P., Kuzyakov, Y., Xu, X., Xu, M., Zhang, W. Soil carbon balance by priming differs with single versus repeated addition of glucose and soil fertility level. *Soil Biology and Biochemistry*, 2020, 148, 107913. (Co-first author).

(3) **Xu, H**., Cai, A., Wu, D., Liang, G., Xiao, J., Xu, M., Colinet, C., Zhang, W. Effect of biochar application on crop productivity, soil carbon sequestration, and global warming potential controlled by biochar C: N ratio and soil pH: a global meta-analysis. *Soil &Tillage Research*, 2021, 213, 105125.

(4) **Xu, H**., Cai, A., Zhou, H., Colinet, C., Zhang, W., Xu, M. Long-term straw incorporation significantly reduced subsoil organic carbon stock in cinnamon soil. *Journal of Plant Nutrition and Fertilizers*, 2021, 27(5): 768-776. (In Chinese with English abstract)

(5) **Xu, H**., Cai, A., Zhang, W., Yang, X., Huang, S., Wang, B., Zhu, P., Colinet, C., Xu, M. Partial substitution of mineral nitrogen fertilizer by manure promotes carbon and nitrogen accumulation and benefit crop production: evidence from four upland experiments over two decades. Journal of Integrative Agriculture, 2021, (Major revision).

(6) Cai, A., **Xu, H.**, Duan, Y., et al. Changes in mineral-associated carbon and nitrogen by long-term fertilization and sequestration potential with various cropping across China dry croplands. *Soil & Tillage Research*, 2021, 205, 104725.

(7) Abrar, M.M., **Xu, H**., Aziz, T., et al. Carbon, nitrogen, and phosphorus stoichiometry mediate sensitivity of carbon stabilization mechanisms along with surface layers of a Mollisol after long-term fertilization in Northeast China. *Journal of Soils and Sediments*, 2021, 21:705-723.

(8) Mustafa, A., **Hu, X**., Abrar, M.M., et al. Long-term fertilization enhanced carbon mineralization and maize biomass through physical protection of organic carbon in fractions under continuous maize cropping. *Applied Soil Ecology*, 2021, 165.103971.

(9) Mustafa, A., **Hu, X**., Shah, S.A.A., et al. Long-term fertilization alters chemical composition and stability of aggregate-associated organic carbon in a Chinese red soil: evidence from aggregate fractionation, C mineralization, and 13C NMR analyses. *Journal of Soils and Sediments*, 2021, 21: 2483-2496.

(10) Wang, Q., **Xu, H.**, Ma, C., et al. Change of soil fertility and productivity of purple soil in Western China in recent 30 years. *Journal of Plant Nutrition and Fertilizers*, 2018, 24(6): 1492-1499. (In Chinese with English abstract)

(11) Wang, C., Wang, Q., **Xu, H**., et al. Carbon, nitrogen, and phosphorus stoichiometry characteristics of bulk soil, organic matter, and soil microbial biomass under long-term fertilization in cropland. *Acta Ecologica Sinica*, 2018, 38: 3848-3858. (In Chinese with English abstract)

2. Presentations

(1) Characteristic and driving factor of carbon and nitrogen changes in soil profile of China's typical upland soil under long-term fertilization (2018 International Workshop on Long-term Field Experiments and Soil Carbon Sequestration, Zhanjiang, 07/11/2018, Oral Presentation).

(2) Response of carbon and nitrogen in soil and their particles fraction to long-term fertilization in maize continuous cropping area of southern China (Annual meeting of Chinese Society of Plant Nutrition and Fertilizer Science, Jiangsu, 07/08/2018, Oral Presentation).