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Soil organic carbon contents, aggregate stability, and humic acid composition in different alpine grasslands in Qinghai-Tibet Plateau

CAO Zhi-yuan[1] http://orcid.org/0000-0002-6423-8291; e-mail: mingyuan891220@126.com

WANG Yong[1] http://orcid.org/0000-0001-9967-1610; e-mail: wang7236879@163.com

LI Jie[2] http://orcid.org/0000-0002-3849-9383; e-mail: 535889657@qq.com

ZHANG Jin-jing[1*] http://orcid.org/0000-0002-5443-1354; e-mail: zhangjinjing@126.com

HE Nian-peng[2*] http://orcid.org/0000-0002-0458-5953; e-mail: henp@igsnrr.ac.cn

** Corresponding author*

1 College of Resource and Environmental Science, Jilin Agricultural University, Changchun 130118, China

2 Key Laboratory of Ecosystem Network Observation and Modeling, Institute of Geographic Sciences and Natural Resources Research, Chinese Academy of Sciences, Beijing 100101, China

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Abstract: Alpine grassland soils on Qinghai-Tibet Plateau store approximately 33.5 Pg of organic carbon (C) at 0–0.75 m depth and play an important role in the global carbon cycle. We investigated soil organic C (SOC), water-soluble organic C (WSOC), easily oxidizable organic C (EOC), humic C fractions, aggregate-associated C, aggregate stability, and humic acid (HA) composition along an east-west transect across Qinghai-Tibet Plateau, and explored their spatial patterns and controlling factors. The contents of SOC, WSOC, EOC, humic C fractions and aggregate-associated C, the proportions of macro-aggregates (2-0.25) and micro-aggregates (0.25-0.053 mm), and the aggregate stability indices all increased in the order alpine desert < alpine steppe < alpine meadow. The alkyl C, O-alkyl C, and aliphatic C/aromatic C ratio of HA increased as alpine desert < alpine meadow < alpine steppe, and the trends were reverse for the aromatic C and HB/HI ratio. Mean annual precipitation and aboveground biomass were significantly correlated with the contents of SOC and its fractions, the proportions of macro- and micro-aggregates, and the aggregate stability indices along this transect. Among all these C fractions, SOC content and aggregate stability were more closely associated with humic C and silt and clay sized C in comparison with WSOC, EOC, and macro- and micro-aggregate C. The results suggested that alpine meadow soils containing higher SOC exhibited high soil aggregation and aggregate stability. Mean annual precipitation should be the main climate factor controlling the spatial patterns of SOC, soil aggregation, and aggregate stability in this region. The resistant and stable C fractions rather than labile C fractions are the major determinant of SOC stocks and aggregate stability.

Keywords: Soil organic carbon; Aggregate stability; Humic acid; Carbon-13 nuclear magnetic resonance; Latitudinal transect; Qinghai-Tibet Plateau

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Introduction

Soil organic carbon (SOC) plays a vital role in soil fertility and ecological environment because of its large contribution to soil properties, such as soil structure (Bronick and Lal 2005), water retention (Rawls et al. 2003), microbial activity (Degens et al. 2000), and nutrient availability (Oelofse et al. 2015). As a heterogeneous mixture of organic substances, SOC is composed of various fractions with apparent differences in the degree of decomposition, recalcitrance, and turnover rate (Huang et al. 2008). The different SOC fractions have different effects on soil fertility and ecological environment. The labile fractions of SOC, such as water-soluble organic carbon (WSOC) and easily oxidizable organic carbon (EOC), have been considered as more sensitive indicators for land use changes compared to SOC (Yang et al. 2009a). On the other hand, most SOC exists as chemically resistant humic substances, namely, humic acid (HA), fulvic acid (FA) and humin (HU), which constitute approximately 60%-75% of SOC (Grinhut et al. 2011). Owing to their refractory properties, humic substances play a crucial role in soil carbon sequestration (Swift 2001). Soil aggregate stability is an important indicator of soil physical quality (Chen et al. 2009). Formation and stabilization of soil aggregates generally facilitates soil carbon sequestration and provides physical protection for soil carbon (Cheng et al. 2015). Soil aggregate-associated carbon has been used as a major indicator in evaluating soil carbon sequestration (Six et al. 2000; Bronick and Lal 2005). Therefore, the investigation of SOC fractions is important for better understanding the turnover and stabilization mechanisms of SOC. Carbon-13 nuclear magnetic resonance (^{13}C NMR) spectroscopy is a powerful tool for characterizing the chemical composition of soil organic matter, which can clarify quantitatively the structural properties of humic substance fractions at the molecular level (Conte et al. 2004).

The Qinghai-Tibet Plateau is the highest and largest plateau in the world, covering an area of 2.5 × 10^6 km^2 (Zheng 1996) at an average elevation of 4000 m a.s.l. (Shang et al. 2015). Meanwhile, some studies have demonstrated the region is very sensitive to global climate changes (Wang et al. 2007; Zhang et al. 2014). Alpine grasslands, which cover more than 50% of the whole plateau area, are the dominant ecosystem in the region (Chen et al. 2014a; Tang et al. 2015). The alpine grassland soils contain a large amount of organic carbon and thus have an important role in the regional and global carbon cycle. It is estimated that SOC storage in the alpine grassland soils (0–0.75 m depth) across the entire Qinghai-Tibet Plateau is approximately 33.5 Pg C (Wang et al. 2002). Therefore, the estimates of SOC storage and turnover in this region have attracted much attention in the past decades (Yang et al. 2009b; Tan et al. 2010; Wang et al. 2013). However, previous studies have mainly focused on the content or storage of SOC among different types of alpine grasslands on Qinghai-Tibet Plateau (Wang et al. 2002; Cao et al. 2013; Liu et al. 2014b; Shang et al. 2015; Tang et al. 2015). To the best of our knowledge, few studies have been conducted to explore the differences in SOC fractions, soil aggregate stability, and soil HA composition among the different types of alpine grasslands in this region, although they are important (Shang et al. 2016).

In this study, we investigated SOC, WSOC, EOC, humic C fractions and aggregate-associated C, and the chemical composition of HA along a 2200 km east-west transect across Qinghai-Tibet Plateau in order to understand the responses of soil properties to environmental changes. Specifically, the main objectives of this study were to: (1) quantify the variation in the contents of SOC, WSOC, EOC, humic C fractions, aggregate-associated carbon, and HA composition among different alpine grasslands (alpine meadow, alpine steppe, and alpine desert), and (2) explore the influencing factors of soil C fractions and stability in view of precipitation, temperature, and plant biomass, along the transect.

1 Materials and Methods

1.1 Study sites

Our study sites are shown in Figure 1. In detail, a 2200 km east-west transect across different types of grasslands in Qinghai-Tibet Plateau was set up in August 2013. We set up nine sampling sites along this transect, which represented alpine

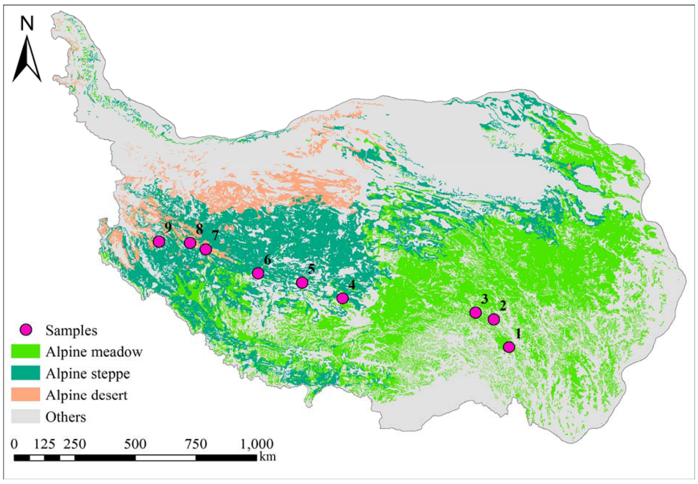

Figure 1 Location of the study sites.

meadow, alpine steppe, and alpine desert, respectively. Within each grassland type, three sampling sites were selected as replicates. In each site, four quadrats (0.5 m × 0.5 m) were established at 20 m intervals along a random transect. All aboveground plants were harvested from these quadrats, dried (60°C), and weighed for aboveground biomass (AGB) measurement. The basic information for the sampling sites, including longitude, latitude, elevation, mean annual precipitation (MAP), mean annual temperature (MAT), grassland type, soil type, soil texture, soil mineralogy, dominant plant species, and AGB were listed in Table 1.

1.2 Soil sampling and analysis

In each sampling site, surface soil samples (0–20 cm) were collected from the same four quadrats used for AGB measurement, and then pooled to form one composite sample. The fresh soil samples were sieved to pass through a 5-mm sieve, and air dried at room temperature. One portion of the samples was used for aggregate determination, and the other part was sieved through a 2- and 0.25-mm sieve for SOC analysis.

SOC contents were measured using the potassium dichromate-sulfuric acid oxidation method (Lu 2000). EOC was determined using the 333 mmol L^{-1} $KMnO_4$ oxidation method (Blair et al. 1995). Water-soluble and humic fractions were obtained following the method of Zhang et al. (2010) by successively extracting soil samples with distilled water and in 0.1 mol L^{-1} NaOH + 0.1 mol L^{-1} $Na_4P_2O_7$ solution, respectively; HA fraction was separated from FA fraction by acidifying the total

Table 1 Basic information of the sampling sites along the east-west grassland transect on the Qinghai-Tibet Plateau, western China

No.	Long.	Lat.	Elev. (m)	MAP (mm)	MAT (°C)	Grassland type	Soil type	Soil texture	Soil mineralogy		Dominant plant species	AGB ($g\ m^{-2}$)
									Fe_d ($g\ kg^{-1}$)	Al_d ($g\ kg^{-1}$)		
1	97° 12′	30° 24′	4207	516.6	0.75	Alpine meadow	Subalpine meadow soil	Sand loam	4.35	0.85	*K. pygmaea*, *K. humilis*	115.6
2	96° 26′	31° 24′	3931	514.4	-0.60	Alpine meadow	Subalpine meadow soil	Sand loam	4.54	1.43	*K. pygmaea*, *K. humilis*	177.6
3	95° 36′	31° 36′	4618	524.7	-1.24	Alpine meadow	Subalpine meadow soil	Sand loam	4.39	1.77	*K. pygmaea*, *K. humilis*	129.2
4	89° 42′	31° 32′	4578	367.5	-3.11	Alpine steppe	Alpine steppe soil	Sand loam	3.34	0.26	*S. purpurea*, *S. pinnate*	86.0
5	87° 50′	31° 52′	4527	327.4	-2.55	Alpine steppe	Alpine steppe soil	Sand loam	3.72	0.27	*S. purpurea*, *S. pinnate*	75.6
6	85° 50′	31° 55′	4907	308.7	-3.45	Alpine steppe	Alpine steppe soil	Sand loam	3.47	0.25	*S. purpurea*, *S. pinnate*	64.0
7	83° 20′	32° 24′	4547	259.2	-3.49	Alpine desert	Alpine desert soil	Sand	3.96	0.52	*S. glareosa*, *O. thoroldii*	31.8
8	82° 36′	32° 30′	4370	299.5	-6.72	Alpine desert	Alpine desert soil	Sand	3.11	0.22	*S. glareosa*, *O. thoroldii*	24.2
9	81° 14′	32° 17′	4527	237.3	-3.46	Alpine desert	Alpine desert soil	Sand	3.67	0.28	*S. glareosa*, *A. wellbyi*	30.2

Note: MAP = Mean annual precipitation; MAT = Mean annual temperature; Grassland is classified according to Chinese Rangeland Resources Distribution Map (ARDOQP 1997); Soil is classified according to Chinese Genetic Soil Classification (Xi 1998); Soil texture is named according to International System (ISSS 1929); Dithionite-citrate-bicarbonate extractable iron (Fe_d) and aluminum (Al_d); AGB = Above-ground biomass.

humic extract (HE) to pH 1.0. The solid residue obtained after alkaline extraction was referred to as HU fraction. A 50 g of soil sample (< 5 mm) was wet sieved to obtain three water-stable aggregate size classes, i.e., macroaggregates (2–0.25 mm), microaggregates (0.25–0.053 mm), and silt + clay-sized fraction (< 0.053 mm), following the methodology reported by Cambardella and Elliott (1993). There were no large macroaggregates (> 2 mm) in the soils. The aggregate size distributions and aggregate-associated carbon contents were expressed as g kg^{-1} soil and g kg^{-1} aggregates, respectively, on a sand-free basis (Chen et al. 2014b).

The extraction and purification of HA for spectroscopic analyses were performed in accordance with the procedure described by Li et al. (2015). Briefly, soil samples were extracted with a solution of 0.1 mol L^{-1} NaOH and 0.1 mol L^{-1} $Na_4P_2O_7$, precipitated with 6 mol L^{-1} HCl solution, purified by three cycles of NaOH-dissolution and HCl-flocculation, dialyzed against distilled water, and then lyophilized. The solid-state ^{13}C cross-polarization magic-angle-spinning and total-sideband-suppression (CPMAS TOSS) NMR spectra were obtained on a Bruker AVANCE III 400 WB spectrometer (Switzerland) at 100.6 MHz under the following conditions: spinning speed, 8 kHz; spectral width, 50 kHz; acquisition time, 20 ms; contact time, 2 ms; recycle delay, 3 s; and number of scans, 7000-10000. Semi-quantification was conducted using MestReNova 5.3.1 (Mestrelab Research S.L., Santiago de Compostela, Spain) by dividing the spectra into four different chemical shift regions representing alkyl C (0–50 ppm), O-alkyl C (50–110 ppm), aromatic C (110–160 ppm) and carbonyl C (160–200 ppm).

1.3 Calculations and statistical analysis

SOC stock was calculated by multiplying SOC content by soil bulk density and by soil depth (Wang et al. 2008; Chen et al. 2009). In the present study, soil bulk density was mean values obtained from eight common pedo-transfer functions (Xu et al. 2015). Mean weight diameter (MWD), geometric mean diameter (GMD), percentage of > 0.25 mm water stable aggregates ($R_{0.25}$), structure deterioration rate (SDR), index of unstable aggregates (E_{LT}), and stability ratio of water stable aggregate (WSAR) were calculated according to the methods described by Fan et al. (2010), Gupta Choudhury et al. (2014), and Liu et al. (2014a). The ratios of alkyl C/O-alkyl C, aliphatic C/aromatic C, and hydrophobic C/ hydrophilic C were calculated according to the method of Zhang et al. (2011).

All statistical analyses were conducted using SPSS 16.0 software (SPSS Inc., Chicago, IL, USA). The comparisons of dependent variables (SOC, soil aggregate stability indices, and soil HA composition) among different grassland types were determined by one-way ANOVA with LSD test. Pearson linear correlation analyses were performed to evaluate the relationships between dependent variables (vide supra) and independent variables (MAP, MAT, and AGB). The $P < 0.05$ level was considered to be significant.

2 Results

2.1 SOC, WSOC, EOC and HFC

The SOC content and stock are different among the three grassland types (Figure 2). SOC content and stock in alpine meadow were highest, followed by alpine steppe and alpine desert. There was no significant difference between alpine steppe and alpine desert.

The WSOC, EOC, HAC, FAC and HUC contents and the HAC/FAC ratios of soils are also distinct among the three grassland types (Table 2). WSOC, EOC, HAC, FAC and HUC, which accounted for 1.56%–2.81%, 5.12%–7.95%, 15.2%–22.7%, 14.1%–27.5%, and 48.3%–68.1% of SOC, respectively, followed similar patterns to SOC content along the transect. Also, there was no significant difference between alpine steppe and alpine desert. In contrast, the HAC/FAC ratios, ranging from 0.84 to 1.52, were highest in alpine desert, followed by alpine steppe and alpine meadow.

2.2 Soil aggregate-associated carbon and aggregate stability

The distribution of aggregate size fractions in soil varied across grassland types (Figure 3a). For alpine meadow and alpine steppe, the dominant aggregate size fraction was 0.25-0.053 mm, followed by 2–0.25 and <0.053 mm size fractions. For alpine desert, the dominant aggregate size fraction was <0.053 mm, followed by 0.25–0.053 and 2–0.25 mm size fractions. Among the three grassland types, the proportions of 2–0.25 and 0.25–0.053 mm aggregates were highest in alpine meadow, then in alpine steppe and alpine desert.

For all three grassland types, the SOC content of 0.25–0.053 mm aggregate was highest (Figure 3b). For alpine meadow, the SOC content of 2–0.25 mm aggregate was higher than that of <0.053 mm aggregate. However, the opposite trend was observed for alpine steppe and alpine desert.

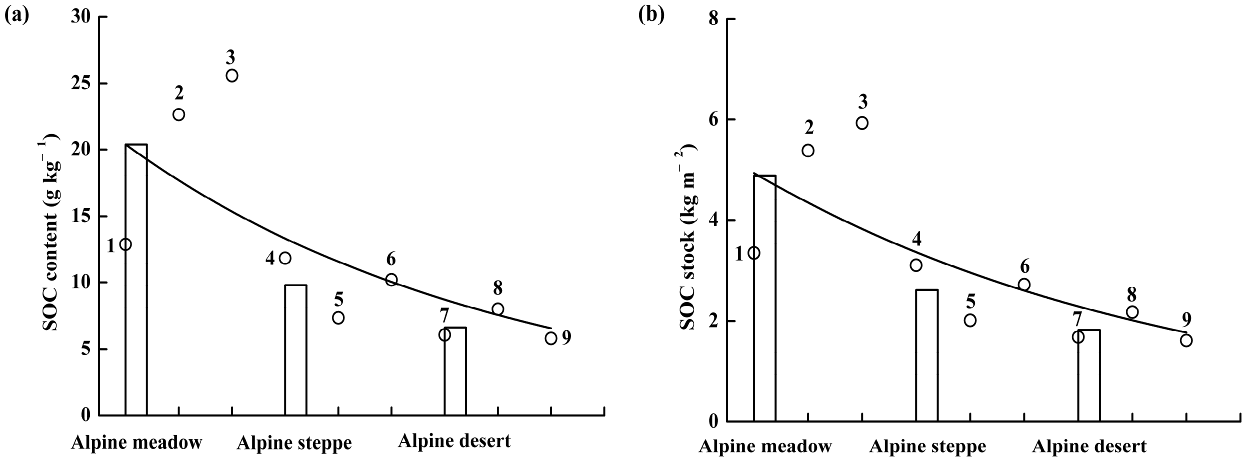

Figure 2 Soil organic carbon (SOC) content and stock from alpine meadow, alpine steppe, and alpine desert on the Qinghai-Tibet Plateau, western China. Circles and columns represent SOC content or stock of each sampling site and each grassland type, respectively. See Table 1 for site number.

Table 2 Soil water soluble organic carbon (WSOC), easily oxidizable organic carbon (EOC), humic acid carbon (HAC), fulvic acid carbon (FAC), humin carbon (HUC), and HAC/FAC ratio among different alpine grasslands on the Qinghai-Tibet Plateau, western China

Grassland type	WSOC (g kg^{-1})	EOC (g kg^{-1})	HAC (g kg^{-1})	FAC (g kg^{-1})	HUC (g kg^{-1})	HAC/FAC
Alpine meadow	0.32 ± 0.03a	1.62 ± 0.33a	4.63 ± 1.79a	5.60 ± 2.33a	9.83 ± 2.79a	0.84 ± 0.12b
Alpine steppe	0.22 ± 0.02b	0.50 ± 0.13b	1.49 ± 0.20b	1.41 ± 0.37b	6.67 ± 1.70ab	1.09 ± 0.14b
Alpine desert	0.19 ± 0.04b	0.40 ± 0.10b	1.40 ± 0.11b	0.93 ± 0.14b	4.10 ± 0.98b	1.52 ± 0.14a

Note: Mean values ± standard deviations of three replicates are presented. Values within the same column followed by the same small letter are not significantly different at $P = 0.05$.

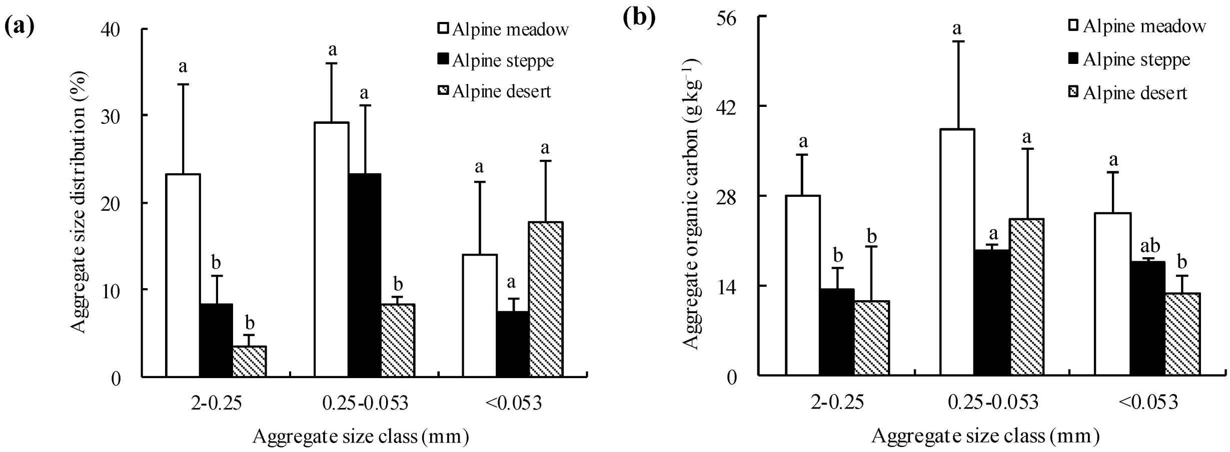

Figure 3 Distribution (a) and organic carbon content (b) of soil aggregate size fractions from alpine meadow, alpine steppe, and alpine desert on the Qinghai-Tibet Plateau, western China. Vertical bars represent standard deviation of the mean (n = 3). Bars with different small letters within the same aggregate size class represent significant differences at $P < 0.05$.

Across all aggregate size fractions, the SOC contents were highest in alpine meadow, then in alpine steppe and alpine desert. Significant differences were found between alpine meadow with alpine steppe and alpine desert for the 2–0.25 mm aggregate, and between alpine meadow and alpine desert for the <0.053 mm aggregate.

The aggregate stability indices in soil also varied among grassland types (Table 3). MWD, GMD, $R_{0.25}$ and WSAR were highest in alpine meadow, followed by alpine steppe and alpine desert. On the contrary, the SDR and E_{LT} were highest for alpine desert, hen alpine steppe and alpine meadow. There were no significant differences between alpine steppe with alpine meadow and alpine desert for MWD and GMD, and between alpine steppe and alpine desert for $R_{0.25}$ and E_{LT}.

2.3 Soil HA composition

The solid-state ^{13}C CPMAS TOSS NMR spectra of HA all exhibited major signal peaks at around 25, 30, 33, 56, 62, 72, 104, 129, 152, and 173 ppm (Figure 4). The peak assignments have been reported in our previous work (Zhang et al. 2011). On the other hand, the relative intensities of different carbon functional groups differed across grassland types (Table 4). Among the three grassland types, the alkyl C and O-alkyl C were highest in alpine steppe, followed by alpine meadow and alpine desert. The aromatic C was highest in alpine desert, followed by alpine meadow and alpine steppe. The carbonyl C was highest in alpine meadow, followed by alpine steppe and alpine desert. Significant differences were found between alpine steppe with alpine

Table 3 Soil aggregate stability indices among different alpine grasslands on the Qinghai-Tibet Plateau, western China

Grassland type	MWD (mm)	GMD (mm)	$R_{0.25}$ (%)	SDR (%)	E_{LT} (%)	WSAR (%)
Alpine meadow	0.47 ± 0.18a	0.52 ± 0.11a	23.2 ± 10.4a	52.7 ± 11.5c	76.8 ± 10.4b	47.3 ± 11.5a
Alpine steppe	0.33 ± 0.03ab	0.46 ± 0.02ab	8.25 ± 3.49b	71.6 ± 7.66b	91.7 ± 3.49a	28.4 ± 7.66b
Alpine desert	0.20 ± 0.08b	0.32 ± 0.04b	3.47 ± 1.32b	94.2 ± 2.66a	96.5 ± 1.32a	5.79 ± 2.66c

Note: Mean values ± standard deviations of three replicates are presented. Values within the same column followed by the same small letter are not significantly different at $P = 0.05$. MWD = Mean weight diameter; GMD = Geometric mean diameter; $R_{0.25}$ = Percentage of >0.25 mm water stable aggregates; SDR = Structure deterioration rate; E_{LT} = Index of unstable aggregates; WSAR = Stability ratio of water stable aggregate.

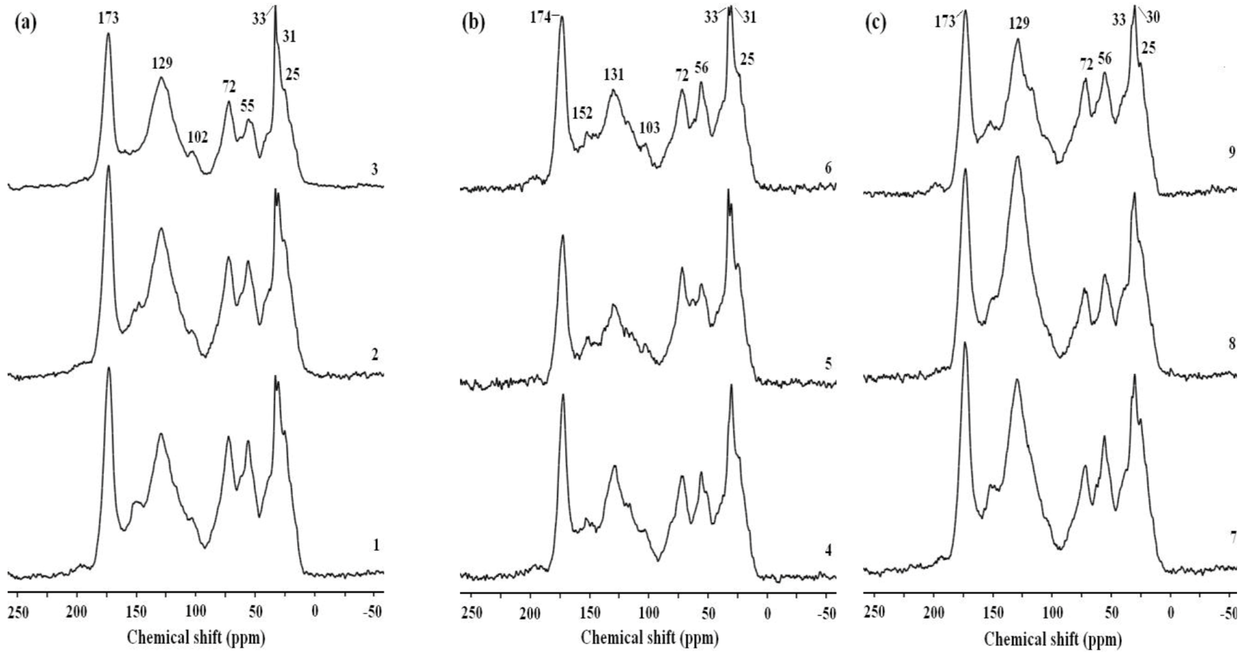

Figure 4 Solid-state ^{13}C CPMAS TOSS NMR spectra of soil humic acid from alpine meadow (a), alpine steppe (b), and alpine desert (c) along the east-west grassland transect on the Qinghai-Tibet Plateau, western China. See Table 1 for site number.

Table 4 Relative intensities of different carbon functional groups from solid-state ^{13}C CPMAS TOSS NMR spectra of soil humic acid among different alpine grasslands on the Qinghai-Tibet Plateau, western China

Grassland type	Alkyl C (%)	O-alkyl C (%)	Aromatic C (%)	Carbonyl C (%)	A/O-A	Alip/Arom	HB/HI
Alpine meadow	23.7 ± 0.47b	27.4 ± 2.15a	30.6 ± 1.16a	18.3 ± 0.86a	0.87 ± 0.08a	1.67 ± 0.13b	1.25 ± 0.08ab
Alpine steppe	26.8 ± 1.87a	29.6 ± 1.49a	26.0 ± 2.33b	17.6 ± 1.09a	0.90 ± 0.02a	2.19 ± 0.34a	1.17 ± 0.04b
Alpine desert	22.4 ± 0.43b	25.6 ± 3.92a	34.5 ± 2.81a	17.5 ± 2.37a	0.89 ± 0.12a	1.40 ± 0.22b	1.38 ± 0.15a

Note: Mean values ± standard deviations of three replicates are presented. Values within the same column followed by the same small letter are not significantly different at $P = 0.05$. A/O-A = Alkyl C/O-alkyl C; Alip/Arom = Aliphatic C/aromatic C; HB/HI = Hydrophobic C/hydrophilic C.

meadow and alpine desert for alkyl C and aromatic C. The above changes in relative intensity of the different carbon functional groups led to the aliphatic C/aromatic C ratio was highest in alpine steppe, followed by alpine meadow and alpine desert; the HB/HI ratio was highest in alpine desert, followed by alpine meadow and alpine steppe. Significant differences were found between alpine steppe with alpine meadow and alpine desert for the aliphatic C/aromatic C ratio, and between alpine steppe and alpine desert for the HB/HI ratio. However, the alkyl C/O-alkyl C ratio was not significantly different among three grassland types.

2.4 Relationships of SOC, aggregate stability, HA composition, and environmental factors

The relationships between SOC, aggregate stability, and HA composition with environmental factors (i.e., MAP, MAT and aboveground biomass) are shown in Table 5. There were significantly positive correlations between SOC and its fractions with MAP and aboveground biomass, with the exception of the SOC content of 0.25-0.053 mm aggregate which had no significant correlation with MAP and aboveground biomass. There were significantly negative correlations between the HAC/FAC ratio with MAP and AGB. However, no significant correlations were found between SOC and its fractions with mean annual temperature (MAT), with the exception of the WSOC and EOC contents which had significantly positive correlations with MAT. There were significantly positive correlations between the proportions of 2–0.25 mm aggregates with MAP and aboveground biomass, and between the

Table 5 Pearson's coefficients of correlation between soil organic carbon, aggregate stability and humic acid composition with mean annual precipitation (MAP), mean annual temperature (MAT), and above-ground biomass (AGB) along the east-west grassland transect on the Qinghai-Tibet Plateau, western China

	MAP (mm)	MAT (°C)	AGB (g/m²)
SOC (g kg^{-1})	0.879**	0.581	0.875**
SOC (kg m^{-2})	0.892**	0.591	0.886**
WSOC (g kg^{-1})	0.872**	0.825**	0.923**
EOC (g kg^{-1})	0.926**	0.818**	0.911**
HAC (g kg^{-1})	0.838**	0.588	0.881**
FAC (g kg^{-1})	0.870**	0.605	0.862**
HUC (g kg^{-1})	0.842**	0.504	0.811**
HAC/FAC	-0.890**	-0.653	-0.826**
2–0.25 mm AOC (g kg^{-1})	0.854**	0.474	0.812**
0.25–0.053 mm AOC (g kg^{-1})	0.651	0.158	0.583
<0.053 mm AOC (g kg^{-1})	0.848**	0.532	0.883**
2–0.25 mm aggregates (%)	0.849**	0.599	0.888**
0.25–0.053 mm aggregates (%)	0.792*	0.721*	0.714*
<0.053 mm aggregates (%)	-0.050	0.067	-0.269
MWD (mm)	0.719*	0.509	0.840**
GMD (mm)	0.733*	0.541	0.861**
$R_{0.25}$ (%)	0.849**	0.599	0.888**
SDR (%)	-0.890**	-0.706*	-0.898**
E_{LT} (%)	-0.849**	-0.599*	-0.888**
WSAR (%)	0.890**	0.706*	0.898**
Alkyl C in HA	0.008	0.176	0.148
O-alkyl C in HA	0.016	0.490	0.156
Aromatic C in HA	-0.132	-0.447	-0.297
Carbonyl C in HA	0.339	0.028	0.319
A/O-A in HA	-0.031	-0.503	-0.088
Alip/Arom in HA	0.019	0.324	0.172
HB/HI in HA	-0.173	-0.631	-0.337

Note: $n = 9$, * $P < 0.05$, ** $P < 0.01$. AOC = Aggregate organic carbon. For other abbreviations, see Table 1 to Table 4.

proportions of 0.25–0.053 and all the environmental factors. Among all the aggregate stability indices, the MWD, GMD, $R_{0.25}$ and WSAR were significantly positively correlated with MAP and aboveground biomass, whereas the SDR and E_{LT} were significantly negatively correlated with MAP and aboveground biomass. The SDR and E_{LT} were significantly negatively correlated with MAT. No significant correlations were observed between HA composition and all the environmental factors. Furthermore, a significantly positive correlation was observed between SOC content and dithionite-citrate-bicarbonate (DCB) extractable iron (Fe_d) ($r = 0.689$, $P < 0.05$) and aluminum (Al_d) ($r = 0.930$, $P < 0.01$).

The relationships between SOC and its fractions and aggregate stability are shown in Table 6. There were significantly positive correlations between SOC and its corresponding fractions including WSOC, EOC, HAC, FAC, HUC and aggregate-associated C. Significantly positive correlations were also generally found among WSOC, EOC, HAC, FAC, HUC and aggregate-associated C. The correlation coefficients were lower between SOC with WSOC, EOC, and macro- and micro-aggregate C than between SOC with HAC, FAC, HUC, and silt and clay sized C. In general, there were significant correlations between SOC and its corresponding fractions with aggregate stability indices. Among all these C fractions, aggregate stability indices showed a higher correlation with HAC, FAC, HUC, and silt and clay sized C in comparison with WSOC, EOC, and macro- and micro-aggregate C. In addition, there were no significant correlations between HA composition and the amounts of SOC and its fractions as well as the indices of soil aggregate stability (data not shown).

3 Discussion

3.1 SOC and its fractions

In the present study, the contents of SOC and its fractions, including SOC, labile organic C fractions (WSOC and EOC), resistant organic C fractions (HAC, FAC and HUC) and aggregate-associated organic C fractions, were all highest in alpine meadow, followed by alpine steppe and alpine desert (Table 2, Figure 2, Figure 3b). Similar results have been reported by other researchers, who found that SOC and WSOC contents were the higher in alpine meadow than in alpine steppe and alpine desert in Qinghai-Tibet Plateau (Wang et al. 2002; Cao et al. 2013; Liu et al. 2014b; Shang et al. 2015; Tang et al. 2015). Considering alpine meadow on the Qinghai-Tibet Plateau is one of the most sensitive vegetation types to climate change (Shen et al. 2015), their importance in the global carbon cycle should receive more attention. With respect to the SOC stocks at the 0–20 cm depth, our estimation (4.89 kg m^{-2} for alpine meadow and 2.61 kg m^{-2} for alpine steppe) is lower than the one of Yang et al. (2010) (6.23 kg m^{-2} for alpine meadow and 3.00 kg m^{-2} for alpine steppe) and Chang et al. (2014) (5.37 kg m^{-2} for alpine meadow and 3.28 kg m^{-2} for alpine steppe). In this study, the bulk density for the calculation of the SOC stocks were obtained by using pedotransfer functions (Xu et al. 2015), which may explain the difference. Furthermore, the difference could also be due to the underrepresentation of sampling sites in our present study, which would increase the uncertainty in regional SOC stock estimation (Chang et al. 2014).

In this study, the spatial distribution of HA/FA ratio (Table 2) indicated that HA fraction predominated in alpine desert whereas FA fraction predominated in alpine meadow, which could also be attributed to the different precipitation and plant biomass among the grassland types (Table 5). Previous studies had reported that a high precipitation promoted the formation of FA fraction (Tardy et al. 1997; Abril et al. 2009) by facilitating soil microbial activity (Abril et al. 2009), which could explain the low HA/FA ratio in alpine meadow in our present study. The HA/FA ratio has been used as indicator to describe the humification degree of SOM, with a larger value indicating a higher degree of humification (McCallister and Chien 2000; Rivero et al. 2004; Aranda and Oyonarte 2006). Thus, our results suggested that the humification degree of SOM was higher in alpine desert than in the other two grassland types The positive correlations between the contents of SOC and its fractions with aboveground biomass (Table 5) indicated that grass productivity was an important factor influencing SOC distribution along this transect. This could be attributed to the.

Table 6 Pearson correlation coefficients between soil organic carbon and its fractions and aggregate stability along the east-west grassland transect on the Qinghai-Tibet Plateau, western China

	SOC	WSOC	EOC	HAC	FAC	HUC	MWD	GMD	$R_{0.25}$	SDR	E_{LT}	WSAR	2–0.25 mm AOC	0.25–0.053 mm AOC	<0.053 mm AOC
SOC	1														
WSOC	0.872**	1													
EOC	0.797*	0.908**	1												
HAC	0.957**	0.871**	0.849**	1											
FAC	0.988**	0.872**	0.813**	0.977**	1										
HUC	0.958**	0.799**	0.684*	0.838**	0.907**	1									
MWD	0.910**	0.754*	0.642	0.880**	0.886**	0.879**	1								
GMD	0.875**	0.748*	0.618	0.807**	0.827**	0.887**	0.975**	1							
$R_{0.25}$	0.990**	0.877**	0.789*	0.964**	0.982**	0.936**	0.944**	0.902**	1						
SDR	-0.919**	-0.857**	-0.751*	-0.809**	-0.873**	-0.953**	-0.886**	-0.924**	-0.917**	1					
E_{LT}	-0.990**	-0.877**	-0.789*	-0.964**	-0.982**	-0.936**	-0.944**	-0.902**	-1.000**	0.917**	1				
WSAR	0.919**	0.857**	0.751*	0.809**	0.873**	0.953**	0.886**	0.924**	0.917**	-1.000**	-0.917**	1			
2–0.25 mm AOC	0.894**	0.834**	0.831**	0.886**	0.881**	0.840**	0.691*	0.668*	0.849**	-0.754*	-0.849**	0.754*	1		
0.25–0.053 mm AOC	0.831**	0.524	0.566	0.845**	0.848**	0.748*	0.720*	0.623	0.798**	-0.593	-0.798**	0.593	0.809**	1	
<0.053 mm AOC	0.958**	0.787*	0.739*	0.923**	0.940**	0.920**	0.940**	0.929**	0.951**	-0.900**	-0.951**	0.900**	0.842**	0.838**	1

Note: $n = 9$, * $P < 0.05$, ** $P < 0.01$. For abbreviations, see Table 2, Table 3 and Table 5.

fact that SOC is derived mainly from the decomposition and turnover of plant residues, and thus higher biomass could lead to higher SOC content. Apart from plant biomass, precipitation has been considered as one of the key climate factors controlling SOC contents (Liu et al. 2011). In a temperate grassland climosequence, Martin-Neto et al. (1998) reported a positive correlation between SOC content and MAP, which was in agreement with our present results. Although temperature is also a key climate factor affecting SOC contents, the present study indicated that temperature affected only the distribution of labile carbon organic fractions (Table 5). In previous studies, some researchers have also reported that the distribution of SOC largely depended on plant communities and soil moisture rather than soil temperature across different types of grasslands on the Qinghai-Tibet Plateau (Baumann et al. 2009; Liu et al. 2014b), which were in accordance with our present results.

In the present study, the labile, resistant and aggregate-associated organic C fractions were all significantly correlated with SOC contents (Table 6). In general, the labile, resistant and aggregate-associated organic C fractions were also significantly correlated with each other, indicating that they were closely interrelated. On the other hand, our results indicated that the correlation coefficients were lower between SOC with labile C and macro- and micro-aggregate C than between SOC with resistant C and silt and clay sized C. It has been reported that silt and clay sized C was more stable than macro- and micro-aggregate C (Buyanovsky et al. 1994; Six et al. 2000; Saha et al. 2010). Therefore, although labile C fractions have been suggested as early indicators of SOC changes (Haynes et al. 2000), the resistant and stable C forms (i.e., humic C and silt and clay sized C) were the main determinant of SOC contents and stocks present in our study region. In previous study, three main mechanisms of SOC stabilization, i.e., chemical stabilization, physical protection, and biochemical stabilization, have been proposed (Six et al. 2002). Our results suggested that SOC in alpine grasslands on the Qinghai-Tibet Plateau was mainly stabilized by chemical and biochemical mechanisms.

Our results indicated that the free Fe and Al oxides were important for the preservation of SOC, which was consistent with the previous findings (Bruun et al. 2010). Considering the importance of soil mineral phase in SOC stabilization (Doetterl et al. 2015), further studies should be carried out to examine the relationship between SOC and mineral phase in our future works.

3.2 Soil aggregate stability

The formation of soil water-stable macro- and micro-aggregates was more beneficial in alpine meadow than in other two alpine grasslands (Figure 3a). The MWD, GMD, $R_{0.25}$, SDR, E_{LT} and WSAR have been used as indices to assess soil aggregate stability. The integrated usage of these indices can provide a more comprehensive evaluation for aggregate stability. As expected, MWD, GMD, $R_{0.25}$, SDR, E_{LT} and WSAR were significantly correlated with each other (Table 6). A larger MWD, GMD, $R_{0.25}$ and WSAR and a smaller SDR and E_{LT} indicated the stability of soil aggregates was higher (Fan et al. 2010; Gupta Choudhury et al. 2014; Liu et al. 2014a). Thus, our results (Table 3) suggested that the stability of soil water-stable aggregates was the highest for alpine meadow, followed by alpine steppe and alpine desert. The reason could be higher SOC that is the main binding agent for the formation and stability of soil aggregates in alpine meadow (Bronick and Lal 2005). The significant correlations between the SOC contents and the aggregate stability indices were also observed in this study (Table 6).

All the aggregate indices had significant correlations with MAP and aboveground biomass (Table 5). The positive correlations between soil aggregate stability with MAP (Lavee et al. 1996; Boix-Fayos et al. 1998; Cerdà 2000) and aboveground biomass (Bronick and Lal 2005) have also been reported by other researchers. The decrease in soil aggregate stability with decreasing MAP may result from the reduced vegetation cover in arid environments and the increased erosion (Lavee et al. 1996; Boix-Fayos et al. 1998).

Apart from SOC, labile and resistant C fractions also play important roles in aggregate stabilization. Many researchers have found that the amounts of WSOC (Chen et al. 2009; Padbhushan et al. 2016) and humic C (Chaney and Swift 1984; Piccolo and Mbagwu 1990; Bouajila and Gallali 2008) in the soil were significantly correlated with aggregate stability, which were consistent with our present result (Table 6). Table 6 clearly represented that among all the C fractions, aggregate stability was more closely associated with HAC, FAC, HUC, and silt and clay sized C in comparison with WSOC, EOC, and macro- and micro-aggregate C, suggesting that resistant and stable C forms were the main determinant of soil aggregate stability in this region.

3.3 Soil HA composition

Generally, alkyl C and aromatic C represented the recalcitrant and hydrophobic fractions, whereas O-alkyl C was a relatively easily decomposable and hydrophilic fraction in HA (Ussiri and Johnson 2003; Spaccini et al. 2006). The larger values of the alkyl C/O-alkyl C, aliphatic C/aromatic C, and hydrophobic C/hydrophilic C ratios indicated that HA were more decomposed, aliphatic, and hydrophobic (Zhang et al. 2011). Therefore, our results suggested that the aliphaticity degree of HA was the highest for alpine steppe and the lowest for alpine desert, the hydrophobicity degree was the highest for alpine desert and the lowest for alpine steppe. However, the decomposition degree of HA was similar among the three grassland types.

Previous studies showed that precipitation, temperature and biomass all influenced HA composition in soil (Zech et al. 1997; Martin et al. 1998; Martin-Neto et al. 1998). However, these were not supported by our present study. Undoubtedly, some other factors controlled the HA composition in the grassland soils. The chemical composition of the starting materials has been considered a vital factor controlling humification process and, in turn, the HA composition, by altering the turnover rates of organic matter (Zech et al. 1997; Senesi et al. 2007). According to Swift et al. (1979), the chemical composition of the primary materials controlled the composition, activity and distribution of decomposer community in soil. In the present study, the dominant plant species were different among the three grassland types, which may result in the different HA composition for the different types of grasslands.

4 Conclusions

The contents of SOC, WSOC, EOC, HAC, FAC, HUC and aggregate-associated carbon fractions, the proportions of soil macro- and micro-aggregates, and the soil aggregate stability were highest in alpine meadow, followed by alpine steppe and alpine desert on Qinghai-Tibet Plateau. The aliphaticity and hydrophobicity degrees of HA from alpine meadow soils are between those of HA from alpine steppe and alpine desert soils. MAP and aboveground biomass are key environmental factors to impact SOC contents, soil aggregation, and aggregate stability across different grasslands types on Qinghai-Tibet Plateau. Among all the C fractions, the resistant and stable C forms, i.e., humic C and silt and clay sized C, are the main determinant of SOC stocks and aggregate stability.

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