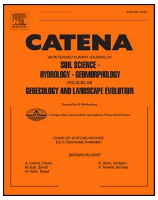
Catena 204 (2021) 105405

Contents lists available at ScienceDirect

Catena

journal homepage: www.elsevier.com/locate/catena



Long-term afforestation accelerated soil organic carbon accumulation but decreased its mineralization loss and temperature sensitivity in the bulk soils and aggregates

Yan Zhang [a,b], Nannan Ge [b,c], Xiaolin Liao [b,d], Zhao Wang [b], Xiaorong Wei [b,c,e,*], Xiaoxu Jia [b,c]

[a] College of Pastoral Agriculture Science and Technology, Lanzhou University, Lanzhou 730020, PR China
[b] State Key Laboratory of Soil Erosion and Dryland Farming on the Loess Plateau, Northwest A&F University, Yangling 712100, PR China
[c] CAS Center for Excellence in Quaternary Science and Global Change, Xi'an 710061, PR China
[d] College of Biology and the Environment, Nanjing Forestry University, Nanjing 201137, PR China
[e] College of Resources and Environment, University of Chinese Academy of Sciences, Beijing 100049, PR China

ARTICLE INFO

Keywords:
Abandoned farmland
Afforestation
Organic carbon mineralization
Soil aggregates
Temperature sensitivity

ABSTRACT

The conversion of land use from agricultural land to forests is considered an effective measure of mitigating atmospheric CO_2, but the impacts of long-term afforestation on soil organic carbon mineralization (C_m) and its temperature sensitivity (Q_{10}) remain uncertain. In this study, we aimed to investigate the effects of different afforestation ages on OC contents and C_m and Q_{10} in bulk soils and aggregates. Soils were collected from 0–10 cm and 10–20 cm depths in afforested woodlands after 10, 20, 30 and 40 yrs of establishment of *Robinia pseudoacacia* on abandoned farmlands on the Loess Plateau, China. C_m and Q_{10} were measured in an 83-day incubation experiment at 25 °C and 15 °C. The results showed that long-term afforestation accelerated soil OC accumulation but decreased its C_m and Q_{10} in bulk soils and aggregates, and the effects were greater at the 0–10 cm soil depth. Macroaggregates contributed most of the OC content (62%), but microaggregates and silt + clay contributed most of the OC mineralized (40% and 36%) in the bulk soils. The increased OC content and decreased C_m in aggregates suggested an increase in the sequestration of OC in fine soil particles. The temperature sensitivity of OC mineralization increased with increasing particle size, with a higher Q_{10} value for macroaggregates (1.81 ± 0.44) than for microaggregates (1.42 ± 0.35) and silt + clay (1.31 ± 0.14). Our results indicated that long-term afforestation would be conducive to the accumulation of OC and would decrease the release of CO_2 from soils under future climate warming scenarios. The findings highlighted the OC dynamics in abandoned farmland were more sensitive to the temperature changes than those in forests, and the stability of OC in aggregates increased as the aggregate size decreased. This study contributed to bridging current knowledge gapes about the process underlying the observed OC budget and its response to warming scenarios in rehabilitated ecosystems.

1. Introduction

Land-use change, e.g., afforestation, has been acknowledged as an effective measure of carbon (C) sequestration (Rahman et al., 2017), thereby mitigating greenhouse gas emissions (Davidson and Janssens, 2006). The soil C sequestration potential is usually determined by the inputs (e.g., litterfall and rhizodeposition) and outputs (e.g., heterotrophic respiration) of organic matter (Jandl et al., 2007). Many studies have reported that afforestation affects soil C storage. However, the soil C biogeochemical processes regulated by the input and output of organic matter (e.g., mineralization) during afforestation practices are not yet fully understood.

Mineralization, i.e., the conversion process of organic to inorganic via microorganisms, has been extensively used to study soil C dynamics (Rahman et al., 2017). Generally, the changes in soil physical and chemical properties as well as the quantity and quality of litter caused by land-use changes significantly affect soil C mineralization. For instance, the changes in soil pH caused by land-use change directly regulated soil C turnover by affecting the composition and activity of microbial communities (Li et al., 2019). Additionally, the size distribution and connectivity of pores under different land uses could influence the contact of microorganisms with soil nutrients and hence affect OC

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* Corresponding author at: State Key Laboratory of Soil Erosion and Dryland Farming on the Loess Plateau, Northwest A&F University, Yangling 712100, PR China.
E-mail address: xrwei78@163.com (X. Wei).

https://doi.org/10.1016/j.catena.2021.105405
Received 9 April 2020; Received in revised form 14 April 2021; Accepted 19 April 2021
Available online 7 May 2021
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mineralization (Franzluebbers, 1999). Therefore, the effects of afforestation on soil C mineralization vary in different studies (Côté et al., 2000; Rahman et al., 2017). For example, Côté et al. (2000) found that C mineralization in the soil organic layer was higher in clay soils than in till soils, while the opposite was found in the mineral layer in northwestern Quebec, Canada, and C mineralization in both organic and mineral layers was higher in older forest stands (124 yrs) than in young forest stands (50 yrs). Rahman et al. (2017) reported that soil N mineralization increased while C mineralization did not change at the 0–5 cm soil depth after afforestation on farmland in western Copenhagen, Denmark, and these processes were affected by tree species and afforestation time.

Aggregate-associated organic carbon can respond quickly to land-use changes and can usually indicate changes in nutrients and structure of soils (Nie et al., 2014). Generally, afforestation changes the fractions of soil aggregates, with an increase in the coarse fraction and a decrease in the fine fraction, and drives the accumulation of soil C, mainly in macroaggregates (Qiu et al., 2015; Zhang et al., 2020), due to less disturbance, more organic matter inputs, and lower decomposition compared to other land uses (e.g., farmland) (Deng and Shangguan, 2017; Rahman et al., 2017; Zhang et al., 2018). The majority of soil OC is concentrated within aggregates, which can physically protect microbial decomposition and mineralization (Liu et al., 2020; Von Lutzow and Kogel-Knabner, 2009). Therefore, it is expected that soil OC mineralization will differ between aggregate size classes. However, the effects of long-term afforestation on OC mineralization in aggregates have not been well examined (Feng et al., 2018), which is critical for understanding OC sequestration and dynamics in forest soil. Moreover, it has been reported that OC in bulk soils and aggregates is sequestered during the beginning stage of afforestation and then becomes stable after several decades of afforestation (Deng and Shangguan, 2017; Zhang et al., 2018). Whether soil OC mineralization changes with afforestation age, however, is not yet clear (Rahman et al., 2017).

A change in temperature significantly affects soil C mineralization, which usually increases with the exponential function of temperature (Froseth and Bleken, 2015; Knorr et al., 2005). Temperature sensitivity is often used to express the increase in the C mineralization rate as the temperature increases by 10 °C, commonly referred to as Q_{10}, which is often affected by the availability and quality of the mineralized substrate (Davidson and Janssens, 2006; Wei et al., 2016). Afforestation practices can change the sources, composition, and stability of soil organic matter and can thus affect Q_{10} (Fang et al., 2005; Gershenson et al., 2009; Giardina and Ryan, 2000; Schütt et al., 2014). Therefore, it is critical to examine the temperature sensitivity of C mineralization after land-use change to accurately predict the soil C budget under the context of global warming.

To quantify the response of soil OC mineralization to long-term afforestation and its temperature sensitivity in bulk soils and aggregates, a laboratory incubation experiment at two temperatures (15 °C and 25 °C) was conducted using soils collected from 0–10 cm and 10–20 cm depths in afforested woodlands 10, 20, 30 and 40 yrs after the establishment of *Robinia pseudoacacia* on abandoned farmlands on the Loess Plateau, China. We hypothesized that (H1) long-term afforestation would increase soil OC accumulation but decrease its mineralization loss and temperature sensitivity in the bulk soils and aggregates due to the sufficient substrate for microbes via the accumulation of organic matter during the afforestation process (Ma et al., 2019; Hadas et al., 2004) and (H2) compared with fine soil particles (e.g. microaggregate and silt + clay), coarse soil particles (e.g. macroaggregates), would contribute most of the OC mineralized in the bulk soils because soil OC sequestration caused by afforestation occurs mainly in the macroaggregates (Qiu et al., 2015; Zhang et al., 2020).

2. Materials and methods

2.1. Study site description

We carried out our experimental study in the afforested area of Fu County (E109°37′, N36°38′, 1267 m, a.s.l.), located in northern of Shaanxi Province on the Loess Plateau, China. The study site has a subtropical warm-temperature monsoon climate; with an average annual rainfall of 577 mm. Rainfall is primarily concentrated between June and September. The mean annual temperature is 9.0 °C, with a monthly maximum of 38.7 °C (in July) and a minimum of −25.2 °C (in January). The average sunshine duration and frost-free period are 2468.8 h and 170 days, respectively. The topography is dominated by hilly and gully. The soil type in this area is a Cambisol according to the FAO (2015), with a clay loam texture.

The land use type at the site before afforestation was farmland. Crop grow is very diverse owing to the long history of agriculture. More than 20 crops are currently cultivated, including winter wheat, maize, potato, buckwheat, miller, sorghum, pearl miller, and soybean. Among them, wheat and maize are the two major crops, accounting for 35% and 30%, respectively, of the total cultivated area (Tsunekawa et al., 2014). Most cultivars have high tolerance to drought and other abiotic stresses, such as heat, cold and low fertility. Although the traditional agricultural system has been well developed, there are still certain constraints on further agricultural development, for example, soil degradation in agricultural fields. Because most agricultural fields are on slopes, surface soils with relatively high fertility and organic matter content are easily eroded by water and wind. The concentrated rainfall in summer and strong wind in early spring often remove surface soils from agricultural fields. It has been reported that 0.2–1.0 cm of surface soils on cultivated sloping land have been eroded by water, and the total soil erosion modulus is 2019 t throughout a whole year, which is dominated by water and wind erosion (Tsunekawa et al., 2014; Zhang and Schwärzel, 2017). As a result, large areas of land have been abandoned and agricultural production activities have ceased, so manure fertilizer has not been used since the 1970 s. In the adjacent farmland, the main planting systems are monoculture crops of winter wheat (*Triticum aestivum* L.) or summer maize (*Zea mays* L.). To meet the needs of crops for N and P, chemical fertilizers, urea and calcium superphosphate are applied annually to the farmland at rates of 224–350 kg N $ha^{-1}yr^{-1}$ and 89–120 kg P $ha^{-1}yr^{-1}$, and equal amounts of fertilizer are applied to wheat and maize. To prevent soils from degrading on the Loess Plateau, many farmlands have been abandoned and converted to forests. Today, afforested land includes a variety of arbors, shrubs and herbs. Among them, the tree species are mainly black locust (*Robinia pseudoacacia* L.), Chinese pine (*Pinus tabuliformis* Carr.) Liaodong oak (*Quercus wutaishansea Mary*) and white birch (*Betula platyphylla Suk.*). The shrubs are mainly apricot and mountain peaches. All tree species following afforestation are monocultures with natural growth. These forestry resources are protected and developed by the state and local forestry administrations.

2.2. Soil sampling

In September 2014, we selected an adjacent abandoned farmland and four black locust forests with stand ages of 10, 20, 30, and 40 yrs to compose a chronosequence site and established 3 replicated plots (10 m × 10 m) less than 40 m apart at each site. A chronosequence is composed of a range of sample sites and is spread out over time to study the temporal variation in soil development at multiple time scales. The basis of this method is that each site in the sequence has similar natural and historical conditions and only differs in age. The stand ages of all forests were determined by drilling technology with the help of the local forest management departments. In this method, we selected wood with obvious bark and sapwood to drill the wood core of the tree using a 4.3-mm diameter growth cone. The wood core was placed on paper straw for

Table 1
The basic information of the study sites in Fu County, Loess Plateau.

Depth (cm)	Afforestation (yrs)	Slope degree	DBH (cm)	Height (m)	Sand (%)	Silt (%)	Clay (%)	pH	Ks (mm h^{-1})	BD (g cm^{-3})	OC (g kg^{-1})	N (g kg^{-1})	P (g kg^{-1})	Min-N (mg kg^{-1})	C/N
0–10	0	4°	NA	NA	48.67	33.07	18.26	8.59	43.40	1.10	11.03	1.04	0.49	18.02	10.63
	10	4°	12.5	13.0	51.97	31.08	16.95	8.40	25.83	0.94	20.78	1.88	0.63	37.03	11.04
	20	5°	14.2	14.6	54.00	31.18	14.83	8.51	33.25	1.04	18.72	1.77	0.61	25.86	10.59
	30	5°	23.0	15.4	59.54	25.64	14.82	8.35	38.06	1.07	24.72	2.19	0.65	30.01	11.26
	40	5°	30.1	16.4	61.17	22.29	16.55	8.28	104.92	0.92	26.53	2.32	0.57	35.27	11.43
10–20	0	4°	NA	NA	48.54	33.15	18.30	8.67	12.63	1.29	5.33	0.54	0.45	10.46	9.72
	10	4°	12.5	13.0	49.06	34.13	16.81	8.57	12.69	1.14	12.54	1.17	0.59	58.26	10.50
	20	5°	14.2	14.6	47.54	36.79	15.66	8.52	15.88	1.23	10.43	1.07	0.54	24.78	9.78
	30	5°	23.0	15.4	60.47	24.25	15.28	8.54	23.49	1.15	10.48	1.07	0.59	17.63	10.66
	40	5°	30.1	16.4	61.30	22.71	15.99	8.45	15.73	1.18	11.41	1.15	0.52	19.63	10.38

DBH: Diameter at breast height; Height: the height of tree; Afforestation age 0: the farmland; Ks: soil saturated hydraulic conductivity; BD: soil bulk density; OC: soil organic carbon content; N: soil total nitrogen content; P: soil total phosphorus content; Min-N: soil mineral nitrogen content (NH_4^+ + NO_3^-); C/N: the ratios of organic carbon to total nitrogen.

air drying and polishing. The polished sample was marked every 10 years from the pith to the bark, and the age was estimated based on the number of annual rings using the cross-dating method of the Tree Ring Research Laboratory of the University of Arizona (Cook and Holmes, 1996; Holmes, 1983).

The area of each site was 5 ha. There was approximately 1–3 km between each site. The sampling plots have similar physical and geographical conditions, and the main information about the forests and farmland is shown in Table 1. We collected 3 replicated samples for each soil layer (0–10, 10–20 cm depths) in each plot using a stainless-steel ring cutter (5 cm diameter × 5 cm height) to measure soil bulk density (BD). In addition, we randomly collected 7 representative undisturbed samples from the 0–10 and 10–20 cm depths of each plot to form a composite bulk clod sample (ca. 1 kg) to analyze soil physical and chemical properties. In total, 30 composite soil samples (5 sites × 3 plots × 2 soil depths) were collected. Each sample was placed in an aluminum box to maintain the original structure of the soil. All soil samples were protected from vibration or tipping during loading and transportation. When returned to the laboratory, all undisturbed soil samples were gently stripped into small clods with a diameter of 10–12 mm along the natural structure. Visible organic material and small stones were removed. The moist soil samples were naturally dried and preserved at room temperature for laboratory analysis.

2.3. Soil analysis

In this study, soil samples before the incubation experiment were used to analyze the aggregate distribution and basic physical and chemical properties. The soil BD was determined by the original volume and dry mass (dried at 105 °C) of each soil core using the soil bulk sampler method (Jia et al., 2005). The aggregates were separated into 3 fractions, macroaggregates (8–0.25 mm, MA), microaggregates (0.25–0.053 mm, MI), and silt + clay (<0.053 mm, SC), with a wet-sieving method (Cambardella and Elliott, 1993). In this method, a 100 g soil sample was placed on the top of a nest of sieves with opening sizes of 0.25 and 0.053 mm arranged from top to bottom. Wet sieving was performed in distilled water using a shaker for 5 min at a frequency of 50 times every two minutes. After wet sieving, the soil remaining on each sieve was collected separately and dried to a constant weight at 40 °C. The bulk soils were passed through 2-mm sieves for the measurement of soil particle composition (i.e., sand, silt and clay proportions), pH, mineral N (NO_3^-, NH_4^+), and OC mineralization. The bulk soil and aggregate samples were passed through 0.25 mm sieves to measure OC, total N and total P concentrations. Soil OC and N concentrations were measured using the Walkley–Black method and the Kjeldahl method, respectively (Page et al., 1982). The soil total P concentration was measured colorimetrically after digestion with $HClO_4$-H_2SO_4. Soil particle composition was measured using the sieve pipette method (Day, 1965). Soil pH was measured in a soil:water (1:5) extract with a pH meter. Mineral N was measured using a continuous flow analyzer (AutoAnalyzer-AA3, Seal Analytical, Norderstedt, Germany) after extraction with 2 mol L^{-1} KCl.

2.4. Soil incubation

To determine soil OC mineralization, we incubated approximately 10 g of bulk soil and aggregate size class samples in 250-ml jars. Other studies showed that 10 g of soil could be used to study soil OC mineralization (Fierer and Schimel, 2002; Gentsch et al., 2018; Yu et al., 2012; Zhao et al., 2019). The soil water content was adjusted to 60% of the field water-holding capacity (WHC) by adding deionized H_2O. We had six replicates of the blank (without soils). The mouth of the jars was coated with silicone to prevent air leakage. All samples were pre-incubated for 5 days at 25 °C and 15 °C to activate microorganisms and to minimize the “pulse effect” (Fierer and Schimel, 2002; Liu et al., 2017) or to remove the flush of OC mineralization caused by rewetting.

After preincubation, the bulk soil and aggregate samples were incubated under dark conditions for 83 days at their initial incubation temperatures. The gases in the jars of soil samples were extracted with a 1 mL gas-tight syringe after 2, 4, 7, 14, 21, 28, 35, 42, 49, 56, 63, 76, and 83 days of incubation and were immediately injected into a buffer volume of CO_2-free air flowing into an infrared gas analyzer (LI-6262 CO_2/H_2O Analyzer, LI-COR Biosciences, Lincoln, NE) to measure the CO_2 concentration. After each sampling, the glass jars were opened and the headspace was flushed with atmospheric air for approximately 20 min to allow the replenishment of O_2 and a uniform starting condition of air composition. Moreover, soil moisture was measured by weighing the jars and adjusting them to their initial value (60% WHC). After the lids were tightly reclosed, the CO_2 concentration was immediately recorded again as the initial value for the next incubation, and then the sealed glass jars were placed in the incubator and allowed to continue incubating. The soil in Fu County contained a large amount carbonate, which increased after afforestation (Jia et al., 2019). However, the increased soil inorganic carbon was stable in this arid and semiarid area, which had a small exchange with the atmosphere and a long turnover time (Jia et al., 2019; Raheb et al., 2017). Therefore, in our study, the short-term laboratory incubation experiment suggested that the released CO_2 all originated from organic carbon.

2.5. Calculations

We used a first-order model (natural growth equation) to fit the OC mineralized over time (Wang et al., 2014) as follows:

$$C_t = C_p \times (1 - e^{-k\times t})$$

where C_t is the cumulative mineralized OC (g kg^{-1} soil or aggregate) at time t (day), which was calculated as the sum of OC mineralized at each sampling time; C_p is the potentially mineralized OC (g kg^{-1} OC); k is the rate constant of OC mineralization (day^{-1}), indicating the potential mineralization rate; and specific OC mineralized (C_m) was calculated as the percent of C_t relative to the initial OC concentration.

We used the relative temperature sensitivity of OC mineralization (Q_{10}) to evaluate the response of OC mineralization to temperature changes. Q_{10} was calculated by dividing the time taken to mineralize a given amount of soil OC (0.5 g kg^{-1} soil or aggregate, which is the minimum amount of OC mineralized in this study) at 15 °C (t_{15}) by the time taken at 25 °C (t_{25}) (Conant et al., 2008; Wei et al., 2016). The process of drying, wet sieving, and drying again may disturb the OC composition and microbial activity of soils, making the result different from natural soils; however, due to the comparative nature of this work and the uniform analysis of all soils as well as the calculation of Q_{10} values from rate constants derived from a longer incubation period (83 days) rather than from instantaneously derived activities at different temperature intervals (Reichstein et al., 2000), this approach is applicable (Yu et al., 2012).

We used the proportion of OC mineralized in each aggregate to the predicted OC mineralized in bulk soils to express the contribution of each aggregate to OC mineralization in bulk soils as follows:

$$f_i = \frac{C_{mi} \times W_i}{\sum C_{mi} \times W_i} \times 100$$

C_m is the OC mineralization in aggregate size class i. W_i is the aggregate proportion (%) in aggregate size class i.

2.6. Statistical analysis

All statistical analyses were conducted using JMP software (version 10.0, SAS Institute, Cary, USA). First, we tested the data for normality and homogeneity of variance. Then, two-way analysis of variance (ANOVA) was used to determine the direct and interaction effects of

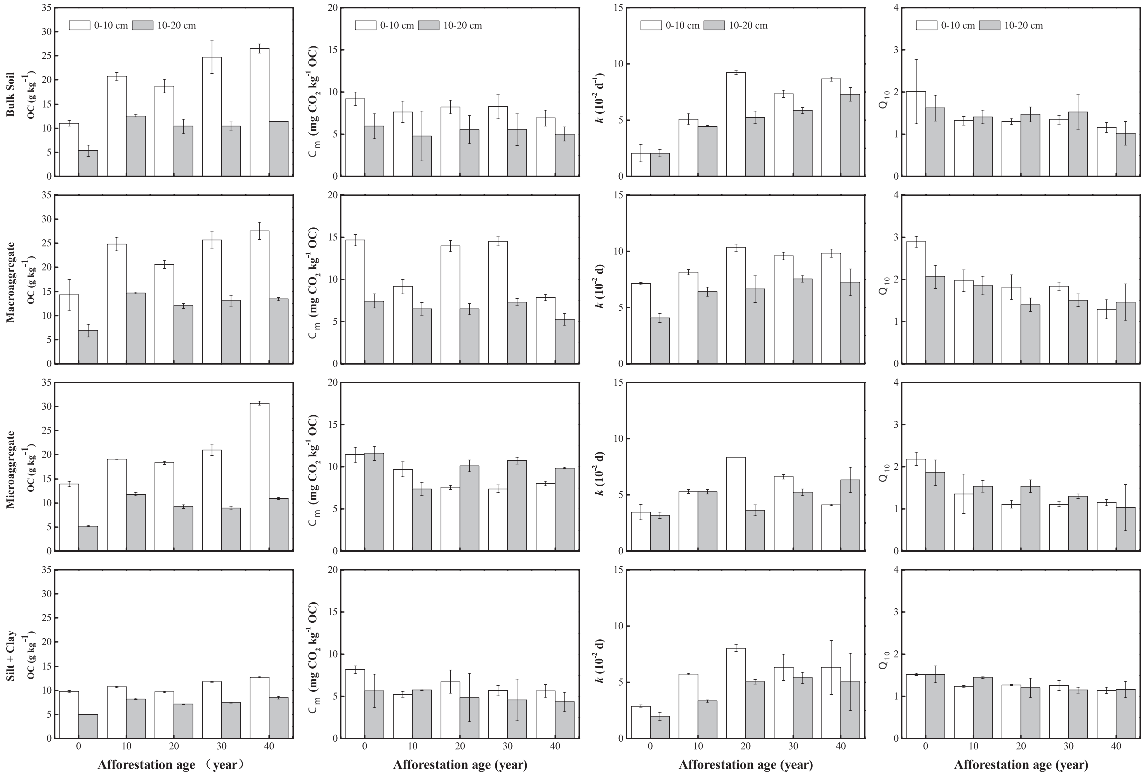

Fig. 1. Effects of afforestation and soil depth on the organic carbon content, mineralizationand their temperature sensitivityin bulk soils and aggregatesat 0–10 cm and 10–20 cm soil depth. Afforestation age 0 means the farmland. OC: organic carbon content; C_m: cumulative OC mineralization; k: rate constant of OC mineralization; Q_{10}: the temperature sensitivity of OC mineralization; BS: bulk soil; MA: macroaggregates; MI: microaggregates; SC: silt + clay. Error bars are two standard errors of the means.

Table 2
The two-way ANOVA analysis was carried out to test the direct and interactive effects of afforestation (A) and soil depth (D) on the OC content, cumulative OC mineralization (C_m), rate constant of OC mineralization (k), the contribution of each size class to OC mineralization in bulk soils (f), and the temperature sensitivity (Q_{10}).

Variations	Afforestation		Depth		D × A	
	F	P > F	F	P > F	F	P > F
OC-BS	20.19	**<0.0001**	137.54	**<0.0001**	4.73	**0.0087**
OC-MA	8.18	**0.0006**	127.58	**<0.0001**	2.05	0.1300
OC-MI	14.25	**<0.0001**	56.39	**<0.0001**	4.63	**0.0096**
OC-SC	16.85	**<0.0001**	0.05	0.8189	5.98	**0.0030**
C_m-BS	6.83	**0.0109**	17.50	**0.0001**	0.69	0.6027
C_m-MA	2.85	**0.0060**	12.13	**0.0011**	1.35	**0.2647**
C_m-MI	7.60	**0.0125**	13.80	**0.0376**	2.42	0.0620
C_m-SC	3.35	**0.0173**	21.88	**<0.0001**	1.18	0.3314
k-BS	80.34	**<0.0001**	28.65	**<0.0001**	14.46	**<0.0001**
k-MA	17.31	**<0.0001**	78.56	**<0.0001**	0.51	0.7272
k-MI	62.63	**<0.0001**	3.42	0.0808	1.20	0.3437
k-SC	10.85	0.0001	0.34	0.5668	1.67	0.1997
f-MA	0.58	0.6764	0.08	0.7731	2.37	0.0661
f-MI	4.21	**0.0055**	11.69	**0.0013**	2.26	0.0762
f-SC	3.35	**0.0173**	14.72	**0.0004**	0.65	0.6307
Q_{10}-BS	1.06	0.4050	1.42	0.2497	1.64	0.2083
Q_{10}-MA	4.86	**0.0078**	0.01	0.9119	0.60	0.6661
Q_{10}-MI	3.50	**0.0277**	2.14	0.1609	0.67	0.6251
Q_{10}-SC	15.91	**<0.0001**	4.01	0.0606	4.78	**0.0083**

BS: bulk soils, MA: macroaggregates, MI: microaggregates, SC: silt + clay.

afforestation and soil depth on the OC contents and mineralization and its temperature sensitivity in all samples. Three-way analysis of variance (ANOVA) was also applied to assess the effects of aggregate size class, afforestation and soil depth on OC contents and mineralization associated with aggregates. Regression analysis was conducted to analyze the relationship between different parameters in all samples. In all comparisons, $P = 0.05$ was used as the significance level.

3. Results

3.1. OC contents in the bulk soils and aggregates after long-term afforestation

Afforestation and soil depth and their interaction significantly affected the OC content in the bulk soil (Fig. 1, Table 2). In general, afforestation on abandoned farmland could result in OC accumulation, and the accumulation increased as the forest stand age increased. These increases were greater at the 0–10 cm soil depth than at the 10–20 cm soil depth. When averaged across all soil depths, the respective of OC increased by 70%, 88%, 124% and 141% at the 0–10 cm soil depth and by 135%, 96%, 97% and 114% at the 10–20 cm soil depth after 10, 20, 30 and 40 years of afforestation establishment, with accumulation rates of 0.39 g OC kg^{-1} yr^{-1} and 0.15 g OC kg^{-1} yr^{-1} in the two soil layers.

The OC content varied greatly with aggregate size ($P < 0.0001$, Table 3), increased with increasing aggregate size and was highest in the coarse fractions (MA). The OC content in a given aggregate was affected by the direct and indirect effects of soil depth and afforestation (Table 2). 40 years of afforestation resulted in 94%, 115% and 50% increases in the MA, MI and SC fractions, respectively. Furthermore, the OC contents in a given aggregate, particularly MA and MI, were higher at the 0–10 cm soil depth than at the 10–20 cm soil depth (Fig. 1).

3.2. OC mineralization in the bulk soils after long-term afforestation

C_m in the bulk soil was affected directly by afforestation and soil depth, but the k value predicted by the first-order model was affected by the direct or indirect effects of afforestation and soil depth (Table 2). Our results showed that afforestation on abandoned farmland significantly decreased the C_m in the bulk soils at all soil depths (Fig. 1), with 22%, 10%, 10% and 27% decreases, after 10, 20, 30 and 40 yrs of afforestation, respectively, and the k value was between 2.06 and 9.22 10^{-2} d^{-1}, indicating that long-term afforestation could decrease OC mineralization and therefore decrease CO_2 emissions. The decrease in C_m after afforestation varied with soil depth ($P = 0.0001$, Fig. 1), with greater decreases at the 0–10 cm depth (+0.96 to + 2.30 g CO_2 kg^{-1}) than at the 10–20 cm depth (+0.40 to + 1.18 g CO_2 kg^{-1} OC). The k value was higher at the 0–10 cm soil depth (2.06–9.22 10^{-2} d^{-1}) than at the 10–20 cm depth (2.05–7.30 10^{-2} d^{-1}), and its response to afforestation was affected significantly by soil depth ($P < 0.0001$, Table 2).

3.3. OC mineralization in the water-stable aggregates after afforestation

The mineralization of OC varied greatly with aggregate size fraction across or within afforestation and soil depths (Table 3). When averaged across all afforestation ages and soil depths, the MA fraction had a higher C_m of OC; however, the C_m was lower in the MI and SC fractions. Similarly, the k in the MA fraction was higher but was lower in the MI and SC fractions ($P < 0.0001$; Fig. 1).

In addition, the C_m in each aggregate fraction was affected by afforestation and soil depth or the interaction between these two factors (Fig. 1, Table 2). After 10, 20, 30 and 40 yrs of afforestation, C_m decreased by 35%, 30%, 27%, and 29%, respectively, in the MA fraction, by 26%, 19%, 34%, and 38%, respectively, in the MI fraction, and by 41%, 8%, 2%, and 69%, respectively, in the SC fraction, across the 0–20 cm soil profile, with the highest C_m occurring in the abandoned farmland. Moreover, the OC mineralization in aggregates varied with soil depth after afforestation ($P < 0.05$, Table 2, Fig. 1). Usually, C_m in the MA, MI and SC fractions was 13%, 25% and 82% higher, respectively, at the 0–10 cm soil depth than at the 10–20 cm soil depth. The k values for the MA and MI fractions were affected by afforestation and soil depth, but those for the SC fraction were not affected by the direct or indirect effects between the two factors ($P < 0.05$, Table 2, Fig. 1). Compared to abandoned farmland, the k values in the MA, MI and SC fractions in afforested soil were 68%, 47% and 39% higher, respectively. In addition, the k values of the MA, MI and SC fractions were 15%, 14% and 5% higher, respectively, at the 0–10 cm depth than at the 10–20 cm soil depth.

Table 3
The three-way ANOVA analysis was carried out to test the direct and interactive effects of aggregate and afforestation and soil depth on organic carbon content (OC), cumulative mineralization (C_m), rate constant of OC mineralization (k); and the temperature sensitivity of OC mineralization (Q_{10}).

Variations	OC		C_m		k		Q_{10}	
	F	F > P	F	F > P	F	F > P	F	F > P
Aggregate	397.71	**<0.0001**	36.41	**<0.0001**	153.56	**<0.0001**	22.45	**<0.0001**
Afforestation	14.39	**<0.0001**	10.19	**<0.0001**	55.91	**<0.0001**	5.84	**0.0010**
Depth	170.67	**<0.0001**	0.84	0.3668	65.71	**<0.0001**	1.39	0.2581
Aggregate × Afforestation	3.14	**0.0260**	1.53	0.2139	13.45	**<0.0001**	0.44	0.5100
Aggregate × Depth	70.87	**<0.0001**	12.53	**<0.0001**	33.82	**<0.0001**	2.06	0.1603
Afforestation × Depth	3.67	**0.0132**	1.67	0.1773	1.04	0.4011	0.75	0.5661
Aggregation × Afforestation × Depth	0.93	0.4575	1.74	**0.0520**	0.52	0.7241	0.57	0.6859

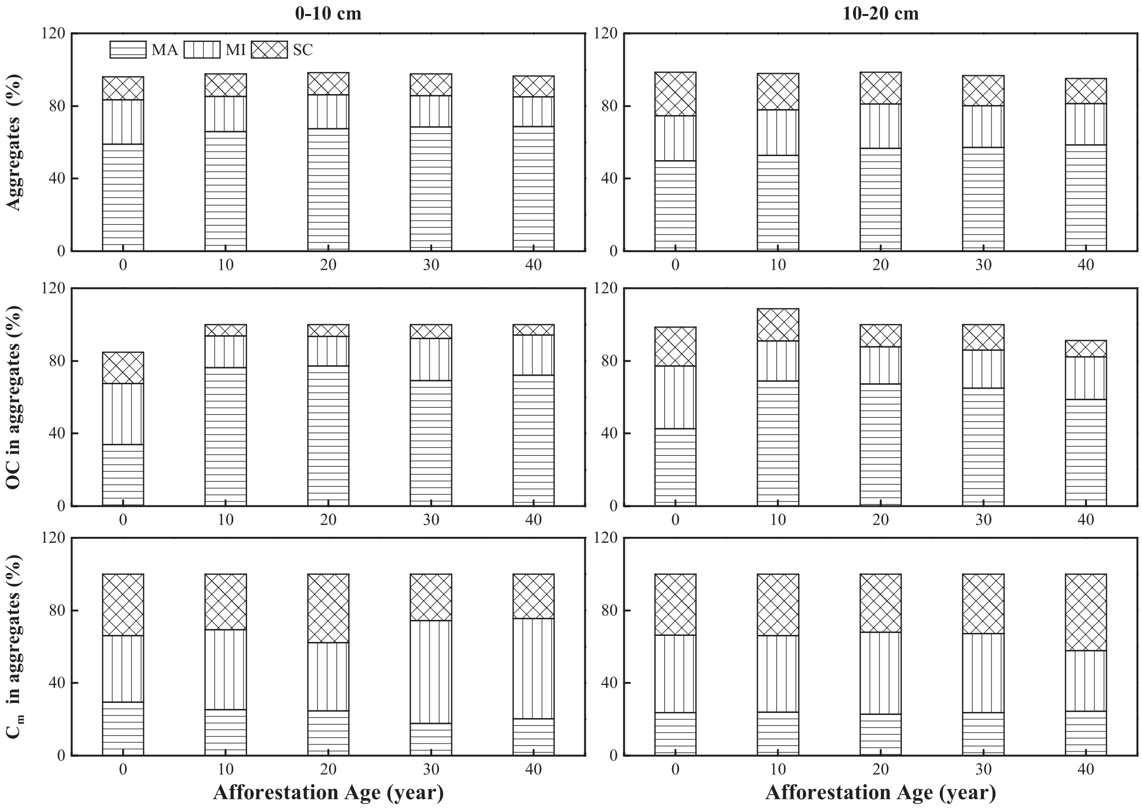

Fig. 2. The contribution of each size class to OC content and mineralization in bulk soils. MA: macroaggregates; MI: microaggregates; SC: silt + clay. Error barsare two standard errors of the means.

3.4. *The contribution of the OC content and mineralization in aggregates to bulk soils*

When averaged across afforestation age and soil depth, MA contributed most of the OC; for example, the OC content in MA, MI and SC accounted for 63%, 21% and 16%, respectively ($P < 0.05$, Fig. 2). Throughout the whole soil, the proportion of OC mineralized was highest in the MI fraction (40%, $P < 0.05$, Fig. 2), indicating that MI contributed most of the OC mineralized in the bulk soil. The effect of afforestation and soil depth on the contributions of OC mineralized in the MI and SC fractions to OC mineralized in bulk soils was significant ($P < 0.05$, Table 2). However, afforestation and soil depth did not affect f_{MA} ($P > 0.05$, Table 2, Fig. 2). All afforestation treatments decreased f_{MA} but increased f_{MI} and f_{SC} across the two soil depths. These results suggested that afforestation has strong potential to change contribution of the aggregates to OC mineralization in bulk soils.

3.5. *Temperature sensitivity of soil OC mineralization*

Long-term afforestation had no effect on the temperature sensitivity of OC mineralization in the bulk soils ($P = 0.4074$, Table 2), but compared to the abandoned farmland, the afforested bulk soil had a lower Q_{10} of C_m (Fig. 1), indicating that OC dynamics in the abandoned farmland were more sensitive to the temperature change. The Q_{10} of bulk soils was not affected by soil depth and was similar at the 0–10 cm (1.43 ± 0.63) and 10–20 cm soil depths (1.41 ± 0.28).

The Q_{10} of C_m varied with aggregate size and was significantly affected by afforestation ($P < 0.0001$, Table 3). When comparing the difference in the Q_{10} of C_m for each aggregate size, we found that Q_{10} changed in the following order: MA (1.81 ± 0.44) > MI (1.42 ± 0.35) > SC (1.31 ± 0.14). MA had the highest temperature sensitivity of C_m (Fig. 1). Similarly, the MA, MI and SC fractions had lower Q_{10} values of C_m in the afforested soil than in the abandoned farmland. Soil depth had no effect on the temperature sensitivity, and Q_{10} for MA was higher at the 0–10 cm (1.96 ± 0.51) soil depth than at the 10–20 cm soil depth (1.65 ± 0.26) but was higher at the 10–20 cm soil depth (1.45 ± 0.28) than at the 0–10 cm soil depth (1.38 ± 0.41) for MI and was similar between the two soil depths for SC.

4. Discussion

Examining the contents and mineralization of OC and its temperature sensitivity of bulk soils and aggregates is essential for accurately predicting OC dynamics and storage in forest soils in the context of global warming. We demonstrated that long-term afforestation increased OC sequestration but decreased OC mineralization and temperature sensitivity, verifying the first hypothesis. Macroaggregates contributed most of the OC content (63%), but microaggregates contributed most of the OC mineralized in the bulk soil (40%), refuting the second hypothesis, suggesting an increase in sequestration of OC in fine soil particles. These results contributed important information for evaluating soil C sequestration and its response to warming scenarios in this region.

4.1. *Increased OC accumulation but reduced CO_2 emissions in the bulk soils and aggregates after long-term afforestation*

Our results demonstrated that long-term afforestation increased OC accumulation but decreased mineralization loss (Fig. 1, Table 2) in bulk soils and aggregates. The accumulation of soil OC was mainly due to the greater input of organic matter (including litter, above- and belowground biomasses and exudates) (Markewitz et al., 2002; Tian et al., 2016; Rumpel and K€ogel-Knabner, 2011) and the higher abundance and diversity of microbial communities (Chavarria et al., 2018; Li et al., 2018) during afforestation, which provides a direct resource for mineralization (Chen et al., 2017; Jandl et al., 2007; Nadal-Romero

Table 4
The changes in aggregates proportion and the ratios of organic carbon to total nitrogen (C/N) in aggregate after afforestation at two soil depth. MA: macroaggregates; MI: microaggregates; SC: silt + clay.

Afforestation (yrs)	0		10		20		30		40	
Depth (cm)	0–10	10–20	0–10	10–20	0–10	10–20	0–10	10–20	0–10	10–20
MA (%)	68.44 ± 4.79	56.65 ± 7.44	65.79 ± 2.06	58.42 ± 1.87	68.68 ± 3.62	57.09 ± 4.08	58.93 ± 1.50	52.77 ± 6.37	67.54 ± 1.78	49.84 ± 3.62
MI (%)	17.24 ± 2.49	24.71 ± 4.94	19.42 ± 0.73	23.10 ± 1.91	16.38 ± 1.95	22.78 ± 3.91	24.41 ± 2.60	25.16 ± 3.36	18.53 ± 0.64	24.43 ± 2.26
SC (%)	12.32 ± 2.16	16.62 ± 3.37	12.51 ± 1.37	13.92 ± 2.63	12.46 ± 2.00	17.54 ± 1.21	13.88 ± 2.23	19.89 ± 3.43	11.51 ± 1.17	23.92 ± 1.72
C/N_{MA}	10.10 ± 0.12	10.05 ± 0.32	10.76 ± 0.34	10.70 ± 0.06	10.04 ± 0.30	9.80 ± 0.21	11.00 ± 0.23	9.82 ± 0.64	10.92 ± 0.27	9.93 ± 0.44
C/N_{MI}	10.08 ± 0.24	8.97 ± 0.87	10.59 ± 0.05	10.15 ± 0.03	10.09 ± 0.27	9.43 ± 0.32	10.48 ± 0.42	9.14 ± 0.11	10.98 ± 0.21	10.19 ± 0.17
C/N_{SC}	8.89 ± 0.15	7.87 ± 0.27	8.75 ± 0.01	8.54 ± 0.02	8.74 ± 0.12	8.42 ± 0.12	9.09 ± 0.22	8.35 ± 0.21	9.37 ± 0.17	8.31 ± 0.02

Table 5
The relationship ($Y = b + a \times \ln(X)$) between cumulative mineralization (C_m), rate constant of OC mineralization (k) and soil OC content, the ratio of OC and N within bulk soils and aggregates across soil depths and afforestation age.

		Bulk soils and aggregates (n = 112)			Bulk soils (n = 28)			Macroaggregates (n = 28)			Microaggregates (n = 28)			Silt + Clay (n = 28)		
X	Y	a	R^2	P	a	R^2	P	a	R^2	P	a	R^2	P	a	R^2	P
OC	C_m	−1.59	0.23	**<0.0001**	−2.38	0.34	**<0.0001**	−2.35	0.33	**<0.0001**	−0.52	0.05	0.2323	−5.34	0.12	**<0.0001**
	k	0.05	0.27	**<0.0001**	0.04	0.31	**<0.0001**	0.05	0.33	**<0.0001**	0.11	0.17	**<0.0001**	−0.04	0.10	**<0.0001**
C/N	C_m	−14.41	0.17	**<0.0001**	−1.09	0.33	**<0.0001**	−0.62	0.24	**<0.0001**	−1.03	0.16	**<0.0001**	−0.01	0.04	0.4436
	k	0.38	0.13	**<0.0001**	0.10	0.03	**<0.0001**	0.26	0.08	0.0786	0.50	0.11	**<0.0001**	−0.01	0.02	0.1105

et al., 2016; Rahman et al., 2017; Schulze, 2014). Moreover, the decreased decomposition of organic matter in afforested soil was conducive to soil organic carbon accumulation, as indicated by the increased soil C/N ratio (Tables 1 and 4). These results are consistent with previous studies in various regions (Baddeley et al., 2017; Zhang et al., 2018, 2020). The variations in OC mineralization among the afforestation ages could likely be a result of the difference in the biodegradability of OC, determined by the quality and quantity of mineralized substrates. Long-term afforestation resulted in significant variations in the OC content, C/N ratio in bulk soil and aggregates (Tables 1 and 4), and degradability of leaves and litter and therefore in the quality and quantity of the soil substrates for microbial decomposition. This explanation was supported by the decreases in C_m with soil OC and the C/N ratio increases (Table 5) among most soils in our study. C_m decreased with increasing soil OC content, indicating that the proportional quantity of substrates for microbial decomposition decreased with soil OC content. This might occur because a higher OC content promotes soil aggregation (in our study, the proportion of macroaggregates showed an increasing trend due to the cessation of interference activities, including tillage, fertilizer and agricultural machines in the afforested soil, Table 4), which could physically decrease the mineralization loss of OC due to microbial decomposition. The quality of substrates generally differs between abandoned farmland and forest, as indicated by the increased C/N ratios in the bulk soils and aggregates along the afforestation chronosequence (Tables 1 and 4). The leaf litter and roots of trees generally have higher C/N ratios and more recalcitrant C components than the litter from farmland. Hence, soil organic matter derived from farmland litter is considered to decompose more readily than that derived from forest litter. C_m (g CO_2 kg^{-1} OC) decreased with increasing soil OC content and C/N ratio during afforestation, which suggested that the stability of soil OC increased along the afforestation chronosequence and therefore had lower mineralization. However, the effects of the OC content rather than the C/N ratio on C_m were larger, because the coefficient of determination (R^2) between OC and C_m was higher than that between C/N and C_m in most soils (Table 5).

It was expected that OC mineralization varied with aggregate size (Fig. 1) because the content, chemical composition and stability of the organic matter varied among different aggregates (Cookson et al., 2005; Wei et al., 2017; Zhao et al., 2014). Based on a comparison of MA and MI, SC had the lowest OC mineralization and k value (Fig. 1), indicating that OC was more labile and susceptible to decomposition in the coarse fraction. Coarse aggregates (e.g., MA) usually contain enriched labile SOM (fresh OC, O/N-alkyl C, lignin, and hemicellulose), which has a faster turnover rate and is easier to be mineralized (Arevalo et al., 2012; Nadal-Romero et al., 2016; Steffens et al., 2009; Six et al., 2000; Wu et al., 2012; Yu et al., 2012). Therefore, k increased with increasing aggregate size and was highest in the coarse fractions (Fig. 1, Sainju et al., 2009). This finding is consistent with the current understanding that the stability of OC in aggregates increases as aggregates size decreases (Six et al., 2000) and concurs with previous findings (Yu et al., 2012). In contrast to expectation, higher C/N and k for the MA and MI fractions were observed in our study (Table 4), implying that the OC mineralization in the coarse fraction is thus controlled by the decomposition of labile OC, and OC mineralization in the fine fraction is controlled by the decomposition of relatively stable OC (Yu et al., 2012).

In addition, we also found that the mechanism that drives OC sequestration with afforestation age could be due to the temporal dynamics of the input and decomposition of litter (Clark et al., 2012). For example, the OC content in most soils showed remarked increases in the first 10 yrs of afforestation at the two soil depths because the litter in the forests was in direct contact with the soil and the input of organic matter into the soil was comparatively greater than the decomposition, thus leading to higher OC from plant litter (Wainkwa Chia et al., 2017). The OC content at the 0–10 cm depth in most soils was lower at the 20 yrs afforestation site than at other sites, which was related to the highest OC mineralization rate (k, Fig. 1). However, the OC content at 10–20 cm showed a slight decreasing trend after afforestation for 20 yrs, perhaps because of the decreased newly input OC rate and increased k after 20 yrs of afforestation. OC mineralization also shows temporal dynamics; for example, Rahman et al. (2017) reported that soil OC mineralization increased with time during 45 years of afforestation and that lower mineralization occurred in a 200-yr-old forest in western Copenhagen, Denmark. In the initial stages of afforestation, OC mineralization is determined by the OC concentration due to the greater input of organic matter into the soil than decomposition. In an older afforested ecosystem, the soil C/N ratio determines OC mineralization, because the rate of newly input OC would thus be decreased, and the soil pH and organic matter reach the levels more common in forest ecosystems (Ritter et al., 2003). Therefore, our results suggested that the increased OC in older forests was stable, thereby leading to lower OC mineralization.

Higher OC mineralization in bulk soils and aggregates was observed at the 0–10 cm depth than at the 10–20 cm depth, which has been widely reported worldwide (Kemmitt et al., 2008; Rumpel and Kögel-Knabner, 2011; Tian et al., 2016). This result was expected because most processes affecting soil particle aggregation and OC mineralization (e.g., microbial activity, litter and root inputs, and land-use changes) are more active in topsoil. Compared to the deeper soil layer, greater and more labile litterfall and root inputs, which decompose easily, remain in the upper soil layer after afforestation (Jandl et al., 2007; Nadal-Romero et al., 2016; Rahman et al., 2017; Schulze, 2014; Wild et al., 2015), hence accelerating soil OC mineralization. In addition, C resources and energy deficiency in subsoils strongly affect soil OC availability and the demand for microbial OC, thereby limiting the mineralization rate (Fierer et al., 2003; Fontaine et al., 2007; Karhu et al., 2016; Tian et al., 2016; Wild et al., 2015).

4.2. *The temperature sensitivity of OC mineralization*

In our study, long-term afforestation decreased the Q_{10} of OC mineralization in the bulk soils ($P = 0.4050$) and aggregates ($P < 0.5$). Substrate quality and availability are known to influence the temperature sensitivity of OC mineralization. Compared to farmland, the long-term afforested soils had a lower Q_{10} of OC mineralization, indicating that OC in abandoned farmland would be sensitive to a predicted increased in temperature (Tian et al., 2016). This result is supported by the theory (Arrhenius, 1889) that a stronger temperature dependency of mineralization of low-quality substrates due to average substrate quality generally in abandoned farmland decreased as a result of the enrichment of humified, recalcitrant organic substrates and declining production of easily decomposable compounds. In addition, due to substrate limitations in abandoned farmland (lower OC content and availability), OC had higher temperature sensitivity. The temperature sensitivity of OC mineralization in older forests showed a declining trend in most soils, which might be related to higher organic matter contents. Together, the quality and availability of substrates influenced the temperature sensitivity of organic matter decomposition in the field. In our study, the interaction between the OC content and C/N ratio determined the change in the Q_{10} of OC mineralization based on the three-dimensional scatter gram (Fig. 3, $P < 0.05$).

The mineralization rate increases with temperature, but the effects of temperature on substrate availability and the ability of microorganisms and enzymes to use substrates vary with aggregate size class, resulting in a difference in Q_{10} of OC mineralization among aggregates. In our study, higher Q_{10} values of OC mineralization were observed for MA (Fig. 1). This was expected because most labile OC is physically occluded in macroaggregates (Six et al., 2000), and the availability of such OC to decomposers is not affected by temperature. However, the activities and abundances of microorganisms and enzymes in macroaggregates increase with temperature (Price and Sowers, 2004), which led to the higher Q_{10} in macroaggregates among the three size classes. The association of substrates with fine particles and the adsorption of microorganisms or enzymes on fine particles increases with temperature (Kim et al., 2014), leading to a smaller increase in C_m with temperature and thus a lower Q_{10} compared to other aggregates.

4.3. *Implications for forest soil C stabilization and future global change*

Experiments on OC mineralization and its temperature sensitivity have been conducted in different ecosystems, but the effects of long-term afforestation on C_m and Q_{10} in both bulk soils and aggregates have not been well examined to our knowledge. Our study therefore provides experimental evidence that long-term afforestation decreases OC mineralization and temperature sensitivity. These results suggested that long-term afforestation would accelerate OC accumulation and decrease OC mineralization and thus decrease the release of CO_2 from soils under future climate warming scenarios relative to abandoned soils, because soil OC mineralization provides positive feedback to climate warming. Increased OC contents but decreased C_m in bulk soils and aggregates after afforestation (Fig. 1) suggested that increased OC in forest was stable and was not susceptible to temperature changes. Macroaggregates contributed the highest OC content in bulk soil (60%), but the microaggregates and silt + clay contributed most of the OC mineralized in the bulk soil ($f_{MI} = 40\%$, $f_{SC} = 36\%$), increased the OC content and decreased C_m after long-term afforestation in aggregates, suggesting an increase in the sequestration of OC in fine soil particles. The findings in this study provide new insights into the response of soil OC mineralization and its temperature sensitivity to land-use change on the Loess Plateau, contributing important information for evaluating soil C sequestration and its response to warming scenarios in this region.

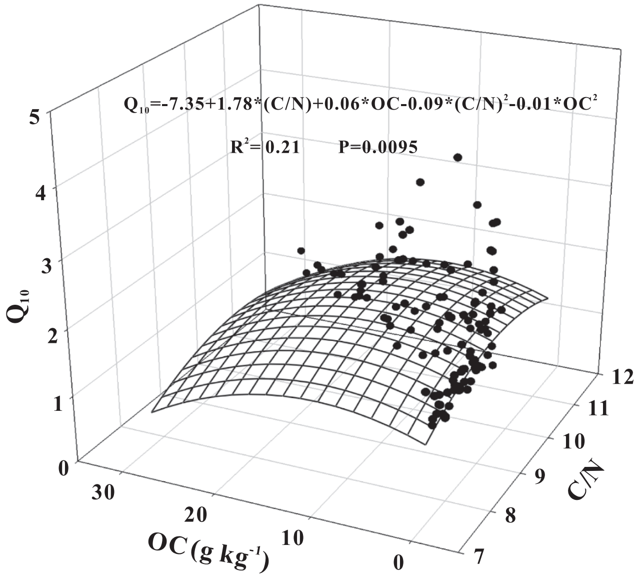

Fig. 3. Binary relationship between OC, C/N, and Q_{10}. OC: soil organic carbon content; C/N: the ratios of soil organic carbon to total nitrogen; Q_{10}: temperature sensitivity of organiccarbon mineralization (Cm).

5. Conclusion

In our study, we examined the changes in the OC content and its mineralization and temperature sensitivity in bulk soils and aggregates after long-term afforestation on abandoned farmland. We found that long-term afforestation accelerated soil OC accumulation and decreased OC mineralization and temperature sensitivity in bulk soils and aggregates, and the effects were higher at the 0–10 cm depth than at the 10–20 cm depth. Macroaggregates contributed most of the OC content (62%), but microaggregates contributed most of the OC mineralized (40%) in the bulk soil. The increased OC content and decreased C_m in aggregates suggested an increase in the sequestration of OC in fine soil particles. The temperature sensitivity of OC mineralization increased with increasing particle size, with a higher Q_{10} value for macroaggregates (1.81 ± 0.44) than for microaggregates (1.42 ± 0.35) and silt + clay (1.31 ± 0.14). We concluded that long-term afforestation would accelerate OC accumulation and decrease the release of CO_2 from soils in future scenarios of climate warming relative to abandoned soils. C_m and its temperature sensitivity under the rehabilitated ecosystems were controlled mainly by the availability (e.g., OC content) and quality (e.g., the ratio of OC to total N) of substrates. This study contributed to bridging current knowledge gaps about the processes underlying the observed OC budget and its response to warming scenarios in rehabilitated ecosystems.

Declaration of Competing Interest

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

Acknowledgements

The authors thank Drs. Xuezhang Li and Qingyin Zhang for their help in field sampling. This study was supported by the Strategic Priority Research Program of the Chinese Academy of Sciences (XDA23070200, XDB40000000, XDB40020100), the National Natural Science Foundation of China (41571296, 41622105, 41571130082), the Program from Chinese Academy of Sciences (QYZDB-SSW-DQC039), and the Program from Northwest A & F University (2452017028).

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.catena.2021.105405.

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