Soil Organic Carbon and Its Fractions Across Vegetation Types: Effects of Soil Mineral Surface Area and Microaggregates^{*1}

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ABSTRACT

Soil organic carbon (SOC) can act as a sink or source of atmospheric carbon dioxide; therefore, it is important to understand the amount and composition of SOC in terrestrial ecosystems, the spatial variation in SOC, and the underlying mechanisms that stabilize SOC. In this study, density fractionation and acid hydrolysis were used to assess the spatial variation in SOC, the heavy fraction of organic carbon (HFOC), and the resistant organic carbon (ROC) in soils of the southern Hulun Buir region, northeastern China, and to identify the major factors that contribute to this variation. The results showed that as the contents of clay and silt particles (0–50 µm) increased, both methylene blue (MB) adsorption by soil minerals and microaggregate contents increased in the 0–20 and 20–40 cm soil layers ($P < 0.05$). Although varying with vegetation types, SOC, HFOC, and ROC contents increased significantly with the content of clay and silt particles, MB adsorption by soil minerals, and microaggregate content ($P < 0.05$), suggesting that soil texture, the MB adsorption by soil minerals, and microaggregate abundance might be important factors influencing the spatial heterogeneity of carbon contents in soils of the southern Hulun Buir region.

Key Words: carbon fractions, microaggregates, soil mineral surface area, soil organic carbon, vegetation

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INTRODUCTION

The Kyoto Protocol has drawn attention to terrestrial ecosystems as potential sinks for atmospheric carbon dioxide (O'Brien *et al.*, 2003). Because soils contain nearly twice as much carbon (C) as the vegetation, a better understanding of soil organic carbon (SOC) distribution and its stabilization mechanisms is important (Mann, 1986). Soil organic matter is often categorized into physico-chemically protected fractions, such as organo-mineral particles, and the occluded particulate organic matter in microaggregates, biochemically protected fractions, such as recalcitrant soil organic matter (SOM) compounds, and free particulate organic matter (Six *et al.*, 2002; Peng *et al.*, 2004). The light fraction organic matter and particulate organic matter, which are not included in the organo-mineral particles because of their nonassociation with soil minerals, can be removed by density fractionation or acid hydrolysis (Rovira and Vallejo, 2002; Six *et al.*, 2002). Clay and silt particles, with high specific surface area and numerous reaction sites, are crucial to the formation of organo-mineral particles (Caravaca *et al.*, 1999; Eustehues *et al.*, 2003). Organo-mineral particles and some microbial polysaccharides can further form microaggregates by mutual binding (Angers, 1998; Andreu *et al.*, 2001). Presumably, the organo-mineral particles and microaggregates may increase SOC residence time and pool size by reducing the accessibility of microbes to SOC, through chemical complexation and/or physical protection (Powers and Schlesinger, 2002).

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Density fractionation can separate soil organic matter into the light and heavy fractions, the latter being mostly in the organo-mineral particles or microaggregates. Not all of the organic matter associated with soil minerals is chemically recalcitrant (Six *et al.*, 2002). For example, some microbial polysaccharides that are not accessible by microorganisms because of their association with mineral surfaces or physical protection inside soil aggregates are chemically labile (McLauchlan and Hobbie, 2004). Therefore, a combination of density fractionation and acid hydrolysis may be useful in assessing the storage of SOC and its stabilization mechanisms.

In the present study, density fractionation and acid hydrolysis were used to assess the spatial variation in SOC, heavy fractions of organic carbon (HFOC), and resistant organic carbon (ROC) in soils of the southern Hulun Buir region, China. The objectives were to identify the dominant factors that affect the spatial variation in SOC, HFOC, and ROC contents in the region and to compare the differences between the HFOC contents derived by density fractionation and the ROC contents obtained by acid hydrolysis.

MATERIALS AND METHODS

Site description

Six distinct vegetation patterns exist in the southern Hulun Buir region ($47^{\circ} 30' - 48^{\circ} 30' \text{ N}$ and $115^{\circ} 31' - 126^{\circ} 04' \text{ E}$), northeastern China (Fig. 1), and the distance from east to west of this region is about 600 km. In the eastern part are the Daxingan Mountains and in the western part lies the Hulun Buir great steppe. As influenced by the Siberia-Mongolia anticyclone in winter and moist Pacific air masses in summer, the climate varies from warm and moist in the east to cold and dry in the west. The lofty Daxingan Mountains also have some effects on the spatial pattern of the climate, and the highest annual precipitation and the lowest annual mean temperature occur at their summit (Zhang, 1998; Ni and Zhang, 2000). Under the influence of the climate, natural vegetation types from east to west are as follows: oak (*Quercus mongolica*) forest (OF), larch (*Larix gmelini*) forest (LF), forest-steppe mosaic (FS), pine (*Pinus sylvestris*) forest (PF), semi-humid steppe (HS), and semi-arid steppe (AS). OF, on the eastern foothills of the Daxingan Mountains, is deciduous forest. LF represents the southernmost part of the global boreal forest biome (known as the boreal forest) and is distributed in the Daxingan Mountains (Jiang *et al.*, 2002). FS is dominated by birch (*Betula pendula*) and poplar (*Populus deltoides*) forest on shaded slopes and by grassland on sunny slopes. PF, on the western foothills of the Daxingan Mountains, is drought-resistant forest. HS is dominated by *Stipa grandis* and *Aneurolepidium chinense*,

Fig. 1 Vegetation types and sampling sites in the southern Hulunbeier region, China. OF = oak forest; LF = larch forest; FS = forest-steppe; PF = pine forest; HS = semi-humid steppe; AS = semi-arid steppe.

Fig. 2 Soil organic carbon (SOC) contents in the soils under various vegetation types. OF = oak forest; LF = larch forest; FS = forest-steppe; PF = pine forest; HS = semi-humid steppe; AS = semi-arid steppe.

and AS is dominated by *Stipa baicalensis* and *Aneurolepidium chinense* (Wang *et al.*, 1979; Zhou, 1997). Soil types from east to west vary, ranging from dark-brown soils (brown loamy soils) to brown coniferous forest soils (Semi-Pedossols), gray forest soils, aeolian soils, sandy black soils, and chestnut soils (Inner Mongolia Soil Survey Office, 1994).

Soil sampling and analyses

For each vegetation type, four typical sites were selected and sampled in May 2004. At each site, 10 random soil cores (6 cm diameter) were collected at 0–20 and 20–40 cm depths and mixed into one composite sample for each of the two depths. The soil samples were air-dried and then passed through a 2 mm sieve before analysis.

To quantify the clay and silt particles (0–50 μm), the soil was first oxidized with H_2O_2 to remove the organic matter. About 50 g air-dried soil (passed through 2 mm sieve) was placed in a 1 L plastic beaker, 300 mL L^{-1} H_2O_2 was gradually added and agitated gently with a glass stick to prevent foaming, and the mixture was subsequently treated in an oven at 60–70 $^{\circ}\text{C}$ for about 5 h. This process was repeated at least six times. The contents of clay and silt particles were determined in 20 g H_2O_2 -treated soil through ultrasonic dispersion, followed by wet-sieving through a 50 μm sieve. The residues (fine and coarse sand) were collected, oven-dried at 105 $^{\circ}\text{C}$, and weighed; the contents of clay and silt particles (0–50 μm) were given by the difference in the oven-dried weight between the total H_2O_2 -treated soil mineral particles (0–2000 μm) and the wet-sieved residues (50–2000 μm).

The methylene blue (MB) adsorption by soil minerals has been used to quantify the surface reaction sites of soil minerals. It can be used to define the potential binding capacity of soil minerals with humic substances, by the formation of organo-mineral particles (Gürses *et al.*, 2004). In brief, 0.5 g of the H_2O_2 -treated soil was placed in a 100 mL plastic centrifuge tube (duplicate) and 50 mL aqueous MB solution (0.2 g L^{-1}) was added. The mixed suspension in the centrifuge tube was capped and shaken vigorously (120 times per minute) for 1 h, and then the supernatant was obtained by centrifugation (5 min, 3000 r min^{-1}). The content of remaining MB in the supernatant solution was determined with an ultraviolet (UV) spectrophotometer (UNICO, UV-2102 PC) at 666 nm. Finally, the MB adsorption by soil minerals was calculated and expressed as mg g^{-1} (Gürses *et al.*, 2004).

Water-stable microaggregates were defined as the clay and silt organo-mineral particles that existed in 50–250 μm soil fractions after 24 h water saturation. The microaggregates excluded the fine sand and particulate organic matter (iS + POM) in 50–250 μm fractions of the soil (Six *et al.*, 2000). Briefly, 10 g air-dried soil was placed in a 100 mL beaker, 50 mL distilled water was added, the soil remained saturated for 24 h, and then it was carefully wet-sieved through 250 and 50 μm sieves sequentially. The materials that passed through the 250 μm sieve and remained on the 50 μm sieve were collected, oven-dried at 105 $^{\circ}\text{C}$, and weighed as microaggregates, including the fine sand and particulate organic matter (MA1). Then MA1 was immersed and wet-sieved through a 50 μm sieve by agitating with a plastic tip until the water passing through was clear. The material remaining on the 50 μm sieve was collected, oven-dried at 105 $^{\circ}\text{C}$, and weighed as iS + POM. The microaggregate content was calculated as the difference between MA1 and iS + POM.

The contents of SOC, HFOC, and ROC were determined by the Walkey Black method (Lu, 1999). The heavy fractions of organic carbon (HFOC) were obtained by density fractionation. Briefly, 20 g soil was placed into a 100 mL plastic centrifuge tube, after which about 50 mL 1.59 g mL^{-1} carbon tetrachloride (CCl_4) was added (Wu and Xu, 2004), to remove the lighter parts by shaking and centrifugation (3000 r min^{-1} , 10 min). This process was subsequently repeated thrice. Then 25 mL 950 g mL^{-1} alcohol (analytical reagent) was used to dissolve the carbon tetrachloride in the tube. Finally, 50 mL distilled water was used repeatedly to rinse the soil by centrifugation, to remove the carbon tetrachloride and to yield HFOC. The resistant organic carbon fractions (ROC) were obtained by acid hydrolysis, which preferentially removed proteinaceous, polysaccharide-type organic carbon, and other labile organic compounds (Eustehues *et al.*, 2003). Briefly, about 20 g of air-dried soil was placed in a

100 mL glass tube with addition of 30–40 mL 6 mol L⁻¹ HCl. The tube capped with a glass funnel was then placed in an oven at 80 °C for 24 h and shaken gently at 5–8 h intervals until the visible organic particulates disappeared. Next, distilled water was used repeatedly to wash the soil, to remove the excess HCl by centrifugation, and the solids were oven-dried at 50 °C to yield ROC. Differences in SOC, HFOC, and ROC contents between vegetation types were first tested by a one-way analysis of variance (ANOVA). The SOC, HFOC, and ROC mean values among vegetation types were then compared with the least significant difference (LSD) method (post hoc test) (SPSS 10.0).

RESULTS

Soil texture, MB adsorption, and microaggregates

The clay and silt particle content, the microaggregate content, and the MB adsorption by soil minerals varied among the sampling sites and vegetation types (Table I). Generally, higher contents of clay and silt particles were found in OF, BF, and FS than in PF, HS, and AS. The higher the clay and silt particle content, the higher the MB adsorption by soil minerals and microaggregate content ($P < 0.05$) (Table II). The MB adsorption by soil minerals and the clay and silt particle content in the 20–40 cm layer were similar to those in the 0–20 cm layer, whereas most of the values of the microaggregates in the 20–40 cm soil layer were lower than those in the 0–20 cm soil layer.

TABLE I

Some physical properties of the 0–20 and 20–40 cm soil layers under various vegetation types

Vegetation Type ^{a)}	Sampling site	Clay and silt particles (0–50 µm)		Microaggregates (50–250 µm)		Methylene blue adsorption by soil minerals	
		0–20 cm	20–40 cm	0–20 cm	20–40 cm	0–20 cm	20–40 cm
		g kg ⁻¹				mg g ⁻¹	
OF	1	700.5	723.5	420.7	280.7	20.32	20.36
	2	643.5	562.0	310.1	172.7	21.32	21.98
	3	778.0	784.5	443.8	399.9	24.93	25.84
	4	873.5	862.0	411.6	350.6	31.62	35.87
LF	1	812.0	803.5	426.5	429.5	34.80	35.84
	2	861.0	869.5	439.3	417.7	34.55	34.69
	3	864.5	873.5	420.6	380.9	36.49	35.04
	4	812.5	821.0	363.4	253.7	24.81	24.29
FS	1	554.0	532.0	329.2	278.6	19.49	19.84
	2	885.5	873.0	417.5	379.9	24.02	24.07
	3	701.0	690.5	368.2	355.7	25.39	25.25
	4	241.0	226.0	52.6	26.3	9.48	8.74
PF	1	24.5	18.5	19.5	13.5	2.36	2.38
	2	157.5	122.0	71.9	51.5	6.58	6.32
	3	223.0	171.0	122.6	120.3	9.65	9.42
	4	168.0	155.5	56.7	43.1	8.42	8.45
HS	1	223.5	345.5	146.6	186.5	4.83	4.98
	2	180.0	162.0	63.7	15.1	9.01	8.99
	3	55.0	21.0	42.3	18.0	2.44	2.32
	4	238.5	235.5	114.6	80.3	3.26	3.26
AS	1	291.0	340.5	199.0	228.2	13.19	15.43
	2	196.5	226.0	85.2	83.6	12.47	14.10
	3	438.5	522.0	296.6	178.1	13.19	17.45
	4	276.5	294.0	137.4	120.4	13.33	11.06

^{a)}OF = oak forest; LF = larch forest; FS = forest-steppe; PF = pine forest; HS = semi-humid steppe; AS = semi-arid steppe.

TABLE II

Correlations among the soil organic carbon (SOC), heavy fraction organic carbon (HFOC), resistant organic carbon (ROC), clay and silt particles (CSP), methylene blue adsorption by soil minerals (MBA), and microaggregates (MA) in the 0–20 and 20–40 cm soil layers ($n = 24$)

	Soil layer											
	0–20 cm						20–40 cm					
	SOC	HFOC	ROC	MBA	CSP	MA	SOC	HFOC	ROC	MBA	CSP	MA
SOC	1.000						1.000					
HFOC	0.998**	1.000					0.992**	1.000				
ROC	0.981**	0.981**	1.000				0.985**	0.992**	1.000			
MBA	0.837**	0.840**	0.785**	1.000			0.816**	0.795**	0.799**	1.000		
CSP	0.915**	0.918**	0.862**	0.945**	1.000		0.855**	0.851**	0.858**	0.932**	1.000	
MA	0.909**	0.905**	0.864**	0.909**	0.973**	1.000	0.876**	0.870**	0.872**	0.908**	0.941**	1.000

**Significant at the 0.01 level.

SOC, HFOC, and ROC contents

Significant differences were found in SOC contents among vegetation types in the 0–20 and 20–40 cm soil layers ($P < 0.05$) (Fig. 2). SOC contents ranged from 2.1 to 66.6 g kg⁻¹ in the 0–20 cm layer and from 0.8 to 46.1 g kg⁻¹ in the 20–40 cm layer. In the 0–20 cm soil layer, SOC contents in the eastern part of the region (OF, LF, and FS) ranged from 11.9 to 66.6 g kg⁻¹, being 2–3 times higher than those in the west part (PF, HS, and AS) ($P < 0.05$). Similarly, mean SOC contents in the 20–40 cm soil layer in LF and FS were approximately twice those in PF, HS, and AS. Although the mean SOC in the 20–40 cm layer in OF was higher than those in PF and HS ($P < 0.05$), it was similar to that in AS ($P > 0.05$).

The HFOC pool that is mostly associated with soil minerals was the major SOC component, accounting for 75%–95 % of the SOC (Figs. 2 and 3). The variation in HFOC among vegetation types followed the same pattern as the SOC ($P < 0.05$) (Fig. 3). HFOC contents ranged mostly from 1.8 to 65.0 g kg⁻¹ in the 0–20 cm layer and from 0.7 to 45.8 g kg⁻¹ in the 20–40 cm layer. Generally, HFOC contents in the eastern part (OF, LF, and FS) were higher than those in the west part (PF, HS, and AS). The HFOC contents in the 20–40 cm layer were lower than those in the 0–20 cm layer.

Fig. 3 Heavy fraction organic carbon (HFOC) contents in the soils under various vegetation types. OF = oak forest; LF = larch forest; FS = forest-steppe; PF = pine forest; HS = semi-humid steppe; AS = semi-arid steppe.

Fig. 4 Resistant organic carbon (ROC) contents in the soils under various vegetation types. OF = oak forest; LF = larch forest; FS = forest-steppe; PF = pine forest; HS = semi-humid steppe; AS = semi-arid steppe.

Significant differences were observed in ROC among vegetation types at soil depths of 0–20 and 20–40 cm ($P < 0.05$) (Fig. 4). ROC contents ranged from 0.7 to 54.4 g kg⁻¹ in the 0–20 cm layer and

from 0.3 to 37.3 g kg⁻¹ in the 20–40 cm layer. The proportions of ROC to SOC were 25%–83% (Figs. 2 and 4). The spatial variation in ROC content was similar to that of SOC. Mean values of ROC in the eastern part (OF, LF, and FS) were 2–3 times higher than those in the west part (PF, HS, and AS).

Relations of SOC, HFOC, and ROC to clay and silt particles, MB adsorption, and microaggregates

The HFOC pool or ROC pool was the major part of SOC content, and the HFOC or ROC content was significantly ($P < 0.01$) correlated to the SOC content (Table II). In the 0–20 cm layer, SOC, HFOC, and ROC contents were significantly ($P < 0.01$) positively correlated with MB adsorption, clay and silt particle content, and microaggregate content (Table II). Similar relationships were found in the 20–40 cm soil layer (Table II). These significant relationships indicated that MB adsorption, clay and silt particle content, and microaggregate content were important factors influencing the spatial variation of SOC, HFOC, and ROC contents in the southern Hulun Buir region.

DISCUSSION

It has been widely recognized that the spatial variability of SOC content is influenced by factors including climate, land cover, and soil texture (Peng *et al.*, 2004). The higher heterogeneity of SOC content on account of changes in land cover or climatic condition in northeast China has been reported elsewhere (Sun *et al.*, 2004; Xie *et al.*, 2004). According to Xie *et al.* (2004), the increasing trend in SOC content from west to east in the northern part of China is primarily associated with the increase in precipitation and the change of land cover. This trend also apparently occurred in the present study. Meanwhile, the soil texture may be an important factor influencing the spatial variation of SOC contents in the southern Hulun Buir region. The higher contents of clay and silt particles occurred in OF, LF, and FS where soils were derived from granite and arenose rock (Table I). Lower contents of clay and silt particles occurred in HS and AS where the soils formed from flooding sand and sediments (Zhang, 1998) and where the weathering rate was low owing to the arid climate. Therefore, SOC, HFOC, and ROC contents were lower in the west part (PF, HS, and AS) than in the east part (OF, LF, and FS) (Figs. 2–4). Higher clay and silt contents tend to form more organo-mineral particles and aggregates, which increases the HFOC and ROC contents (Chaney and Swift, 1986; Angers, 1998; Zhang and Horn, 2001).

HFOC and ROC contents have been widely used to interpret the stabilization mechanism of soil organic carbon (Eustehues *et al.*, 2003; Wu and Xu, 2004). Generally, the HFOC content determined by density fractionation was slightly higher than the ROC content determined by acid hydrolysis (Figs. 3 and 4). Conventionally, the density fractionation method can only remove the free light particulate organic matter that is not occluded in the microaggregates (Angers, 1998; Peng *et al.*, 2004). Some labile enzymes, microorganisms or aggregate-forming polysaccharides are still preserved in the heavy organic matter or on the surface of the microaggregates (Huang *et al.*, 2003; Taubaso *et al.*, 2004). However, the acid hydrolysis method can largely remove the labile components mentioned above, and the residues (ROC) are the chemically recalcitrant organic carbon (Six *et al.*, 2002). Hence, density fractionation or acid hydrolysis should be used with caution when studying the mechanisms of soil organic carbon stabilization.

In conclusion, significant differences were observed in the SOC, HFOC, and ROC contents among vegetation types within each soil layer (0–20 and 20–40 cm). The SOC, HFOC, and ROC contents in LF and FS were significantly higher than those in PF, HS, and AS ($P < 0.05$). Meanwhile, SOC, HFOC, and ROC contents positively increased with clay and silt particles, the MB adsorption by soil minerals, and microaggregate abundance, suggesting that soil texture, the MB adsorption by soil minerals or microaggregate abundance might be important in the spatial variation of soil carbon content and its stability.

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