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# The quantity and stability of soil organic carbon following vegetation degradation in a salt-affected region of Northeastern China

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### ABSTRACT

Understanding the influence of vegetation conversion induced by land degradation on soil organic carbon (SOC) dynamics provides a basis for the sustainable use of grasslands. While labile carbon fractions often vary with the type of vegetation, little is known about whether and how vegetation degradation affects the recalcitrant carbon fraction that is highly resistant to oxidation. Here, we studied the influence of vegetation degradation on changes in SOC and four oxidizable fractions with different stabilities in Northeastern China. The soil was collected from a vegetation degradation sequence, including Leymus chinensis (LEY, native grassland), Puccinellia tenuiflora (PUC, light degradation), Chloris virgata (CHL, moderate degradation), and Suaeda heteroptera (SUA, severe degradation). Contents of total SOC and four oxidizable SOC fractions extracted under a gradient of oxidation (F1, very labile; F2, labile; F3, less labile; F4, oxidizable resistant) were measured using a modified Walkley-Black method. Results showed that the contents of total SOC and oxidizable SOC fractions were significantly different under different vegetation types and soil depths. Under PUC, CHL, and SUA, contents of total SOC (29.0%, 34.3%, and 55.4%, respectively), F1 (22.5%, 20.8%, and 40.8%, respectively), F2 (28.2%, 53.8%, and 72.1%, respectively), F3 (52.0%, 40.6%, and 66.2%, respectively), and F4 (15.0%, 21.9%, and 42.8%, respectively) were lower than those under LEY. Vegetation degradation had a significant effect on the F4 fraction as well as the F1, F2, and F3 fractions. Regression coefficients of the relationship between oxidizable SOC fractions as a function of total SOC showed that these fractions, as a proportion of total SOC, were 22% for F1 and F2, 26% for F3, and 31% for F4. There were no significant differences in the percentages of active carbon fractions (F1 + F2) in total SOC and the lability of total SOC among the four vegetation types. These findings suggest that the recalcitrant carbon fraction can be affected by land degradation as well as the labile carbon fraction, and that shifts in land use affect the contents of total SOC and its fractions, but have no effect on the stability of SOC in semi-arid areas.

### 1. Introduction

Grassland ecosystems cover a large portion of the Earth's surface and contain approximately 343 Pg soil organic carbon (SOC) in the top 1 m depth (Conant et al., 2017). The SOC stocks in grasslands account for 12% of the global total organic carbon pool (Adewopo et al., 2015; Bai et al., 2020), or nearly 50% more than that stores in forests worldwide (Conant et al., 2017). Grassland degradation usually causes the conversion of vegetation types (Peng et al., 2020). Changes in vegetation types can directly influence the quantity and quality of above- and below-ground biomass, root depth, and microbial activity, thereby significantly affecting the SOC content (Lal, 2004; Rey et al., 2011; Lorenz et al., 2019). Hu et al. (2018) found that the SOC content in the karst area under vegetation with high above- and below-ground biomass was higher than that of the vegetation with lower vegetation biomass. Different vegetation communities differ in their ability to capture, store, and release carbon, and the collective functional characteristics of vegetation communities can be expected to be a significant driver of carbon sequestration in grassland ecosystems (Yu et al., 2013; Bargali et al., 2018). Vegetation tissue quality (e.g., vegetation nutrient content and lignin content) can influence the residence time of both living tissues and litter, and therefore indirectly influence the SOC content (Yu et al., 2013). Additionally, the effects of vegetation type are relevant in controlling SOC by modifying the physical and chemical properties of

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the soil. Deng et al. (2018) reported that conversion from grass community to a secondary forest in the Loess Plateau improved the physical and chemical properties of soil and thus increased the SOC content.

Different organic compounds entering the soil have highly varying compositions, making it difficult to understand the nature of SOC. Recent studies report that SOC is a continuum of progressively decomposing organic compounds ranging from intact plant material to highly oxidized carbon fraction, and this soil continuum model focuses on the ability of decomposer organisms to access SOC and on the protection of organic carbon from decomposition by soil minerals (Lehmann and Kleber, 2015). Therefore, a thorough description of the overall SOC continuum that exists in nature is vital to help soil scientists in understanding SOC stability. The SOC stability can be defined as the resilience and resistance of organic carbon to disturbances (Lehmann and Kleber, 2015; Yang et al., 2020). Recent research on SOC stability has concentrated mainly on the response of SOC in different ecosystems to land-use change, land degradation, warming, and nitrogen deposition, among other factors (Liu et al., 2018; Sprunger and Robertson, 2018; Yang et al., 2020). Land degradation altered the plant species, reduced the organic carbon inputs and microbial activity, and thus decreased the SOC decomposition, which was beneficial for accumulating stable carbon fraction and increased the SOC stability (Peng et al., 2020; Xu et al., 2020). However, Bai et al. (2020) reported that grassland degradation decreased the SOC stability in the southeast Tibetan Plateau because the high soil pH in the degraded grassland increased organic matter quality and bioavailability, with a consequent faster SOC decomposition. The controversial results in different studies indicate that the response of SOC stability to land degradation or other factors still requires further in-depth investigations (Yang et al., 2020).

Quantification of labile and recalcitrant organic carbon fractions obtained by physical, chemical, or biological approaches have been used for decades and are traditional metrics of SOC stability (Soucémarianadin et al., 2018). The recalcitrant organic carbon fraction is highly resistant to disturbance and has a relatively long residence time than the labile carbon fraction (Su et al., 2020). Therefore, it plays an important role in determining long-term SOC storage (Liu et al., 2020). Robust evidence from recent studies indicates that recalcitrant carbon fraction decomposes more quickly than previously anticipated under suitable conditions (Lehmann and Kleber, 2015). However, information on the size and response of recalcitrant or stable carbon fractions to different management practices is still limited (Yu et al., 2017; Liu et al., 2018). The lack of information on the change in the recalcitrant carbon fraction leads to an inaccurate understanding of SOC stability (Plante et al., 2011; Kučerík et al., 2018).

Compared with recalcitrant carbon, labile carbon has high bioavailability, relatively quick turnover time, and is sensitive to environmental changes (Plante et al., 2011; Li et al., 2018; Diederich et al., 2019). Therefore, studies that measure the carbon fractions and discuss the effects of management practices mainly focus on the labile carbon fractions, such as microbial biomass carbon, dissolved organic carbon, particulate organic carbon (>53  $\mu$ m), permanganate oxidizable carbon (0.333 mol L<sup>-1</sup> KMnO<sub>4</sub>), and light fraction organic carbon (Bhattacharyya et al., 2011; Wang et al., 2014; Li et al., 2018). As the living component of SOC, microbial biomass carbon plays a critical role in nutrient cycling and SOC decomposition and transformation. The dissolved organic carbon, originating from soil microbial biomass, root exudates, and lysates, is a measure of carbon that is easily transportable within ecosystems and forms of SOC (Wang et al., 2014). The particulate organic carbon and light fraction organic carbon are composed of more recently deposited organic matter particles and respond more rapidly to the influences of different management practices (Ramírez et al., 2020). The permanganate oxidizable carbon has been suggested to reflect a biologically active soil carbon fraction across a wide range of diverse soils and has been shown to be sensitive to recent environmental changes (Diederich et al., 2019).

The amount of labile and recalcitrant carbon fractions in the soil is a

key aspect when we consider the long-term storage and possible sequestration of SOC (Lorenz and Lal, 2010; Liu et al., 2020). In addition, understanding the effects of disturbances on the SOC fractions with different stabilities, rather than the total SOC is vital because it helps us to better understand the SOC responses to disturbances and the SOC protection mechanisms (Gabarrón-Galeote et al., 2015; Liu et al., 2018). Despite its wide interest, to date, single or standard techniques to assess SOC stability from the perspective of viewing SOC as a continuum are yet to be developed (Plante et al., 2011; Yu et al., 2017). The modified Walkley-Black method has been recently used to separate SOC that is envisaged as a SOC continuum into four sequential fractions (Fraction 1, very labile carbon; Fraction 2, labile carbon; Fraction 3, less labile carbon; and Fraction 4, oxidizable resistant organic carbon), which has provided promising results (Chan et al., 2001; Barreto et al., 2011; Benbi et al., 2015; Batista et al., 2018; Liu et al., 2018). The fraction of organic carbon that is susceptible to partial oxidation has been suggested to reflect biologically active soil carbon fractions (Diederich et al., 2019). Fractions 1 and 2, the most easily oxidizable carbon fractions, contribute to the formation of macro-aggregates and nutrient cycling, which can be defined as labile carbon (Barreto et al., 2011; Liu et al., 2018). Fractions 3 and 4 are highly resistant to oxidation, and have a high resistance to disturbance or microbial decomposition, which can be defined as recalcitrant carbon. The storage and stability of SOC can be changed by the alteration in the size of the four sequential oxidizable fractions. Therefore, a comprehensive assessment of the response of different SOC fractions envisaged as a SOC continuum to environmental changes is vitally important for a better understanding of the mechanisms of carbon sequestration and stability.

In this study, we hypothesized that vegetation conversion induced by grassland degradation would reduce the content of total SOC and labile carbon fraction, thus increasing SOC stability. The primary objectives of this study were to (1) compare the changes in the four oxidizable SOC fractions (very labile carbon, labile carbon, less labile carbon, and oxidizable resistant organic carbon) under the four vegetation types (*Leymus chinensis, Puccinellia tenuiflora, Chloris virgate,* and *Suaeda heteroptera*), and (2) evaluate the effect of vegetation degradation on SOC stability.

### 2. Materials and methods

### 2.1. Study area

The research site is located at the Changling Grassland Farming and Ecological Research Station (44°33' N, 123°31' E) in the north Songnen plain (Northeastern China, Fig. 1). The research site covers an area of 300 ha, and this area is relatively flat with an elevation of approximately 145 m a.s.l. The study area has a temperate, semiarid continental climate. The mean annual precipitation and air temperature were 427 mm and 5.9 °C, respectively, as recorded from 1980 to 2013. The pan evaporation was approximately 1600 mm. The soil is classified as Aqui-Alkalic Halosols in the Chinese soil taxonomic system or as Solonetz in the WRB soil taxonomic system (Yu et al., 2018b). The natural vegetation in the study area is meadow steppe, dominated by Leynus chinensis. Because of the impact of human activity (e.g., overgrazing, grassland cultivation, mowing, and collecting surface soil), more than 60% of the grassland area in the Songnen plain has suffered from substantial degradation with high soil salinization and alkalization (Yu et al., 2018b). Grassland degradation can result in significant changes in the vegetation types. Consequently, the dominant vegetation of Leymus chinensis has been gradually replaced by salt-tolerant species such as Puccinellia tenuiflora, Chloris virgata, and Suaeda heteroptera.

*Leymus chinensis* is a rhizomatous perennial grass with a broad ecological amplitude. It is a long-day plant that usually returns to green in early April, flowers in early June, and seeds mature in mid- or late July in Songnen plain. The species has two reproductive modes: sexual reproduction and vegetative propagation. *Puccinellia tenuiflora* is a



Fig. 1. The geographical setting of the study area.

sparsely tufted perennial grass that can grow in mildly saline-alkali soil. The tillering capacity of *P. tenuiflora* is strong, and one plant is capable of tillering more than 20 times. In addition, *P. tenuiflora* can also propagate through seeds. The species usually returns to green in mid-April, flowers in mid-June, and seeds mature in mid- or late July in Songnen plain. *Chloris virgata* and *Suaeda heteroptera* are annual plants that reproduce by seeds. The salt-tolerant ability of *C. virgata* is better than that of *P. tenuiflora*. *C. virgata* usually sprouts in early June, flowers in late July, and seeds mature in late August in Songnen plain. *Suaeda heteroptera* is a halophyte, and has strong salt-tolerance ability. The species usually sprouts in early June, flowers in late July or early August, and seeds mature in September in Songnen plain.

### 2.2. Experimental design and soil sampling

Based on previous research on grassland degradation and the detailed investigation in the study area (e.g., the salt-tolerant order of vegetation types and the soil chemical properties under these vegetation types) in August 2018, vegetation communities in a sequence of degradation with *L. chinensis* (LEY, native grassland without degradation), *P. tenuiflora* (PUC, light degradation), *C. virgata* (CHL, moderate degradation), and *S. heteroptera* (SUA, severe degradation) were selected for this study. The cover of the dominant species in the four vegetation communities was greater than 90%.

Field sampling was conducted during the typical period (August 2018) when the above-ground biomass attained its peak value. In each vegetation community, five sampling quadrats (each  $1 \text{ m} \times 1 \text{ m}$ ) were

established along a random transect at 20 m intervals. The detailed sampling methods of above- and below-ground biomass are described in Yu et al. (2020). Soil samples were collected to a depth of 50 cm at five intervals with a 5-cm diameter soil corer at four points in each quadrat. Four soil cores taken from each quadrat were mixed at each soil depth to produce a composite sample. A total of 100 composite soil samples were collected. Soil samples were air-dried, and separated from visible plant materials. Subsequently, these composite soil samples were sieved through a 2-mm sieve, and one soil sample was divided into two sub-samples: one subsample was used to determine soil pH and electrical conductivity (EC), and the other subsample was grounded to pass through a 0.25-mm sieve to determine the total SOC content and the oxidizable SOC fractions.

Soil pH and EC were measured in a 1:5 soil/water solution (Yu et al., 2018a). The total SOC content was determined using the  $K_2Cr_2O_7$ -H<sub>2</sub>SO<sub>4</sub> oxidation method (Yu et al., 2017). The oxidizable SOC fractions were determined using a modified Walkley-Black method (Walkley and Black, 1934; Chan et al., 2001). This method involved mixing 10 ml of 1 N K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub> with 2.5, 5, and 10 ml of 36 N H<sub>2</sub>SO<sub>4</sub> that resulted in three acid-aqueous solutions with ratios of 0.25:1, 0.5:1, and 1:1 (which corresponded respectively to 6.0 N, 12.0 N, and 18.0 N of H<sub>2</sub>SO<sub>4</sub>). More concentrated H<sub>2</sub>SO<sub>4</sub> added to 10 ml of K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub> resulted in a higher temperature, and more organic carbon could be oxidized. The oxidizable organic carbon determined using 2.5, 5, and 10 ml of concentrated H<sub>2</sub>SO<sub>4</sub> allowed the separation of total SOC into four fractions with decreasing oxidizability. Organic carbon with higher stability was more difficult to oxidize. Therefore, the total SOC was divided into the

following four carbon fractions with different stabilities according to their oxidizability (Barreto et al., 2011; Batista et al., 2018; Liu et al., 2018).

- 1. Very labile carbon (F1): Organic carbon oxidizable under 6.0 N  $\rm H_2SO_4.$
- 2. Labile carbon (F2): Difference in oxidizable organic carbon extracted between 6.0 and 12.0 N  $H_2SO_4$ .
- 3. Less labile carbon (F3): Difference in oxidizable organic carbon extracted between 12.0 and  $18.0\ N\ H_2SO_4.$
- 4. Oxidizable resistant organic carbon (F4): Difference between total SOC and organic carbon oxidizable under 18.0 N H<sub>2</sub>SO<sub>4</sub>. The oxidizable resistant organic carbon was used to represent the SOC component that could not be oxidized by 18.0 N H<sub>2</sub>SO<sub>4</sub>.

### 2.3. Statistical analysis

As reported by Chan et al. (2001), Barreto et al. (2011), and Liu et al. (2018), the F1 and F2 fractions represent the active carbon pool, whereas the F3 and F4 fractions represent the passive carbon pool. Therefore, the percentage of active carbon (F1 + F2) in total SOC was calculated using the equation that follows to show the changes in the active carbon pool in soils under different vegetation types:

$$PAC = \left(\frac{F1 + F2}{totalSOC}\right) \times 100$$

where PAC is the percentage of active carbon in total SOC (%), F1 is the very labile carbon, and F2 is the labile carbon.

In addition, the ratio of labile carbon to stable carbon (defined as the total SOC-labile carbon) could represent the stability of SOC. According to the calculation method of the lability of SOC defined in the calculation process of carbon management index, the lability of SOC was calculated using the equation that follows to show the changes in the stability of SOC under different vegetation types (Yu et al., 2017; Liu et al., 2018).

Lability of SOC = 
$$\frac{F1}{\text{total SOC} - F1}$$

According to previous studies (Culman et al., 2013; Diederich et al., 2019), the labile carbon fraction was usually <20% of the total SOC. To better compare with other studies, we used F1 as the labile carbon to calculate the lability of SOC.

The SPSS 13.0 software package (SPSS 13.0, Inc., Chicago, IL, USA) was used for the statistical analyses. The lability of SOC and the percentage of active carbon in total SOC under different vegetation types were compared using one-way analysis of variance (ANOVA). Two-way ANOVA was used to analyze the effects of different vegetation types and soil depths on total SOC and four oxidizable SOC fractions. Means of vegetation types and soil depths for each variable were compared using Fisher's least significant difference test (LSD). The analyses were tested for a significance level of P < 0.05. To reveal the relationships between total SOC and its oxidizable organic carbon fractions, a simple linear regression analysis was used.

### 3. Results

### 3.1. Soil pH, electrical conductivity and vegetation biomass under different vegetation types

Soil pH (F = 8.15, P < 0.01) and EC (F = 158.51, P < 0.001) were significantly different under the four vegetation types (Table 1). The soil pH and EC under the SUA community were significantly higher than that under LEY and PUC communities. However, the difference of soil pH and EC between LEY and PUC community was not significant. The EC under the SUA community was significantly higher than that under the CHL community, while the difference of soil pH between the two communities was not significant. Vegetation types had significant effects on the above- (F = 52.43, P < 0.001) and below-ground (F = 540.90, P < 0.001) 0.001) biomass. The vegetation biomass under the LEY community was highest, while the lowest value was found under the SUA community. The vegetation biomass including the above- and below-ground biomass were 929, 616, 442, and 359 g m<sup>-2</sup> for the LEY, PUC, CHL, and SUA communities, respectively. The above-ground biomass carbon content was significantly different (F = 9.20, P < 0.01) under the four vegetation types, while no significant difference was found in the below-ground biomass carbon content (F = 1.22, P = 0.33). The above-ground biomass carbon content under the SUA community was significantly lower than that in the LEY, PUC, and CHL community (Table 1).

### 3.2. Total soil organic carbon content under different vegetation types

The total SOC content significantly decreased from the surface soil to the subsoil for each vegetation type (Fig. 2). The average total SOC contents in the four communities were 9.51, 6.50, 4.45, 3.28, and 2.35 g kg<sup>-1</sup> for the 0–10 cm, 10–20 cm, 20–30 cm, 30–40 cm, and 40–50 cm depths, respectively. Compared with the LEY community, the total SOC contents under PUC, CHL, and SUA community were 3.14, 4.39, and 6.61 g kg<sup>-1</sup> lower at the 0–20 cm depth and were 1.49, 1.31, and 2.44 g kg<sup>-1</sup> lower at the 20–50 cm depth. The range of total SOC content among the four vegetation types was higher at the 0–10 cm depth (8.39 g kg<sup>-1</sup>) than that at the 10–20 cm (4.83 g kg<sup>-1</sup>), 20–30 cm (3.18 g kg<sup>-1</sup>), 30–40 cm (2.32 g kg<sup>-1</sup>), and 40–50 cm (1.83 g kg<sup>-1</sup>) depths. A higher total SOC content was observed in the LEY community than in the PUC  $\approx$  CHL > SUA community (Fig. 2). The average total SOC contents at the 0–50 cm depth in the LEY, PUC, CHL, and SUA communities were 7.42, 5.27, 4.88, and 3.31 g kg<sup>-1</sup>, respectively.

## 3.3. The oxidizable soil organic carbon fractions under different vegetation types

For all vegetation types, the contents of oxidizable SOC fractions (F1

### Table 1

Soil pH, electrical conductivity and vegetation biomass in the different vegetation types. Values with the same lowercase letters within columns (vegetation types) are not significantly different at P < 0.05. The results are shown as the mean (±SE). LEY, *Leymus chinensis* (Trin.) Tzvel; PUC, *Puccinellia tenuiflora* (Griseb.) Scribn; SUA, *Suaeda heteroptera*; CHL, *Chloris virgata Swartz*.

Vegetation type	Electrical conductivity	pН	Biomass (g m <sup>-2</sup> )		Biomass carbon	(g kg <sup>-1</sup> )	Companion Species	
	(dS m <sup>-1</sup> )		Aboveground	Belowground (0–50 cm)	Aboveground	Belowground (0–50 cm)		
LEY PUC	0.438(±0.045)c 0.515(±0.005)c	9.5(±0.1)c 9.8(±0.1) bc	411.4(±8.5)a 263.6(±12.8) c	518.1(±11.3)a 352.3(±12.5)b	472.5(±2.8)a 445.3(±23.1) a	356.1(±15.4)a 348.3(±9.3)a	Chloris virgata Chloris virgate, Polygonum aviculare	
SUA	1.496(±0.055)a	10.2(±0.1) a	273.1(±4.6)c	86.2(±2.3)d	370.7(±6.2)b	382.0(±12.1)a		
CHL	0.827(±0.029)b	10.1(±0.1) ab	316.2(±9.6)b	126.3(±3.7)c	443.7(±15.8) a	358.8(±14.8)a	Puccinellia tenuiflora, Polygonum aviculare	





**Fig. 2.** Soil organic carbon content in different vegetation types. Values with the same uppercase letters within vegetation type and lowercase letters within soil depth are not significantly different at P < 0.05. The bars represent standard errors. LEY, *Leymus chinensis* (Trin.) Tzvel; PUC, *Puccinellia tenuiflora* (Griseb.) Scribn; SUA, *Suaeda heteroptera*; CHL, *Chloris virgata Swartz*.

to F4) at the 0–10 cm depth were significantly higher than those at the 20-30 cm, 30-40 cm, and 40-50 cm depth, except for the 20-30 cm depth of the CHL and SUA communities (Tables 2 and 3). At the 10-20 cm depth, the contents of oxidizable SOC fractions (F1 to F4) were significantly higher than that at the 40–50 cm depth, except for the F2 under the CHL and SUA communities and the F3 under the PUC community. However, no significant differences were found among the 20-30 cm, 30-40 cm, and 40-50 cm depth for F1, F2, and F3, irrespective of the vegetation type (Table 2). For the oxidizable resistant organic carbon fraction (F4), the content at the 20-30 cm depth was significantly higher than that at the 30-40 cm and 40-50 cm depth under the LEY, CHL, and SUA communities. At the 0-10 cm, 10-20 cm, 20-30 cm, 30-40 cm, and 40-50 cm depths, contents of oxidizable SOC fractions were 2.43, 1.73, 1.26, 0.94, and 0.70 g kg<sup>-1</sup> for F1 (P < 0.01), 1.97, 1.19, 1.16, 0.90, and 0.68 g kg<sup>-1</sup> for F2 (P < 0.01), 2.21, 1.32, 0.58, 0.69, and 0.45 g kg<sup>-1</sup> for F3 (P < 0.01), and 2.91, 2.27, 1.45, 0.75, and 0.53 g kg<sup>-1</sup> for F4 (P < 0.01), respectively.

The LEY community had the highest oxidizable SOC fractions content, except for F4 at 30-50 cm depth among the four vegetation types. The contents of oxidizable SOC fractions (F1 to F4) in the SUA community were significantly lower than those in the LEY community. Under the PUC, CHL, and SUA communities, the contents of F1 (0.40, 0.37, and 0.73 g kg<sup>-1</sup>, respectively), F2 (0.54, 1.03, and 1.38 g kg<sup>-1</sup> respectively), F3 (0.90, 0.71, and 1.15 g kg<sup>-1</sup>, respectively), and F4  $(0.30, 0.43, and 0.84 \text{ g kg}^{-1}, respectively)$  were lower than those under the LEY community (Table 2). The average contents of F4 at the 0-50 cm depth were not significantly different among the LEY, PUC, and CHL communities. Vegetation degradation significantly decreased the content of F1. Compared with the LEY community, the average contents of F1 were 0.41, 0.37, and 0.73 g kg $^{-1}$  lower under the PUC, CHL, and SUA communities, respectively. Contents of F3 in the LEY community at each soil depth were significantly higher than those under the SUA, PUC, and CHL communities, except those in the PUC community at 40-50 cm depth and the CHL community at 30-40 cm depth. For F2, the contents in the LEY community were significantly higher than those in the CHL, PUC, and SUA communities.

Significant linear relationships were found between the four

#### Table 2

Contents of soil oxidizable organic carbon fractions at the 0–50 cm depth. Values with the same uppercase letters within rows (vegetation types) and lowercase letters within columns (soil depths) are not significantly different at P < 0.05. F1, very labile carbon, which is the organic carbon oxidizable under 6.0 N H<sub>2</sub>SO<sub>4</sub>; F2, labile carbon, which is the difference in oxidizable organic carbon extracted between 6.0 N and 12.0 N H<sub>2</sub>SO<sub>4</sub>; F3, less labile carbon, which is the difference in oxidizable organic carbon extracted between 12.0 N and 18.0 N H<sub>2</sub>SO<sub>4</sub>; and F4, oxidizable organic carbon oxidizable under 18.0 N H<sub>2</sub>SO<sub>4</sub>. LEY, *Leymus chinensis* (Trin.) Tzvel; PUC, *Puccinellia tenuiflora* (Griseb.) Scribn; SUA, *Suaeda heteroptera*; CHL, *Chloris virgata Swartz*.

Soil depths	Vegetation types							
(cm)	LEY	PUC	CHL	SUA				
	$F1 (g kg^{-1})$							
0–10	$3.14(\pm 0.18)$	$2.45(\pm 0.40)$	$2.32(\pm 0.21)$	$1.81(\pm 0.26)$				
	Aa	ABa	ABa	Ba				
10–20	2.00(±0.23)	$1.70(\pm 0.62)$	1.83(±0.16)	$1.39(\pm 0.37)$				
00.00	Ab	Aab	Ab	Aab				
20-30	$1.74(\pm 0.10)$	$1.37(\pm 0.10)$	0.99(±0.18)	$0.95(\pm 0.17)$				
20.40	ADC	ABDC	BC	BDC				
30-40	$1.20(\pm 0.12)$	$0.82(\pm 0.13)$	1.00(±0.09)	$0.69(\pm 0.07)$				
40.50	$0.86(\pm 0.28)$	$0.59(\pm 0.07)$	ADC $0.88(\pm 0.10)$	$0.45(\pm 0.04)$				
40-30	0.80(±0.28) Ad	0.39(±0.07)	0.00(±0.10) Ac	0.43(±0.04)				
0-50	1.79(+0.08)	1.38 (+0.15)B	1.42(+0.08)	1.06(+0.14)				
	A		В	В				
0.10	$F2 (g kg^{-1})$	0.00(1.0.01)	1 50(10.00)	0.51(1.0.05)				
0-10	3.36(±0.43)	2.22(±0.21)	1.59(±0.20)	$0.71(\pm 0.07)$				
10.00	Aa	Ba	Ba					
10-20	2.04(±0.26)	1.87(±0.08)	0.50(±0.08) Bb	$0.34(\pm 0.05)$				
20-30	$1.60(\pm 0.18)$	$122(\pm 0.18)$	1 19(+0 20)	$0.66(\pm 0.08)$				
20 00	Abc	Ab	Aa	Bab				
30-40	1.59(±0.10)	0.87(±0.06)	0.70(±0.05)	0.45(±0.07)				
	Abc	Bbc	Bb	Cbc				
40–50	1.01(±0.22)	0.71(±0.15)	0.46(±0.06)	0.52(±0.01)				
	Ac	ABc	Bb	Babc				
0–50	1.92 (±0.07)	1.38 (±0.07)B	0.89 (±0.02)	0.53 (±0.03)				
	Α		С	D				
	F3 (g $kg^{-1}$ )							
0–10	3.71(±0.27)	1.76(±0.43)	2.14(±0.26)	1.22(±0.12)				
	Aa	BCa	Ba	Ca				
10-20	$2.00(\pm 0.21)$	$1.24(\pm 0.32)$	$1.24(\pm 0.04)$	0.78(±0.15)				
	Ab	Bab	Bb	Bb				
20–30	$1.25(\pm 0.12)$	$0.21(\pm 0.06)$	0.46(±0.05)	0.38(±0.05)				
00.40	Ac	Cc	Bc	BCc				
30–40	$1.01(\pm 0.15)$	$0.39(\pm 0.06)$	$0.94(\pm 0.11)$	$0.42(\pm 0.11)$				
40 50	AC $0.72(+0.21)$	DC = 7(10.04)	AD 0.29(10.07)	DC = 0.14(+0.02)				
40-30	$0.72(\pm 0.21)$	ABbc	$0.38(\pm 0.07)$	$0.14(\pm 0.02)$				
0-50	1.74 (+0.06)	0.83 (+0.11)B	1.03 (+0.07)	0.59 (+0.05)				
0.00	A	0100 (±0111)2	B	C				
	1							
0.10	F4 (g kg <sup>-1</sup> )	0.00(1.0.05)	0.50(1.0.0.0)	0.05(1.0.10)				
0–10	3.97(±0.17)	3.02(±0.35)	2.59(±0.34)	2.05(±0.19)				
10_20	Aa 2 87(+0 25)	Da 2 55(+0.98)	$2.09(\pm 0.21)$	$Ca = 1.57(\pm 0.13)$				
10-20	Ab	Aab	Aa	Ab				
20-30	$1.72(\pm 0.25)$	$1.45(\pm 0.22)$	$1.48(\pm 0.06)$	$1.13(\pm 0.12)$				
	Ac	ABbc	ABb	Bc				
30-40	0.71(±0.06)	1.02(±0.17)	0.64(±0.04)	0.64(±0.06)				
	Bd	Ac	Bc	Bd				
40–50	0.60(±0.05)	$0.35(\pm 0.03)$	$0.91(\pm 0.09)$	$0.26(\pm 0.03)$				
	Bd	Cc	Ac	Ce				
0–50	1.97 (±0.11)	1.67 (±0.25)	1.54 (±0.10)	1.13 (±0.06)				
	A	A	AB	в				

oxidizable SOC fractions and total SOC content (Fig. 3). Among the four oxidizable SOC fractions, the regression coefficients between total SOC content and F1, F2, F3, and F4 were 0.22, 0.22, 0.26, and 0.31, respectively.

#### Table 3

Two-way ANOVA results for the influence of vegetation type and soil depth on total SOC and soil oxidizable organic carbon fractions. SOC, soil organic carbon; F1, very labile carbon, which is the organic carbon oxidizable under  $6.0 \text{ N} \text{ H}_2\text{SO}_4$ ; F2, labile carbon, which is the difference in oxidizable organic carbon extracted between  $6.0 \text{ N} \text{ H}_2\text{SO}_4$ ; F3, less labile carbon, which is the difference in oxidizable organic carbon, which is the difference in oxidizable organic carbon extracted between  $12.0 \text{ N} \text{ H}_2\text{SO}_4$ ; F3, less labile carbon, which is the difference in oxidizable organic carbon extracted between  $12.0 \text{ N} \text{ H}_2\text{SO}_4$ ; and F4, oxidizable resistant organic carbon, which is the difference between total SOC and organic carbon oxidizable under  $18.0 \text{ N} \text{ H}_2\text{SO}_4$ .

		SOC		F1		F2		F3		F4	
	df	F	Р	F	Р	F	Р	F	Р	F	Р
Vegetation type (VE)	3	127.99	< 0.001	7.90	< 0.001	63.62	< 0.001	38.89	< 0.001	7.78	< 0.001
Soil depth (SD)	4	291.32	< 0.001	33.60	< 0.001	33.52	< 0.001	67.43	< 0.001	51.35	< 0.001
VE*SD	12	12.12	< 0.001	0.69	0.76	5.96	< 0.001	4.51	< 0.001	1.71	0.09



Fig. 3. Liner relationships between total SOC and different soil oxidizable organic carbon fractions. F1, very labile carbon, which is the organic carbon oxidizable under 6.0 N  $H_2SO_4$ ; F2, labile carbon, which is the difference in oxidizable organic carbon extracted between 6.0 N and 12.0 N  $H_2SO_4$ ; F3, less labile carbon, which is the difference in oxidizable organic carbon extracted between 12.0 N and 18.0 N  $H_2SO_4$ ; and F4, oxidizable resistant organic carbon, which is the difference between total SOC and organic carbon oxidizable under 18.0 N  $H_2SO_4$ .

### 3.4. The stability of soil organic carbon under different vegetation types

Vegetation type had no significant effect on the percentage of active carbon in total SOC content (Fig. 4) or the lability of SOC (Fig. 5). Results of one-way ANOVA showed that no significant difference among the four vegetation types was found at the 0–10 cm (F = 0.61, P = 0.62), 10–20 cm (F = 0.63, P = 0.61), 20–30 cm (F = 2.43, P = 0.12), 30–40 cm

(F = 1.29, P = 0.32), and 40–50 cm (F = 2.63, P = 0.10) depth for the percentage of active carbon in total SOC content. Similarly, the differences in the lability of SOC among the vegetation types were not significant at the 0–10 cm (F = 1.38, P = 0.30), 10–20 cm (F = 1.20, P = 0.35), 20–30 cm (F = 1.22, P = 0.35), 30–40 cm (F = 0.69, P = 0.58), and 40–50 cm (F = 0.58, P = 0.64) depth.

The lability of SOC and the percentage of active carbon in total SOC

Fig. 4. Percentage of active carbon content in total soil organic carbon content in different vegetation types. The bars represent the standard errors. The active carbon is the sum of very labile carbon (F1) and labile carbon (F2). Organic carbon oxidizable under 6.0 N H<sub>2</sub>SO<sub>4</sub> corresponds to the very labile carbon (F1); the difference in oxidizable organic carbon extracted between 6.0 N and 12.0 N H<sub>2</sub>SO<sub>4</sub> is defined as the labile carbon (F2). LEY, Leymus chinensis (Trin.) Tzvel; PUC, Puccinellia tenuiflora (Griseb.) Scribn; SUA, Suaeda heteroptera; CHL, Chloris virgata Swartz. There is no significant difference within vegetation types at P < 0.05. Values with the same lowercase letters within soil depths are not significantly different at P < 0.05. NS represents no significant differences among the treatments.





**Fig. 5.** Lability of soil organic carbon in the different vegetation types. The lability of soil organic carbon is the content of very labile carbon (F1) divided by the difference in content between very labile carbon and total soil organic carbon. Organic carbon oxidizable under 6.0 N H<sub>2</sub>SO<sub>4</sub> corresponds to the very labile carbon (F1). LEY, *Leymus chinensis* (Trin.) Tzvel; PUC, *Puccinellia tenuiflora* (Griseb.) Scribn; SUA, *Suaeda heteroptera*; CHL, *Chloris virgata Swartz*. The bars represent the standard errors. There is no significant difference within soil depth and vegetation type at *P* < 0.05.

content under the four vegetation types remained relatively stable at the soil profile of 0–50 cm depth, except for the percentage of active carbon in total SOC content at the 0–20 cm depth under the LEY community and at the 40–50 cm depth under the SUA community (Figs. 4 and 5). The percentages of active carbon in total SOC content in the LEY community at the 0–10 cm and 10–20 cm depth were significantly lower than that at the 30–40 cm and 40–50 cm depth. Under the SUA community, the percentage of active carbon in total SOC content at the 40–50 cm depth was significantly higher than that at the 0–10 cm, 10–20 cm, 20–30 cm, and 30–40 cm depth.

### 4. Discussion

Soil salinization is the dominating driving factor resulting in grassland degradation, which causes significant changes in the vegetation types in Songnen plain (Yu et al., 2018a). In the present study, vegetation type and soil depth had significant effects on total SOC content (Fig. 2 and Table 3). The downward trend of total SOC contents along the vegetation degradation sequence from LEY (native grassland without degradation) to SUA (severe degradation) was mainly attributed to the vegetation degradation and high soil salinity under the three degraded vegetation types compared with the LEY community (Raiesi, 2017; Li et al., 2018). Firstly, soil salinization under the degraded community usually reduced the plant biomass compared with the native grassland without degradation in Songnen plain (Yu et al., 2013). The lower plant biomass and biomass carbon content (Table 1) decreased the absolute amount of organic carbon inputs from the vegetation into soils, resulting in a lower SOC content under the three degraded vegetation types. Secondly, the higher electrical conductivity and soil pH under the PUC, SUA, and CHL communities (Table 1) compared to the LEY community indicated that the soil condition was poor in the study area. Our previous study in the same area showed that the high soil salinity decreased the vegetation cover under the degraded vegetation types (Yu et al., 2013). The low vegetation cover increased the wind and rain erosion under the degraded grasslands, and thus promoted the loss of SOC content in fine soil fractions and dissolved organic carbon. The soil in degraded grassland was significantly affected by rain and wind erosion, and overlooking soil erosion resulted in an underestimation of 50% of soil carbon loss in the surface soil of the Songnen plain (Li et al., 2014a, 2014b). Thirdly, the perennials of the LEY community tend to have higher root productivity than the annual plants under the degraded vegetation communities, for example, in the SUA and CHL communities (Table 1). The high below-ground carbon inputs resulting from extensive root systems may be another important driver for the high total SOC content under the LEY community (Sprunger and Robertson, 2018). In addition, soil microorganisms play an important role in the decomposition of plant and animal remains, and they also perform important steps in various nutrient cycles and in the solid, liquid, and gaseous phases of the soil–plant root system. Our previous study indicated that microbial biomass was relatively small due to the high soil salinity, and the differences among different plant communities were not significant in the Songnen plain (Yu et al., 2018b). Therefore, the role of microbes in SOC changes varied little in different communities in the present study.

A more obvious change in total SOC content at the surface soil (0-20 cm) than at the subsoil (20-50 cm) resulting from vegetation degradation was observed in the present study. This result was generally consistent with those of studies conducted by Yu et al. (2017) in Northeastern China and Deng et al. (2018) in the Loess Plateau at the same soil depth. Compared with the LEY community, the higher reduction in total SOC content under the PUC, CHL, and SUA communities at the surface soil (0-20 cm) than at the subsoil (20-50 cm) likely resulted from the differences in the above- and below-ground biomass allocation patterns and the root distribution patterns in shallow and deep soil (Jobbágy and Jackson, 2000; dos Santos et al., 2019). In general, the accumulation of above- and below-ground biomass inputs usually occurred in the surface soil at 0-20 cm depth (Wang et al., 2014). The difference in SOC contents at the surface soil and at the subsoil could also be related to the erosion of the original upper soil horizons and exposure of a former B horizon with lower SOC content (Yu et al., 2017). Soil erosion intensified with an increase in vegetation degradation in the Songnen plain because of the low vegetation cover and plant biomass (Li et al., 2014a, 2014b). These results indicated that vegetation degradation reduced the SOC content and storage, and the reduction in total SOC content mainly stemmed from the SOC decrease in the surface soil in the semi-arid regions.

Similar to the total SOC content, the higher supply of above- and below-ground biomass in the surface soil resulted in a decreasing trend with increasing soil depth for the four oxidizable carbon fractions, irrespective of the vegetation type (Table 2). Li et al. (2014a, 2014b) investigated the below-ground biomass (0–100 cm depth) under different vegetation types in the Songnen plain, and found that more than 80% of below-ground biomass was distributed at a depth of 0–30 cm. Vegetation degradation significantly decreased the content of F1, F2, F3, and F4 fractions by 28.12%, 51.39%, 53.06%, and 26.57%, respectively, compared with the LEY community in this study (Table 2). The percentage of labile carbon fraction in total SOC is usually <20% as reported by researchers (Culman et al., 2013; Diederich et al., 2019),

which is similar to F1 fraction defined in our study. These results indicated that the relatively recalcitrant carbon compound was also decomposed in the same manner as the labile carbon in the Songnen plain. That is, the inherently stable and chemically unique compounds of SOC may not be the primary factors that influence the decomposition of SOC. Instead, the ability of decomposer organisms to access SOC and the protection of organic carbon provided by soil minerals and aggregates are the main factors affecting SOC decomposition (Lehmann and Kleber, 2015). Although the difference in the F4 contents among the LEY, PUC, and CHL communities was not significant, the F4 content in the PUC and CHL communities decreased by 18.45% compared with the LEY community. In addition, the F4 content in the LEY community was significantly higher than that in the SUA community (Table 2). This result was in line with the finding obtained by Liu et al. (2020), that the recalcitrant carbon content increased by 25.9% after the conversion from cropland to grassland in the Ili River valley. These results reconfirmed that landuse change could also influence the content of the recalcitrant carbon fraction, and thus affect the carbon sequestration and storage in a specific region. Therefore, the change in the recalcitrant carbon fraction should be considered when evaluating the variation in SOC sequestration and stability after land-use change. The significantly lower F4 content in the SUA community than in the LEY community mainly depended on the size of the input fluxes of the organic matter as much as on the soil process. The severe soil salinization in the SUA community was not conducive to vegetation growth, and thus decreased the vegetation cover and plant biomass. The lower input fluxes of organic matter in the SUA community from plant biomass to soil reduced the absolute input flux of F4 compared with the higher input fluxes of organic matter in the LEY community. Furthermore, the strong wind and rain erosion caused by vegetation degradation in the SUA community removed the original upper soil horizon with higher F4 content and led to the exposure of a former subsoil with lower F4 content.

The oxidizable SOC fractions increased significantly in response to increasing total SOC (Fig. 3), which was consistent with the results of Benbi et al. (2015) in the semiarid subtropical regions of Northern India and those of Yu et al. (2017) in the semiarid temperate zone of Northeastern China. The oxidizable carbon fractions and the total SOC were closely interrelated properties, and the total SOC was a primary determinant of the amount of the four oxidizable carbon fractions (Yu et al., 2017). The percentage of passive carbon pool (F3 + F4) in total SOC at the 0-50 cm depth was 57% (26% for F3 and 31% for F4), indicating that SOC in the present study was characterized by predominantly passive carbon. High percentage of passive carbon in total SOC was mainly due to the selection of soil microbes because soil microbes preferred to decompose the labile carbon fraction (Zhang et al., 2018). This finding was in line with the previous findings reported by Wang et al. (2014) in the Songnen plain of Northeastern China and those of Zhang et al. (2018) in an alpine meadow of the Tibet Plateau that the percentage of recalcitrant carbon fraction in total SOC at the 0-20 cm and 20-40 cm depth were all more than 60%.

A wide range of labile carbon fractions determined using chemical or physical separation methods, such as microbial biomass carbon, dissolved organic carbon, and light fraction organic carbon, are measured to better understand how management practices affect short-term and long-term carbon dynamics and stabilities in soils (Lehmann and Kleber, 2015; Ramírez et al., 2020). Organic carbon oxidizable under 12.0 N H<sub>2</sub>SO<sub>4</sub> was defined as the 'very labile carbon fraction' by Chan et al. (2001), Barreto et al. (2011), Datta et al. (2015), and Liu et al. (2018) using a similar determination method as used in this study. However, the percentages of the very labile carbon fraction in total SOC were more than 30% in these studies, which was higher than the normal value (<20% of total SOC) acknowledged by researchers (Culman et al., 2013; Diederich et al., 2019). The differences in amount and type of soil labile carbon fractions will result in different responses of labile carbon fractions to soil management practices, and thus preventing our understanding on the change and stability of SOC. In the modified WalkleyBlack method used in this study, a higher  $H_2SO_4$  concentration will oxidize more organic carbon than a lower concentration. That is, the concentration of  $H_2SO_4$  determines the quantity of oxidizable SOC fraction. Consequently, the organic carbon oxidizable under 6.0 N  $H_2SO_4$  concentration was termed as very labile carbon in our study. The result showed that the percentage of very labile carbon in total SOC was 22%, which was very close to the normal value. Therefore, we suggest that the concentration of  $H_2SO_4$  should be <6.0 N when using the same modified Walkley-Black method to determine the labile carbon fraction in future studies.

In our study, there were no significant differences in the percentages of active carbon fraction in total SOC (Fig. 4) and the lability of total SOC (Fig. 5) among the four vegetation types, indicating that vegetation degradation did not change the proportion of labile carbon to total SOC. That is, vegetation degradation did not change the stability of SOC in the Songnen plain. It was not surprising that the SOC stability was not affected by vegetation degradation in the present study, because vegetation degradation reduced both contents of the recalcitrant carbon and labile carbon as shown in Table 2. These results confirmed the findings of previous studies conducted by Bhattacharyya et al. (2011) in the Indian sub-Himalayas and Liu et al. (2018) in the Loess Plateau that the proportion of labile carbon to total SOC and the stability of SOC were not affected by soil management practices. Therefore, land-use change or land degradation can alter the absolute amount of total SOC and its oxidizable carbon fractions, while the stability of SOC remains unchanged. However, it should be noted that SOC fractions are thoroughly mixed with and often adhere to soil minerals, and merely making an inventory of several SOC fractions is not sufficient to evaluate the effect of land-use change on SOC dynamics and stability. Therefore, more experiments on the changes of SOC and its fractions after land-use conversion in various ecosystems are necessary, and the role played by soil microbes and soil minerals in this process should be taken into account.

### 5. Conclusion

Our results clearly indicated that vegetation degradation significantly decreased the contents of total SOC and oxidizable SOC fractions during the progressive stages of degradation from LEY (native grassland without degradation) to SUA (severely degraded grassland) in the Songnen plain. Vegetation degradation had a significant influence on the F4 fraction as well as the F1, F2, and F3 fractions, indicating that the recalcitrant carbon fraction could also be influenced by vegetation degradation. Therefore, we suggested that the recalcitrant carbon fraction should be taken into account when evaluating the effect of land-use change on SOC changes in future studies. The higher regression coefficient of the relationship between F4 as a function of total SOC and the higher percentages of passive carbon pool (F3 + F4) in total SOC indicated that the SOC in the study area was characterized by a predominantly recalcitrant carbon fraction. Vegetation degradation had no significant effect on the percentage of active carbon in total SOC content and the lability of SOC, suggesting that vegetation degradation did not change the stability of SOC in the Songnen plain. These results obtained in this study provide a useful framework for understanding the changes in SOC content and stability under changing environmental conditions.

### **Declaration of Competing Interest**

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

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