Elevational gradient affect functional fractions of soil organic carbon and aggregates stability in a Tibetan alpine meadow

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ABSTRACT

The alpine meadow in the Tibetan plateau is predicted to be sensitive to climate change. Understanding soil organic carbon (SOC) characteristics and soil aggregate stability is essential for scientifically evaluating their effect as a carbon (C) sink or source under a climate change scenario. In this study, the spatial variations of the concentrations of SOC and its fractions, the chemical composition of SOC, and the water stability of aggregates and their relationships with climatic, vegetational and edaphic factors were investigated along an elevational gradient of an alpine meadow (4400–5200 m above sea level) on the Tibetan Plateau. The results showed that with increasing elevation, the concentrations of SOC, water-soluble organic carbon (WSOC), easily oxidizable organic carbon (EOC), humic acid carbon (HAC), fulvic acid carbon (FAC), humin carbon (HUC), and macroaggregate- and microaggregate-associated carbon increased gradually, reached a peak at 4800 or 4950 m, and subsequently decreased. Similarly, the proportion of alkyl C, the ratios of alkyl C/O-alkyl C, aliphatic C/aromatic C and hydrophobic C/hydrophilic C, and the mean weight diameter (MWD) of water-stable aggregates were higher at 4950 m than at other elevations. SOC, WSOC, EOC, HAC, FAC, HUC, macroaggregate- and microaggregate-associated carbon, alkyl C, and MWD were positively correlated with each other and with aboveground biomass (AGB) and free/amorphous Fe- or Al-oxides. The above results indicated that SOC and aggregate stability presented an unimodal pattern along the elevational gradient. The observed unimodal distribution of AGB associated with an optimal combination of temperature and precipitation was responsible for the distribution of SOC and aggregate stability. The physical protection in microaggregates, chemical interactions with Fe- or Al-oxide minerals, and biochemical protection by recalcitrant alkyl C may be the primary mechanisms for SOC preservation in alpine meadows. Our results suggest that climate change in the future can impact SOC and structural stability by regulating the distribution of AGB on this vertical transect. Given the low SOC and structural stability at lower elevations, we recommend that these sites be specifically protected from anthropogenic disturbance to sustain high grassland ecosystems and address future climate change in this region.

1. Introduction

Soil is the largest carbon (C) pool in terrestrial ecosystems, with approximately 2344 Gt organic C being stored in the top three meters of soil (Stockmann et al., 2013). Soil organic carbon (SOC) plays an important role in soil fertility and ecological process, such as C sequestration (Dinakaran et al., 2014), soil structure (Bronick and Lal,

2005), and microbial activity (Leinweber et al., 2008). In theory, the functions of SOC depend to a large extent on their quantity and quality. Therefore, understanding the amount and chemical composition of SOC will be of great importance for elucidating its response to actual conditions and underlying mechanisms of climate change in the future.

Soil organic matter (SOM) is a heterogeneous mixture of organic compounds and it consists of various fractions varying in stability,
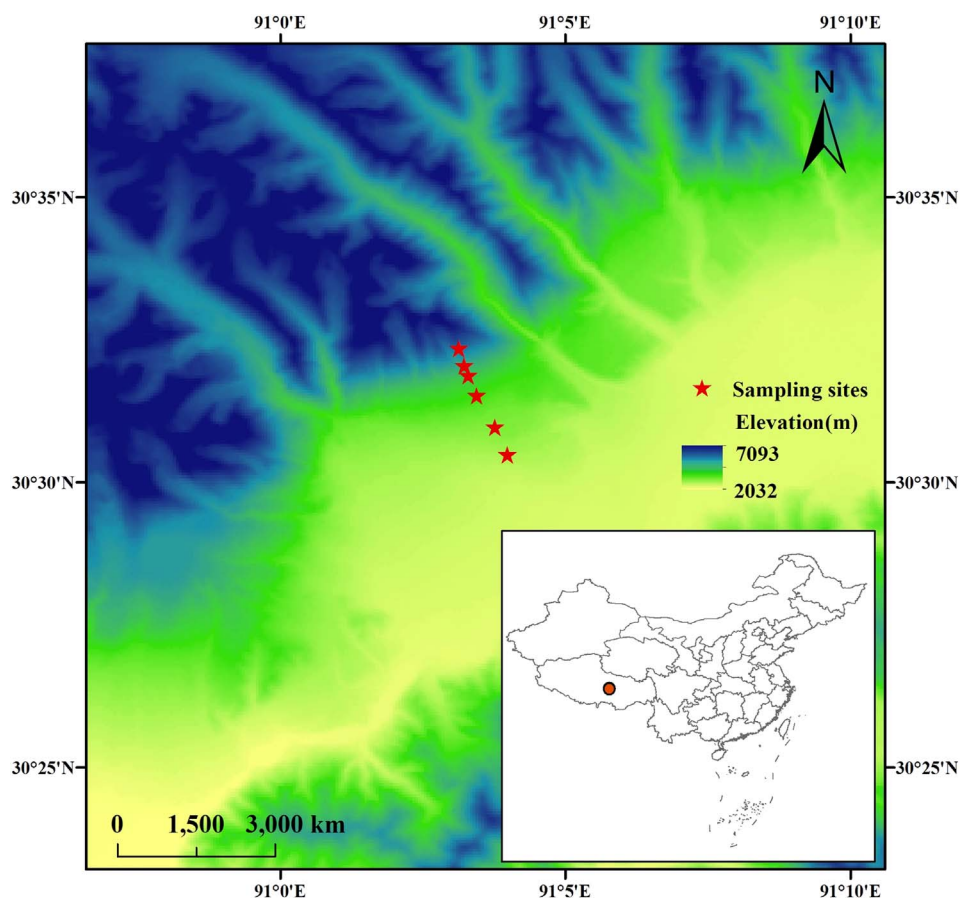
Fig. 1. Topographical map showing the sampling locations on the Tibetan Plateau.

decomposition degree and turnover rate (Huang et al., 2008). The different fractions of SOM exhibit different effects on soil fertility and environmental quality (Yang et al., 2009). Knowledge of SOM fractions is thus important for understanding the turnover and stabilization of SOC under climate change scenarios (Cao et al., 2016). Labile SOC fractions [e.g., water-soluble organic carbon (WSOC) and easily oxidizable organic carbon (EOC)] and chemically resistant SOC fractions [e.g., humic acid carbon (HAC), fulvic acid carbon (FAC) and humin carbon (HUC)] are two major fractions of SOM. They have both been reported to act as potential indicators of climate change (Steinberg, 2003; Cao et al., 2016; Wang et al. 2016a; Hu et al., 2017). Moreover, aggregate-associated C is also often considered as a key indicator in evaluating SOC sequestration and stability (Bronick and Lal, 2005). Microaggregate-associated C is generally more stable than macroaggregate- and silt + clay-associated C (Yu et al., 2012; Huang et al., 2016). Soil mineral phases (e.g., free/amorphous Fe- and Al-oxides) can not only act as binding agents for the formation and stability of soil aggregates (Bronick and Lal, 2005; Peng et al., 2015; Wang et al. 2016b) but can also protect SOC by chemical interaction (Six et al., 2002; Bruun et al., 2010; Adhikari and Yang, 2015). In addition to SOC contents, the knowledge of SOC chemical composition is essential for understanding the biochemical mechanism of accumulation and stabilization of SOM (Six et al., 2002; Plaza et al., 2013). Solid-state ^{13}C nuclear magnetic resonance (NMR) spectroscopy has proven to be a powerful tool to characterize the chemical composition of SOC (Simpson et al., 2011).

The Tibetan Plateau is the largest grassland unit on the Eurasian continent (Hu et al., 2016), with an area of 2.5×10^6 km^2 (Zheng, 1996), a mean elevation of 4000 m above sea level (Shang et al., 2016), and an elevation range from 500 to 8848 m above sea level (Dai et al., 2011). It is estimated that SOC storage at 0–0.75 m depth in the alpine

grassland soils across the Plateau is approximately 33.5 Pg C, representing 2.5% of the global soil C pool (Wang et al., 2002) and > 20% of the SOC storage in China (Shang et al., 2016). The Plateau has been an ideal region for the study of C cycles and corresponding feedback interactions to climatic change in high-elevation ecosystems (Wang et al., 2002; Yang et al., 2008; Liu et al., 2012). On the mountainous Plateau, environmental conditions (e.g., climate, vegetation biomass, and soil mineralogy) change sharply along the elevational gradient of the mountains. Therefore, an investigation of the variation in quantitative and qualitative characteristics of SOC along the elevational gradient within this region is essential for scientifically evaluating their effect as a C sink or source under climate change scenarios. The alpine meadow, with elevations ranging from 3000 to 5400 m above sea level (Guo et al., 2012), is a major grassland type on the Tibetan Plateau (Fu et al., 2012). Meanwhile, the alpine meadow is considered extremely sensitive and vulnerable to climate change (Chen et al., 2014a). In previous studies, some researchers have reported on the variation of SOC concentrations (Xu et al., 2014; Yuan et al., 2014) and SOC pool (Ohtsuka et al., 2008) along the elevational gradient on the Tibetan Plateau. However, to our knowledge, no study has been conducted to explore the variations in SOC fractions concentrations, SOC chemical composition, and aggregate size and stability along the elevational gradient in alpine meadows on the Tibetan Plateau. Moreover, it is still unclear how the differences in environmental factors influence the variations in the qualitative and quantitative characteristics of SOC and aggregate stability at different elevations in this region.

In this study, along an elevational gradient (4400–5200 m above sea level) across the southern slope of the Nyainqentanglha Mountains on the Tibetan Plateau, we evaluated the variation in the concentrations of SOC and any chemical and physical fractions (i.e., WSOC, EOC, humic carbon, and water-stable aggregate-associated carbon), as well as the

chemical composition of SOC and the water stability of aggregates. Furthermore, we explored the factors influencing SOC and aggregate stability in view of climate (i.e., temperature, precipitation), vegetation (i.e., aboveground biomass) and edaphic factors (i.e., pH, particle size distribution, and Fe- and Al-oxides). We hypothesized that the functional forms of SOC and aggregate stability varied along the elevational gradient due to changing environmental conditions.

2. Materials and methods

2.1. Site description

The study was conducted at the Lhasa Plateau Ecosystem Research Station of Chinese Academy of China, near Damxung County, Tibet (30°30′–30°32′N, 91°03′E) (Fig. 1). The climate was subfrigid semiarid continental monsoon, with a mean annual air temperature (MAT) of 1.3 °C and a mean annual precipitation (MAP) of 476.8 mm. A southern slope of the Nyainqentanglha Mountains was selected for the present study. The elevation of the slope varies from 4300 m to 5600 m, with the turf line at approximately 5200 m. The low and middle part of the slope (4300–5200 m) is covered by pasture, whereas the high part of the slope (above 5200 m) is bare land. In June 2015, six sampling sites were set in the pasture along the elevational gradient, i.e., at 4400, 4500, 4650, 4800, 4950, and 5200 m. The rangeland type is alpine meadow, which is dominated by *Kobresis pygmaea*, *Stipa capillacea*, and *Androsace tapete*. The soil is classified as alpine meadow soil (Chinese Genetic Soil Classification), Cambosol (Chinese Soil Taxonomy), Inceptisol (USDA Soil Taxonomy), or Cambisol (WRB Soil Classification). Since 2005, meteorological stations (HOBO weather station, Onset Computer Corporation, MA, USA) have been established at these sampling sites. Basic meteorological information for the six sampling sites is listed in Table 1.

2.2. Soil sampling and analysis

Soil samples (0–20 cm) at each sampling site were taken from three randomly selected locations around the meteorological stations. All aboveground plants were harvested from three 1 m × 1 m quadrats at approximately 5 m intervals, oven-dried (60 °C), and weighed for the measurement of AGB. The fresh soil samples were sieved through a 5 mm sieve, homogenized, and air-dried. One portion of the samples was used for soil aggregates analysis, and the remaining was sieved to 2 mm for SOC and other soil properties analysis.

2.2.1. SOC measurement

The organic C concentrations in bulk soil samples were measured using sulfuric acid-potassium dichromate oxidation at 170–180 °C followed by titration with 0.1 M ferrous sulfate (Lu, 2000).

2.2.2. EOC measurement

EOC was determined as described by Blair et al. (1995). Briefly, soil samples containing approximately 15 mg C were oxidized with 333 mM $KMnO_4$ solution for 1 h, and the amount of EOC was measured spectrophotometrically from the amount of $KMnO_4$ reduced.

2.2.3. WSOC and humic C measurements

WSOC and humic C were determined according to the method of Zhang et al. (2010). Briefly, water soluble and humic fractions were obtained by extracting soil samples with distilled water and 0.1 M $NaOH + 0.1$ M $Na_4P_2O_7$ solution, respectively. HA was isolated from FA by acidifying the alkaline supernatants to pH 1.0, and the alkaline-insoluble residue was HU. The concentrations of WSOC, HAC and FAC were measured using a TOC-VCPH analyzer (Shimadzu, Kyoto, Japan), whereas that of HUC was calculated by subtracting the sum of WSOC, HAC and FAC from SOC.

Table 1
Basic information of the sampling sites along the elevational gradient on the Tibetan Plateau.

Elevation (m)	MAT (°C)	MAP (mm)	AGB (g m^{-2})	pH	Fe$_d$ (g kg^{-1})	Al$_d$ (g kg^{-1})	Fe$_o$ (g kg^{-1})	Al$_o$ (g kg^{-1})	Sand (%)	Silt (%)	Clay (%)
4400	1.60	468.6	64.2 ± 10.8	6.33 ± 0.06 a	4.16 ± 0.15 b	1.68 ± 0.45 cd	1.72 ± 0.13 cd	0.42 ± 0.05 e	55.8 ± 6.88 c	20.1 ± 1.88 cd	24.1 ± 5.39 a
4500	1.26	410.2	49.7 ± 8.25	6.35 ± 0.02 a	4.13 ± 0.07 b	1.97 ± 0.08 c	1.40 ± 0.15 e	0.46 ± 0.13 de	67.0 ± 0.99 b	18.0 ± 1.18 d	15.1 ± 1.98 cd
4650	0.88	451.4	171.6 ± 29.7	6.41 ± 0.05 a	4.29 ± 0.09 b	2.38 ± 0.07 b	1.98 ± 0.29 bc	0.72 ± 0.10 bc	59.1 ± 1.51 c	22.0 ± 0.93 bc	18.9 ± 0.59 bc
4800	0.62	490.3	296.4 ± 25.3	6.02 ± 0.12 b	4.48 ± 0.12 a	2.94 ± 0.14 a	2.20 ± 0.13 b	0.76 ± 0.06 b	57.4 ± 1.47 c	22.5 ± 0.73 b	20.0 ± 1.32 abc
4950	-0.01	529.1	379.5 ± 13.2	6.01 ± 0.13 b	4.48 ± 0.01 a	2.92 ± 0.08 a	3.64 ± 0.28 a	1.72 ± 0.13 a	54.6 ± 0.37 c	25.0 ± 0.62 a	20.4 ± 0.25 ab
5200	-1.11	506.3	133.1 ± 30.3	5.92 ± 0.12 b	3.80 ± 0.06 c	1.33 ± 0.04 d	1.65 ± 0.07 de	0.59 ± 0.03 cd	77.3 ± 7.13 a	11.5 ± 3.07 e	11.2 ± 4.16 d

Different lowercase letters within the same column indicate significant differences at $P < 0.05$ (mean ± standard deviation, $n = 3$).
MAT: mean annual temperature; MAP: mean annual precipitation; AGB: aboveground biomass; Fe$_d$ and Al$_d$: free Fe and Al oxides, which are measured by dithionite-citrate-bicarbonate method (Lu, 2000); Fe$_o$ and Al$_o$: amorphous Fe and Al oxides, which are measured by oxalate ammonium method (Lu, 2000). Sand (2–0.02 mm), silt (0.02–0.002 mm) and clay (< 0.002 mm) are quantified by pipet method (Lu, 2000).

2.2.4. Soil aggregates analysis

Water-stable aggregates were separated into three size classes, i.e., macroaggregates (2–0.25 mm), microaggregates (0.25–0.053 mm) and silt + clay fractions (< 0.053 mm), using a regular wet-sieving method (Cambardella and Elliott, 1993).

The distribution of aggregate-size classes and the concentration of aggregate-associated organic C were expressed on a sand-free basis (Chen et al., 2014b). However, C concentration in the macroaggregates at the highest elevation site (i.e., 5200 m) was not corrected for sand due to the very high sand content (approximately 93%) in this sample (Lugato et al., 2010). The mean weight diameter (MWD) of water-stable aggregates was calculated (Gupta Choudhury et al., 2014).

2.2.5. Solid-state ^{13}C NMR analysis

After treatment with 10% HF-HCl solution (Schmidt et al., 1997), solid-state ^{13}C cross-polarization magic-angle-spinning and total-side-band-suppression (CPMAS TOSS) NMR spectra of soil samples were obtained on an AVANCE III 400 WB spectrometer (Bruker BioSpin AG, Fällanden, Switzerland) at 100.6 MHz. The conditions used were as follows: spinning rate, 8 kHz; acquisition time, 20 ms; recycle time, 3 s; contact time, 2 ms; spectral width, 50 kHz; line broadening, 80 Hz; and zero-filled to 131,072 data points. Chemical shift values were referenced externally to the methylene resonance of adamantane standard at 38.4 ppm.

The spectra were divided into four common chemical shift regions representing alkyl C (0–50 ppm), O-alkyl C (50–110 ppm), aromatic C (110–160 ppm), and carbonyl C (160–190 ppm). The relative intensity of each chemical shift region was obtained by integration using MestReNova 5.3.1 software (Mestrelab Research S.L., Santiago de Compostela, Spain). The ratios of alkyl C to O-alkyl C (alkyl C/O-alkyl C), aliphatic C to aromatic C (aliphatic C/aromatic C), and hydrophobic C to hydrophilic C (hydrophobic C/hydrophilic C) were calculated (Zhang et al., 2009).

2.3. Statistical analysis

Statistical analyses were performed using SPSS 16.0 software (SPSS Inc., Chicago, IL, USA). One-way analysis of variance (ANOVA) with a least significant difference (LSD) test was used to evaluate the differences of dependent variables (SOC contents, soil aggregate stability, and SOC chemical compositions) among different elevations. Pearson correlation analysis was used to examine the relationships between dependent variables (vide supra) with environmental factors (i.e., elevation, MAT, MAP, and AGB) and other soil properties (i.e., particle size distribution, pH, and Fe- and Al-oxides). The $P < 0.05$ level was considered to be significant.

3. Results

3.1. SOC, WSOC, EOC, and humic C

The SOC concentration gradually increased with elevation from 19.7 g kg^{-1} at 4400 m up to 50.6 g kg^{-1} at 4800 m and 46.7 g kg^{-1} at 4950 m, and then decreased to 16.8 g kg^{-1} at 5200 m (Fig. 2). The elevations at 4800 and 4950 m had significantly higher SOC concentration than the other elevations. On the other hand, the SOC concentration at the lowest (4400 m) elevation was not significantly different than that at 5200 m.

The concentrations of WSOC, EOC, HAC, FAC, and HUC in soils showed similar trends to SOC concentration along the elevational gradient (Fig. 2). The concentrations of these SOC fractions were also highest at the middle elevation, which were significantly higher than those at lower and higher elevations. The WSOC and EOC concentrations were significantly higher at the lowest elevation than at the highest elevation, whereas the HAC, FAC and HUC concentrations were not significantly different between the two elevations. The ratio of HAC

to FAC (HAC/FAC) was higher at 4800 m than at other elevations (Fig. 2).

3.2. Soil aggregate-associated organic C and aggregate stability

The size distribution of water-stable aggregates in soils varied along the elevational gradient (Fig. 3). Across all elevations, the proportion of microaggregates was larger than that of macroaggregates and silt + clay fractions at 4400, 4500, 4650 and 4800 m, while the proportions of macroaggregates and silt + clay fractions were larger at 4950 and 5200 m, respectively. With increasing elevation, the proportions of macroaggregates, microaggregates and silt + clay fractions reached their maximums at 4950, 4500 and 5200 m, respectively, larger than at other elevations.

Across all elevations, the concentration of microaggregate-associated organic C was larger than those of the macroaggregate- and silt + clay-associated organic C (Fig. 3). Similarly to SOC, the concentrations of macroaggregate-, microaggregate-, and silt + clay-associated organic C were also significantly larger at 4800 and 4950 m than at other elevations. Moreover, the ratio of macroaggregate- to microaggregate-associated organic C was significantly lower at 5200 m than at other elevations (Fig. 4).

The MWD of water-stable aggregates increased gradually with elevation, reached a peak at 4950 m, and then decreased (Fig. 4). The elevation at 4950 m had significantly larger MWD than others.

3.3. Solid-state ^{13}C NMR spectra of soil samples

The solid-state ^{13}C CPMAS TOSS NMR spectra of soil samples exhibited major resonance peaks at 21, 23, 30, 32, 54, 61, 72, 104, 128, 152, and 172 ppm (Fig. 5). The peak assignments have been reported in our previous work (Zhang et al., 2009; Zhang et al., 2013). The peaks at 21, 23, 30 and 32 ppm in the alkyl C region were assigned as $-CH_3$, $-CH_2-$, amorphous $-(CH_2)_n-$ and crystalline $-(CH_2)_n-$, respectively. The peak at 54 ppm in the O-alkyl C region was assigned as methoxyl C in lignin, and the peaks at 61, 72 and 104 ppm were assigned as $-CH_2OH-$, $-CHOH-$ and anomeric C in carbohydrate, respectively. The peaks at 128 and 152 ppm in the aromatic C region were ascribed to aryl C and phenolic C, respectively. The peak at 172 ppm in the carbonyl C region was indicative of carboxylic acid, amide and ester.

On the other hand, the relative intensities of different resonance peaks varied along the elevational gradient (Table 2). Across all elevations, the intensity of O-alkyl C was the largest (36.3–39.9%), followed by alkyl C (23.2–31.2%) and aromatic C (21.4–26.3%), and the intensity of carbonyl C was the lowest (10.6–12.5%). With elevation, the intensity of alkyl C gradually increased, and peaked at 4950 m, and then decreased. The intensity of carbonyl C also first increased and then decreased but peaked at 4500 m. In contrast, the intensities of O-alkyl C and aromatic C gradually decreased to 4950 m and then increased. The ratios of alkyl C/O-alkyl C, aliphatic C/aromatic C, and hydrophobic C/hydrophilic C all reached the maximum at 4950 m, larger than other elevations.

4. Discussion

4.1. Variations in SOC and aggregate stability along the elevational gradient

SOC and its fractions, including labile SOC fractions (i.e., WSOC and EOC), resistant SOC fractions (i.e., HAC, FAC and HUC), and water-stable aggregate-associated SOC fractions, were significantly larger at elevations 4800 and 4950 m than at lower and higher elevation sites (Fig. 2 and Fig. 3). It suggested that the distribution of SOC and its fractions along the elevational gradient presented an unimodal pattern. Our results were consistent with previous findings on the Tibetan Plateau (Ohtsuka et al., 2008; Yuan et al., 2014). For example, Ohtsuka
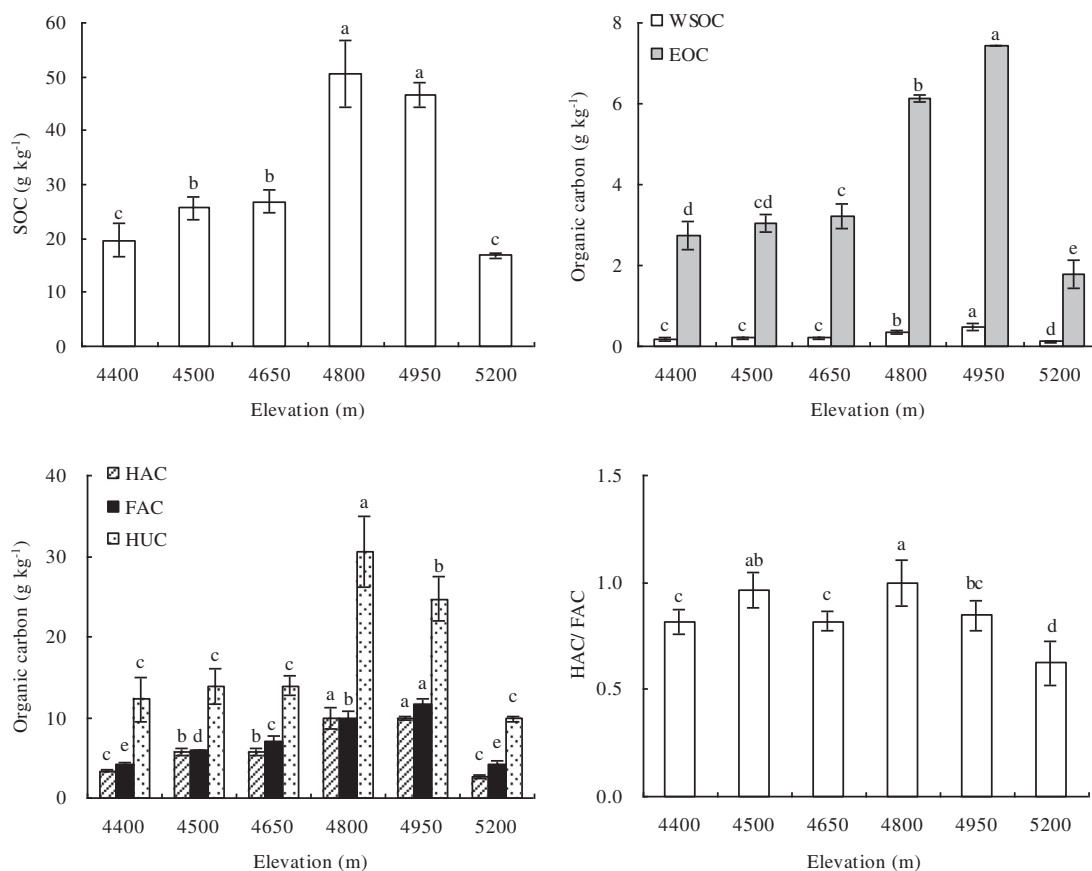
Fig. 2. Concentrations of soil organic carbon (SOC) and its fractions along the elevational gradient on the Tibetan Plateau. The bars having same letters among different elevations are not significantly different (*P* < 0.05). WSOC: water-soluble organic carbon; EOC: easily oxidizable organic carbon; HAC: humic acid carbon; FAC: fulvic acid; HUC: humin carbon.
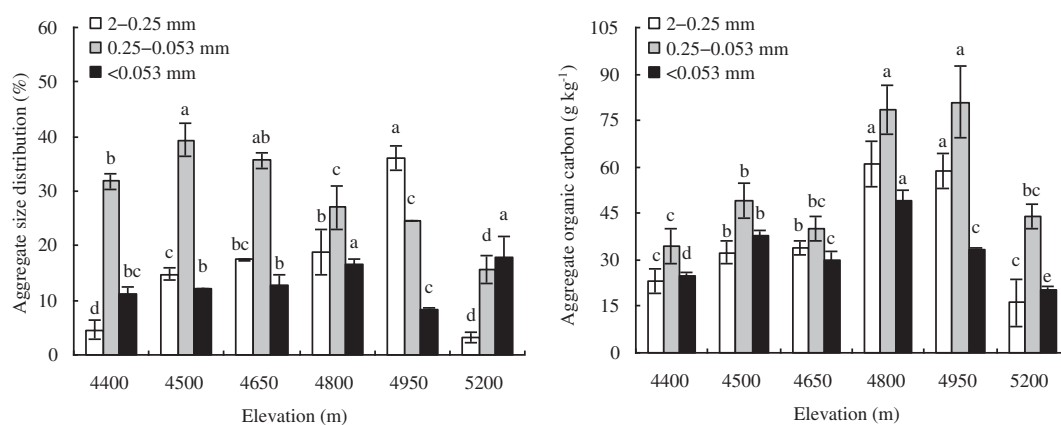

Fig. 3. Distribution and organic carbon concentration of water-stable aggregate size fractions in soils along the elevational gradient on the Tibetan Plateau. The bars having same letters among different elevations are not significantly different (*P* < 0.05).

et al. (2008) found that SOC storage at elevations 4800 and 4950 m were significantly higher than those of other elevations along the elevational gradient. Moreover, Fu et al. (2012, 2014) showed that SOC and WSOC tended to increase along the elevational gradient from 4313 to 4693 m in the same transect. The HAC/FAC ratio has been used as an index of the degree of humification (McCallister and Chien, 2000; Rivero et al., 2004). The variation of HAC/FAC ratio (Fig. 2) suggested that the degree of humification also presented an unimodal pattern along the elevational gradient, with a peak at 4800 m elevation.

The distribution of functional groups of SOC presented an unimodal pattern along the elevational gradient (Table 2). In general, alkyl C and aromatic C represented the recalcitrant and hydrophobic carbon forms, whereas O-alkyl C and carbonyl C were relatively easily decomposable

and hydrophilic carbon forms. The higher values of alkyl C/O-alkyl C and hydrophobic C/hydrophilic C ratios indicated that SOC was more decomposed (Mathers and Xu, 2003) and more hydrophobic (Cao et al., 2016) which, in turn, implied that SOC was more stable (Spaccini et al., 2006; Wu et al., 2014). Thus, our results suggested that SOC at elevations 4800 and 4950 m had higher degrees of decomposition and hydrophobicity and exhibited higher stability than that at lower and higher elevations. In previous studies, a high proportion of alkyl C (von Lützow et al., 2007) and a low proportion of aromatic C (Mathers and Xu, 2003) were related to young SOM, implying that SOC at elevations 4800 and 4950 m was younger than at other elevations. The higher aliphatic C/aromatic C ratio suggested that SOC was simpler at elevation 4950 m (Wu et al., 2014).
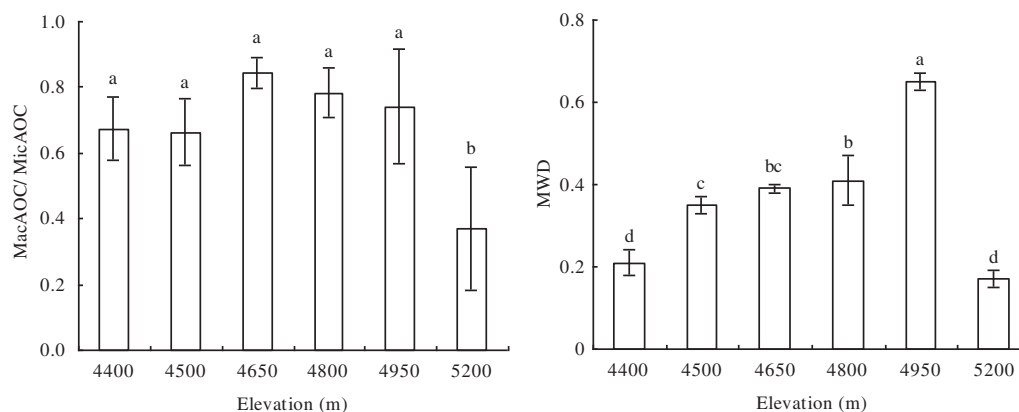
Fig. 4. Ratio of water-stable macroaggregate- to microaggregate-associated organic C (MacAOC/MicAOC) and mean weight diameter (MWD) of water-stable aggregates in soils along the elevational gradient on the Tibetan Plateau. The bars having same letters among different elevations are not significantly different ($P < 0.05$).
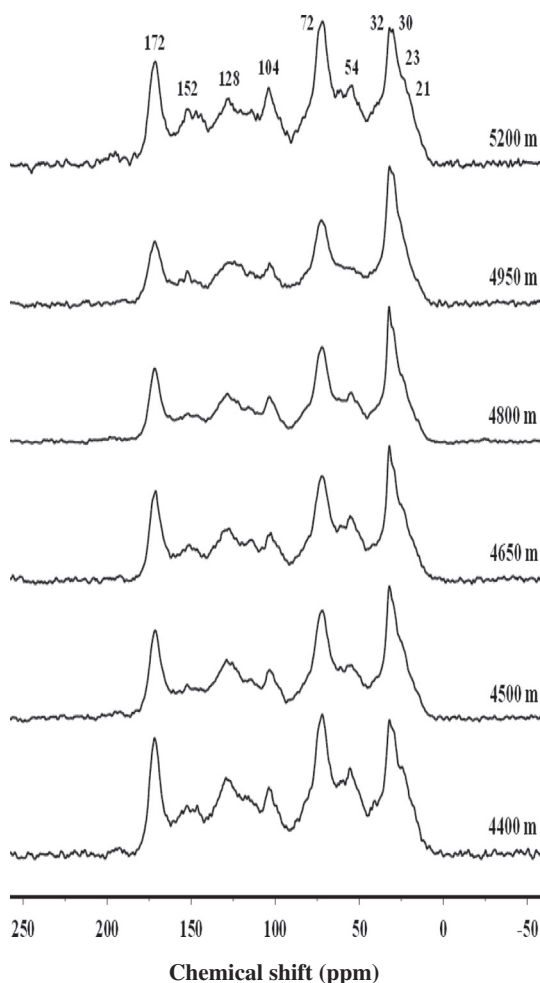

Fig. 5. Solid-state ^{13}C CPMAS TOSS NMR spectra of soil samples along the elevational gradient on the Tibetan Plateau.

Table 2
Relative intensities of different carbon functional groups from solid-state ^{13}C CPMAS TOSS NMR spectra of soil samples along the elevational gradient on the Tibetan Plateau.

Elevation (m)	Alkyl C (%)	O-alkyl C (%)	Aromatic C (%)	Carbonyl C (%)	A/O-A	Alip/Arom	HB/HI
4400	23.2	39.5	26.3	10.9	0.59	2.38	0.98
4500	24.9	38.0	24.6	12.5	0.66	2.56	0.98
4650	26.8	39.0	22.7	11.6	0.69	2.90	0.98
4800	27.6	37.7	23.0	11.7	0.73	2.83	1.02
4950	31.2	36.3	21.4	11.1	0.86	3.16	1.11
5200	24.7	39.9	24.9	10.6	0.62	2.60	0.98

A/O-A: alkyl C/O-alkyl C; Alip/Arom: aliphatic C/aromatic C; HB/HI: hydrophobic C/hydrophilic C.

(Duiker et al., 2003; Six et al., 2004; Bronick and Lal, 2005; Peng et al., 2015; Wang et al. 2016b). The significant correlations between MWD with SOC and free and amorphous Fe- and Al- oxides contents were also observed here (Table 4). In addition to SOC, MWD were also significantly correlated with labile (WSOC and EOC) and resistant (HAC and FAC) SOC fractions (Table 4), suggesting that these SOC fractions contributed to aggregate stabilization. Some studies have reported that WSOC (Tisdall and Oades, 1982) and humic fractions (Chaney and Swift, 1984; Piccolo and Mbagwu, 1999; Bouajila and Gallali, 2008) were major bonding agents responsible for the formation of microaggregates into macroaggregates. On the other hand, the higher aggregate stability at the two elevations could be related to the chemical composition of SOC. Piccolo and Mbagwu (1999) reported a close relationship between aggregate stability and hydrophobic C/hydrophilic C ratio in soil, with a larger value implying a higher aggregate stability. Thus, the high aggregate stability at the two elevations could be attributed to the large degree of hydrophobicity of SOC.

4.2. Correlations of SOC and aggregate stability with environmental factors along the elevational gradient

In the alpine meadow, plant biomass is a major source of SOC. A larger input of biomass into the soil could lead to a higher SOC concentration. Our results demonstrated that SOC and its fractions, with the exception of the silt + clay-associated organic C, were positively correlated with AGB along the elevational gradient (Table 3). The interactive effects of temperature and precipitation could be optimal for plant growth at elevations 4800 and 4950 m than at lower elevation sites with low MAP and at higher elevation sites with low MAT (Li et al., 2013; Wang et al., 2013).

The significant correlations between alkyl C and aromatic C with AGB (Table 3) suggested that substrate quality could partly contribute

The MWD has been widely used as an indicator of soil aggregate stability, with a larger MWD indicating a higher aggregate stability (Chen et al., 2014b). Our results (Fig. 4) suggested that aggregate stability also exhibited an unimodal pattern along the elevational gradient, with higher aggregate stability at elevations of 4800 and 4950 m than at other elevations. On the one hand, the higher aggregate stability at elevations 4800 and 4950 m could be attributed to the higher SOC and free and amorphous Fe- and Al-oxides, which could act as bonding agents for the formation and stability of soil aggregates

Table 3

Pearson correlation coefficients between soil organic carbon contents, soil aggregate stability, and soil organic carbon chemical compositions with environmental factors and other soil properties along the elevational gradient on the Tibetan Plateau.

	Elevation (m)	MAT (°C)	MAP (mm)	AGB (g m^{-2})	pH	Sand (%)	Silt (%)	Clay (%)	Fe$_d$ (g kg^{-1})	Fe$_o$ (g kg^{-1})	Al$_d$ (g kg^{-1})	Al$_o$ (g kg^{-1})
SOC (g kg^{-1})	0.162	−0.011	0.407	0.872*	−0.383	−0.594	0.746	0.366	0.882*	0.721	0.950**	0.679
WSOC (g kg^{-1})	0.137	−0.011	0.491	0.883*	−0.332	−0.674	0.812*	0.452	0.866*	0.907*	0.895*	0.874*
EOC (g kg^{-1})	0.168	−0.031	0.509	0.908*	−0.381	−0.662	0.799	0.443	0.881*	0.874*	0.922**	0.833*
HAC (g kg^{-1})	0.106	0.039	0.310	0.846*	−0.284	−0.599	0.781	0.339	0.901*	0.726	0.972**	0.703
FAC (g kg^{-1})	0.250	−0.113	0.462	0.930**	−0.364	−0.572	0.769	0.300	0.860*	0.858*	0.950*	0.849*
HUC (g kg^{-1})	0.144	0.008	0.406	0.827*	−0.413	−0.575	0.693	0.386	0.849*	0.635	0.906*	0.576
Macroaggregates C (g kg^{-1})	0.091	0.058	0.346	0.853*	−0.298	−0.640	0.797	0.402	0.916*	0.729	0.971**	0.690
Microaggregates C (g kg^{-1})	0.388	−0.258	0.538	0.887*	−0.607	−0.370	0.549	0.140	0.707	0.756	0.827*	0.752
Silt + clay C (g kg^{-1})	−0.192	0.319	−0.147	0.436	−0.046	−0.426	0.559	0.239	0.731	0.198	0.771	0.168
Macroaggregates (%)	0.145	−0.037	0.339	0.822*	−0.177	−0.573	0.784	0.287	0.805	0.879*	0.872*	0.897*
Microaggregates (%)	−0.893*	0.892*	−0.853*	−0.399	0.911*	−0.402	0.396	0.362	0.308	−0.296	0.155	−0.312
Silt + clay (%)	0.447	−0.455	0.042	−0.166	−0.394	0.642	−0.638	−0.572	−0.468	−0.533	−0.316	−0.509
MWD (mm)	0.113	−0.003	0.329	0.820*	−0.159	−0.605	0.808	0.324	0.828*	0.877*	0.884*	0.888*
Alkyl C (%)	0.403	−0.291	0.554	0.935**	−0.405	−0.452	0.673	0.170	0.726	0.924**	0.845*	0.947**
O-alkyl C (%)	−0.063	−0.045	−0.258	−0.745	0.231	0.535	−0.725	−0.274	−0.793	−0.783	−0.844*	−0.795
Aromatic C (%)	−0.377	0.264	−0.399	−0.868*	0.276	0.386	−0.643	−0.074	−0.701	−0.792	−0.848*	−0.832*
Carbonyl C (%)	−0.520	0.552	−0.761	−0.167	0.504	−0.116	0.261	−0.050	0.327	−0.245	0.333	−0.196

n = 6, * P < 0.05, ** P < 0.01.

SOC: soil organic carbon; WSOC: water-soluble organic carbon; EOC: easily oxidizable organic carbon; HAC: humic acid carbon; FAC: fulvic acid; HUC: humin carbon; MWD: mean weight diameter; MAT: mean annual temperature; MAP: mean annual precipitation; AGB: aboveground biomass; Fe$_d$ and Al$_d$: free Fe and Al oxides; Fe$_o$ and Al$_o$: amorphous Fe and Al oxides.

Table 4

Pearson correlation coefficients between soil organic carbon contents, soil organic carbon chemical composition, and soil aggregate stability along the elevational gradient on the Tibetan Plateau.

	SOC	WSOC	EOC	HAC	FAC	HUC	Macroaggregates C	Microaggregates C	Silt + clay C	Alkyl C	O-alkyl C	Aromatic C	Carbonyl C	MWD
SOC	1													
WSOC	0.913*	1												
EOC	0.957**	0.991**	1											
HAC	0.985**	0.925**	0.954**	1										
FAC	0.952**	0.960**	0.971**	0.970**	1									
HUC	0.986**	0.853**	0.914*	0.948**	0.889*	1								
Macroaggregates C	0.995**	0.928**	0.963**	0.995**	0.961**	0.969**	1							
Microaggregates C	0.945**	0.897*	0.931**	0.926**	0.926**	0.923**	0.924**	1						
Silt + clay C	0.806	0.573	0.641	0.814*	0.653	0.833*	0.812*	0.692	1					
Alkyl C	0.822*	0.909*	0.896*	0.856*	0.954**	0.726	0.835*	0.843*	0.419	1				
O-alkyl C	−0.855*	−0.942**	−0.918**	−0.909*	−0.915*	−0.777	−0.886*	−0.872*	−0.646	−0.860*	1			
Aromatic C	−0.770	−0.790	−0.789	−0.822*	−0.906*	−0.668	−0.789	−0.745	−0.457	−0.955**	0.764	1		
Carbonyl C	0.238	0.103	0.106	0.347	0.183	0.210	0.295	0.130	0.681	0.034	−0.364	−0.164	1	
MWD	0.820*	0.940**	0.907*	0.884*	0.943**	0.715	0.859*	0.794	0.511	0.949**	−0.943**	−0.897*	0.246	1

n = 6, * P < 0.05, ** P < 0.01.

SOC: soil organic carbon; WSOC: water-soluble organic carbon; EOC: easily oxidizable organic carbon; HAC: humic acid carbon; FAC: fulvic acid; HUC: humin carbon; MWD: mean weight diameter.

to the chemical composition of SOC (Zech et al., 1997; Senesi et al., 2007). As reported in a previous study, the chemical composition of SOM can be associated with physiological plant adaptation to environmental conditions and this can be maintained as footprint in SOM (Campo and Merino, 2016). Thus, further studies are necessary to examine the relationship between SOM chemical composition and substrate quality along the elevational gradient. Furthermore, the higher decomposition degree of SOC at elevations 4800 and 4950 m may result from higher litter input, which could stimulate microbial activity and thus increase the decomposition (Fang et al., 2015).

In the present study, the MWD was also significantly correlated with AGB (Table 3), suggesting that AGB was also a major determinant of soil aggregate stability. The positive correlation between soil aggregate stability and AGB has also been reported by other researchers (Bronick and Lal, 2005). On the one hand, the input of biomass into soil could alter the quantity and quality of SOC which, in turn, could impact the aggregate stability of the soil. On the other hand, the high vegetation cover could decrease soil leaching and erosion (Boix-Fayos et al., 1998; Ohtsuka et al., 2008), which subsequently could result in a high soil aggregate stability at 4950 m elevation.

As mentioned above, AGB was a major determinant of SOC concentrations, SOC chemical composition, and soil aggregate stability along the elevational transect. The observed unimodal distribution pattern of AGB (Table 1) associated with an optimal combination of temperature and precipitation along the elevational gradient (Li et al., 2013; Wang et al., 2013) was in line with the distribution of SOC and aggregate stability. Moreover, the low AGB at lower elevation could also result from climate warming as reported by a previous study (Du et al., 2012). At the same site studied here, Du et al. (2012) observed that MAT has increased approximately 2 °C between 1962 and 2010, with a larger increase at low elevation. The negative effect of warming on AGB (Fu et al., 2013) could explain low SOC and aggregate stability at lower elevation. The climate warming at low elevation was generally due to overgrazing (Du et al., 2004; Ohtsuka et al., 2008; Du et al., 2012). Therefore, we suggest that these sites should be specifically protected from anthropogenic disturbance to maintain high grassland ecosystems and tackle future climate change in this region. However, further studies are still necessary to examine the relationship between overgrazing and climate warming on the elevational transect.

4.3. Preservation mechanisms of SOC on the elevational transect

According to Six et al. (2002), SOC could be physically protected by aggregate formation. In this study, the higher microaggregate-associated organic C across all elevations (Fig. 4) suggested that physical protection in microaggregates acted as a main mechanism of SOC preservation on this elevational transect. Generally, SOC associated with microaggregates is more stable than with macroaggregate and silt + clay fractions (Yu et al., 2012; Huang et al., 2016). The significantly lower ratio of macroaggregate- to microaggregate-associated organic C at elevation 5200 m compared to other elevations (Fig. 4) suggests that microaggregate-associated organic C contributed relatively more to SOC preservation at the highest elevation.

The importance of the mineral matrix in the accumulation and stabilization of SOC has been reported previously (Torn et al., 1997; Six et al., 2002). Alpine soils on the Tibetan Plateau contained a certain amount of Fe and Al, ranging from 7.50 to 61.3 g kg^{-1} and from 4.68 to 64.9 g kg^{-1}, respectively (Wang et al. 2016c). However, little is known about the role of Fe/Al oxides for SOC preservation in the alpine meadow. In the present study, no correlations were observed between SOC and its fractions with sand, silt and clay fractions, except for WSOC that was positively correlated with the silt-sized fraction (Table 3). But, we found that SOC and its fractions were generally positively correlated with free Fe- and Al-oxides contents, and the correlation coefficients were larger with free Al oxides than with free Fe oxides (Table 3). It indicated that free Fe- and Al-oxides, especially free Al oxides, played

an important role for the preservation of SOC. On the other hand, labile SOC fractions were positively correlated with amorphous Fe- and Al-oxides contents, and the correlation coefficients were higher with amorphous Fe oxides than with amorphous Al oxides (Table 3). This indicated that amorphous Fe- and Al-oxides, especially amorphous Fe oxides, were important for the preservation of labile SOC fractions. According to previous studies, SOC could be chemically protected against biodegradation through association with mineral fractions (Torn et al., 1997; Six et al., 2002; Bruun et al., 2010). Thus, our results suggested that chemical interactions with Fe- and Al-oxide minerals are a main mechanism of SOC preservation on this elevational transect. As shown in Table 3, alkyl C was positively correlated with free Al oxides and amorphous Fe- and Al-oxides contents, which implied that it is the recalcitrant alkyl C group in the SOC molecule that is selectively preserved by Fe- and Al-oxides, and thereby protected SOC against biodegradation. The selective stabilization of aliphatic biomacromolecules by Fe oxides has been reported by other researchers (Adhikari and Yang, 2015).

In addition to physical and chemical protection, SOC could also be biochemically protected through the formation of recalcitrant compounds (Six et al., 2002). According to Lorenz et al. (2007), biochemically recalcitrant SOC fractions are enriched in alkyl C groups. In this study, with the exception of the HUC and silt + clay-associated SOC, SOC and its fractions were positively correlated with alkyl C and negatively correlated with O-alkyl C. This finding indicated that the change in SOC concentration was consistent with the change in alkyl C. Therefore, biochemical protection by forming recalcitrant alkyl C was considered as a mechanism of SOC preservation.

5. Conclusions

The concentrations of SOC and its labile, resistant, and water-stable aggregate-associated fractions increased gradually with elevation, reaching maximum concentrations at 4800 or 4950 m. Similarly, the degrees of decomposition, aliphaticity and hydrophobicity of SOC, and the water stability of aggregates also presented an unimodal distribution pattern along the elevational gradient. The SOC, WSOC, EOC, HAC, FAC, HUC, macroaggregate- and microaggregate-associated organic C, alkyl C, and MWD were positively correlated with each other and with AGB and free/amorphous Fe- or Al-oxides. The physical protection in microaggregates, chemical interactions with Fe- and Al-oxide minerals, and biochemical protection by recalcitrant alkyl C may be the main mechanisms for SOC preservation on the vertical transect of this alpine meadow.

Appendix A. Supplementary data

The following KMZ file contains the Google map of the most important areas described in this article.

Map KMZ file containing the Google map of the most important areas described in this article.

Appendix B. Supplementary data

Supplementary data associated with this article can be found in the online version, at doi:http://dx.doi.org/10.1016/j.catena.2017.04.007.

These data inlcude the Google map of the most important areas described in this article.

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