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Chemical and Isotopic Characterization of Size-Fractionated Organic Matter From Cryoturbated Tundra Soils, Northern Alaska

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Chemical and isotopic characterization of size-fractionated organic matter from cryoturbated tundra soils, northern Alaska

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[1] Recent studies indicate a second layer of organic matter often accumulates in the lower active layer and upper permafrost in arctic tundra soils as a result of cryoturbation. In this study, cryoturbated organic matter was characterized using a combination of physical size fractionation and modern analytical techniques for elemental composition (C and N), stable isotopes (δ^{13}C and δ^{15}N), radiocarbon content (Δ^{14}C), and molecular fingerprinting (pyrolysis-gas chromatography/mass spectrometry, Py-GC/MS). The results indicated that cryoturbated organic matter could be highly bioavailable. Soil organic matter (SOM) associated with fine sand particles was considered to be the organic carbon pool most sensitive to the changing climate. More organic matter is stabilized on clay minerals in arctic tundra soils compared to those in temperate and tropical soils. The bioavailable soluble organic matter extracted from cryoturbated soil was found to have significant long-term effects on carbon cycling. The similar molecular composition between cryoturbated and surface soil organic matter suggests that the vegetation cover has not significantly changed since the early Holocene. Furthermore, the SOM quality in moist acidic tundra was found to be higher than that of wet nonacidic tundra. With thawing permafrost and a deepening of the active layer, cryotrubated organic matter could reenter the biogeochemical cycles in the Arctic, resulting in a positive feedback to climate change.

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1. Introduction

[2] The importance of the arctic tundra and boreal regions in the global carbon cycle has long been recognized because of the large soil carbon stores in these regions [*Post et al.*, 1982; *Oechel and Billings*, 1992; *Hobbie et al.*, 2000; *Ping et al.*, 2008a]. Owing to the cold temperature and low decomposition rate of soil organic matter (SOM), the arctic tundra ecosystems alone contain up to 30% of the global soil C pool of the entire pedon, including the upper permafrost [*Michaelson et al.*, 1996; *Bockheim and Tarnocai*, 1998; *Ping et al.*, 1998].

[3] According to the morphological features, a second layer of SOM often accumulates in the lower active layer (Bg) and upper permafrost (Cf) at a depth of 60–120 cm [*Ping et al.*, 1998]. This second OM layer is created by cryoturbation, mainly through frost churning from a series of thawing and freezing cycles. Because of cryoturbation, soil carbon stores are redistributed, and soil horizons are warped, broken, or discontinuous [*Ping et al.*, 1998]. In arctic Alaska, the nonsorted circles, commonly called frost boils, are the dominant patterned ground resulting from cryoturbation through differential frost heave. Cryoturbation is thus considered the dominant process leading to cryogenic soil morphological features and SOM redistribution within the profile [*Walker et al.*, 2004; *Ping et al.*, 2008b].

[4] Mounting evidence and models all suggest a significant polar amplification of the global warming signal [*Chapman and Walsh*, 1993; *Arctic Climate Impacts Assessment* (ACIA), 2004]. Under warming climate conditions, this zone of frozen soils will most certainly be affected, leading to the gradual loss of circumpolar permafrost [*Hinzman and Kane*, 1992]. Hence, the portion of SOM in presently frozen layers could be released and have a significant impact on the global carbon balance. The existence of this deep carbon has been ignored in the past and its recognition could significantly improve estimates of global terrestrial carbon pools and climate model simulations [*Ping et al.*, 2008a]. In addition, this carbon is not totally sequestered and passive under the extreme cold environment. Field measurements and observations suggest biological activities occur in the lower active layers and the upper permafrost, in spite of near freezing to subzero soil temperatures [*Oechel et al.*, 1997]. Because of the relatively

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high quality of substrates and available unfrozen water, soil respiration in cryoturbated organic layers could account for significant net flux of CO_2 to the atmosphere [*Fahnestock et al.*, 1998, 1999; *Oechel et al.*, 2000; *Romanovsky and Osterkamp*, 2000; *Michaelson and Ping*, 2003] affecting also land-ocean and air-sea CO_2 fluxes [*Chen and Gao*, 2007]. Laboratory studies involving incubation of tundra soil samples from the active layer and upper permafrost layers at low temperature (−2 to 4°C) showed that obvious microbial respiration occurred via CO_2 flux [*Michaelson and Ping*, 2003]. With the warming and thawing permafrost [*Osterkamp and Romanovsky*, 1999], these deep soil organic carbon pools become even more available for decomposition. Current soil C models estimate that the potential loss of organic carbon from permafrost would be two to three times greater than simulated C losses from mineral soils [*Davidson and Janssens*, 2006]. Thus, it is critical to understand the characteristics of this deep carbon preserved in upper permafrost.

[5] Size fractionation has proven to be particularly useful for identifying specific SOM pools with different biogeochemical functions and describing the SOM dynamics [*Anderson et al.*, 1981; *Tissen and Stewart*, 1983; *Elliott and Cambardella*, 1991; *Christensen*, 1992; *Six et al.*, 1998]. Particle size fractionation is based on the principle that OM associated with particles of different sizes differs in chemical composition and bioavailability, thereby playing different roles in SOM dynamics [*Tisdall and Oades*, 1982; *Christensen*, 1992]. Compared to chemical fractionation, particle size fractionation techniques cause less destruction of structure and function of SOM [*Christensen*, 1992]. Furthermore, the use of stable isotope ($\delta^{13}C$ and $\delta^{15}N$) and radiocarbon (^{14}C) methods, coupled with particle size fractionation further advances SOM turnover studies, and has been well suited to the study of SOM dynamics with different timescales [*Ågren et al.*, 1996; *Gerzabek et al.*, 2001a; *Solomon et al.*, 2002].

[6] In this study, the quality and quantity of SOM in a cryoturbated arctic tundra soil profile were characterized by using isotope composition ($\delta^{13}C$, $\delta^{15}N$ and ^{14}C) and pyrolysis-gas chromatography/mass spectrometry (GC/MS) coupled with particle size fractionation. In addition to the traditional size fractionation of soils, ultrafiltration techniques were used to separate soluble soil organics into high (HMW) and low molecular weight (LMW) organics. Size-fractionated SOM was further analyzed for elemental (C and N), molecular (based on Py-GC/MS) and isotopic ($\delta^{13}C$ and $\delta^{15}N$) composition for a better understanding of biogeochemical characteristics of SOM in arctic tundra soils.

2. Materials and Methods

2.1. Study Sites and Soil Sampling

[7] Soil was studied from the two major physiographic regions of northern Alaska: the Arctic Foothills (Sagwon Hills) and the Arctic Coastal Plain (Franklin Bluffs). The physical environments of the sites are presented in Table 1.

[8] The major land cover types in the study area include moist acidic tundra (MAT) and moist nonacidic tundra (MNT), defined by substrate pH values and moisture content [*Walker et al.*, 1998]. The soil of the MAT site was poorly drained and has discontinuous surface organic horizons. It is classified as a Ruptic Histoturbel [*Soil Survey Staff*, 2006] or a Histic Gleyic Cryosol [*IUSS Working Group WRB*, 2006]. The soil of the MNT site was poorly drained and had thin surface organic horizons in between the nonsorted circles. It is classified as Aquic Molliturbel [*Soil Survey Staff*, 2006] and Gleyic Mollic Cryosol [*IUSS Working Group WRB*, 2006].

[9] Soil pits of ca. 1 m^2 were excavated to a depth of 1.2 m depth at each site using a shovel in the active layer and a gasoline-powered chisel for the frozen layers. The excavations were made so that the vertical face exposed a complete cycle of the surface microrelief patterns (<2 m). Profiles were jointly described and sampled with a representative from the USDA National Soil Survey Laboratory according to the Soil Survey Manual [*Soil Survey Staff*, 1993]. Samples were collected in August 2003 from undisturbed genetic horizons using a serrated knife and kept frozen until analysis. Results of the characterization analyses were entered in the National Soil Survey Center database (http://www.soils.usda.gov). The Lab Pedon number for the MAT site is 04N0274 and that of the MNT is 04N0276. Horizons in the upper permafrost and cryoturbated layers were designated by lower case *f* and *jj*, respectively. In cryoturbated soils, the horizons were broken and warped, so the depth increment could not be used to estimate carbon density. Instead, a normalized horizon thickness was calculated by estimating the relative proportion of each "horizon" in a 1.2 m^2 soil profile [*Michaelson et al.*, 1996].

2.2. Particle Size Fractionation

[10] The size fractions were separated using dry and wet sieving, centrifugation, filtration and ultrafiltration with methods modified from previous studies [*Cambardella and Elliott*, 1992, 1993; *Guo and Santschi*, 1996; *Six et al.*, 1998; *Guo et al.*, 2000]. The bulk soils from the upper permafrost were fractionated into five particulate and two dissolved fractions, including the 1000–2000 μm (coarse sand size equivalent); 250–1000 μm (macroaggregates); 53–250 μm (fine sand size equivalent); 2–53 μm (silt size equivalent); 0.45–2 μm (clay size); 1 kDa-0.45 μm (HMW-DOC); and the <1 kDa (LMW-DOC) fractions. Details of size fractionation were described by *Xu et al.* [2009]. Only samples from the MAT site were fractionated to show the effect of size fractionation on the carbon balance. Our soil samples may not represent soils from different sampling sites from Arctic tundra due to their heterogeneous nature. Further studies are needed to compare different sampling sites with different soil types.

2.3. Analyses of Bulk and Size-Fractionated SOM

2.3.1. DOC and TDN Measurements

[11] Concentrations of dissolved organic carbon (DOC) were determined using the high-temperature combustion method with a Shimadzu TOC-V analyzer [*Guo et al.*, 1995]. Immediately after sample collection, all DOC samples, including the bulk DOC (<0.45 μm), HMW-DOC (1 kDa-0.45 μm) and LMW-DOC (>1 kDa), were acidified with concentrated HCl to pH $\leq$ 2 (normally 2 drops of concentrated HCl to a 20 ml solution). The DOC blank, including Milli-Q water and instrument blank, was on the order of 2–6 μM. The water blank was subtracted to correct sample DOC concentration. Analytical precision, in terms

Table 1. Sampling Locations and Physical Environments of Soils From Arctic Tundra, Alaska[a]

Site	Latitude and Longitude	Elevation (m)	Landform	Microrelief	Parent Material	Drainage Condition
MAT	69°23.708′N 148°44.165′W	261	Rolling hills (Arctic Foothills)	nonsorted circle	Loess/Moraine	poor
MNT	69°59.046′N 148°41.619′W	37	Flood plain (Arctic Coastal Plain)	low-center polygon	Alluvium	poor

[a]MAT, moist acidic tundra; MNT, moist nonacidic tundra.

of coefficient of variation, was within 1–4%, depending on concentrations. Total dissolved nitrogen (TDN) was measured with a TNM-1 total nitrogen unit interfaced with the Shimadzu TOC analyzer [*Guo and Macdonald*, 2006].

2.3.2. Measurements of Total Organic C, N, and Isotope Composition ($\delta^{13}C$, $\Delta^{14}C$, and $\delta^{15}N$)

[12] Bulk and size-fractionated SOM were pretreated with 1N HCl before TOC, TN and stable isotopic ($\delta^{13}C$ and $\delta^{15}N$) measurements. Stable isotope ^{13}C and ^{15}N and TOC and TN contents were measured using continuous flow isotopic ratio mass spectrometry [*Schell et al.*, 1998]. Stable carbon and nitrogen isotope ratios were calculated in terms of $\delta^{15}N$ and $\delta^{13}C$, using the formula $(R_{sample}/R_{standard} - 1) \times 1000$, where R is the ratio of $^{13}C/^{12}C$ or $^{15}N/^{14}N$ in soil samples or standard materials (PDB for carbon and atmospheric N_2 for nitrogen). Peptone was used as a standard for both $\delta^{13}C$ and $\delta^{15}N$ analysis (with 44.3% of C and 15.8% of N; $\delta^{13}C = -15.8‰$ and $\delta^{15}N = 7.0‰$). The precision and accuracy of $\delta^{13}C$ and $\delta^{15}N$ analysis were ±0.1‰ and ±0.2‰, respectively, as determined by replicate analysis of standards and samples.

[13] Radiocarbon ($\Delta^{14}C$) of bulk soil samples was measured using accelerator mass spectrometry (AMS) at the National Ocean Science AMS Facility [*Guo and Macdonald*, 2006]. One-sigma errors are given in $\Delta^{14}C$ values and ^{14}C ages.

2.3.3. Py-GC/MS of Size-Fractionated SOM

[14] Freeze-dried bulk and size-fractionated SOM samples as well as DOC samples, were ground to a fine powder in a Wig-L-Bug ball mill. About 150 μg C were pyrolyzed in a quartz sample tube using Py-GC/MS techniques [*White et al.*, 2002; *Guo et al.*, 2003]. Py-GC/MS was conduced with a CDS Analytical Pyroprobe 2000/AS 2500 connected to an HP 6890 gas chromatograph in tandem with an HP 5973 mass selective detector (MSD) operating in electron ionization (EI) mode. Samples were held in the interface chamber at 280°C for 15 s, then heated in the pyrolysis chamber from 280°C to 700°C at 10°C ms^{-1} and held at 700°C for 10 s. Products were separated using a Restek Rtx35-MS column (30 m × 0.32 mm × 0.25 μm). The chromatograph was run for 1 min with pulsed splitless injection at 25 psi. The oven temperature program was 40°C for 30 min, ramp at 1°C min^{-1} to 120°C, ramp at 2°C min^{-1} to 280°C and hold for 10 min prior to the next run. Helium was the carrier gas at a constant 2 ml min^{-1} flow rate. The spectrometer scanned from *m/z* 45 to 650. Spectra were identified using the Wiley 275 mass spectral library.

[15] A total of 30 compounds from each chromatogram were selected, identified, and grouped into 8 classes (Table 2) reported previously [*White et al.*, 2002, 2004] and known to be derived from specific fractions of the SOM. The sum of the 30 compounds was referred to as the index. The dominant peak areas of the 30 compounds were identified and summed to find the total index area for that chromatogram. For each sample, the relative percent of each selected class was calculated by summing the areas of all compounds in the class and dividing by the total index area. The relative abundance analysis was used to compare selected classes of compounds among different samples [*Dai et al.*, 2002], so the results are not intended to represent the SOM composition in its entirety, but rather the relative percent among index compounds.

3. Results

3.1. Abundances of C and N in Bulk and Size-Fractionated SOM

[16] The soil organic carbon (SOC) content in the bulk soils differed considerably among horizons, ranging from 40 to 408 g kg^{-1} (Table 3). Large variability in SOC was also observed for size-fractionated soils, ranging from 33 to 362 g kg^{-1}. Total N concentration ranged from 2.1 to 20.3 g kg^{-1} in the bulk soils of different horizons and from 1.0 to 31.3 g kg^{-1} in the size fractionated soils and dissolved

Table 2. Index Compounds and Index Classification Used in Py-GC/MS[a]

Index Classification	Index Compounds
Primary polysaccharide	Furfural, hydroxyfuran, methyl hydantoin, 1,4:3,6-dianhydro-alpha-gluco
Secondary polysaccharide	Methylfurfural, 2-proply furan
Polypeptide and protein	Indole, pyridine
Lignin	2-methoxyphenol, 4-ethyl-2-methoxy phenol, 4-vinyl-2-methoxy phenol, dimethoxy propenyl phenol
Phenols	Phenol, 2-methyl phenol, 4-methyl phenol, dimethyl phenol
Lipids	1-tridecene,1-pentadecene,1-hexadecene,1- heptadecene,1-octdecene
Alkanes	Decane, undecane, dodecane, tridecane, pentadecane, hexadecane, naphthalene
Cyclopentenones	Methylcyclopentenone, dimethylcyclophentenone

[a]*White et al.* [2002, 2004].

Table 3. Contents of Soil Organic Carbon, Total Nitrogen, and C/N Ratio in Bulk and Particle Size-Fractionated Soils From MAT Site[a]

Horizon (Depth, cm)	Bulk Density (g/cm^3)	Bulk Soil			1000–2000 μm			250–1000 μm			53–250 μm			2–53 μm			0.45–2 μm			1kDa-0.45 μm			<1 kDa		
		SOC	TN	C/N	SOC	TN	C/N	SOC	TN	C/N	SOC	TN	C/N	SOC	TN	C/N	SOC	TN	C/N	SOC	TN	C/N	SOC	TN	C/N
Oi (0–9 cm)	0.14	408	20.3	23	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
Bg1 (9–36)	1.16	40	2.1	21	n/a	n/a	n/a	40	1.9	25	77	4.1	22	47	2.1	25	80	3.4	27	288	15.1	22	n/a	n/a	11
Bg2 (8–42)	1.16	41	2.1	23	33	1.0	38	45	2.2	24	35	1.6	26	47	2.2	25	69	3.1	26	338	21.2	19	n/a	n/a	17
Bg/Oajj (32–55)	0.71	56	3.1	21	n/a	n/a	n/a	78	4.0	23	48	2.1	26	53	3.0	21	81	3.9	24	325	20.4	19	156	11.4	16
Oajj/Bg (36–50)	0.53	88	4.9	21	109	5.4	23	140	6.7	24	87	4.7	22	60	3.5	20	106	5.7	22	348	31.3	13	n/a	n/a	n/a
Bg/Oajjf (50–90)	0.44	116	6.8	20	362	12	35	164	8.7	22	67	3.3	24	75	4.0	22	121	6.8	21	332	30.4	13	n/a	n/a	n/a
Oajj/Cf (90–110)	0.53	79	5.4	17	140	6.1	27	153	8.5	21	68	3.5	23	55	3.7	17	76	4.9	18	309	28.1	13	n/a	n/a	n/a
Mean		118	6.4	21	161	6.1	31	103	5.3	23	64	3.2	24	56	3.1	22	89	4.6	23	323	24.4	16	-	-	15
±SD		±131	±6.4	±2	±141	±4.5	±7	±56	±3.1	±1	±19	±1.2	±2	±11	±0.8	±3	±20	±1.4	±3	±22	±6.5	±4			±3

[a]SOC, soil organic carbon (in g C kg^{-1}); TN, total nitrogen (in g N kg^{-1}); n/a, not available.

fractions (Table 3). The C/N ratio of SOM associated with particles of different sizes ranged from 11 to 38 (Table 3).

[17] In the soil DOC fractions, the C/N values of HMW-DOC ranged from 13 to 22, while in the LMW-DOC fraction values ranged from 11 to17. These C/N ratios are much lower than for those of bulk soils and other size fractionated soil fractions (Table 3), likely resulting from the enrichment of dissolved inorganic nitrogen and other LMW N-containing organic compounds such as amino acids during soil leaching [*van Hees et al.*, 2005].

3.2. Carbon Partitioning Between SOM Size Fractions

[18] The OC stores of each of the size fractions from different horizons in the MAT soil are shown in Table 4. The dominant size fraction was the fine sand size fraction (53–250 μm), followed by silt (2–53 μm), coarse sand (1000–2000 μm), macroaggregates (250–1000 μm), clay (0.45–2 μm), HMW (1 kDa-0.45 μm) and LMW (<1 kDa). However, in the Bg/Oajjf horizon, the dominant fraction was the coarse sand size fraction, followed by fine sand, silt, macroaggregates and clay, then HMW- and LMW-DOC (Table 4). The HMW-DOC comprised 0.21–1.6% of the total SOC while the LMW fraction comprised 0.05–0.16% (Table 4).

[19] On average, 18% of the total soil organic carbon was partitioned in the 1000–2000 μm size fraction, 8% in the 250–2000 μm fraction, 48% in the 53–250 μm fraction, 23% in the 2–53 μm fraction, 0.5% in the HMW-DOC fraction and 0.07% in the LMW-DOC fraction (Figure 1).

[20] The contribution from DOC fractions, including HMW- and LWM-DOC, could account for 0.25–1.7% of total SOC, with an average of 0.6% in the whole pedon. Within the bulk soil DOC, the HMW-DOC comprised up to 87% while the LMW-DOC comprised <13% of the bulk DOC pool. The low fraction of LMW-DOC in the soil DOC pool is in agreement with previous observations [e.g., *van Hees et al.*, 2005].

3.3. Stable Isotope and Radiocarbon Composition

[21] The $\delta^{13}C$ values of bulk SOM ranged from −26.0 to −27.4‰ (Table 5), in agreement with typical values for C3 plants and soil organic carbon in the arctic region [*Guo et al.*, 2007]. Size fractionated SOM fractions also had similar $\delta^{13}C$ values compared to bulk soils (Table 5). The average $\delta^{13}C$ values of SOM in bulk soil (−26.5‰) from cryoturbated horizons (i.e., Oajj/Bg, Bg/Oajjf and Oajj/Cf) was similar to that of the surface SOM (−26.4‰) (Table 5). Similar $\delta^{13}C$ values for deep horizons suggest that the deep SOM is cryoturbated from the surface SOM. In addition, $\delta^{13}C$ values of HMW-DOC varied from −23.8‰ to −25.7‰ with an average of −24.6‰ (Table 5), which were relatively higher. For the LMW-DOC fraction, only one sample was measured due to the very low DOC mass and the $\delta^{13}C$ value was −22.2‰.

[22] The natural abundance of ^{15}N of bulk soils ranged from 1.0 to 2.4‰ (Table 5). In the particle size fractions, there were only small variations in $\delta^{15}N$ values, while HMW-DOC (1 kDa-0.45 μm) fractions were highly enriched in ^{15}N, with a $\delta^{15}N$ value ranging from 3.3‰ to 6.2‰ (Table 5). The $\delta^{15}N$ values of coarse fractions (1000–2000 μm) ranged from −1.8‰ to 0.84‰, close to those of

Table 4. Carbon Partitioning in the Moist Acidic Tundra Soil

Horizon	1000–2000 μm (%)	250–1000 μm (%)	53–250 μm (%)	2–53 μm (%)	0.45–2 μm (%)	1 kDa-0.45 μm (%)	<1 kDa (%)
Bg1	n/a	6.4 ± 1.8	73.3 ± 3.0	16.9 ± 0.3	3.1 ± 0.1	0.21 ± 0.02	0.04 ± 0.02
Bg2	n/a	8.7 ± 1.5	54.5 ± 4.2	32.5 ± 0.4	3.6 ± 0.1	0.52 ± 0.11	0.08 ± 0.06
Bg/Oajj	n/a	6.0 ± 0.8	67.2 ± 11.8	24.8 ± 1.8	1.4 ± 0.0	0.39 ± 0.08	0.07 ± 0.04
Oajj/Bg	16.5 ± 0.7	9.4 ± 0.3	53.3 ± 12.7	19.1 ± 1.8	1.2 ± 0.0	0.43 ± 0.10	0.10 ± 0.12
Bg/Oajjf	33.7 ± 3.4	10.9 ± 0.8	28.4 ± 12.9	25.4 ± 9.2	1.3 ± 0.02	0.26 ± 0.06	0.05 ± 0.04
Oajj/Cf	7.9 ± 1.9	10.7 ± 1.7	43.5 ± 4.7	35.1 ± 1.1	1.1 ± 0.0	1.6 ± 0.2	0.16 ± 0.13
Mean ± SD	19.4 ± 13.1	8.7 ± 2.1	53.4 ± 16.2	25.7 ± 7.1	1.9 ± 1.1	0.56 ± 0.51	0.08 ± 0.04

living plants (−3.6 to −1.5‰ for *Salix* spp. [*Barnett*, 1994]).

[23] For the surface SOM (Oi), the radiocarbon composition (Δ^{14}C value) was −20 ± 7‰ or 105 ± 35 years B.P. (in ^{14}C age). The Δ^{14}C value was −338 ± 4‰ corresponding to a ^{14}C age of 3260 ± 40 for SOM in Oajj/Bg (36–55 cm), and was −596 ± 5‰ (or a ^{14}C age of 7220 ± 55 years B.P.) in Oajj/Cf (90–110 cm) (Table 6).

3.4. Molecular Compositions of SOM in Bulk and Size-Fractionated Soils

[24] The relative percentage indices for primary polysaccharides in bulk SOM ranged from 34.5% to 54.7% in MAT and 14.8% to 30.1% in MNT (Table 6), and decreased with decreasing particle size, except for the HMW-DOC fraction. In general, polysaccharides are preferentially decomposed during SOM degradation and thus could serve as an index for evaluating the quality of SOM [e.g., *Bracewell et al.*, 1989; *Dai et al.*, 2002; *White et al.*, 2002, 2004]. The higher relative percentage of index for primary polysaccharides in the MAT soil compared to that in the MNT soil supports the hypothesis that acidic soils preserve SOM. In addition, the higher percentage of index for primary polysaccharides in the larger fraction sizes would indicate a fresher less decomposed organic component in these size fractions. Indeed, the highest percentage of index for primary polysaccharides was measured in the coarse SOM (44%) and fine sand particles (38%) in the lower active layer and upper permafrost (Table 6). The higher percentage of index for primary polysaccharides in the HMW-DOC (26%) compared to the clay size fraction (18%) was presumably caused by microbial processes [*Kracht and Gleixner*, 2000]. The result was supported by higher isotope δ^{13}C values and low C/N ratio (Tables 3 and 5).

[25] As shown in Table 6, the coarse SOM fractions (e.g., 250–1000 μm, 53–250 μm) had higher percentages of index for lignin compared to finer SOM fractions (e.g., 2–53 μm, 0.45–2 μm). The highest percentage of index for lignin (15.8%) was found in the coarse sand fractions in both the lower active layer and upper permafrost, indicating that SOM in deep horizons was poorly degraded. Interestingly, lignin components did not occur in the HMW-DOC fraction (Table 6). The lack of lignin in soluble organics suggests that lignin contents in the deep horizons were not translocated by leaching, but rather by burial or mechanical movement, i.e., in this case soil morphology, indicating frost churning of OM from the surface to deep horizons.

[26] The relative percentage of index for phenols in bulk SOM increased from the lower active layer (15.3%) to the upper permafrost (30.1%). The percentage of index for phenols in bulk SOM from the upper permafrost was similar to that in the surface organic horizon (30.7%, Table 6).

[27] Polypeptides and proteins are pyrolysis products of amino acid parent material [*Bracewell et al.*, 1989]. During the degradation of SOM, the percentage of index for polypeptide increases while primary polysaccharide decreases (D. M. White *et al.*, unpublished results). Higher percentages of index for polypeptides and proteins were observed in the clay size fraction, 10.9% in the lower active layer and 13.2% in the upper permafrost, compared to other particle size fractions and the HMW-DOC fraction (Figure 2). The HMW-DOC fraction had a lower percentage of index for polypeptides and proteins (5.9%), likely due to microbial consumption of bioavailable amino acids [e.g., *Kracht and Gleixner*, 2000].

[28] Lipids comprised a relatively minor proportion of all organic compound classes, ranging from 0.1% to 3.2% for both bulk and size fractionated soils, with a higher percentage of index in the lower active layer and upper permafrost but lower in the surface organic layer (Table 6). In the particle size fractions, the percentage of index for lipid increased with decreasing particle size, with the highest values in clay fractions. However, no lipids were found in the HMW-DOC fraction because lipids are insoluble in water [*Kögel-Knabner*, 2002]. The mirror relation between lipids and primary polysaccharides suggests that the loss of primary polysaccharide results in an increase in the relative percentage of index for lipids in SOM. Thus, the percentage of index for lipids could also be used as an index of the

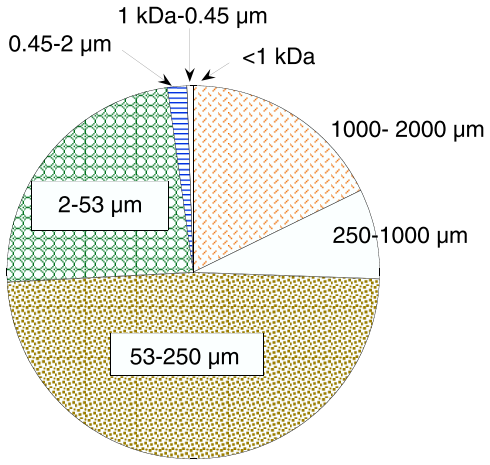

Figure 1. Size distribution of soil organic carbon in the moisture acidic tundra soil.

Table 5. Stable Isotope Composition of Soil Organic Matter Associated With Bulk and Size Fractionated Soils[a]

	Bulk Soil		1000–2000 μm		250–1000 μm		53–250 μm		2–53 μm		0.45–2 μm		1 kDa-0.45 μm		<1 kDa	
Horizon	$\delta^{13}C$	$\delta^{15}N$	$\delta^{13}C$	$\delta^{15}N$	$\delta^{13}C$	$\delta^{15}N$	$\delta^{13}C$	$\delta^{15}N$	$\delta^{13}C$	$\delta^{15}N$	$\delta^{13}C$	$\delta^{15}N$	$\delta^{13}C$	$\delta^{15}N$	$\delta^{13}C$	$\delta^{15}N$
Oi	−26.4 ± 0.2	1.2 ± 0.1	n/a													
Bg1	−27.4 ± 0.1	1.7 ± 0.1	n/a	n/a	−27.1 ± 0.2	0.8 ± 0.1	−27.6 ± 0.1	1.4 ± 0.3	−27.5 ± 0.1	1.1 ± 0.3	−27.8 ± 0.1	0.7 ± 0.8	−25.2 ± 0.2	3.3 ± 0.2	n/a	n/a
Bg2	−27.3 ± 0.1	2.1 ± 0.2	−29.0 ± 1.1	−1.8 ± 0.8	−27.4 ± 0.1	0.7 ± 0.6	−27.4 ± 0.0	0.0 ± 0.3	−27.8 ± 0.1	0.2 ± 0.4	−27.8 ± 0.0	0.8 ± 0.1	−25.7 ± 0.02	4.3 ± 0.03	n/a	n/a
Bg/Oajj	−26.8 ± 0.0	2.4 ± 0.1	n/a	n/a	−27.0 ± 0.0	2.0 ± 0.1	−27.1 ± 0.1	0.5 ± 0.7	−27.7 ± 0.1	1.1 ± 0.6	−27.5 ± 0.1	1.1 ± 0.2	−25.1 ± 0.2	4.6 ± 0.03	−22.2 ± 0.2	−0.5 ± 1.9
Oajj/Bg	−26.8 ± 0.0	1.8 ± 0.2	−26.9 ± 0.1	0.8 ± 0.1	−27.2 ± 0.1	1.1 ± 0.4	−27.1 ± 0.3	1.1 ± 0.4	−27.3 ± 0.1	0.1 ± 0.5	−27.4 ± 0.0	0.4 ± 0.2	−23.9 ± 0.2	5.0 ± 0.3	n/a	n/a
Bg/Oajjf	−26.5 ± 0.2	2.1 ± 0.1	−27.7 ± 1.1	0.7 ± 0.5	−27.2 ± 0.0	1.1 ± 0.2	−27.1 ± 0.1	0.7 ± 0.4	−27.4 ± 0.0	−0.5 ± 0.3	−27.3 ± 0.0	−0.6 ± 0.2	−24.0 ± 0.1	6.2 ± 0.3	n/a	n/a
Oajj/Cf	−26.0 ± 0.0	1.0 ± 0.1	−26.3 ± 0.1	−1.2 ± 0.5	−26.1 ± 0.3	0.0 ± 0.3	−26.3 ± 0.0	−0.7 ± 0.4	−26.7 ± 0.0	−0.2 ± 0.2	−26.5 ± 0.0	−0.8 ± 0.2	−23.8 ± 0.1	3.9 ± 0.1	n/a	n/a

[a]Units are in per mil; n/a, not available.

degree of SOM decomposition. Similar to lipids, the alkane percentage of index also increased with decreasing particle size, including the HMW-DOC fraction, indicating that as the particle size decreases the organic matter in that size class shows evidence of increasing microbial decomposition. The highest percentage of index for alkanes in the HMW-DOC fraction likely resulted from intense microbial activity, consistent with the findings of the lowest C/N value and higher isotope signatures (^{13}C and ^{15}N).

4. Discussion

4.1. Variations in C/N Ratio and Organic Carbon Store

[29] In general, older or highly degraded soil organic matter is of lower C/N ratio [*Ping et al.*, 1998] due to the escape of CO_2 during organic matter degradation. Interestingly, the C/N ratio of bulk soils from the upper permafrost was indeed relatively lower, ranging from 17 to 21, compared to that in the surface soil, indicating older SOM in the deeper layer, as supported by radiocarbon composition (Table 6, and see discussion below). The C/N ratio of the size-fractionated soils generally decreased with deceasing particle size, suggesting that evidence of microbial alteration of SOM was greater in the finer fractions than in the coarser fractions. This result is consistent with the fact that soil organic geochemistry is distinct between different soil size fractions [e.g., *Christensen*, 1992; *Hedges and Oades*, 1997; *Solomon et al.*, 2002]. Higher C/N ratios in the coarse SOM fractions indicated a higher percentage of SOM from plant debris and little decomposed components in coarser fractions, consistent with the variations in percentage of index for organic compound classes, such as polysaccharides, proteins, lipids and alkanes (see previous section and Table 6). Moreover, large variability in SOC suggests that soil organic matter is highly heterogeneous and size fractionation may provide more detailed information for identifying different SOM pools [*Christensen*, 1992].

[30] For size-fractionated particulate soils and DOC, the averaged SOC and TN contents increased generally with increasing particle size (e.g., between 2 to 2000 μm), while averaged SOC and TN increased with decreasing DOC size, with the highest OC and TN content found in the HMW (1 kDa-0.45 μm) fraction (Table 3). In addition, the concentration of HMW-DOC changed little with depth, with an average concentration of 323 ± 22 mg OC kg^{-1} and a C/N ratio of 16 ± 4. This variation pattern is somewhat different from that found for forest soils without permafrost [e.g., *Certini et al.*, 2004], and likely due to the effect of cryoturbation process and well preserved SOC within the permafrost, especially for the soluble fraction.

[31] On average, 43% of OC was measured in the upper permafrost (Bg/Oajif, Oaji/Cf), 20.4% in the lower active layer (Bg/Oajj, Oajj/Bg), 30.4% in the mineral subhorizons (Bg1, Bg2), and 10.2% in the surface organic layer (Table 7). In contrast to temperate and tropical soils, arctic tundra soils contained higher OC contents in the deep horizons (56–116 g kg^{-1}) than the subsurface horizons (40–41 g kg^{-1}), consistent with the cryoturbation effect. When carbon storage was calculated to 1.1 m depth, the lower active layer (i.e., Bg/Oajj, Oajj/Bg, with 10.3 kg C m^{-2}) and upper permafrost (i.e., Bg/Oajjf, Oajj/Cf, with 21.8 kg C m^{-2}) accounted for 63.8% of the total OC store

Table 6. Molecular Composition Indices of Bulk and Size Fractionated Arctic Tundra Soils

Sample	First Polysaccharide (%)	Second Polysaccharide (%)	Polypeptide and Protein (%)	Lignin (%)	Phenols (%)	Lipids (%)	Alkanes (%)	Cyclopentenones (%)
Moist Acidic Tundra (Sagwon Hill Site)								
Oi 0–9 cm, ^{14}C age = 105 ± 35 years B.P.[a]								
Bulk soil	40.8	10.0	4.8	10.9	30.7	0.2	1.2	1.5
Bg/Oajj 32–55 cm								
Bulk soil	54.7	16.1	5.3	4.1	15.3	0.8	1.2	2.6
250–1000 μm	44.5	11.5	5.0	9.6	24.1	0.6	1.6	3.1
53–250 μm	44.0	13.9	5.7	6.5	24.1	0.9	2.4	2.5
2–53 μm	30.3	10.4	7.7	4.8	36.0	2.6	4.8	3.5
0.45–2 μm	19.9	11.1	10.9	6.1	39.3	3.2	5.5	4.0
1 kDa-0.45 μm	28.4	26.9	5.4	0.0	22.7	0.0	10.7	6.0
Oajj/Bg 36–55 cm, ^{14}C age = 3260 ± 40 years B.P.								
1000–2000 μm	34.5	8.0	3.4	16.1	32.7	0.5	2.3	2.6
53–250 μm	41.4	12.9	5.7	5.6	27.7	0.7	2.5	3.5
2–53 μm	39.5	14.3	6.9	4.8	27.4	1.0	2.5	3.6
Oajj/Cf 90–110 cm, ^{14}C age = 7220 ± 55 years B.P.								
Bulk soil	37.7	12.7	6.1	9.0	30.1	0.3	1.5	2.5
1000–2000 μm	38.8	7.0	2.9	21.9	27.6	0.1	0.5	1.2
250–1000 μm	37.5	8.6	4.6	16.6	29.1	0.2	1.4	2.0
53–250 μm	40.1	11.4	6.2	10.6	28.5	0.3	1.1	1.8
2–53 μm	30.7	10.4	8.1	4.9	38.7	1.0	2.7	3.6
0.45–2 μm	16.1	8.6	13.2	6.1	43.5	1.8	3.7	7.0
1 kDa-0.45 μm	24.2	28.8	4.8	0.0	19.3	0.0	15.1	7.8
Moist Nonacidic Tundra (Franklin Bluffs Site)								
Oe 2–18 cm								
Bulk soil	30.1	9.5	7.3	10.3	35.9	0.1	1.3	5.5
Cf/Oejj 60–70 cm								
Bulk soil	14.8	4.4	6.6	16.9	50.4	0.4	1.7	4.8

[a]B.P., before present.

(Table 7). This again indicates that cryoturbation plays a key role in the redistribution of soil carbon stocks [*Michaelson et al.*, 1996; *Ping et al.*, 1998].

4.2. Variations in Stable Isotope Composition of Soil Organic Carbon

[32] The δ^{13}C values of SOM in the size-fractionated particulate fractions were broadly similar to those of bulk soils (Table 5), and did not show significant variation, indicating similar organic carbon sources and a low decomposition rate of SOM. However, the HMW-DOC had higher δ^{13}C values compared to the bulk and size fractionated particulate SOM (Table 5). In the LMW-DOC fraction, the δ^{13}C value was also relatively high (−22.2‰) compared to particulate fractions. Higher δ^{13}C values and low C/N ratio (Table 3) in the LMW-DOC fraction could indicate that this fraction contained highly microbially transformed materials [*Smolander and Kitunen*, 2002].

[33] The δ^{15}N values of bulk soils show a general increase with depth, except for the upper permafrost horizon (Oajj/Cf). This is similar to patterns in grasslands [*Kerley and Jarvis*, 1997]. Lower δ^{15}N values were also found in the upper permafrost (Oajj/Cf), suggesting that the OM sequestrated in permafrost has undergone less decomposition and alteration, likely a result of the cryoturbation of SOM into permafrost conditions. Similar δ^{15}N values between coarse SOM fractions and fresh plant materials suggest less transformation in the coarse SOM fractions. The δ^{15}N values of SOM did not show a general increase with decreasing particle size, as found for temperate and tropical soils [e.g., *Tissen and Karamanos*, 1984; *Gerzabek et al.*, 2001b]. This difference likely resulted from the extreme cold climate and saturated soil conditions in tundra soils, significantly retarding the decomposition of SOM, leading to slow enrichment in ^{15}N abundance in SOM. However, the δ^{15}N values of HMW-DOC (3.3 to 4.95‰) in tundra soils were much higher than those in plant residues, presumably the result of more intense microbial activity.

4.3. Climate-Sensitive Soil Fractions in Arctic Tundra

[34] SOM in the fine sand size fraction contained the largest percentage of the OC store, with averages from 28% to 73% (Table 5). Except for the Bg/Oaf2 and Oajj/Cf horizons, all of the visible horizons had > 50% of their OC store in the fine sand size (53–250 μm) fractions. *White et al.* [2002, 2004] and *Anderson and White* [2006] found that percentage of index for primary polysaccharides, due to their freshness and lability, could serve as an important measure for evaluating the quality and bioavailability of SOM. Hence, having both the largest OC stores and a high percentage of index for primary polysaccharides, fine sand fractions are likely the OC pool most sensitive to climate change in arctic tundra soils should the amplified warming continue. Furthermore, compared to the finer particles (i.e., clay and silt), fine sands have less potential for physical protection of SOM against microbial attack [*Cambardella and Elliott*, 1992]. Therefore, OC stores in the fine sand fraction have the greatest potential to mobilize with climate warming. Even under current climate conditions, the OM associated with fine sands could be altered or remineralized through microbial respiration or mass movement.

4.4. Comparison Between MAT and MNT Soils

[35] The relative percentage of index for selected compounds from both MAT and MNT SOM are compared in Figure 2. In the surface organic horizon, MAT soils contain a higher percentage of index for primary and secondary

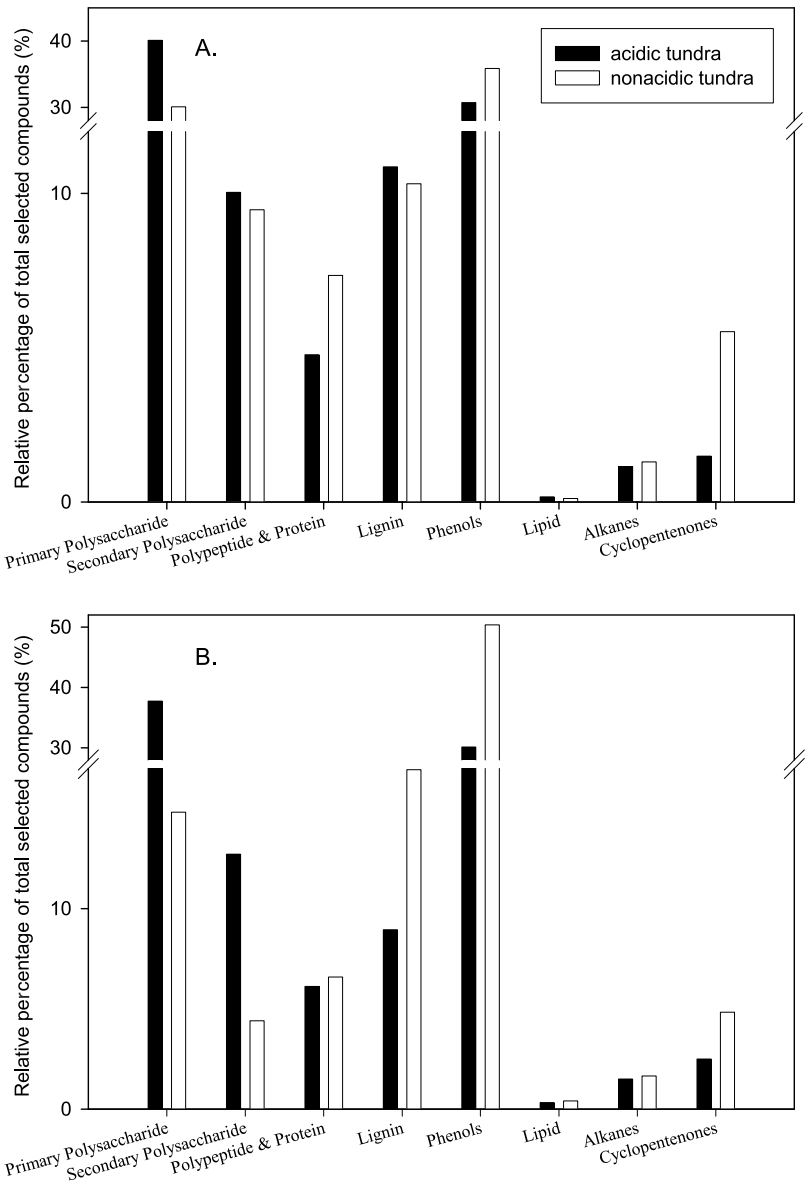

Figure 2. Comparison of molecular composition between MAT and MNT soils for both (a) surface organic and (b) upper permafrost layers.

polysaccharides, lignin and lipids than MNT soils. In the upper permafrost, however, MAT soils have a higher percentage of index for primary polysaccharides and a lower relative percentage of index for phenols. Acidic tundra soils generally have thick, poorly degraded organic horizons and a shallow active layer with relatively high quality and quantity of SOM compared to nonacidic tundra soils. Using polysaccharides in the index [e.g., *White et al.*, 2002; *Anderson and White*, 2006], SOM in the MAT soil seemed to have higher quality of SOM compared to MNT soils, consistent with earlier studies [*White et al.*, 2004]. Because of a favorable pH for microbial activity, high thermal conductivity and dominance of herbaceous vegetation with a low C/N ratio [*Walker et al.*, 1998], the nonacidic tundra

Table 7. Soil Organic Carbon Storage to 1.1 m Depth at the MAT Site

Horizon	Normalized Thickness (cm)	Bulk Density (g cm^{-3})	OC (%)	OC Storage (kg C m^{-2})	Proportion OC Stock (%)
Surface organic layer (Oi)	9	0.14	40.8	5.1	10.2
Mineral subhorizons (Bg1, Bg2)	33	1.16	4.0	15.3	30.4
Lower active layers (Bg/Oajj, Oajj/Bg)	23	0.62	7.2	10.3	20.4
Upper permafrost (Bg/Oajjf, Oajj/Cf)	45	0.48	10.1	21.8	43.4

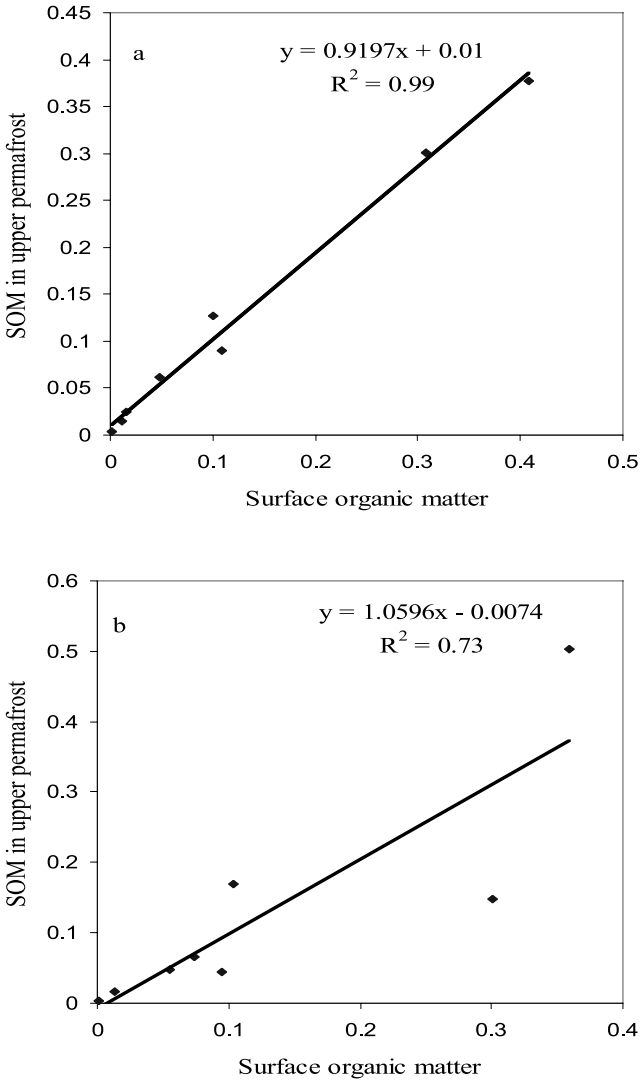

Figure 3. Correlation between molecular composition indices of cryoturbated organic matter (upper permafrost, Oajj/Cf) and surface organic matter (Oi or Oe) for (a) MAT and (b) MNT.

soils could have faster rates of soil C and N cycling than acidic tundra soils [*Hobbie et al.*, 2002]. As shown in Figure 2, MNT soils contained higher percentages of index for polypeptides, protein and cyclopentenones, suggesting a heterogeneous nature of soil organic matter and that a higher quality of SOM appeared to be preserved in the acidic tundra.

[36] The relative percentage of index for primary polysaccharides in surface OM was significantly higher than that in the upper permafrost (Table 6). This was especially true for MNT, in which surface OM had 30% of the index for primary polysaccharides, while the upper permafrost had only ~15%. In MAT, SOM in upper permafrost contained 34%, which was also lower than that in the surface organic layers (41%). Our results indicated that the SOM quality of cryoturbated horizons was significantly higher than that in the mineral horizons (i.e., Bg) within the soil profile. With the deepening active layer followed by thawing permafrost and hydrological change, release of cryoturbated organic matter could result in significant biogeochemical consequences for the arctic region.

4.5. Chemical Characteristics of Cryoturbated Organic Matter

[37] The apparent ^{14}C age of SOM in upper permafrost horizons (Oajj/Cf) was much older than that in surface OM (Oi). However, a high correlation coefficient ($r^2 = 0.99$) was found for the percentage of index of compound classes between surface and upper permafrost OM in MAT soil (Figure 3). The results of linear regression analysis showed that under the significance level of 0.05, the p value was 0.1287; thereby, H_0 (y = b1*x + b2; H_0: b1 = 1, b2 = 0; x, y: relative percentage of index for selected compound classes in surface and upper permafrost layer) cannot be rejected. Thus, the Py-GCMS index classes for the surface OM was significantly related to that in the upper permafrost. The measured percentage of index for compound classes in SOM from the surface soil horizon was similar to that in upper permafrost. In MNT, the correlation coefficient (r^2 = 0.73) and p value (0.1185) also indicate a similar pattern. Even though this OM has been sequestrated for thousands of years, the similar chemical composition to surface OM indicated that vegetation cover types on the acidic and nonacidic tundra soils have not changed since the early Holocene.

[38] The molecular compositions indicated that the cryoturbated OM (Oajj/Cf) was highly bioavailable even though it has been sequestered for more than 7000 years. In the lower active layer, the relative percentage of index for primary polysaccharides was higher than that in surface OM (Table 6), even though the cryoturbated OM had been preserved for more than 3000 years. Furthermore, the percentage of index values obtained using Py-GC/MS supported the hypothesis of the presence of a high quality of cryoturbated OM in arctic tundra soils. The presence of high-quality "deep" SOM was attributed to the saturated and reducing conditions, cold climate, and acidic soil microenvironments (i.e., in MAT [*Ping et al.*, 1997]).

5. Summary

[39] The results of the comprehensive analysis of SOM in different size fractions showed distinct elemental (C and N), stable isotopic ($\delta^{13}C$ and $\delta^{15}N$) and molecular composition. On the basis of the distinct chemical characteristics between the clay and DOC fractions, it is recommended that DOC be separated from the clay fraction during physical fractionation. Results from a combination of dry and wet sieving, filtration and ultrafiltration approach could provide more specific information on SOM dynamics and bioavailability. Arctic tundra soils in northern Alaska contain a great quantity of SOM sequestrated in deep, cryoturbated horizons, that is of high quality. Hence, arctic tundra soils have a greater potential for contributing to greenhouse gas emissions than soils from other regions under a warmer climate, through increased microbial respiration, mass movement (e.g., DOC export and solifluction) and stream bank and coastal erosion. This study has established a baseline data set for understanding biogeochemical characteristics of such deep cryoturbated OM in the arctic region,

which could aid in model development and consequently improve prediction of the global carbon budget and balance.

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