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Changes in soil organic carbon and its fractions under grassland reclamation in alpine-cold soils, China

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Abstract: Grasslands are the main land use types in China, but their reclamation into croplands can influence the terrestrial carbon and, consequently, impact the global carbon balance. The long-term reclamation of alpine-cold grasslands to croplands are expected to decrease the soil organic carbon (SOC) and its fractions. Here, we conducted an *in situ* systematic study to measure the SOC and its fraction in soils sampled in an alpine-cold grassland with a gradient of cultivation history from 0 to 40 years. The SOC and its fractions significantly decreased after reclamation ($P < 0.05$), and the changes in the 0–20 cm soil layer were the greatest among the three sampling depths. After 40 years of reclamation, the SOC content and storage at 0–20 cm decreased by 74 and 60%, respectively. The decreases in the soil labile carbon fractions were more rapid and apparent than the SOC, especially the particular organic carbon (POC), which decreased by 82%. The soil humus carbon fractions also decreased, particularly the humic acid carbon (HAC), which decreased by 81%. The reduction rates of SOC and its fractions gradually decreased with an increase in the cultivation history. Besides, the ratios of the optical densities or absorbances of humic acid (HA) and fulvic acid (FA) solutions at 465 and 665 nm (E_4/E_6 ratios) and the hue coefficient (Δlog K values) which is the logarithm disparity between the 400 and 600 nm absorbance of the HA (FA) substance in the solution gradually decreased, indicating that the quality of the soil humus decreased. The reclamation significantly decreased the SOC and its fractions in the alpine-cold soils, which should not be underestimated in the impact on the terrestrial carbon cycles and balance in the long run.

Keywords: land use change; Qinghai-Tibet Plateau; soil carbon; soil humus carbon; soil labile carbon

Soil organic carbon (SOC) is a measurable component of the soil organic matter, which results from the interplay of the net primary producers, decomposers, and mineralogy (Horwath & Kuzyakov 2018). The dynamics of the SOC can influence the bio-physiochemical processes that govern the sustainability of food production in agroecosystems. Based on the vulnerability to decomposition, SOC is generally divided into more sensitive labile fractions and stable organic fractions. The sensitive labile carbon fractions include the microbial biomass carbon (MBC), dissolved organic carbon (DOC), particulate organic carbon (POC), and easily oxidised organic carbon (EOC). MBC plays an important role in soil organic carbon dynamics and is an important indicator in detecting the soil carbon stability and nutrient dynamics after soil management practices (Liu et al. 2016). Although DOC only accounts for

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< 0.25% of the total organic carbon, the water-soluble characteristics of DOC makes it readily available to microorganisms and, thus, has an important impact on organic carbon cycling in terrestrial ecosystems (Liu et al. 2021). POC is an important carbon pool in soils, mainly composed of structural plant (i.e., lignin) and fungal matter (Rocci et al. 2021; Witzgall et al. 2021). As a soil unstable carbon component, EOC is fast-moving, unstable, easy to decompose, and is easily affected by plants and microorganisms (Cao et al. 2021). The sensitive labile carbon fractions are very sensitive to changes in the soil environment and are, therefore, often used to indicate the soil organic carbon pool dynamics (Li et al. 2010, 2012; Zhang et al. 2018). Besides, the stable soil humus carbon fractions are major components of SOC, such as humic acid carbon (HAC), fulvic acid carbon (FAC) and humin carbon (HUC), which are critical for stabilising the SOC pool (Navarrete et al. 2010; Andreas & Zhang 2014; Wagner et al. 2016).

Soil is the largest terrestrial carbon (C) reservoir, which stores approximately 2 344 Gt of organic carbon that is as many as 3.5 times greater than the C in all living plants and about 2.3 times higher than the atmospheric CO_2-carbon (Lal 2004; Yang et al. 2019). Currently, approximately 1.2 billion acres of soils are being transformed by humans for food, fibre and livestock production (Sanderman et al. 2017), which has induced substantial losses of carbon from soils globally. The prolonged agricultural cultivation could account for 20–67% of the soil C loss mainly due to the promotion effects on the decomposition of soil organic matter in the field (Davidson & Ackerman 1993; Lal 2001; Wei et al. 2014). The current global annual CO_2 emissions from agricultural soils can be as much as 13 times higher than the other terrestrial sources (Sanderman et al. 2017), so even a minor change of SOC and its fractions caused by the intensive use of land resources could impact the global C balance (Grace et al. 2014; Li et al. 2017; Hu et al. 2017b; Shi et al. 2018).

The unsustainable reclamation of grasslands to agricultural lands is a common practice for increasing food production and economic growth all over the world. Grasslands cover about one-quarter of the earth's land surface, in which the stored C accounts for 15 % of the global C pool and sequestrates 89% of the C of grassland ecosystems (Soussana et al. 2010; Wen et al. 2013). The intensive use of grasslands can disturb the processes associated with maintaining the C rich and stability in grasslands, especially the reclamation of grasslands into croplands which makes grasslands lose their active rhizodeposition and high earthworm activity of promoting the formation of stable aggregates. (Six et al. 2002; Jones & Donnelly 2004; Bossuyt et al. 2005). In China, approximately 18% of the current arable lands originated from the cultivation of grassland, which has a great impact on the C cycle and balance especially in the Qinghai-Tibet Plateau. The alpine-cold grasslands there could hold as much as 55% of China's total grassland C (in both vegetation and soils) (Ni 2002; Qi et al. 2007). However, the reclamation of alpine-cold grassland does not receive as much attention as reclamation in (semi) arid areas mainly due to its inaccessible geographic location and harsh climatic conditions. Very little is known about the changes in the SOC and its fractions under long-term grassland reclamation acts in alpine-cold soils. Therefore, this study systematically investigated the changes in the SOC and its fraction at different depths of 0–20, 20–40, 40–60 cm, as affected by the long-term reclamation after 3, 10, 16, 27 and 40 years in alpine-cold grasslands at the eastern edge of the Qinghai-Tibet Plateau, China.

MATERIAL AND METHODS

Study site and land history. The study site is located in the Hongyuan County of Sichuan Province (33°1'N and 102°37'E), at the eastern edge of the Qinghai-Tibet Plateau, China. It covers 19 600 km^2 at an average altitude of 3 400~3 500 m in the upper reaches of the Yellow River. The target Zoige grassland is in this area, which straddles the boundary between Sichuan province and Gansu province (Yan & Wu 2005). This site is cold subhumid monsoon climate characterised by a short spring and autumn, a long winter without a summer. The solar radiation is strong with ~6 194 (MJ/m^2)/year; the duration of the sunshine is 2 417 h/year; (Hu et al. 2017a). The annual mean temperature is 0.7–1.1 °C, and the annual average wind speed is 1.6–2.4 m/s (Hu et al. 2017a). The annual rainfall is about 647–753 mm, with most of the precipitation occurring from May to September (Hu et al. 2017a). The landform of the study area has typical characteristics of a transition from a mountain plain to a hilly plateau. The terrain is relatively flat with a slope of about 6°. The dominant plant species are the perennials *Blysmus sinocompressus*, *Elymus sibiricus*, *Kobresia setchwanensis*, *Leymus secalinus* and *Kobresia capillifolia* (Hu et al. 2017a).

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The vegetables grown in the experimental site were potatoes and Chinese cabbage. The tillage depth of the sample plot was 35 cm, the planting depth was 8 and 5 cm, the row spacing was 50 and 70 cm, and the plant spacing was 25 and 50 cm, respectively. The potatoes were usually planted in April and harvested in July each year. The Chinese cabbage was generally planted in July and harvested in October each year. Due to the fertile soil and high organic matter content of the natural alpine-cold grassland, the local farmers did not apply fertiliser during the agricultural cultivation, so the growth of the crops only relied on the natural fertility of the alpine soil.

Soil sampling and parameters analysis. The soil type is a Histosol (Schad 2016). The parent material is Quaternary river sediment, and there is almost no skeleton in the soil. Soil samples were collected in November 2015. To ensure that the basic conditions (e.g., topography, slope, aspect, soil types, soil parent material, etc.) of the research site were relatively consistent between the different years, the sampling method of the space-for-time substitution was applied. The vegetable lands in the alpine-cold grassland which had been reclaimed for 3, 10, 16, 27 and 40 years were chosen as the treatment groups, and the natural grassland adjacent to the cultivated grassland was taken as the control group (CK), which was uncultivated and without agricultural management measures. Three 10 m × 10 m plots were chosen in each type of alpine-cold grassland after 0, 3, 10, 16, 27 and 40 years of reclamation, and five subplots were randomly selected in each plot for the soil sampling. We collected soil samples at depths of 0–20, 20–40 and 40–60 cm using a drill (10 cm in diameter). The collected soil samples were air-dried after removing any roots and gravel. A portion of the fresh soil sample was used within 2 days to measure the MBC and DOC. The other fresh samples were ground, sieved (0.2 mm) and stored in polyethylene sealed bags for the subsequent measurement for the other soil labile C fractions, soil humus C fractions and physicochemical properties.

The SOC content was analysed by the vitriol acid-potassium dichromate oxidation method, with 0.8 mol/L 1/6 $K_2Cr_2O_7$ at 170–180 °C for 5 min, followed by titration of the digestates with $FeSO_4$ (Lu 2000). The soil HAC and FAC were determined using a Multi 3100 N/C total organic content (TOC) analyser (Analytik Jena, Germany). The soil HUC was determined by potassium dichromate oxidation followed by titration with ferrous ammonium sulfate (Walkley & Black 1934). The soil MBC was determined by the fumigation-extraction method (Vance et al. 1987), fresh soils were fumigated with alcohol-free chloroform for 24 h at 25 °C, extracted with 0.5 M K_2SO_4, and then filtered. The non-fumigated soil was similarly extracted prior to fumigation. The soil MBC was calculated by E_C/k_{EC}, where E_C is the difference between the organic carbon extracted from the fumigated and non-fumigated soils; and k_{EC} is a conversion coefficient (0.45). The soil POC was determined using the sodium hexametaphosphate dispersion method (Six et al. 1999). Briefly, 10 g of an air-dried soil sample was sieved with a 2 mm sieve, added to 30 mL of an $NaPO_3$ solution (concentration of 5.0 g/L), and shaken for 15 h. The soil suspension was then filtered with a 53 μm filter membrane and rinsed repeatedly with distilled water. The remaining material was dried on a filter membrane at 60 °C for 12 h for the particulate carbon fraction determination. The soil DOC was extracted by mixing 2.5 g of field-moist soil with 25 mL of 2 M KCl. We shook the mixed solutions for 15 min, centrifuged (10 min) it to get a supernatant, finally injected the sample to the automated TOC analyser (Multi N/C®3100, Analytik Jena, Germany) for the soil DOC determination (Jones & Willett 2006). The soil EOC was determined as described by Blair et al. (1995). Briefly, 15–30 mg of the air-dried soil was reacted with 333 mmol/L of a $KMnO_4$ solution for 1 h. The soil EOC was obtained from the reduction of $KMnO_4$ determined by a spectrophotometer.

The moist soil sample was oven-dried at 105 °C for 24 h to measure the soil water content (SWC). The soil bulk density (BD) was measured by the cutting ring method. The soil pH (soil : water; 1 : 2.5) was determined by a combination electrode (Chen et al. 2006). The clay, silt and sand contents were determined using the sedimentation method (Kölbl et al. 2014). The particle size distribution of the < 2 mm fraction was determined after pre-treatment with HCl to dissolute the carbonates, with H_2O_2 to remove the organic matter, and $Na_4P_2O_7$ to disperse it. The sand content was obtained via wet sieving with a > 63 μm screen, and the silt and clay contents were determined by the sedimentation rate and X-ray absorption (Sedigraph 5100, Micromeritics GmbH, Germany). The ratios of the optical densities or absorbances of the humic acid (HA) and fulvic acid (FA) solutions were measured at 465 and 665 nm (E_4/E_6 ratios) on a Beckman model B spectrophotometer (Chen et al. 1977), respectively. The Δlog K values were the hue coefficient, which

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was the logarithm disparity between the 400 and 600 nm absorbance of the HA (FA) substance in the solution (Cui et al. 2017).

Data analysis. The SOC storage (t C/hm^2) refers to the total mass of the SOC at a certain depth within a region, which was calculated using the below formula:

$$\text{SOC storage (t C/hm}^2\text{)} = \text{SOC content (g/kg)} \times \text{bulk density (g/cm}^3\text{)} \times \text{soil depth (cm)} \times 0.1 \quad (1)$$

Statistical analysis. All the statistical analyses were performed using SPSS 26.0 software (SPSS, Inc., Chicago, IL, USA). To evaluate the effect of the reclamation time on the SOC and its fractions, groups were compared using a one-way analysis of variance (ANOVA) with a least significant difference (LSD) test for the post-hoc comparison (α = 0.05 as the significance threshold).

RESULTS

Effects of the reclamation on the soil physiochemical properties. The reclamation of grassland to agricultural land influenced the soil physiochemical properties, depending on the reclamation years (Table 1). After 40 years of reclamation acting on the grassland, almost all the soil properties at different depths showed significant differences (P < 0.05) between the reclaimed land (treatment) and non-reclaimed land (control). Generally, the SWC, clay and silt contents showed a decreasing trend, whereas the BD, pH and sand content increased with the reclamation years at the different soil depths. The final reductions of the SWC, clay and silt contents were 45, 40 and 23%, and the final promotion rate of BD, pH and sand content were 36, 3 and 18%, respectively, after 40 years reclamation compared with the CK. At the surface soil (0–20 cm), the pH increased at a significant level (P < 0.05) after 10 years of reclamation, and its final value was 1.3 higher than the control. The pH had no significant difference between the treatment and control at a depth of 20–60 cm under the different reclamation years, although its mean increased.

Effects of the reclamation on the SOC content and storage. The SOC contents and the storages at the different soil depths showed a significant decreasing trend with an increase in the reclamation

Table 1. Soil physicochemical properties in the different reclamation years

	Depth (cm)	Reclamation (years)					
		CK	3	10	16	27	40
BD (g/cm^3)	0–20	0.80 ± 0.08^d	0.96 ± 0.09^c	1.08 ± 0.07^{bc}	1.09 ± 0.07^{bc}	1.16 ± 0.11^b	1.24 ± 0.09^a
	20–40	0.97 ± 0.10^b	**0.97 ± 0.09**^{b}	**1.15 ± 0.08**^{ab}	1.21 ± 0.09^a	1.22 ± 0.12^a	1.34 ± 0.13^a
	40–60	1.12 ± 0.12^b	1.08 ± 0.10^c	1.21 ± 0.11^a	1.28 ± 0.13^a	1.29 ± 0.21^a	1.35 ± 0.15^a
SWC (%)	0–20	53.91 ± 1.76^a	38.26 ± 1.29^c	31.42 ± 1.88^c	31.15 ± 1.32^c	28.50 ± 1.64^d	26.15 ± 1.72^d
	20–40	42.84 ± 2.11^a	34.29 ± 2.21^c	26.55 ± 1.74^c	23.42 ± 1.98^d	22.72 ± 2.13^d	21.52 ± 1.47^d
	40–60	25.20 ± 2.18^a	**22.69 ± 1.97**^{ab}	20.20 ± 2.01^b	**21.89 ± 1.42**^{ab}	20.79 ± 2.14^b	19.54 ± 1.86^b
pH	0–20	6.05 ± 0.13^d	**6.28 ± 0.11**^{cd}	6.43 ± 0.14^{bc}	6.64 ± 0.27^b	7.23 ± 0.20^a	7.36 ± 0.13^a
	20–40	6.02 ± 0.21^a	**6.13 ± 0.05**^{a}	**6.27 ± 0.09**^{a}	**6.24 ± 0.28**^{a}	**6.51 ± 0.73**^{a}	**6.72 ± 0.41**^{a}
	40–60	6.24 ± 0.17^a	**6.29 ± 0.23**^{a}	**6.41 ± 0.31**^{a}	**6.23 ± 0.15**^{a}	**6.37 ± 0.51**^{a}	**6.60 ± 0.53**^{a}
Clay content (%)	0–20	22.31 ± 1.32^a	21.04 ± 0.71^b	17.59 ± 1.07^c	16.7 ± 1.66^d	12.47 ± 1.09^e	12.97 ± 0.54^e
	20–40	19.34 ± 0.67^a	18.43 ± 0.61^b	15.76 ± 1.14^c	12.2 ± 1.20^d	11.23 ± 1.00^d	13.23 ± 1.03^c
	40–60	17.45 ± 0.92^a	**17.20 ± 0.60**^{a}	14.41 ± 0.54^b	11.3 ± 0.64^c	9.2 ± 0.35^c	9.25 ± 2.89^c
Silt content (%)	0–20	17.41 ± 1.71^a	**16.66 ± 0.81**^{a}	**16.43 ± 1.23**^{a}	**15.1 ± 1.95**^{ab}	14.04 ± 0.60^b	13.54 ± 0.60^b
	20–40	16.39 ± 0.88^a	**15.47 ± 1.40**^{a}	**15.15 ± 0.94**^{ab}	**14.73 ± 0.53**^{ab}	13.3 ± 0.87^{bc}	12.3 ± 1.72^c
	40–60	15.11 ± 1.42^a	**15.40 ± 0.74**^{a}	**14.28 ± 1.76**^{ab}	**14.4 ± 1.03**^{ab}	**13.03 ± 1.25**^{ab}	12.03 ± 1.03^b
Sand content (%)	0–20	60.28 ± 3.85^d	**62.3 ± 3.72**^{cd}	65.98 ± 2.36^{bc}	68.2 ± 2.13^b	73.49 ± 1.79^a	73.49 ± 0.31^a
	20–40	64.27 ± 2.24^d	**66.1 ± 2.99**^{cd}	69.09 ± 1.91^{bc}	73.07 ± 1.83^{ab}	75.47 ± 2.89^a	74.47 ± 1.20^a
	40–60	67.44 ± 2.56^d	**67.4 ± 2.22**^{d}	72.31 ± 1.17^c	74.3 ± 2.36^{bc}	77.77 ± 2.13^{ab}	78.72 ± 1.14^a

BD – soil bulk density; SWC – soil water content; CK – control group; values represent mean ± SD; different lowercase letters indicate significant differences among the reclamation years at P < 0.05; the values in bold means there is no significant difference between the treatment (reclaimed land) and the control group (non-reclaimed land)

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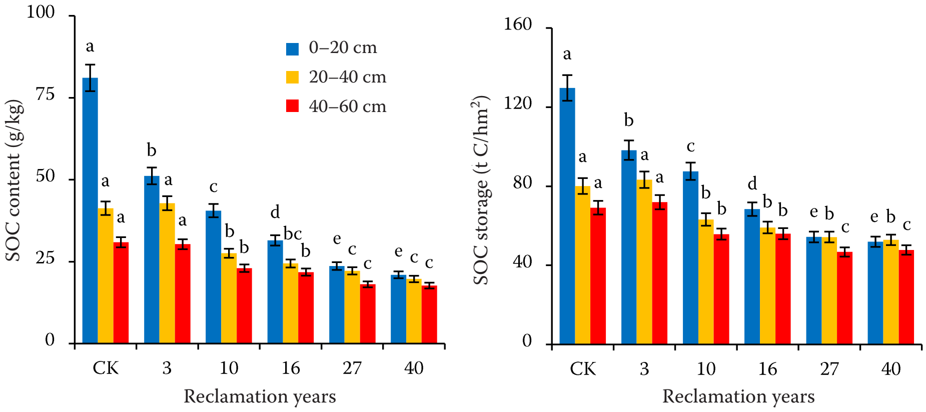

Figure 1. The soil organic carbon (SOC) content and storage at the different soil depths and years of reclamation

CK – control group; error bars indicate standard errors of the means (n = 3); means in the same colour (depth) followed by different lowercase letters are significantly different at a 5% level (P < 0.05)

years (P < 0.05) (Figure 1). The SOC contents and storages were significantly different in the different reclamation years, and the declining rate showed a slowing trend with an increase in the reclamation years. In addition, the SOC contents and storages changed most significantly in the 0–20 cm soil layer.

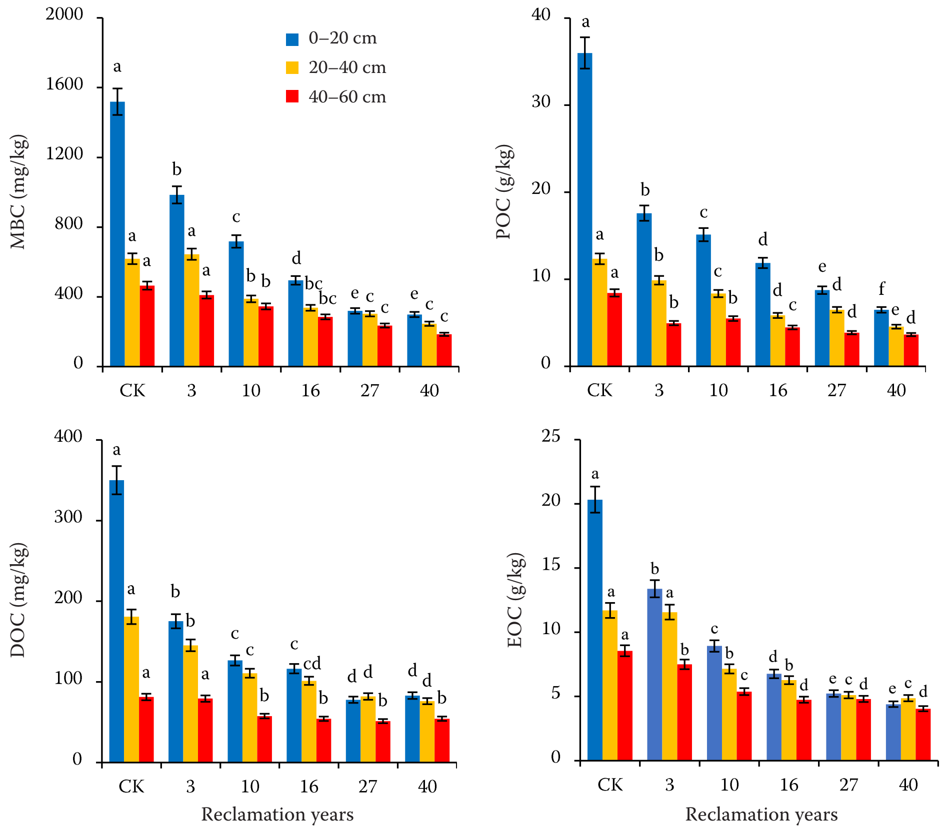

Figure 2. The contents of the soil labile organic carbon fractions at the different soil depths and years of reclamation

MBC – microbial biomass carbon; DOC – dissolved organic carbon; POC – particulate organic carbon; EOC – easily oxidised organic carbon; CK – control group; error bars indicate standard errors of the means (n = 3); means in the same colour (depth) followed by different lowercase letters are significantly different at a 5% level (P < 0.05)

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After 40 years of reclamation, the SOC contents and storages at 0–20 cm decreased by 74% and 60%, respectively, compared with the CK ($P < 0.05$).

Effects of the reclamation on the soil labile organic carbon fractions. The contents of the soil labile organic C fractions at the different soil depths showed a significant decreasing trend with an increase in the reclamation years ($P < 0.05$) (Figure 2). The contents of the soil labile organic C fractions were significantly different in the different reclamation years, and the declining rate showed a slowing trend with an increase in the reclamation years. In addition, the contents of the soil labile organic C fractions changed most significantly in the 0–20 cm soil layer. After 40 years of reclamation, the contents of the POC, MBC, EOC and DOC at 0–20 cm decreased by 82, 80, 78 and 76%, respectively, compared with the CK ($P < 0.05$).

Effects of the reclamation on the soil humus carbon fractions. The contents of the soil humus organic C fractions at the different soil depths showed a significant decreasing trend with an increase in the reclamation years ($P < 0.05$) (Figure 3). The contents of the soil humus organic C fractions were significantly different in the different reclamation years, and the declining rate showed a slowing trend with an increase in the reclamation years. In addition, the contents of the soil humus organic C fractions changed most significantly in the 0–20 cm soil layer. After 40 years of reclamation, the contents of the HAC, FAC and HUC at 0–20 cm decreased by 81, 71 and 73%, respectively, compared with the CK ($P < 0.05$).

Changes in the characteristics of soil humus. The E_4/E_6 ratios and Δlog K values of the HA and FA in the different soil depths showed a significant decreasing trend with an increase in the reclamation years ($P < 0.05$) (Figure 4). The E_4/E_6 ratios and Δlog K values of the HA and FA were significantly different in the different reclamation years, and the declining rate showed a slowing trend with an increase in the reclamation years. In addition, the E_4/E_6 ratios and Δlog K values of the HA and FA changed most significantly in the 0–20 cm soil layer. After 40 years of reclamation, the E_4/E_6 ratios and Δlog K values of the HA at 0–20 cm decreased by 13 and 10%,

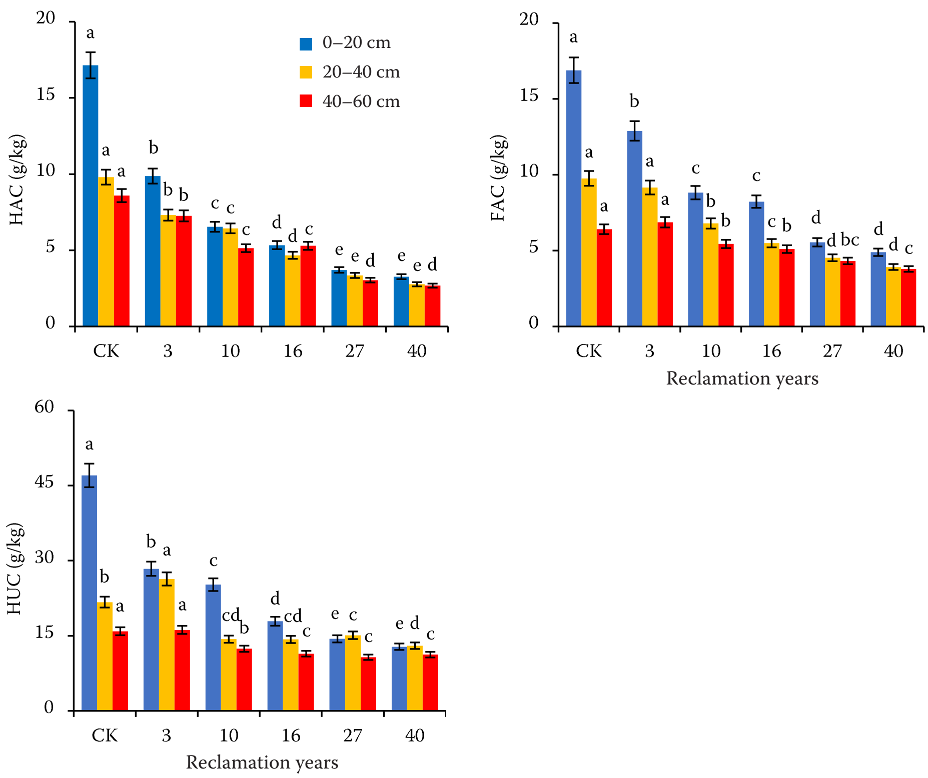

Figure 3. The contents of the humus carbon fractions at the different soil depths and years of reclamation

HAC – humic acid carbon; FAC – fulvic acid carbon; HUC – humin carbon; CK – control group; error bars indicate standard errors of the means ($n = 3$); means in the same colour (depth) followed by different lowercase letters are significantly different at a 5% level ($P < 0.05$)

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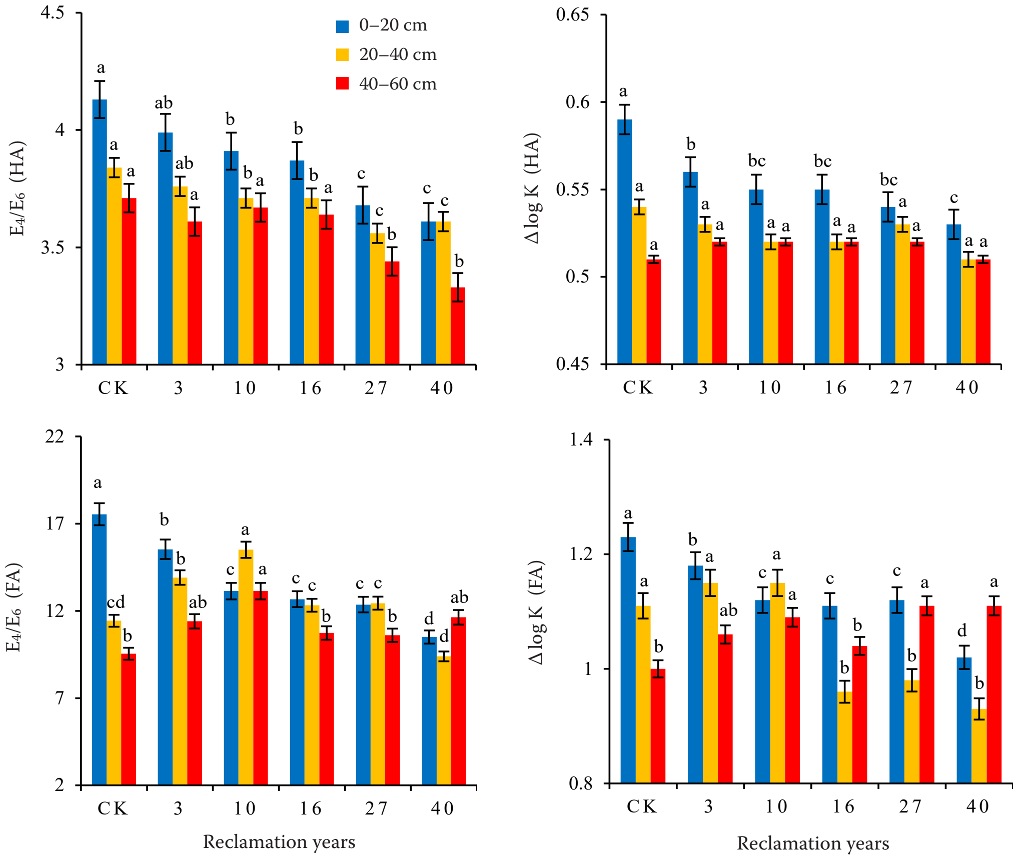

Figure 4. The E_4/E_6 ratios for the humic acid (HA) and fulvic acid (FA), and the Δlog K value for the HA and FA at the different soil depths and years of reclamation

CK – control group; error bars indicate standard errors of the means (n = 3); means in the same colour (depth) followed by different lowercase letters are significantly different at a 5% level (P < 0.05)

respectively, and FA decreased by 40 and 17%, respectively, compared with the CK (P < 0.05).

DISCUSSION

Reclamation decreased the SOC content and storage. The conversion from grassland into agricultural land could destroy the topsoil structure, destabilise the soil macroaggregates, and accelerate the loss of SOC (Six et al. 2000). In this study, the SOC content and storage decreased after the reclamation and the abrupt decline happened early in the reclamation time, in line with previous findings, but at a higher decreasing rate (Davidson & Ackerman 1993; Bu et al. 2015; Rasouli-Sadaghiani et al. 2018). The reason in explaining this discrepancy is mainly due to the differences in the study locations. In the alpine-cold soil, the lower temperature inhibited the litter composition rates, resulting in a higher accumulation of SOC in the grassland before the reclamation (Post et al. 1982; Hu et al. 2014). Moreover, a previous study suggested a positive correlation between the SOC stock and the average annual rainfall under various land use systems (Singh & Sharma 2017). The rainfall in the Qinghai-Tibet Plateau is less than that in the warm areas, which could lead to a smaller C stock. Furthermore, the soil pH altered by the reclamation is also a critical factor in affecting the SOC turnover rates and decomposition. On the one hand, the soil pH dominates the anion exchange between the carboxylic and phenolic OH groups of the SOC and the mineral surface hydroxyl groups that creates strong organo-mineral associations (Gu et al. 1994). With an increase in the pH, the adsorption of the SOC by the ligand exchange tends to decrease, thus an increase in the soil pH may weaken the binding of the SOC with the mineral surface, which consequently cause the loss of the SOC in this study (Gu et al. 1994). On the other hand, the optimal pH for microbial growth ranges from 6.5 to 7.5 (Wang et al.

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2014), the pH increased from 6.1 to 7.4 at 0–20 cm after 40 years of reclamation in this study, which enhanced the microbial activity and consequently promoted the decomposition of the SOC.

Some studies reported different effects of reclamation on the SOC. Among them, Li et al. (2006) and Zhang et al. (2017) showed significantly increased contents, while Goodrick et al. (2014) showed no significant increase. However, Li et al. (2006) applied manures and fertilisers, which can offset the loss of the SOC and consequently increased the SOC level. Besides, the organic and chemical fertilisation promoted the crop biomass production and, thus, increased the inputs of the crop residue (Meng et al. 2005; Steiner et al. 2007). In addition, Cui et al. (2014) found the slow recovery of the SOC with the cultivation after the SOC decreased in the first 16 years. It may be because Cui et al. (2014) based the empirical data on 120, 200 and 500 years, but not based on the long-term field monitoring. Besides, the sampling depth of their study was only 8 cm, which could not reflect the soil actual conditions appropriately.

Reclamation decreased the soil labile organic carbon. Currently, there is little information on the impacts of grassland reclamation on the soil labile organic C fractions. In this study, the contents of the POC, MBC, EOC and DOC significantly decreased after the reclamation, which were more obvious than the SOC. This confirms previous views that the soil labile organic C fractions are more sensitive to minor soil environmental changes than the SOC (Song et al. 2012; Li et al. 2016; Ramesh et al. 2019). Moreover, our study found that the POC decreased most significantly among all the soil labile organic C fractions. Ensinas et al. (2016) found that the POC was the most labile fraction of the SOC, which easily changed in the soil throughout the mineralisation and tillage. The greatly decreased POC can be mainly explained by the decrease in the soil nutrients and the increase in the microbial activity caused by the reclamation. On the one hand, the crops continuously absorbed the organic nutrients from the soil, then people took most of the nutrients out of the soil through crop harvesting during reclamation. On the other hand, insoluble POC is a substrate for soil microorganisms (Oduor et al. 2018). The increased pH caused by reclamation promoted the activity of microorganisms, which consumed more POC. The MBC content depends on the concentration of the soil microorganisms. During the cultivation, plant residue and litter provide necessary food sources for microorganisms, and their mass is positively correlated with the microorganism abundance (Hu et al. 2017a). In contrast, we found that the MBC content decreased after the reclamation, which was mainly due to the fact that there was little plant residue and litter left because crops were removed from the ecosystem. The loss of food sources could negatively affect the soil microorganisms, resulting in the decrease of the MBC during the reclamation. The DOC was more easily eroded by water, and its mobility makes it be involved in the transport of carbon in the soil profiles (Fröberg et al. 2011). In this study, the tillage destroyed the structure of the soil vertical profile, which would lead to an increase in the water loss and soil erosion. Besides, the reclamation increased the sand fraction, which increased the soil permeability. Consequently, the DOC tends to lose more under poor soil conditions. The EOC can participate in the soil material exchange process and move with solvents. Agricultural cultivation practices during reclamation can increase the area and frequency of the subsurface soils contacting with atmospheric oxygen, which consequently accelerate the loss of EOC through oxidation.

Reclamation affected the soil humus carbon, depending on the soil depths. In this study, the contents of the soil humus C fractions decreased significantly after the reclamation. Cultivation decreases the soil humic substances through the disruption of the equilibrium between the competing processes of humus formation and mineralisation (Saviozzi et al. 1994). Specific to the individual fraction, the decrease in the soil humus C fractions at 0–20 cm was greater from the HAC (81%) > FAC (71%) > HUC (73%). In line with our study, Spaccini et al. (2006) found that deforestation and the subsequent cultivation generally decreased the relative HA content whereas the FA and HU content were less affected in Ethiopia. Moreover, Sun et al. (2012) found that the HAC decreased by 38%, while the FAC decreased slightly by 7% after 200 years of cultivation in northeast Jilin. The HA may have poor stability under cultivation conditions or may not be easily formed to explain why more HAC tended to be lost than the FAC after the reclamation. Additionally, the E_4/E_6 ratios and Δlog K value of FA were higher than the HA, which indicated that the FA had smaller molecular weights than the HA, consistent with the study of Wagner et al. (2016). In addition, the humus C in the surface soil (0–20 cm) was more sensitive to reclamation than the deep soil (20–60 cm) with a more

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significant decline. The surface soil was exposed to climatic conditions, aeration and the temperature increased, which may accelerate the consumption of SOC through mineralisation or water and wind erosion (Villarino et al. 2017). The lower reduction of the SOC in the deep soil was probably due to the SOC content of the deep soil was lower than that of the surface soil, which cannot provide enough energy to maintain the active microbial population, resulting in an inefficient use of the SOC (Fontaine et al. 2007). Moreover, the result showed that the soil humus C fractions in the soil profile tend to be uniform from the topsoil to subsoil with the years of reclamation, which may be related to the downward leaching of the soil humus (Zavarzina et al. 2004).

CONCLUSION

The SOC and its fractions tend to decrease with time after the reclamation of alpine-cold grassland into cropland within 0–60 cm over 40 years, in the eastern Qinghai-Tibet Plateau, China, with a deterioration in the soil quality. The rate and amount of the reduction in the SOC concentrations gradually decreased with the increase in the reclamation years and soil depths. The soil changes most violently at 0–20 cm, which shows that the soil labile C fractions, such as POC, MBC, EOC and DOC, are more sensitive than the SOC; the soil humus C fractions, such as HAC, FAC and HUC. also decreased; the soil humus quality decreased after the reclamation. In conclusion, our study advanced the knowledge about the effect of grassland reclamation on the SOC and its fraction in alpine-cold soils with high altitudes, which has great implications for maintaining the global carbon balance in the long run.

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