#### ORIGINAL PAPER

# Soil carbon stocks and quality across intact and degraded alpine wetlands in Zoige, east Qinghai-Tibet Plateau

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Abstract The wetlands on the Qinghai-Tibet Plateau are experiencing serious degradation, with more than 90,000 hectares of marshland converted to wet meadow or meadow after 40 years of drainage. However, little is known about the effects of wetland conversion on soil C stocks and the quality of soil organic carbon (SOC) (defined by the proportion of labile versus more resistant organic carbon compounds). SOC, microbial biomass carbon, light fraction organic carbon (LFOC), dissolved organic carbon, and the chemical composition of SOC in the soil surface layer (0–10 cm), were investigated along a wetland degradation gradient (marsh, wet meadow, and meadow). Wetland degradation caused a 16 % reduction in the carbon stocks from marsh  $(178.7 \pm 15.2 \text{ kg C m}^{-2})$  to wet meadow  $(150.6 \pm 15.2 \text{ kg C m}^{-2})$ 21.5 kg C m<sup>-2</sup>), and a 32 % reduction in C stocks of the 0–10 cm soil layer from marsh to meadow

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 $(122.2 \pm 2.6 \text{ kg C m}^{-2})$ . Wetland degradation also led to a significant reduction in SOC quality, represented by the lability of the carbon pool as determined by a density fractionation method  $(L_{LEOC})$ , and a significant increase in the stability of the carbon pool as reflected by the alkyl-C:O-alkyl-C ratio.  $^{13}$ C NMR spectroscopy showed that the labile form of C (O-alkyl-C) declined significantly after wetland degradation. These results assist in explaining the transformation of organic C in these plateau wetland soils and suggest that wetland degradation not only caused SOC loss, but also decreased the quality of the SOC of the surface soil.

**Keywords** Solid-state  ${}^{13}C$  NMR  $\cdot$  Microbial biomass carbon (MBC) - Dissolved organic carbon (DOC) - Alpine marsh - Meadow - Wetland degradation





## Introduction

Soil organic matter (SOM) is an important part of many terrestrial ecosystems, given its role in regulating the supply of nutrients to plants and microbes, and its influence on soil moisture and long-term carbon (C) storage (McLauchlan and Hobbie [2004](#page-10-0)). The various ecosystem functions associated with SOM, or soil organic carbon (SOC), depend on both its quantity and quality (Smith [2005](#page-11-0)), where the quality of SOC is the ease with which carbon in the SOM can be mineralized (Fissore et al. [2009\)](#page-10-0).

Due to the impact of land use (Six et al. [2002a](#page-11-0); Leifeld and Kögel  $2005$ ; Luan et al.  $2010$ ) and climate change (Kirschbaum  $2006$ ; Agren  $2000$ ) on the vulnerability of SOC stocks, a number of SOC fractionation procedures have been proposed in order to distinguish the more easily decomposed SOC (i.e., relatively low stability, high-quality, labile) from that which is less easily decomposed (i.e., relatively high stability, low-quality, recalcitrant) (Six et al. [2002b](#page-11-0); von Lützow et al. [2007\)](#page-11-0). Labile carbon indicators, such as the light fraction organic carbon (LFOC), particulate organic carbon (POC) (Janzen et al. [1992](#page-10-0); Six et al. [2002a](#page-11-0)), microbial biomass carbon (MBC), and dissolved organic carbon (DOC) (Neff and Asner [2001\)](#page-10-0), were developed relying on physical or chemical fractionation methods. Although solid state  $^{13}$ C CP/ MAS NMR has been developed to provide a semiquantitative evaluation of soil chemical composition nondestructively (Huang et al. [2008](#page-10-0); Dignac et al. [2002\)](#page-10-0), no existing single biological, physical, or chemical fractionation technique adequately describes the continuum of SOM in nature (Paul et al. [2006](#page-10-0)). There is still no consensus as to which method or combination of methods is most suitable to evaluate the impacts of land use changes on the quality of SOC (Skjemstad et al. [2006\)](#page-11-0). Therefore, in order to get a more accurate understanding of the effects of land use change on soil carbon, it is necessary to evaluate the quality of the SOC by employing multiple indices (Luan et al. [2010\)](#page-10-0), particularly since the quality of the SOC is considered to be an important factor influencing the temperature sensitivity of SOC decomposition  $(Q_{10}$  values) both in field studies (Arevalo et al. [2010;](#page-9-0) Luan et al. [2013\)](#page-10-0) and in laboratory studies (Arevalo et al. [2012](#page-9-0); Xu et al. [2012;](#page-11-0) Fierer et al. [2005\)](#page-10-0).

Wetlands store approximately one-third of the world's soil C, despite only occupying approximately 2–6 % of the earth's land surface (Whiting and Chanton [2001](#page-11-0); Kayranli et al. [2010\)](#page-10-0). Thus, wetlands play an important role in global C cycling, particularly in the context of climate change and other human disturbances that may affect the storage and decomposition of carbon (Wu [2012\)](#page-11-0). As the largest wetland on the Eurasian continent, the Qinghai-Tibet Plateau accounts for 51.15 %  $(\sim 48,075 \text{ km}^2)$  of China's natural wetland (excluding lakes and floodplains)(Ding et al. [2004](#page-10-0)). However, wetlands in this region are experiencing a severe decline as a result of intensive human activities (e.g. drainage, grazing, peat harvest etc.) (Xiang et al. [2009;](#page-11-0) Wang et al. [2007a](#page-11-0)). Drainage through ditching has intensely changed the hydrologic condition and caused severe degradation of the Zoige wetland (Bai et al. [2009\)](#page-9-0) in the northeast of Qinghai-Tibet Plateau, which is the largest high altitude wetland in the world. For example, around 1,000 km of channel (covering around  $2,000 \text{ km}^2$ ) was dug between 1965 and 1973, and another 50.5 km (covering  $148 \text{ km}^2$ ) was dug in the early 1990s, to meet the increasing demand for grazing land (Sun [1998a](#page-11-0)), which resulted in more than 905.1993  $\text{km}^2$  of wetland loss through degrading into wet or drier meadows (Bai et al. [2009\)](#page-9-0). There are three stages during wetland degradation in the Qinghai-Tibet plateau, and each stage is mainly determined by changes in hydrology: (1) permanently inundated wetland (marsh, representing intact wetland); (2) seasonally inundated wetland (wet meadow, representing intermediately degraded wetland); and (3) no inundated meadow (dry meadow, representing severely degraded wetland) (Huo et al. [2013](#page-10-0)). The impact of wetland degradation on the quantity and especially the quality of the SOC in these wetlands is unknown.

In this study, both the loss and change in quality of soil carbon were quantified along a degradation gradient from marsh to wet meadow, and then meadow. Our objectives were to: (1) investigate the effect of wetland degradation on the labile organic



Fig. 1 Location of the study sites, Zoige wetland

carbon, chemical composition and the quality of SOC; and (2) identify the similarities or differences among carbon fractionation methods for describing the continuum of SOM.

#### Materials and methods

#### Site description and soil sampling

The study sites were located at the Wetland Ecological Research Station in the Zoige National Wetland Reserve (33°56'N, 102°52'E, 3,430 m.a.s.l.), Sichuan Province, China. The area is characterized by a mild, cold, temperate continental monsoon climate. The annual mean temperature is about  $0.7-1.1$  °C, with the highest monthly mean being  $10.8 \degree C$  in July and the lowest being  $-10.6$  °C in January. The annual mean precipitation is 656.8 mm, with 86 % of this occurring between April and October (Xiang et al. [2009\)](#page-11-0). Based on the three wetland degradation stages (i.e., intact wetland, early degrading, and degraded), we chose five marshes (Gangna-a, Fenqu-a, Shebao, Fenqu-b, Hua lake-b; marsh), three wet meadows (Chuke, Hua lake-a, Gangna-b; wet meadow), and four meadows (Angda, A'Xi-a, A'Xi-b, A'Xi-c; meadow) for the investigations. These wet meadows and meadows were converted from marshes after 20–40 years of drainage. Alpine peat (Histosols, FAO; Fibrists, USDA), and alpine meadow soils (mat cryic cambisols, FAO) (Wang et al. [2007b](#page-11-0)) are common in this area, with perennial or seasonal and temporary water logging as a result of the flat topography. The soil parent material is mainly homogeneous silt and clay (Sun [1998b\)](#page-11-0). All sample sites were exposed to grazing by yak and sheep. The location of the sampling sites is shown in Fig. 1, and a common set of site attributes is summarized in Table [1](#page-3-0).

At each site, three  $5 \times 5$  m<sup>2</sup> plots over 10 m apart from each other were established. Three soil cores from each plot were extracted (7 cm in diameter, 10 cm in depth) and thoroughly mixed, and then each sample was divided into two parts. One part of the sample was air dried for pH, mass-based SOC, TN, LFOC, and solid-state  $^{13}$ C cross polarization with magic angle spinning nuclear magnetic resonance  $(^{13}C)$ CP/MAS NMR) measurement (three replicates from each site were mixed into one sample for  ${}^{13}$ C CP/MAS NMR analysis). The other part was kept at field moisture content at  $4^{\circ}$ C for MBC, microbial biomass nitrogen (MBN), extractable DOC, and  $K_2SO_4$ extractable nitrogen (DON) measurements after

<span id="page-3-0"></span>

Values in parentheses are standard errors (SE),  $n = 3$ 

2 weeks; 100 mL (50.46 mm diameter, 50 mm height) sampling cylinders were used for analyses of bulk density (BD).

#### Soil analysis

#### Physical fractionation

Light fraction SOM was obtained by density fractionation based on a study by Six et al. [\(1998](#page-11-0)), but with a modification using a  $CaCl<sub>2</sub>$  solution (density of 1.5 g mL<sup> $-1$ </sup>) as Luan et al. [\(2011](#page-10-0)) described. The soil structure of a suspension of 10 g of soil and 25 mL  $CaCl<sub>2</sub>$  solution were disrupted by ultrasonication with an energy of 225 J  $mL^{-1}$  applied over 12 min. The supernatant solution containing the light fraction was vacuum filtered through a  $0.45 \mu m$  fiberglass filter (previously dried at 50 $\degree$ C and weighed) after centrifuging. The filter and the retained light fraction were rinsed with distilled water, dried at  $65^{\circ}$ C, ground and analyzed for organic C and N. Bulk SOC and LFOC contents were determined using a wet oxidation method with 133 mmol  $L^{-1}$  K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub> at 170–180 °C (Lu [2000\)](#page-10-0). Soil nitrogen concentration in bulk soil (TN) and light fractions (LFN) were determined using the micro-Kjeldahl method. Soil pH was measured from soil–water suspensions (1:5, v/v).

#### Chemical fractionation

MBC and MBN of each sample was determined using the chloroform fumigation-extraction method (modified from Vance et al. [1987\)](#page-11-0), while the waterlogged soil was fumigated according to Inubushi et al. ([1991\)](#page-10-0) method. Both fumigated and nonfumigated soils were extracted with 50 mL 0.5 M  $K_2SO_4$  and then filtered after removal of the CHCl<sub>3</sub> from the soil by repeated evacuations. The extracts were kept frozen at  $-30$  °C before analysis using a MULTI N/C 3100 (Analytik Jena AG). The total amount of MBC and MBN were determined by the difference between  $K_2SO_4$ -extractable C and N in fumigated and non-fumigated soil, with correction factors of  $K_{EC} = 0.45$  (Sparling et al. [1990\)](#page-11-0) and  $K_{EN} = 0.54$  (Brookes et al. [1985\)](#page-10-0) for MBC and MBN, respectively. Extractable DOC was determined as total organic C in extracts from nonfumigated soil.

Carbon pool 'lability' (or SOC quality) defined by different fractionation methods was calculated using the method described by Luan et al.  $(2010)$ :

$$
L_{\text{LFOC}} = LFOC/(SOC - LFOC) \tag{1}
$$

 $L_{\text{extractableDOC}} = DOC/(SOC - DOC)$  (2)

$$
L_{\rm MBC} = MBC/(SOC - MBC) \tag{3}
$$

where LFOC is the light fraction organic carbon; SOC is the soil organic carbon; DOC is the extractable dissolved organic carbon; and MBC is the microbial biomass carbon.

# Solid-state <sup>13</sup>CP/MAS NMR

To remove paramagnetic compounds and to increase the C content of the mineral soil fractions, soil samples were treated with hydrofluoric acid (10 %, v/v) prior to CPMAS  $^{13}$ C NMR spectroscopy, as previously demonstrated by Schmidt et al. [\(1997](#page-11-0)). Soil samples were packed in a  $ZrO<sub>2</sub>$  rotor (7 mm outside diameter). Solid-state <sup>13</sup>CP/MAS NMR measurements were performed at magic angle spinning (MAS) rates of 5 kHz using a Bruker AVANCE III 400 spectrometer, operating at 100.64 MHz. Single contact time of 1 ms was applied with an acquisition time of 42 ms, and a recycle delay of 1 s. Twenty thousand transients were collected for all samples, and a Lorentzian line broadening function of 50 Hz was applied to all spectra. Chemical shift values were referenced externally to glycine at 176.03 ppm, which is equivalent to tetramethylsilane at 0 ppm (Wang et al. [2010b](#page-11-0)).

The  $^{13}$ C NMR spectra was divided into four chemical shift regions: (i) aliphatic or alkyl-C (0–50 ppm) of lipids, fatty acids, and plant aliphatic polymers (recalcitrant form of C); (ii) O-alkyl-C (50–112 ppm) derived primarily from polysaccharides (cellulose and hemicelluloses), but also from proteins and side chains of lignin (labile form of C); (iii) aromatic or aryl-C (112–163 ppm) derived from lignin and/or protein; and finally, (iv) carbonyl-C (163–190 ppm) derived from aliphatic esters, carboxyl groups and amide carbonyls (Faz Cano et al. [2002\)](#page-10-0). Areas of the chemical-shift regions were measured by integration and were expressed as a percentage of the total area (relative intensity). As recommended by Baldock et al. ([1997\)](#page-9-0), this study considers the ratio of alkyl-C to  $O$ -alkyl-C as an index of the extent of decomposition or substrate quality for <span id="page-5-0"></span>microbes. An aromaticity index has been used to characterize the extent of humification of SOM (Dai et al. [2001](#page-10-0)), with the index defined as:

$$
Aromaticity \, (\%)
$$
\n
$$
= \frac{Aromatic - C}{Alkyl - C + O-alkyl - C + Aromatic - C} \times 100
$$
\n(4)

Statistical analysis

One way analysis of variance (ANOVA) with the least significant difference (LSD) was performed to assess the effect of wetland degradation on labile carbon, nitrogen concentration, C:N ratio, carbon pool lability, and the relative intensity of C in the region, alkyl-C:Oalkyl-C ratio, and aromaticity. Regression analysis was used to examine the relationships among labile carbon indicators, carbon pool lability, and the extent of decomposition. Statistical analyses were performed using the SPSS 13.0 software package for Windows.

#### Results

Soil C stocks

Soil BD was significantly increased after wetland degradation, from  $0.31 \pm 0.01$ (SD) g cm<sup>-3</sup> in the marsh to  $0.58 \pm 0.08$  g cm<sup>-3</sup> in the wet meadow, and  $1.06 \pm 0.06$  g cm<sup>-3</sup> in the meadow at the 0-10 cm soil layer ( $F = 70.4, P < 0.001$ ). However, the SOC stock in the 0–10 cm soil layer was reduced, from 178.7  $\pm$ 15.2 kg C m<sup>-2</sup> in the marsh to  $150.6 \pm 21.5$ kg C m<sup>-2</sup> in the wet meadow, and  $122.2 \pm 2.6$ kg C m<sup>-2</sup> in the meadow ( $F = 4.29$ ,  $P < 0.05$ ). No significant change in soil pH was observed ( $F = 2.22$ ,  $P = 1.64$  $P = 1.64$  $P = 1.64$ ) (Table 1), and a negative exponential model described the relationship between BD and SOC  $(SOC = 1,021.9e^{-2.09 \times BD}, R^2 = 0.94, P < 0.001).$ 

Labile organic carbons and carbon pool lability

Soil MBC, LFOC, MBN, extractable DON, and LFN were reduced, while extractable DOC increased in the 0–10 cm layer after degradation from marsh to wet meadow (Fig. 2). Wetland degradation also led to a significant decrease in LF C:N ratio and a significant increase of DO C:N (Fig. 2). Additionally, an increase



Fig. 2 a Concentrations of soil MBC, MBN; b Concentrations of soil extractable DOC, DON, and DO C:N; c Concentrations of soil LFOC, LFN, and LF C:N for meadow  $(n = 4)$ , wet meadow  $(n = 3)$ , and marsh  $(n = 5)$ . Different *lowercase* letters above each bar indicate a significant difference at  $P = 0.05$  level

of carbon pool lability, as shown by density fractionation method  $(L_{LFOC})$ , was found after wetland degradation (Table [2\)](#page-6-0). Both MBC and LFOC strongly correlated with SOC ( $R^2 = 0.59$  for MBC and 0.94 for LFOC,  $P < 0.001$ ) (Fig. [3a](#page-6-0), c). Significant correlation between DOC and SOC ( $R^2 = 0.66$ ,  $P \lt 0.001$ ) was only found after data from the marsh was excluded (Fig. [3](#page-6-0)b).

<b>Types</b>	Meadow	Wet meadow	Marsh
$L_{\rm MBC}$	0.0017(0.0004)a	0.0019(0.0003)a	0.0020(0.0003)a
$L_{\text{extractable DOC}}$	0.0019(0.0002)a	0.0017(0.0002)a	0.0006(0.0001)b
$L_{LFOC}$	0.1102(0.0133)a	0.2237(0.0404)b	0.5834(0.0326)c
Chemical shift range (ppm)			
Relative intensity of C region $(\%)$			
$0 - 50$ (alkyl-C)	28.3(0.55)a	$24.1(1.59)$ b	20.4(0.72)c
50–112 $(O\text{-alkyl-C})$	44.1(0.72)a	45.1(0.71)a	$50.4(0.41)$ b
112–163 (aromatic C)	16.4(0.50)a	21.2(1.07)b	20.9(0.86)
163–190 (carboxyl C)	11.3(0.20)a	9.6(1.02)b	$8.3(0.27)$ b
alkyl- $C/O$ -alkyl- $C$	0.64(0.02)a	$0.53(0.03)$ b	0.41(0.01)c
Aromaticity (%)	18.5(0.54)a	$23.5(1.43)$ b	$22.8(0.96)$ b

<span id="page-6-0"></span>Table 2 Carbon pool lability (0–10 cm) as reflected by different methods, and composition of organic matter (functional groups) for 12 sites (0–10 cm) as determined by solid-state  $^{13}$ C CP/MAS-NMR spectroscopy

Values in parentheses are standard errors (SE),  $n = 4$ , 3, and 5 for meadow, wet meadow, and marsh respectively

Different lowercase letters in each row indicate a significant difference at  $P = 0.05$  level



Fig. 3 Linear regressions between SOC with a MBC, b extractable DOC (data from marsh were excluded), and c LFOC. Linear regressions between MBC and extractable DOC (d) (data from marsh were excluded); MBC and LFOC (e); LF C:N and  $L_{\text{LFOC}}(f)$ 

Solid-state 13CP/MAS NMR spectroscopy

Soil samples in the 0–10 cm layer showed similar distribution of functional groups across the three sites, in the order of O-alkyl  $>$  alkyl $>$  aromatic  $>$  carboxyl (Table 2). However, wetland degradation significantly increased the alkyl-C  $(\%)$ , while it reduced the O-alkyl-C  $(\%)$  (Table 2). Alkyl-C:O-alkyl-C ratio and the aromaticity of 12 sites varied from 0.37 to 0.69 and 17.35 to 5.71 %, respectively. The alkyl-C:O-alkyl-C ratio increased while the *aromaticity* reduced significantly after degradation (Table 2).

<span id="page-7-0"></span>Table 3 Correlation coefficients between relative intensity of O-alkyl-C with MBC, extractable DOC, and LFOC; as well as between alkyl-C:O-alkyl-C ratio with  $L_{\text{MBC}}$ ;  $L_{\text{extractable DOC}}$ ; LLFOC; extractable DOC/DON; and LF C:N ratio

Factors	Intensity $(\%)$			
	R	F	P	
$O$ -alkyl-C				
MBC	0.74	12.18	0.006	
Extractable DOC	0.17	0.28	0.61	
<b>LFOC</b>	0.89	38.9	< 0.001	
alkyl-C:O-alkyl-C				
$L_{\rm MBC}$	0.35	1.42	0.26	
$L_{\rm{DOC}}$	0.89	41.6	< 0.001	
$L_{\text{LFOC}}$	0.96	120.9	< 0.001	
Extractable DOC:DON	0.45	2.5	0.14	
LF C:N	0.80	17.9	0.002	



Fig. 4 Linear regression between aromaticity  $(\%)$  and  $L_{\text{LFOC}}$ 

Relationships among soil labile carbon indicators

A positive correlation between MBC and LFOC was found  $(R^2 = 0.62, P < 0.001,$  Fig. [3e](#page-6-0)), while a positive correlation between MBC with extractable DOC was only found when data from the marsh were excluded (Fig. [3](#page-6-0)d). The variance of  $O$ -alkyl-C (%) can be explained by the MBC and LFOC ( $R^2 = 0.55$ , and 0.80, respectively) (Table 3), and the variation of alkyl-C:O-alkyl-C ratio can be explained by the  $L_{LEOC}$ , and LF C:N ratio ( $R^2 = 0.43{\text -}0.92$ ) (Table 3). We found a positive linear relationship between the  $L_{\text{LFOC}}$  and LF C:N ratio (Fig. [3](#page-6-0)f). The  $L_{\text{LFOC}}$  explains the variation of *aromaticity* among sites ( $\mathbb{R}^2$  > 0.35) (Fig. 4).

#### **Discussion**

Soil organic carbon and carbon pool lability

Wetland degradation results in a significant reduction in the 0–10 cm soil layer C stocks (e.g., 15.8 and 31.6 % of reduction in the wet meadow and meadow respectively as compared with the marsh). This reduction is mainly attributed to the reduction in the SOC concentration (Table [1](#page-3-0)) because the BD was significantly increased. Both the increase in decomposition and the reduction in biomass input after degradation (e.g., a higher grazing intensity in the meadow and wet meadow because of easier access) can be a consequence of the decrease in the surface soil C stocks.

The MBC concentrations in the meadow (0.20  $\pm$ 0.05 mg  $g^{-1}$ ) and wet meadow (0.54  $\pm$  0.19 mg  $g^{-1}$ ) are comparable to those reported in the Kobresia meadow in Haibei, where they ranged 0.47–0.80 mg  $g^{-1}$  (Wang et al. [2010a](#page-11-0)). In our study, however, the MBC only contributed 0.17, 0.19, and 0.20 % to the SOC in the meadow, wet meadow, and marsh, respectively. These contributions are lower than those reported in other studies, which showed a contribution of 1–5 % (e.g., Haynes  $2005$ ). A lower prevalence of the MBC in the SOC at our sites may be attributed to the lower mean annual temperature at this high elevation, hence the lower microbial community and activity (Plante et al. [2011](#page-11-0)). A significant reduction in the MBC after wetland degradation (Fig. [2](#page-5-0)) could be partly attributed to the reduction in the substrate supply, such as LFOC (Fig. 4b).

The marsh was found to have a lower extractable DOC than the wet meadow (a moderately degraded wetland). Similarly, Song et al. ([2011\)](#page-11-0) observed a greater loss of the DOC in the river from the degraded wetland compared to the pristine marshy rivers. The lower concentrations of the soil extractable DOC in the marsh may be caused by its waterlogged conditions, because the DOC efflux may be increased due to a higher leaching frequency (Christ and David [1996](#page-10-0); Godde et al. [1996\)](#page-10-0). However, no significant difference in the extractable DON between the marsh and wet meadow was detected. This finding suggests that the dissolved OM with a high C:N ratio is lost from the marsh, leading to a significantly lower C:N ratio of the extractable OM in the top soil (Fig. [2b](#page-5-0)). Further, the urine and feces of yaks and sheep, with a high N concentration, also contributes to the lower DO C:N in these grazing sites. Different pathways for the C and N losses may also explain the differences (i.e., the soluble C was respired and the soluble N was immobilized or simply not leached). The wet and dry cycles increase the solubility of OM in the soil (Miller et al. [2005](#page-10-0)), which results in a higher extractable DOC in the wet meadow than in the meadow.

The LFOC concentrations (i.e., 212.2, 56.9, and 11.5 g C  $kg^{-1}$  in the marsh, wet meadow, and meadow, respectively) and proportions (i.e., 36.5, 17.6, and 9.8 % in the marsh, wet meadow, and meadow, respectively) are higher than those reported in the forests (i.e., 6.62–23.09 g C kg<sup>-1</sup>, 9.5–20.4 % (Luan et al. [2010](#page-10-0))), which are also located in the east edge of the Qinghai-Tibet plateau. However, the carbon content of the LFN (e.g., 267.42, 308.09, and 387.82 g C kg<sup>-1</sup> light fraction in the meadow, wet meadow, and marsh, respectively) are similar to the previous studies in the forests (e.g., 270.6–314.4 g C kg<sup>-1</sup> light fraction (Luan et al. [2010](#page-10-0))), indicating a similar composition of partly decomposed OM (i.e., light fraction OM). Interestingly, a significantly higher C:N ratio of LF was found in the marsh than in the meadows (Fig. [2c](#page-5-0)), suggesting that wetland degradation results in a higher decomposition extent of light fraction. The significantly higher  $L_{\text{LOC}}$ and aromaticity in the marsh than in the meadows further confirm our speculation (Table [2,](#page-6-0) 3). Nonetheless, the litter input from different species among our sites may also account for the differences in the LFOC, because the plant trait is a major control factor on the soil C sequestration (Russell et al. [2004](#page-11-0)).

In addition, the variation in the SOC among our sites strongly correlated with both the MBC and LFOC (Fig. [3](#page-6-0)), which confirms that these labile indicators are effective indicators of soil carbon due to land use change. However, the carbon pool lability represented by the different fractionation methods has different sensitivities to degradation effects (Tables [2](#page-6-0), [3](#page-7-0)). For example, significant reductions in the carbon pool lability after degradation can only be detected by the density fractionation method and  $^{13}$ C NMR technique, indicating that different techniques fractionate the soil labile C pool differently (McLauchlan and Hobbie [2004\)](#page-10-0).

# Solid-state <sup>13</sup>CP/MAS NMR spectroscopy

Our study found that wetland degradation significantly affects the chemical composition of the SOC (Table [2](#page-6-0)). The spectra of the top soil in the three sites showed similar structures with various proportions. Among the four C functional groups, O-alkyl-C showed the highest relative intensity, which is consistent with the previous reports in the grasslands (Helfrich et al. [2006\)](#page-10-0) and marshes (Wang et al. [2011](#page-11-0)). High O-alkyl-C signals in the  $^{13}$ C NMR spectra, representing mainly the plant polysaccharides, suggests a high potential of decomposition in the soil C (Almendros et al. [2000](#page-9-0)). Therefore, the highest relative intensity of the O-alkyl-C signal further confirms that a low annual temperature results in an accumulation of the labile organic carbon. Nevertheless, wetland degradation leads to a significant reduction in the relative intensity of O-alkyl-C and a significant increase in the relative intensity of alkyl-C, indicating an increase in the proportion of the recalcitrant SOC (Lorenz et al. [2007\)](#page-10-0). Therefore, the decline in the SOC quality caused by wetland degradation results in a higher risk of C loss in the degraded wetlands under the projected future warming because the fractions of the more recalcitrant (lower quality) soil carbon are more sensitive to changes in temperature (Xu et al. [2012](#page-11-0); Hartley and Ineson [2008;](#page-10-0) Craine et al. [2010](#page-10-0)).

The aryl-C:O-alkyl-C ratio, a useful indicator of the degree of SOM decomposition (Baldock et al. [1997\)](#page-9-0) or substrate quality for microbes, is also significantly affected by the degradation, with the highest value occurring in the meadow and the lowest occurring in the marsh (Table [2](#page-6-0)). The aryl-C:O-alkyl-C ratio trend among the wetland degradation gradient confirms the effect of wetland degradation on the SOC quality. Similarly, *aromaticity*  $(\%)$  has been used to characterize the extent of humification of the SOM (Dai et al. [2001\)](#page-10-0). The lowest aromaticity was found in the meadow (Table [2](#page-6-0)), and this observation reflects the lowest extent of humification of the SOM in the meadow compared with the marsh and wet meadow. A lower redox potential in the marsh  $(-157)$  to  $-130$  mV) than in the wet meadow  $(-114$  to -63 mV) was reported in the same area (Wang et al. [2003;](#page-11-0) Chen et al. [2008\)](#page-10-0), which can potentially help explain the lower humification extent in the marshes and account for why there are significant relationships

<span id="page-9-0"></span>among some site variables only when the marshes are omitted from our analyses (e.g., Fig. [3](#page-6-0)b, d).

#### Carbon pool lability indicators

There are significant correlations among the labile carbon indicators (i.e., the extractable DOC, MBC and LFOC; Fig. [3](#page-6-0)). This finding is consistent with previous reports (Laik et al. [2009](#page-10-0); McLauchlan and Hobbie [2004;](#page-10-0) Sparling et al. [1998](#page-11-0)). A higher proportion of the labile C in the SOM, represented by the labile carbon indicators (e.g., MBC and LFOC), can support a higher microbial biomass, and thus an enhanced microbial activity, which favors the production and/ or accumulation of O-alkyl-C (Chen et al. [2004](#page-10-0)). Therefore, positive linear relationships between the Oalkyl-C intensity and the labile carbon indicators (e.g., MBC and LFOC) were found (Table [3](#page-7-0)).

Recently, the simpler and more rapid indices of SOC stability or quality are increasingly applied (Plante et al. [2011](#page-11-0); Luan et al. [2010](#page-10-0); Baldock et al. 1997). Several studies have compared these methods for measuring the labile SOC with significantly different sizes, and found positive correlations among various SOC quality indicators (McLauchlan and Hobbie [2004;](#page-10-0) Luan et al. [2010;](#page-10-0) Chen et al. [2004](#page-10-0); Plante et al.  $2011$ ). In this study, the  $L_{LEOC}$ ,  $L_{MBC}$ ,  $L_{\text{extractable DOC}}$ , aryl-C:O-alkyl-C ratio, and *aromatic*ity (%) were also employed to evaluate the SOC quality based on different fractionation methods. Most of them can reasonably reflect the decomposition or the SOC quality. These indicators were also found to have tight connections to one another, especially the significantly linear relationship between the aryl-C:Oalkyl-C ratio and  $L_{LFOC}$ , indicating that the density fractionation method and solid state  $^{13}$ C NMR spectroscopy can detect the soil decomposition extent or quality. Mathers et al. ([2003\)](#page-10-0) reported that the aromaticity index is probably a better estimate for the degree of decomposition compared with the aryl-C:O-alkyl-C ratio. However, we did not find a higher sensitivity of *aromaticity* to wetland degradation than that of the aryl-C:O-alkyl-C ratio (Table [2](#page-6-0)). Further, the aryl-C:O-alkyl-C ratio better explained the variation of  $L_{\text{LFOC}}$  among study sites ( $\mathbb{R}^2 = 0.92$ , Table [3\)](#page-7-0) than the *aromaticity* ( $R^2 = 0.36$ , Fig. [4](#page-7-0)). This result suggests that the aryl-C:O-alkyl-C ratio is a better indicator for the SOC quality and decomposition extent.

## **Conclusions**

Our results indicate that the marsh and wet meadow have a larger capacity for storing the SOC in the top 10 cm soil layer, especially those of the labile fractions, compared to the meadow. Also, wetland degradation will lead to a decrease in the carbon pool lability or SOC quality (an increase in the proportion of the recalcitrant organic C). Therefore, there is a high risk of soil carbon loss because both environment change and human activity disturbance can result in an intensive degradation of wetland in the Qinghai-Tibet plateau. In addition, the density fractionation method obtains similar results to the  $^{13}$ C NMR technique in evaluating the SOC quality.

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