

Division - Soil Use and Management | Commission - Land Use Planning

Land-use affecting organic carbon and its active components in soil aggregates in China

Xiaoqian Liu⁽¹⁾ (D), Kaiwen Guo⁽¹⁾ (D), Bin Li⁽¹⁾ (D), Xin Li^{(1)*} (D) and Wei Li⁽¹⁾ (D)

⁽¹⁾ Northeast Agricultural University, College of resources and environment, Harbin Heilongjiang, China.

ABSTRACT: Due to large-scale wetland reclamation, the typical wetland had been conversed to different wetland use types (upland field, paddy field, and artificial forest) in the Sanjiang Plain. However, there are scarce data regarding soil aggregates and active organic carbons during land-use transition. Here, soil aggregates and the changes in content and storage of active organic carbon [total organic carbon (TOC), dissolved organic carbon (DOC), microbial biomass carbon (MBC), and readily oxidized carbon (ROC)] were studied under three land-uses reclaiming wetlands as an upland field, paddy field, and artificial forest in Heilongjiang Province, China. The results showed that soil aggregate structure changed significantly under the three land-uses, of which the largest decrease of mean weight diameter (MWD) occurred in the upland field. Under the three land-use types, the content and storage of TOC and each active organic carbon in soil aggregates with different size fractions significantly decreased compared with that in the wetland. In addition, the proportion of the 1-2 mm soil aggregate was significantly lower than that of other particle sizes, which resulted in the lowest storage of TOC and active organic carbon at 1-2 mm and was found in the wetland, and different land-use types; small aggregates (<0.25 mm) with a small proportion were ignored in calculating organic carbon content and storage. The TOC and active organic carbon content in various soil aggregates varied significantly in different land-use types; the paddy field was most similar to the wetland because of seasonal flooding factors. In soil aggregates with various size fractions, the paddy field presented small changes in the TOC, DOC, and MBC content, and the ROC content was significantly lower than that in the upland field and the artificial forest land-use types. The TOC, DOC, and MBC content in the upland field and the artificial forest were significantly reduced compared with that in the paddy field due to the decrease of water content in the soil. In the artificial forest, which had less human disturbance, organic carbon content was less affected than in long-term cultivated upland fields due to its abundant plant root systems and large input of organic matter. The upland field could seriously affect the structure of soil aggregates and organic carbon in the wetlands and had the most negative impact on the wetland ecosystem.

Keywords: Sanjiang Plain wetland, aggregate, land-use type, active organic carbon.

* Corresponding author: E-mail: swx05256lx@126.com

Received: January 22, 2022 Approved: April 11, 2022

How to cite: Liu X, Guo K, Li B, Li W. Land-use affecting organic carbon and its active components in soil aggregates in China. Rev Bras Cienc Solo. 2022;46:e0220001. https://doi.org/10.36783/18069657rbcs20220001

Editors: José Miguel Reichert () and Milton César Costa Campos ().

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INTRODUCTION

Wetland ecosystems account for merely 4-6 % of the Earth's surface and are the most important carbon sink or carbon source in terrestrial ecosystems, with carbon storage occupying over 20 % of total global organic carbon in soil (Gorham, 1991; Andreetta et al., 2016). Sangjiang Plain wetland is the largest concentrated marsh wetland area in China and has been listed as an Internationally Important Wetlands since 2002 (Xiao et al., 2015). This wetland, which has an important ecological value, is a low plain formed by the confluence of three rivers (Heilongjiang, Songhua, and Wusuli Rivers). Furthermore, it is a wetland resource in northeastern China that plays an important role in maintaining the ecosystem stability in this region. However, due to large-scale wetland reclamation, the wetland ecosystem has been severely damaged and faces severe problems such as a large reduction in the wetland area, severe soil erosion, a decrease in biodiversity, and an increase in environmental pollution (Sui et al., 2017; Xu et al., 2017).

Organic carbon is an important component of soil and plays an important role in the formation and maintenance of soil aggregates and the storage of nutrients and energy sources for microbial activities in soil (Peng et al., 2003). The active organic carbon in soil is characterized by dissolved organic carbon (DOC), microbial biomass carbon (MBC), and readily oxidized carbon (ROC). Although the active organic carbon is a relatively small proportion of total organic carbon (TOC), its change is one of the most important factors affecting the global carbon balance due to its sensitivity and activity to environmental changes (Liang et al., 1998).

As the most basic structural soil unit, the quantity, and quality of soil aggregates determine soil fertility and soil properties. Soil aggregates are carriers of soil organic carbon and also have physical and biological protection effects on soil organic carbon (Six et al., 2000; Mrabet, 2002). In return, soil organic carbon (SOC) promotes the formation of soil aggregates (Sonnletner et al., 2003), yet SOC content shows significant differences in various soil aggregates (Yilmaz and Sonmez, 2017). Therefore, studying the relationship between aggregates and soil organic carbon has important ecological significance for further research on the fixation and emission of soil carbon and organic carbon stability (Barreto et al., 2009; Nigussie et al., 2017).

Previous studies have shown that the change of land-use could directly affect the distribution of wetland vegetation and the composition of plant diversity (Liang et al., 2017; Moges et al., 2017), as well as significantly affect the nutrient cycling of soil carbon and nitrogen (Huang and Song, 2010; Ouyang et al., 2013), and the composition and function of microbial diversity (Zhang et al., 2018). The most obvious and rapid response when the land-use changes usually occurs in the active components of soil (Haynes, 1999). After artificial reclamation, the active organic carbon in wetland soil is heavily oxidized and released, which leads to the increase in CO_2 concentration of greenhouse gases in the atmosphere; therefore, wetland use is also an important cause affecting the global carbon balance (Jenkinson et al., 1991; Bortolotti et al., 2016).

In this study, the typical wetland and different wetland use (upland field, paddy field, and artificial forest) in the Sanjiang Plain wetland reserve, Heilongjiang Province, China, were used to study the effects of different land-uses on mechanically stable aggregates, TOC, and active organic carbon components.

MATERIALS AND METHODS

Site description and soil sampling method

The experimental site was located in the Honghe Nature Reserve (47° 45′ 39″ N, 133° 37′ 04″ E) of the Sanjiang Plain wetland in Heilongjiang Province (Figure 1). The



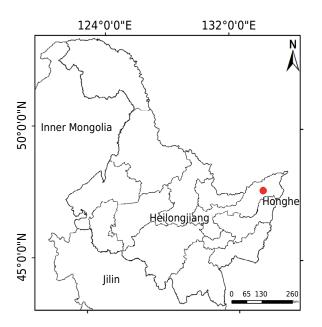


Figure 1. The map Honghe Nature Reserve (47° 45' 39" N, 133° 37' 04" E) of the Sanjiang Plain wetland in Heilongjiang Province.

test plots were located in an area with a humid and semi-humid monsoon climate in the temperate zone and the field sites had an altitude of approximately 55-65 m. Average annual rainfall was 550-600 mm, and rain usually occurs in summer and autumn. The average annual temperature was 1.9 °C, and the frost-free period was about 125 days (Sui et al., 2015). The soil is classified as Mollisols (IUSS, 2015). Sampling was conducted in the core area of the Honghe Nature Reserve, and the dominant plants in this region included *Deyeuxia angustifolia*, *Carex pseudocuraica*, *Glyceriaspiculosa*, *Carexlasiocarpa*, *Phragmites communis* Trin., *Stachys baicalensis Fisch. ex Benth.*, and *Sanguisorba tenuifolia* var. alba.

Test plots of different land-uses were located at the edge of the core areas of the wetlands. A paddy field cultivated with *Oryza sativa* and the upland area planted with *Zea mays* L. and *Glycine max* (Linn.) Merr., used in this study, were reclaimed more than 50 years ago. The artificial forest, with over 30-year-old *Betula platyphylla* Suk., was at the edge of the paddy field and upland field. Soil samples were collected after the crops were harvested from the paddy field and upland field on 20th Oct. 2017. In the wetland and each of the three land-uses, four sampling plots (100 × 100 m) were randomly set up with an interval of 100 m, and then ten soil samples were collected along with the "S" shape in each sampling plot. The ten soil samples were then well mixed. The sampling depth was 0.00-0.20 m and the water depth was excluded in the collection of soil in marshland meadows. To prevent the damage of soil aggregates during the collection and transport process, soil aggregates were carefully collected as undisturbed soil and kept in a hard plastic fresh-keeping box, and then transported to the laboratory for preservation at 4 °C.

Test materials and methods

Dry sieving method was used to determine soil aggregates, which preserve the watersoluble organic matter in soil and protects the biological habitat. The undisturbed soil in different treatments was gently broken into pieces by hand and air-dried at 4 $^{\circ}$ C until approximately 20 % of water content remained in the soil. After screening using an 8 mm sieve and removing plant roots and other remains, soil samples were screened using a sieve set with diameters of 5, 2, 1, and 0.25 mm. Approximately 200 g of soil sample were screened each time for 5 min and aggregates with a size fraction of 5, 2, 1, and <0.25 mm were obtained. Then, the aggregates with the same size fraction after screening were mixed and weighted. Mean weight diameter (MWD; mm) was determined according to Van Bavel (1949).

Soil aggregates obtained by dry sieving were divided into two groups, one of which was preserved in the refrigerator at 4 °C for the determination of water content and soil active organic carbon, such as DOC, ROC, and MBC, while another aggregate was air-dried to measure the content of TOC in soil aggregates with various size fractions. Soil TOC was measured following the Bao titration method (Bao, 2005). The oven drying method (drying at 105 °C for 24 h) was adopted to determine the water content of samples with various size fractions for the measurement of active organic carbon. To determine the content of active organic carbon in various soil aggregates, the Walkley-Black (WB) titration method, potassium permanganate oxidation method, and chloroform fumigation-extraction (CFE) method were used to determine the DOC, ROC, and MBC content, respectively. During the determination of the DOC content, 10 g of fresh samples from soil aggregates with different size fractions were put into a 50 mL centrifuge tube with 25 mL deionized water. A supernatant was obtained by oscillating (180 rpm) for 30 min and centrifuging (4,000 rpm) for 10 min and was filtered using a 0.45 μ m filter membrane. The DOC content in the filtrate was determined using the Walkley-Black (WB) titration method. In the determination of ROC, soil samples containing about 15 mg carbon were oscillated for 1 h and centrifuged (4,000 rpm) for 5 min in 100 mL centrifuge tubes with 25 mL of potassium permanganate solution (333 mmol L⁻¹). The supernatant was diluted to 1:250 with deionized water followed by the colorimetric determination at a wavelength of 565 nm. The ROC content in the soil was calculated by the consumption of potassium permanganate (Wu et al., 2004). In the determination of MBC, fumigated and non-fumigated samples were extracted with K_2SO_4 (0.5 mol L⁻¹) for 30 min and determined using a Shimadzu TOC-VCPH analyzer for its carbon concentration. Then, the MBC was calculated by the formula MBC = EC/0.38, in which EC is the difference of organic carbon between fumigated and non-fumigated extracts (Lu, 2000). The DOC, ROC, and MBC content were calculated using dry soil weight, while the determination of the storage of TOC and active organic carbon in soil aggregates followed methods described in Liu et al. (2006), in which organic carbon storage of aggregate = specific organic carbon content of aggregate $(g kg^{-1}) \times corresponding composition proportion (%).$

Data analysis

The data were statistically analyzed using Excel and SPSS (version 22.0), and the differences between different data groups were compared by one-way analysis of variance (one-way ANOVA) and least significant difference (LSD) tests.

RESULTS

Composition of soil aggregates under different land-uses

In the Sanjiang Plain wetlands, the composition of soil aggregates under different landuses changed significantly (Figure 2). Compared with wetland soil, the proportion of small aggregates (<0.25 mm) in the paddy field increased significantly, while other particles in the aggregate remained stable. In the upland field, the proportion of large aggregates decreased significantly, in which aggregates of >5 and 2-5 mm increased by 3.28 and 3.06 %, respectively. In addition, in the upland field, aggregates of <2 mm rose in varying degrees compared with that in the wetland. As for the artificial forest, the proportion of aggregates (>5 and 2-5 mm) increased significantly compared with that in the wetland, which was also evidently higher than that in the paddy field and the upland field. However, the proportion of small aggregates (0.25-1 mm) was lower than that in the wetland by 3.42 %. Different land-uses affected the mean weight diameter

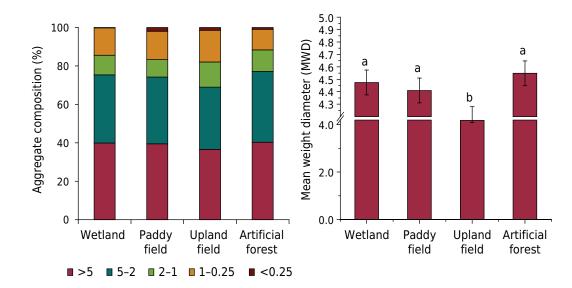


Figure 2. Soil aggregate composition and mean weight diameter (MWD) of different land-uses in the Sanjiang Plain wetlands. Different small letters above the bars represent significant differences (p<0.05).

(MWD) of soil. Compared with wetland soil, the MWD in the upland field significantly decreased by 6.59 % (p<0.05), but there is no significant difference in MWD among other land-uses.

Content and storage of total organic carbon in soil aggregates under different land-use

The three land-uses significantly reduced total organic carbon (TOC) content in various soil aggregates from the wetland (Figure 3). Besides the significantly high TOC content in various soil aggregates from the paddy field compared with those from the upland field and artificial forest. The TOC storage and content in various soil size fractions under the three land-uses were similar, which were both obviously lower than that of wetland soil with the minimum decrease observed in the paddy field. The TOC content of soil aggregates with various size fractions in two types of land-uses, the upland field and artificial forest, demonstrated no significant difference. Moreover, TOC storage decreased first and then increased with the decrease of the size fraction in soil aggregates. The proportion of soil aggregates at 1-2 mm was significantly lower than that of other size fractions, resulting in the lowest TOC storage at aggregates of 1-2 mm, which was observed in both the wetland and different land-use types. Small aggregates (<0.25 mm), which were a small proportion of the soil aggregates, were ignored in calculating organic carbon content and storage.

Content and storage of dissolved organic carbon in soil aggregates under different land-uses

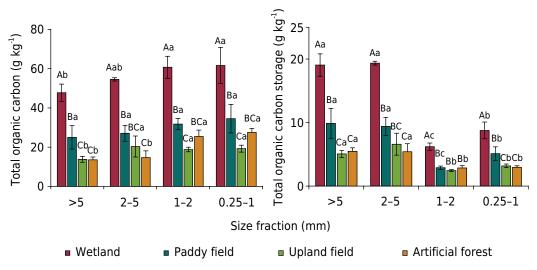
Dissolved organic carbon content in different soil treatments increased significantly with the decrease of the size fraction of soil aggregates, while land-use change significantly decreased the DOC content in the same soil aggregates compared with the wetland (Figure 4). The lowest reduction of DOC content was observed in the aggregates collected from the paddy field. There was a significant difference in aggregates with >5 mm and 2-5 mm between the artificial forest and paddy field, and the largest reduction of DOC content occurred in the upland field. In the 1-2 and 0.25-1 mm aggregates, the DOC content in the artificial forest was similar to that in the upland field.

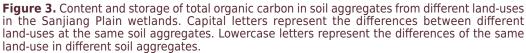
With the decrease of the fraction size of soil aggregates, the change of DOC storage in different treatments showed a "V" trend, meaning the lowest DOC storage was

measured in the aggregate size fraction of 1-2 mm and the DOC storage in the different sized aggregate fractions were reduced to different degrees under different land-uses in the wetland. Compared with wetland, paddy and artificial forest of DOC storage in the aggregates of >5 mm has no significant differences, while the DOC storage showed a significant decreasing in upland field. The results in all land-use types showed no significant difference in DOC storage in the 2-5 and 1-2 mm aggregate, while in the 0.25-1 mm aggregate, the DOC content in the upland field and the artificial forest was significantly lower than that in the wetland and paddy field, which showed similar values.

Content and storage of microbial biomass carbon in soil aggregates from different land-uses

The MBC content showed in figure 5 increased at first and then reduced with the decrease of the fraction size of soil aggregates in both the wetland and different land-uses. The highest content was observed in the 1-2 mm aggregate, but the lowest storage was also





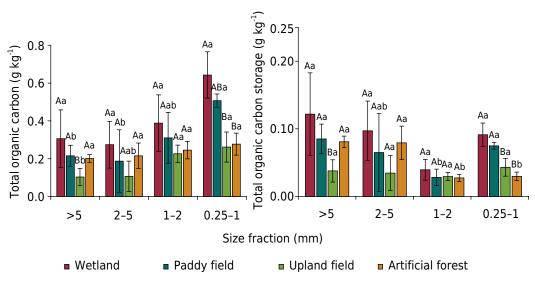


Figure 4. Content and storage of dissolved organic carbon in soil aggregates from different land-uses in the Sanjiang Plain wetlands. Capital letters represent the differences between different land-uses at the same soil aggregates. Lowercase letters represent the differences of the same land-use in different soil aggregates.

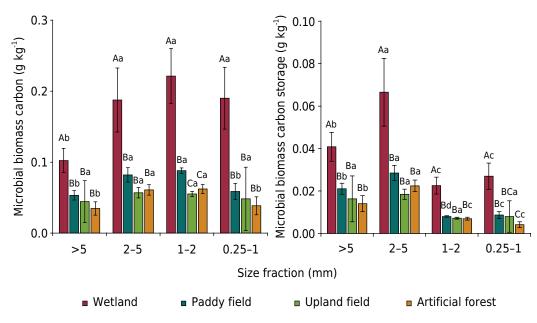
found in this size fraction. The content and storage of MBC in various soil aggregates under different land-uses were significantly reduced compared with those in the wetland. The comparison of different land-uses in the 1-2 mm aggregate showed that the content MBC in the upland and artificial forest field was significantly decreased than those in the paddy field. In the 0.25-1 mm aggregate, the storage of MBC in the artificial forest was significantly lower than in paddy soil.

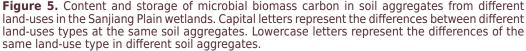
Content and storage of readily oxidized carbon in soil aggregates from different land-uses

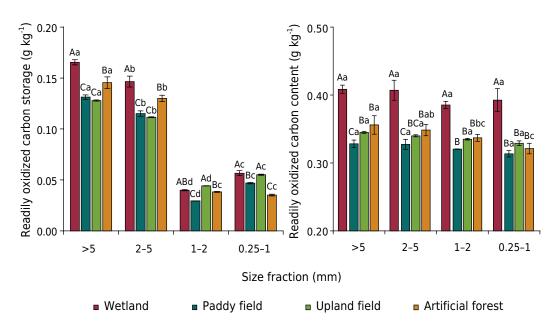
The content of readily oxidized carbon (ROC) in the wetland and different land-use types reduced slightly with the decrease of the soil aggregate fraction size (Figure 6), and the ROC storage in soil aggregates at 1-2 mm and 0.25-1 mm was significantly lower than in the >5 and 2-5 mm aggregates. Compared with the wetland, the ROC content and storage under different land-use types decreased to various degrees. The decrease of the ROC content in the paddy field was obviously greater than that in the upland field and the artificial forest, and the storage of ROC in the aggregates (>5 and 2-5 mm) from the artificial forest were higher than those in the upland field.

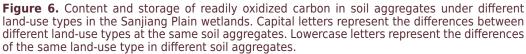
DISCUSSION

As a manifestation of soil degradation, the stability of soil aggregates poses important effects on soil nutrient cycling, water transportation, and microbial community, and the formation and stability of soil aggregates are significantly affected by different land-uses (Pinheiro et al., 2004; Zhuang et al., 2017). In this study, except for the increase in the proportion of micro-aggregates (<0.25 mm), no obvious changes were observed in other micro-aggregates and the wetland, which may be caused by the similar environments in the paddy field and the wetland, and has little influence on large soil aggregates. The increase in the proportion of micro-aggregates in the paddy field may be related to the frequent mechanical vibration of soil caused by agronomic measures such as long-term farming and field sunning. As for the proportion of micro-aggregates in the upland field and artificial forest, the >5 mm and 2-5 mm aggregates increased significantly compared with that in the wetland. A significant increase of large aggregates was found in the









artificial forest, which may be related to the formation of aggregates that benefit from the long-term drought conditions in the upland field and artificial forest.

As an important indicator of soil aggregate stability, the MWD value had a positive correlation with aggregate stability (Li et al., 2005; Pirmoradian et al., 2005). In this study, MWD in upland field decreased compared to that in the wetland, especially the obvious reduction in the upland field, which may be due to the decline of the stability of soil aggregates, resulting from artificial tillage and fertilization in the reclaimed wetlands. Compared with the long-term flooding in the upland field, the condition in the paddy field is more conducive to the accumulation of organic matter, resulting in a higher MWD value in the paddy field. The organic carbon in soil aggregates from the upland field is more vulnerable to oxidation than that from improved soil aeration and frequent microbial activity, resulting in more severe soil aggregate deterioration in the upland field than in the paddy field (Zhao et al., 2003). Zhang et al. (2015) reported that compared to the upland field, the environment in the paddy field is more conducive to soil aggregate stability and the accumulation of organic carbon functional groups, as well as the higher chemical stability of organic carbon, which may lead to more stable soil aggregates in the paddy field than the upland field. The increase of organic matter input is beneficial to the formation of large aggregates in soil (Gao et al., 2010). As a temporary cementing medium of soil aggregates, plant root systems can also promote the formation of aggregates while improving soil stability (Tisdall and Oades, 1982).

As the cementing agent of soil aggregates, the increase of soil organic carbon content improves the quantity and stability of aggregates. However, the differences in structure, aeration, quality, and activity of microorganisms in various soil aggregates led to a different distribution of soil organic carbon in aggregates of different size fractions (Mrabet, 2002). This indicates that soil aggregates have a significant correlation with soil organic carbon content, which has been reported by several previous studies (Eynard et al., 2005; Song et al., 2016).

In this study, the obvious decrease in TOC content and storage in soil aggregates with different size fractions in different land-uses was probably due to the increase in aeration of wetland soil aggregates under different land-uses. Aerobic microorganisms were active, which intensified organic carbon utilization in the soil.

The significant decrease in TOC content and storage in various soil aggregates in the upland field and artificial forest indicates that massive drainage in reclaimed wetlands promotes the oxidation of soil organic carbon. Our results about TOC content after complete drainage are consistent with the research conducted by Zhang et al. (2013), who showed that the TOC content in various soil aggregates of artificial forests was significantly higher than that in the upland field. This is probably associated with the large amount of forest litter and input of organic matter. In addition, long-term human disturbance also reduces soil organic carbon content; therefore, due to less human disturbance in the artificial forest, the TOC content was significantly higher than that in the upland field.

The reasons for the evidently higher content and storage of TOC in various soil aggregates from the paddy field compared to the upland field and the artificial forest are associated with the low aerobic microbial activity and organic carbon utilization under long-term flooding conditions, and the stronger stability of microbial active organic carbon (Zhang et al., 2015). Moreover, in the comparison of various soil aggregates, the TOC content in the 1-2 and 0.25-1 mm aggregates was significantly higher than that of the >5 and 2-5 mm aggregates, which is because the carbon, which was tightly fixed by organic and inorganic colloids into smaller aggregates, is not easily decomposed by microorganisms (Arrouays et al., 1995). Furthermore, the presence of numerous plant root systems and mycelium in big aggregates increases the TOC content in large aggregates due to their breakdown (Zhao et al., 2006). In addition, in small aggregates with small porosity, the degradation of organic carbon can only be carried out by the diffusion of extracellular enzymes when the pore size is smaller than the size limit $(3 \mu m)$ that microorganisms can pass through, which is an energy taxing process for microorganisms, resulting in the reduced ability to utilize organic carbon in small aggregates (An et al., 2018).

The DOC characteristically has strong solubility and can be directly used as an organic carbon source for soil microorganisms; its source includes microbial metabolites, the input of aboveground biomass and litter, and the accumulation of root exudates (Kalbitz et al., 2000; Smolader and Kitunen, 2002; Guggenberger and Kaiser, 2003; Debasish-Saha et al., 2014). Therefore, the level of DOC content could reflect the activity of microorganisms to a certain extent (Gunapala and Scow, 1998).

It has been reported that DOC is a soil component that is sensitive to environmental changes (Singh et al., 2017), and its content was significantly reduced in the reclaimed wetland (Huang et al., 2008). In this study, the DOC content in various soil aggregates decreased significantly under different land-use types, especially in the upland field and the artificial forest, which decreased more than in the paddy field. This is possibly due to the long-term flooding conditions in the wetland and paddy field, and these conditions may be more conducive to the dissolution and precipitation of DOC (Wang and Bettany, 1993). However, in the >5 mm soil aggregates, the content and storage of DOC in the artificial forest were significantly higher than that in the upland field, which may be due to the input of more organic matter in the artificial forest, such as leaf litter, resulting in more DOC sources in the soil (Wang et al., 2016). After the harvest in the upland field, straw was removed, and the land was fertilized mainly by inorganic fertilizer and less organic fertilizer, resulting in a significantly higher DOC content in various soil aggregates from the artificial forest than that in the upland field. In the 1-2 mm and 0.25-1 mm aggregates, there was no significant difference in the content and storage of DOC under the upland field and artificial forest land-use types, which was probably due to the distribution of litter in the artificial forest mainly being the relatively large aggregates.

The MBC, which is beneficial to nutrient transformation and carbon cycling in soil, has become an important indicator to reflect the dynamic changes of the carbon pool due to its short transformation cycle (Roy and Singh, 2003), and it is also one of the most sensitive indicators of land-use change (Gong et al., 2015). Zhang et al.(2007) reported that in the Sanjiang Plain wetlands, the MBC content in soil surface sharply decreased under different land-use types, but others studies suggested that the MBC content increased at the early stage of wetland soil reclamation (Jachson et al., 2003).

In this study, the results showed that the content and storage of MBC in various soil aggregates under different land-uses all significantly decreased, which is related to the fact that after the wetlands with abundant vegetation and soil organic matter sources were reclaimed as farmland and artificial forest, MBC was reduced by the decreased available substrates, resulting from tillage measures and the removal of plant remains. Under different land-use types in reclaimed wetland, MBC storage in >5 mm and 2-5 mm aggregates was significantly higher than that of 1-2 mm and 0.25-1 mm aggregates in both the wetland and different land-uses. This is mainly due to the close relationship between microorganism activity and soil aggregates (Wen et al., 2004).

As one of the organic carbon components, soil ROC, which is easily oxidized and highly active, is an important factor affecting the global carbon balance as it is sensitive to environmental changes (Chan et al., 2001). Unlike DOC and MBC, ROC content in various soil aggregates in the paddy field and upland field was significantly lower than that in the artificial forest despite the decreased ROC content in different land-uses compared to the wetland. The reduction of the ROC content in the paddy field and the upland field may be related to the long-term tillage disturbance, which is consistent with the results described by Conteh et al. (1997). In addition, the ROC content is also associated with organic matter input in soil (Stirling et al., 2005). The higher ROC content in the artificial forest is due to less human disturbance, large organic matter input, developed tree root systems in artificial forest compared with crop root systems, and the removal of straw in the upland field and paddy field after harvest. Elliott and Cambardella (1991) and Puget et al. (2000) have reported that organic carbon in smaller aggregates, which mostly presented as inert components with high humification, was more likely to be mineralized than in larger aggregates. This is consistent with our results that showed that ROC storage in the 1-2 and 0.25-1 mm aggregates was significantly lower than the ROC storage in >5 and 2-5 mm aggregates from different land-uses.

CONCLUSIONS

The composition of soil aggregates in the Sanjiang Plain wetlands was obviously changed when it was reclaimed for different land-uses, resulting in the significant reduction of the content and storage of total organic carbon (TOC) and active organic carbons (dissolved organic carbon (DOC), microbial biomass carbon (MBC), and readily oxidized carbon (ROC)) in soil aggregates with various size fractions. Moreover, the changes of active organic carbons in various soil aggregates varied due to the different interference intensities in the three land-uses. The influence of the paddy field on soil aggregates and active organic carbons was significantly lower than that of the upland field and the artificial forest, while the influence was the strongest, with severe destruction of soil aggregates and organic carbons in the upland field due to long term tillage disturbance. Above all, the land-use with the maximum influence on structure of soil aggregates and organic carbon in the reclaimed wetland is the upland field.

ACKNOWLEDGMENTS

The study was supported by the Opening Project of State Key Laboratory of Tree Genetics and Breeding (K2021103), Postdoctoral Science Foundation (2018M640287) and the National Natural Science Foundation of China (41701289).



AUTHOR CONTRIBUTIONS

Conceptualization: (D) Kaiwen Guo (equal), (D) Wei Li (equal) and (D) Xiaoqian Liu (equal).

Data curation: (D Xiaoqian Liu (lead).

Formal analysis: (D) Xiaoqian Liu (lead).

Funding acquisition: (D) Bin Li (lead).

Investigation: 💿 Xiaoqian Liu (lead).

Methodology: 💿 Xiaoqian Liu (lead).

Project administration: D Xiaoqian Liu (equal) and D Xin Li (equal).

Resources: (D) Xiaoqian Liu (lead).

Software: (D) Bin Li (equal) and (D) Xin Li (equal).

Supervision: (D) Wei Li (equal) and (D) Xiaoqian Liu (equal).

Validation: 🔟 Kaiwen Guo (equal) and 🔟 Xiaoqian Liu (equal).

Writing - original draft: D Xiaoqian Liu (lead).

Writing - review & editing: D Xiaoqian Liu (lead).

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