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Characterizing soil organic matter in Arctic tundra soils by different analytical approaches

X.Y. Dai, C.L. Ping*, G.J. Michaelson

Agricultural and Forestry Experiment Station, University of Alaska Fairbanks, 533E Fireweed, Palmer, AK 99645, USA

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Abstract

Soil organic matter (SOM) of five soil horizons from Arctic tundra and one soil horizon from a temperate region was separated into an extractable fraction (EF) and a non-extractable fraction (NEF) by dilute alkali. The modified classical fractionation method was applied to characterize SOM in the EF; and the wet chemical fractionation scheme, cross-polarization magic angle spinning (CP–MAS) ^{13}C NMR, and pyrolysis–gas chromatography/mass spectrometry (Py–GC/MS) techniques were used to characterize SOM in the NEF. Each of these methods provides information on the chemical composition of SOM at varying angles. The NEF comprises a large amount of SOM in these arctic tundra soils and has great potential to influence carbon cycling in these ecosystems with climate warming.

1. Introduction

Soil organic matter (SOM) can be divided into non-humic and humic substances. Non-humic substances include those with still-recognizable chemical characteristics of their precursors (e.g. polysaccharides, proteins, lipids, etc.), while humic substances are defined as a general category of naturally occurring, biogenic, heterogeneous organic substances that can generally be characterized as yellow to black in color, high molecular weight, and refractory (Aiken et al., 1985). Since the bioavailability of SOM is controlled by the chemical structure of SOM and the physical protection offered by the soil matrix and minerals (Baldock and Skjemstad, 2000), the chemical characteristics of SOM are important to understanding the dynamics of SOM in C cycling.

To characterize SOM, it is usually necessary to separate humic substances from non-humified material, and then characterize them separately. Traditionally, humic substances are extracted with dilute alkali. The residue that remains insoluble following alkali extraction is commonly defined as humin. However, the dilute alkali extractable fraction includes both humic and non-humic substances which are the products of biochemical degradation of plant materials and microbial biomass (Thurman and Malcolm, 1989), and the real humic substances including humic acids, fulvic acids and humin need further fractionation in the extractable fraction. Therefore, we developed a sequential fractionation technique to separate the extractable fraction further into humic acid (HA), fulvic acid (FA), low-molecular-weight acid (LMA), hydrophobic neutral (HON), hydrophilic neutral (HIN), and low-molecular-weight neutral (LMN) (Ping et al., 2001). In this study, we define the alkali-soluble fraction as the extractable fraction (EF) and the alkali-insoluble fraction as the non-extractable fraction (NEF) (instead of humin). Humin (organo-mineral and physically or chemically protected humic material) in this study is defined as a fraction which is not soluble in alkali, and remains behind in the NEF after sequential extraction (toluene/

* Corresponding author. Fax: +1-907-746-2677.
E-mail address: pfclp@uaa.alaska.edu (C.L. Ping).

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ethanol, neutral detergent, 0.5M H_2SO_4, 72% H_2SO_4, acetic anhydride).

In a previous study, SOM in the whole soils and in the EF were characterized using solid-state CP–MAS and liquid-state ^{13}C NMR techniques (Dai et al., 2001a). In this study, we focus on characterizing the SOM in the NEF with different analytical approaches. Wet chemical fractionation protocol (Ping et al., 2001) was used to determine the different fractions of SOM in the NEF, and CP–MAS ^{13}C NMR and Py–GC/MS techniques were applied to measure the chemical structures of SOM in the NEF. Different methods provide information at different levels. The wet chemical fractionation analysis provides information on quantitative determination of different organic matter classes, whereas the CP–MAS ^{13}C NMR and Py–GC/MS provide information on chemical characteristics at a molecular level. Although the NEF comprises a large proportion of the SOM, little is known of its chemical composition and of its role in C cycling due to its macromolecular nature and complexity (Hatcher et al., 1985); this is especially true for the NEF from tundra soils.

Cold, wet, soil environments with short summers slow organic matter decomposition and nutrient mineralization in soils from Arctic ecosystems (Nadelhoffer et al., 1992). These conditions result in the accumulation of SOM that is less humified than that in soils formed in ecosystems of the lower latitudes. Ping et al. (1997) found that most O and A horizons from tundra soils have high C:N ratios, indicating a lower degree of decomposition or humification. The lower degree of humification in the O and A horizons was further supported by the generally lower extractability by dilute alkali solution. The extractability of SOM in the O, A, and Cf horizons averaged 27, 27 and 20% of total organic C (TOC), respectively (Ping et al., 1997). Therefore, the NEF comprised a large proportion (70 to 80% of TOC) of SOM in tundra soils.

In this study, five soil horizons from Arctic tundra ecosystems were used for chemical characterization of SOM, and one soil horizon from a temperate region soil was used for reference. The objectives of this study are: (1) to characterize SOM in soils from Arctic tundra by different analytical approaches, and (2) to relate the chemical composition and structure obtained from each method to the bioavailability of SOM in these soils.

2. Materials and methods

2.1. Soils

Samples used in this study were taken from four soils including three Gelisols (permafrost soil) from Alaska and one Mollisol (Prairie soil) from Nebraska. The site characterization and soil classification are presented in Table 1 and selected properties of soils are presented in Table 2. Site 1 is on the Arctic coastal plain, characterized by frost polygons and thaw lakes with poor to very poor drainage. Sites 2 and 3 are on the Arctic foothills, glaciated uplands characterized by rolling hills with imperfect drainage. The Mollisol, site 4, is on farmland in central Nebraska and is well drained. All soil samples were kept in a cooler in the field right after sampling and then frozen before shipping. The soil samples were thawed in the laboratory, homogenized by hand, and visible roots were removed. Soil sub samples were homogenized by hand and air-dried before total C and N analysis using a LECO 1000 CHN analyzer. Total organic C (TOC) was calculated by subtracting total inorganic C (TIC) from the total C. The TIC was determined by measuring CO_2 evolution using a Perkin-Elmer 8500 gas chromatograph, after acidification by HCl.

2.2. Fractionation of soil organic matter

2.2.1. Extraction of SOM

The SOM was fractionated as described by Ping et al. (2001). Fresh field moist soils of known water content were extracted 3 times with 0.1 M NaOH (Ping et al., 1995; Michaelson and Ping, 1997).

Soils with known water content were weighed (the weight is calculated according to Michaelson and Ping, 1997) into a 35 ml plastic centrifuge bottle, 0.1 M NaOH added to the top of the bottle to exclude all air, and the capped bottles were shaken end to end for 2 h. Suspensions were centrifuged, decanted and supernatants pressure filtered through a 0.45 μm supor filter. Filtrates were acidified to pH 5–6 and the filter cake scraped back into the centrifuge bottle. The extraction–filtration–acidification–scraping was then repeated once with overnight shaking, and again with a deionized water rinse. Acidified extracts were combined and rinsed into a 250 ml centrifuge bottle. This separation creates two parts: extractable SOM (EF), which contains soluble humified and non-humified SOM components, and non-extractable SOM (NEF), which contains minerals and lipids, fibrous, and organo-mineral SOM components.

2.2.2. Fractionation of EF

The EF is further fractionated into its components, humic acid (HA), fulvic acid (FA), low-molecular-weight acid (LMA), hydrophobic neutral (HON), hydrophilic neutral (HIN), and low-molecular-weight neutral (LMN). This fractionation is accomplished by passing the EF through tandem columns of XAD-8/XAD-4 resins (Malcolm and MacCarthy, 1992) according to Malcolm et al. (1995).

The EF is acidified to pH 2 with 6 N HCl and passed through the XAD-8 resin column (Fig. 1). The HA, FA

and HON fractions are retained on the XAD-8 resin. The SOM passing the XAD-8 resin is applied to the XAD-4 resin, which retains the LMA and LMN fractions. The SOM passing the XAD-4 resin is the HIN fraction. The HA and FA fractions are then eluted with NaOH from the XAD-8 resin and separated by solubility at pH 1 (HA precipitates and FA remains in solution). The HON is eluted with ethanol from the XAD-8 resin and determined colorimetrically at 400 nm by comparison to a solution of known HON concentration. The LMA fraction is eluted with NaOH and the LMN fraction is eluted with ethanol from the XAD-4 resin. Dissolved organic C (DOC) samples are taken from each separated fraction and analyzed using an OI Model 700 TOC analyzer.

2.2.3. Fractionation of NEF

The NEF fraction is washed with distilled water until free of salts by monitoring the EC value, and subjected to the sequential extraction procedure as shown in Fig. 2.

A portion of the NEF of known moisture content is weighed into an Alundum thimble, placed in a Soxhlet extractor and boiled in a solution of ethanol/toluene (7:3) followed by a reflux wash (Wu et al., 1995; Ping et al., 2001). The lipid fraction is determined gravimetrically by evaporation of the ethanol/toluene boiling-reflux solution.

The lipid extraction residue is refluxed with a neutral pH detergent solution (Goering and Van Soest, 1975) for 1 h, and the weight loss due to this treatment is

Table 1
Location, classification and site characteristics of samples

Site ID	Area	Land cover class[a] Soil classification[b]	Landform microrelief	Active layer
				cm
Site 1	Prudhoe Bay, AK	Moist nonacidic tundra Euic Sapric Glasistels	Coastal plain, Flat-polygon center	35
Site 2	Sagwon Hills, AK	Moist acidic tundra Fine-loamy, mixed Ruptic-Histic Aquiturbel	Hills Tussock tundra	40
Site 3	Toolik Lake, AK	Moist acidic tundra Loamy, mixed Ruptic-histic Aquiturbel	Hills Tussocks, mid-slope	35
Site 4	Nebraska	Great Plains Fine silty, mesic Calcic Hapludoll	Grassland plain	–

[a] Adapted from Auerbach and Walker, 1995.
[b] Adapted from Soil Survey Staff, 1998.

Table 2
Selected properties of whole soils and non-extractable fractions from three tundra soils of Alaska and one Mollisol from Nebraska

Site	Horizon	Depth (cm)	pH (1:1)	EF–C/C (%)	TOC (%)	TN (%)	C/N	HA/FA[a]
Site 1	Oa1	10–22	5.7	28	14.2	0.9	15	2.5
Prudhoe Bay	Oa1-NEF[b]		5.9[c]		9.4	0.6	17	
	Oa2	22–50	6.6	21	19.9	1.3	16	2.6
	Oa2-NEF[b]		6.9[c]		15.6	1.0	16	
Site 2	Oe	8–16	5.2	27	36.0	1.4	25	2.6
Sagwon Hills	Oe-NEF[b]		5.3[c]		30.3	1.0	32	
	O/A	37–50	5.8	28	11.9	0.8	16	2.3
	O/A-NEF[b]		5.9[c]		7.4	0.5	16	
Site 3	Cf	30–100	5.4	28	6.9	0.4	18	2.4
Toolik Lake	Cf-NEF[b]		5.4[c]		4.4	0.3	18	
Site 4	Ap3	10–26	6.1	24	1.4	0.2	10	5.3
Nebraska	Ap3-NEF[b]		6.0[c]		1.1	0.1	9	

[a] Humic acid to fulvic acid ratio.
[b] Residue insoluble in 0.1 M NaOH.
[c] Phosphate-adjusted pH.

considered to be the neutral detergent soluble carbohydrate fraction (NDSC). The residue from the NDSC dissolution is then boiled with a 0.5 M sulfuric acid solution also containing detergent for 1 h. The weight loss by treatment with the 0.5 M acid is considered to be the hemicellulose fraction (Hcel). Residue from the Hcel is then treated with 72% sulfuric acid for 3 h. The weight loss, by dissolution in the strong acid, is taken as the cellulose fraction (Cel).

The washed residue from the Cel determination above contains the most recalcitrant SOM fractions with regard to both extraction and microbial degradation. These fractions are largely lignin and humin (organo-mineral and physically or chemically protected humic materials) fractions. The Cel residue is dried by washing with acetone and then treated with a mixture of acetic acid and acetic anhydride in the presence of sulfuric acid catalyst to solublize the phenolic lignin components (Beyer et al., 1993). The difference in SOM content before and after treatment is taken as the lignin (Lig) fraction, and the SOM in the residue as the humin (Hn) fraction.

2.2.4. Cross-polarization magic angle spinning (CP–MAS) ^{13}C NMR

Spectra of the NEF were obtained on a Brüker DSX 200 operating at a frequency of 50.3 MHz using zirconia rotors of 7 mm outer diameter with KEL-F-caps. The cross polarization magic angle spinning (CP–MAS) technique (Schaefer and Stejskal, 1976) was applied during magic-angle spinning of the rotor at 6.8 kHz. A contact time of 1 ms and a ramped 90° ^{1}H-pulse width of 4.7 μs were used for all spectra to circumvent mismatch of the Hartmann–Hahn match (Peersen et al., 1993). The ^{13}C-chemical shifts were calibrated to tetramethylsilane (=0 ppm). Between 10,000 and 400,000 scans were accumulated using a pulse delay of 400 ms (Fründ and Lüdemann, 1989; Knicker and Lüdemann, 1995). Prior to Fourier transformation a line broadening between 20 and 75 Hz was applied. Relative carbon distribution was determined by an integration routine supplied with the instrument software.

In general, the ^{13}C NMR spectra of natural organic matter are divided into regions corresponding to specific chemical classes: unsubstituted alkyl C (alkanes, fatty acids) 0–45 ppm, N-alkyl C and methoxyl C (OCH_3, $C–NH_3$) 45–60 ppm, aliphatic C–O (notably carbohydrates) 60–90 ppm, anomeric-C 90–110 ppm, aromatic C (unsubstituted and alkyl substituted) 110–140 ppm, phenolics 140–160 ppm, and carboxyl/carbonyl C (including the carboxylate ion, COO^-, esters, ketones, aldehydes) 160–220 ppm (Kaiser et al., 1997; Stevenson, 1994; Malcolm, 1989).

2.2.5. Pyrolysis–gas chromatography/mass spectrometry (Py–GC/MS)

Twenty-five mg NEF samples were placed in quartz tubes (2 cm×2 mm inside diameter) and quantified using

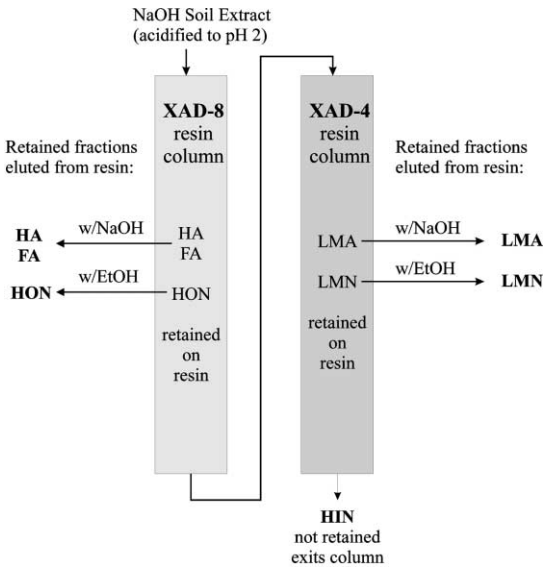

Fig. 1. Fractionation of extractable organic carbon (EF) by the XAD-8 /XAD-4 tandem resin column procedure (HA, humic acid; FA, fulvic acid; HON, hydrophobic neutrals; LMA, low-molecular-weight acid; LMN, low-molecular-weight neutrals; HIN, hydrophilic neutrals).

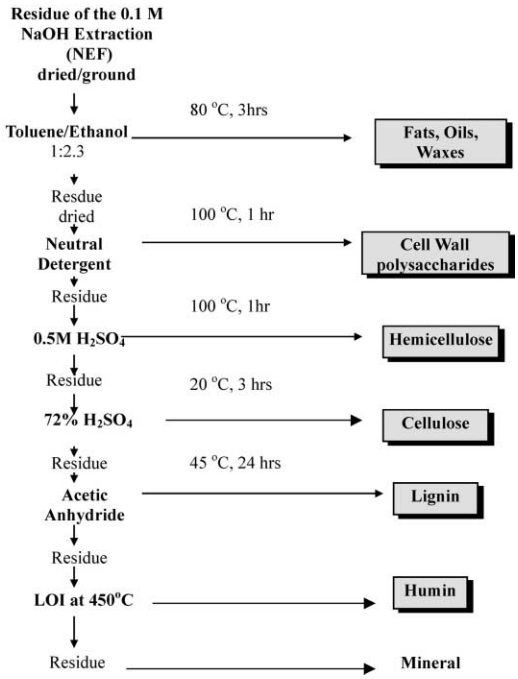

Fig. 2. General flow of the sequential SOM fractionation procedure.

a Mettler microbalance. Py–GC/MS was conducted as described by White and Beyer (1999). The samples were held in place by a plug of quartz wool at each end of the tube. Pyrolysis–GC/MS was conducted on each sample and used to identify as many compounds as possible. Pyrolysis–GC/MS was conducted with a CDS Model 1000 pyrolyzer and a Model 1500 GC interface. The interface temperature was set at 280 °C. During pyrolysis, the sample was heated from 280 to 700 °C in 0.1 seconds and held at 700 °C for 9.9 s. The pyrolysis reactor was mounted on a gas chromatograph, HP 5890 Series II, fitted with a Hewlett Packard HP-1 column (cross-linked methylsiloxane) 25 m×0.2 m i.d.×0.33 μm film thickness. The GC temperature program was 35 °C for 15 min, 2 °C/min ramp to 250 °C and hold for 10 min. The GC was plumbed directly to an HP 5971A Series Mass Selective Detector operations in electron impact mode. The MS scanned mass units 45 to 650. All mass spectra were compared to the Wiley 138 mass spectral library. Helium was used as the carrier gas at 0.5 cc/min. The sample was injected with a split ratio of 1:50.

Following Py–GC/MS, many compounds were identified and a suite of compounds were selected from the biochemical classes: polysaccharides, amino sugars and N-acetyl amino sugars (hereafter referred to as amino carbohydrates), phenol and protein precursors (hereafter referred to as phenols), and lipids (Bracewell et al., 1989). Specific compounds (Table 3), chosen to represent each class, are widely reported in the soil pyrolysis literature and known to be derived from specific fractions of soil organic matter. Comprehensive lists of all compounds in each biochemical class are published (Bracewell et al., 1989; Hempfling and Schulten, 1990; Irwin, 1979). Although these lists were consulted, for the purposes of this work, only the dominant peak (s) (Fig. 3) in each class was (were) chosen for analysis. The dominant peaks could be easily identified and were not subject to complications caused by chromatographic co-elution.

The relative percentage of each selected compound was calculated according to peak height above baseline. For each sample, the total peak height for all identified compounds was calculated and the relative peak height of each identified compound was calculated in percent of the total peak height. The percentage of each compound reported herein, therefore, is the relative percentage of that compound among the compounds identified, not the percentage of that compound of the total SOM.

3. Results and discussion

3.1. Characterization of soil horizons and the extractable fraction (EF)

The C/N ratio, extractability, and HA/FA ratio are shown in Table 2, and the percentage of C in different fractions relative to total C is shown in Fig. 4. The C:N ratio of the tundra soil horizons ranged from 15 to 25, with the highest in the Oe horizon; and that of the Mollisol Ap3 was the lowest at 9.6 (Table 2). The result is in agreement with that of Ping et al. (1997), who indicated that most O and A horizons of the tundra soils have higher C:N ratios than those of the temperate cultivated soils, due to the lower degree of decomposition or humification. Tan (1993) also pointed out that the C:N ratio usually decreased in the humification process from larger than 20 for non-humified organic material, to 8–20 for humus.

Extractability of SOM is the percentage of extractable C (soluble in 0.1 M NaOH) to the TOC. Ping et al. (1997) noted that the extractability of B horizons averaged 52%, almost double that of O, A, and Cf horizons in the Arctic tundra soils, but lower than that of B horizons in Spodosols from southern Alaska. Since the extractable fractions contain the product of biochemical degradation of plant materials and microbial biomass (Thurman and Malcolm, 1989), the extractability of SOM is suggested as an indicator for the degree of decomposition or humification (Ping et al., 1997). The higher extractability of the B horizons implied a higher degree of decomposition or humification. The Cf horizons had similar values as the O and A horizons reflecting that the SOM was probably translocated from O and A to Cf horizons by cryoturbation. In this study, the four O horizons and one Cf horizon had similar extractability, ranged from 21–28% (Table 2), which is consistent with that of Ping et al. (1997); however, the extractability of the Mollisol did not show a higher

Table 3
Biochemical classes selected to represent soil organic materials[a]

Biochemical classes	Post pyrolysis compounds
Polysaccharides	2,4-Dimethylfuran, furancarboxyaldehyde, benzofuran
Phenol (protein and phenol precursors)	Phenol, methylphenol, ethylphenol
Lignin	Methoxyacetaphenone, unresolved methoxyphenolic
Amino carbohydrates (amino sugar and N-acetyl amino sugars)	Unresolved amino sugar
Lipids	Unresolved PAH, unresolved alkene, unresolved alkane

[a] Adapted from Dai et al., 2001b.

value than that of the tundra soils as we expected. This result contradicts the C:N ratio result mentioned above. Therefore, the extractability of SOM may be used as an indicator of the degree of decomposition or humification within tundra soils, not over other regions.

Carbon distribution in different fractions did not show much difference among tundra soil horizons, but had significant differences between the tundra soils and the Mollisol (Fig. 4). The dominant fraction in the tundra soils is HA, followed by HIN, FA, LMA or HON, and LMN; the dominant fraction in the Mollisol, however, is HIN, followed by HA, LMA, LMN and FA (Fig. 4).

Dai et al. (2000) studied the bioavailability of SOM in the whole soil, the NEF, and the EF using laboratory incubation methods (Table 4; Fig. 5). Cumulative CO_2 evolution over the incubation period was used as an index of the bioavailability of SOM. They found that the HIN had the highest amount of cumulative CO_2 evolution, whereas the HA and FA had the lowest amount of CO_2 evolution, indicating that the HIN was more bioavailable than the HA and FA (Fig. 5). Significant correlations between the initial proportion of HIN and the cumulative amount of CO_2 evolved from the whole soil were observed among the tundra soils, the correlation coefficients at 4 and 25 °C are 0.93 and 0.86, respectively (Dai et al., 2000). Based on the lower C/N ratio of SOM in the Mollisol, we assumed that SOM in the Mollisol had a higher degree of humification or decomposition, therefore the higher proportion of HA and FA, and lower degree of bioavailability than that in the tundra soils. However, the results showed that the Mollisol had the highest proportion of HIN, the lowest abundance of

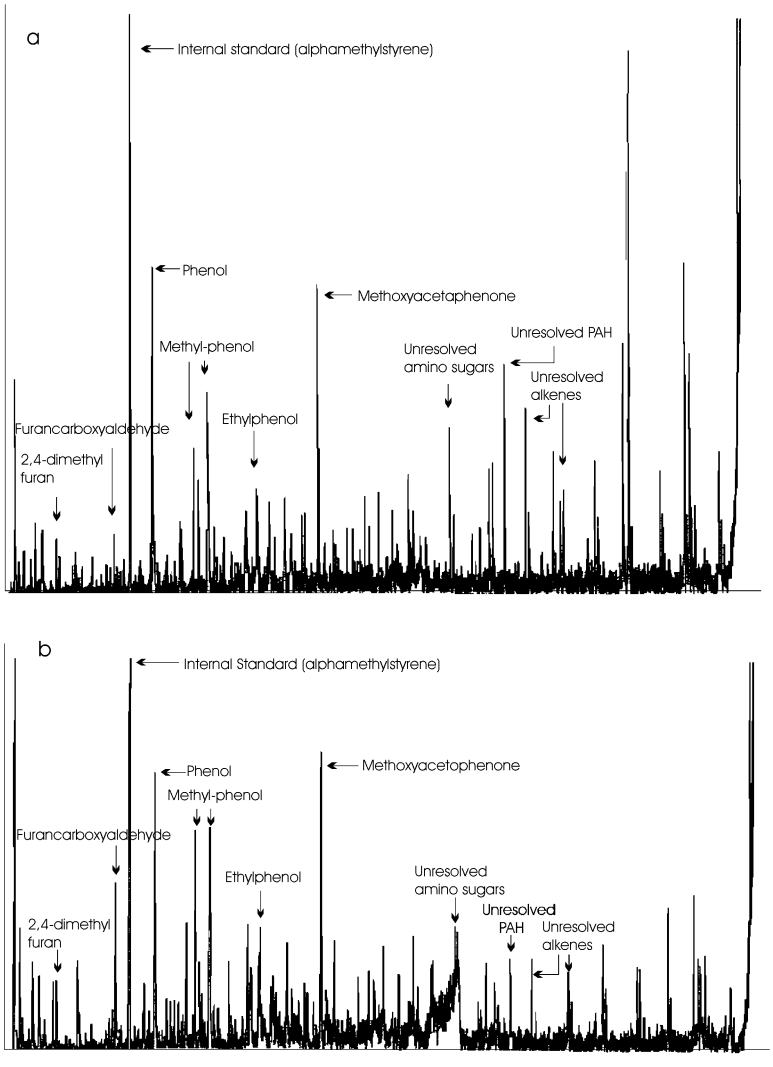

Fig. 3. Examples of pyrograms of site 1 soil Oa1 horizon (a) and site 2 soil Oe horizon (b).

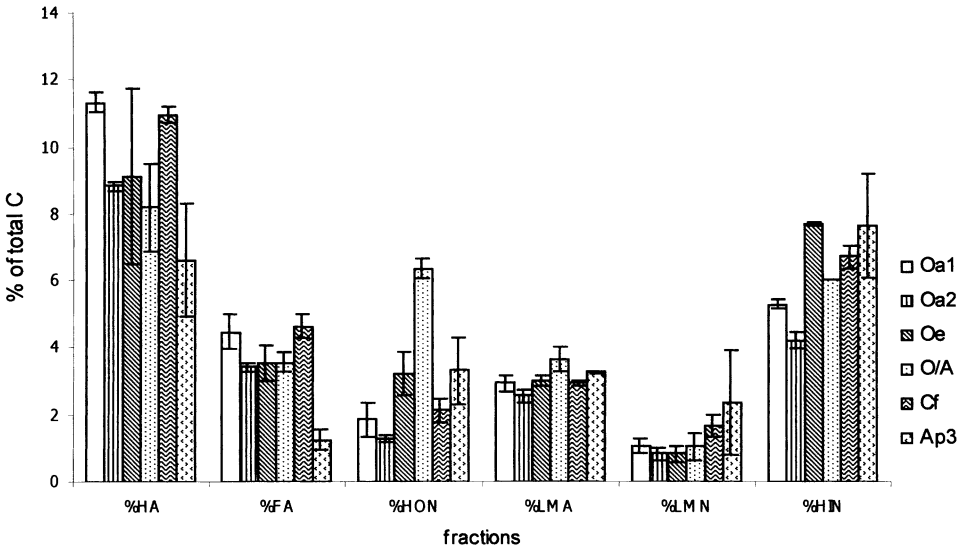

Fig. 4. Distribution of extractable carbon in different fractions to the total organic carbon (%). HA, humic acid; FA, fulvic acid; HON, hydrophobic neutral; LMA, low-molecualr-weight acid; LMN, low-molecular-weight neutral; HIN, hydrophilic neutral.

Table 4
Total cumulative CO_2 evolution from the whole soil and the non-extractable fractions incubated at 4 and 25 °C for 120 days[a]

Site ID	Sample ID	CO_2–C	
		4 °C (mg gOC^{-1})	25 °C (mg gOC^{-1})
Site 1	Oa1	6.3±0.6	23.3±2.3
Prudhoe Bay	Oa1NEF[b]	8.1±0.5	30.5±1.1
	Oa2	4.4±0.4	12.8±0.4
	Oa2 NEF[b]	3.6±0.6	16.1±1.6
Site 2	Oe	9.1±1.9	32.8±6.9
Sagwon Hills	Oe NEF[b]	11.8±1.5	31.0±5.9
	O/A	10.1±2.8	31.5±1.6
	O/A NEF[b]	6.4±1.6	31.8±6.6
Site 3	Cf	11.1±3.8	27.8±3.1
Toolik Lake	Cf NEF[b]	5.4±2.4	27.8±3.1
Site 4	Ap3	43.3±0.9	58.8±8.3
Nebraska	Ap3 NEF[b]	15.4±1.3	23.0±4.4

[a] Adapted from Dai et al., 2000.
[b] Residue insoluble in 0.1 M NaOH.

HA and FA, and the highest amount of CO_2 respired at both temperatures (Table 4), implying that the degree of humification is not necessarily related to the initial proportion of HA and FA and the bioavailability of SOM.

Although the Mollisol had a lower percentage of HA and FA and a higher proportion of HIN than the tundra soils, it showed the highest HA/FA ratio in the soils studied. The HA/FA ratio was 2.3–2.6 in the tundra soils, and 5.3 in the Mollisol (Table 2). This result was in line with those of Kononova (1961) and Ping et al. (1997), who found that the HA/FA ratios were generally lower in less developed soils. Therefore, the C/N ratio and HA/FA ratio might be a better indicator of humification degree of SOM in soil than the relative proportions of different extractable fractions.

3.2. Characterization of the non-extractable fraction (NEF)

3.2.1. Wet chemical fractionation analysis

Results of the wet chemical fractionation of SOM in the NEF are reported in Fig. 6. Neutral detergent soluble C (NDSC) is the dominant fraction accounting for 26–35% of the total C in the tundra soils and 48% in the Mollisol. Hemicellulose (Hcel), cellulose (Cel), lipids (Lp), lignin (Lig) and humin fraction (Hn) accounted for 13–23, 9–13, 0.5–6, 2–4.3 and 3–8% of the total C, respectively (Fig. 6). The tundra soils had relatively higher percentages of Lp, Hcel, and Hn, and lower NDSC compared to the Mollisol. The proportion of Cel and Lig was similar between the tundra soils and the Mollisol. These results disagree with those of Ping et al. (1997), who found that hemicellulose was the dominant fraction accounting for 53% of the total C, and the Arctic tundra soils had higher proportions of hemicellulose than the temperate region soils due to the relatively low degree of decomposition in the Arctic tundra environment. The discrepancy of the dominant fraction in different studies was due to the different analytical procedures and definitions. In the previous study conducted by Ping et al. (1997), the NEF was

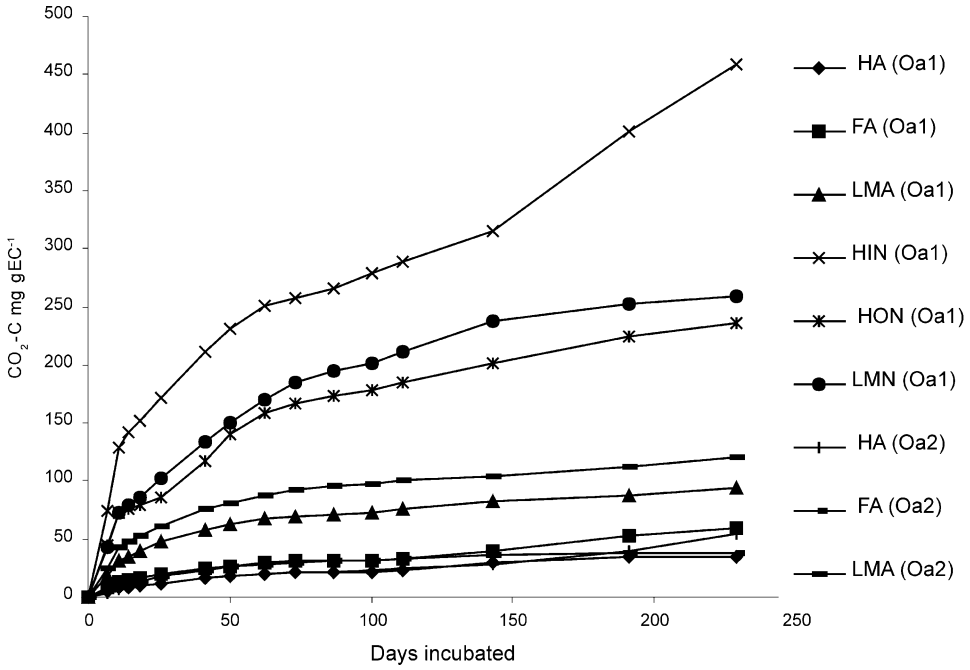

Fig. 5. The cumulative CO_2 evolution from different extractable fractions (EF) of site 1 soil Oa1 and Oa2 horizons.

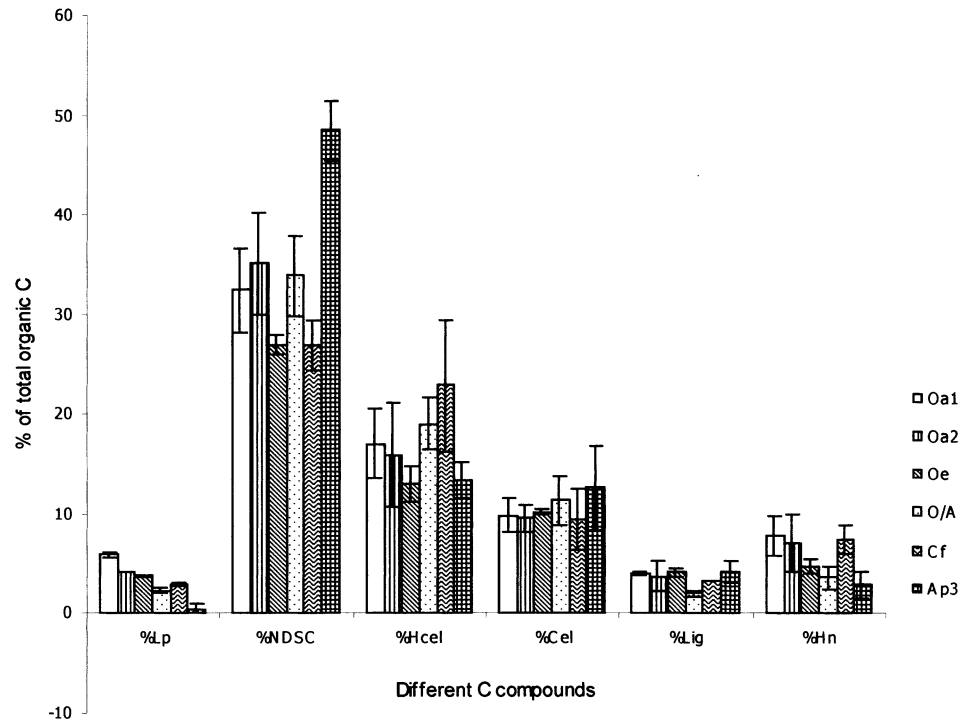

Fig. 6. Distribution of different carbon compounds in the NEF to the total organic carbon (%). Lp, lipids; NDSC, neutral detergent soluble carbon; Hcel, hemicellulose; Cel, cellulose; Lig, lignin; Hn, humin.

fractionated into hemicellulose (neutral detergent soluble C), cellulose (72% H_2SO_4 soluble C), and insoluble SOM (considered humin). In this study, the original fractionation procedure was modified (Fig. 2) based on Beyer et al. (1993). Hemicellulose is the fraction that is soluble in 0.5 M H_2SO_4 and insoluble in neutral detergent solution. Therefore, the hemicellulose in Ping et al.'s study equals the NDSC fraction in this study and the dominant fraction in SOM in both studies agrees.

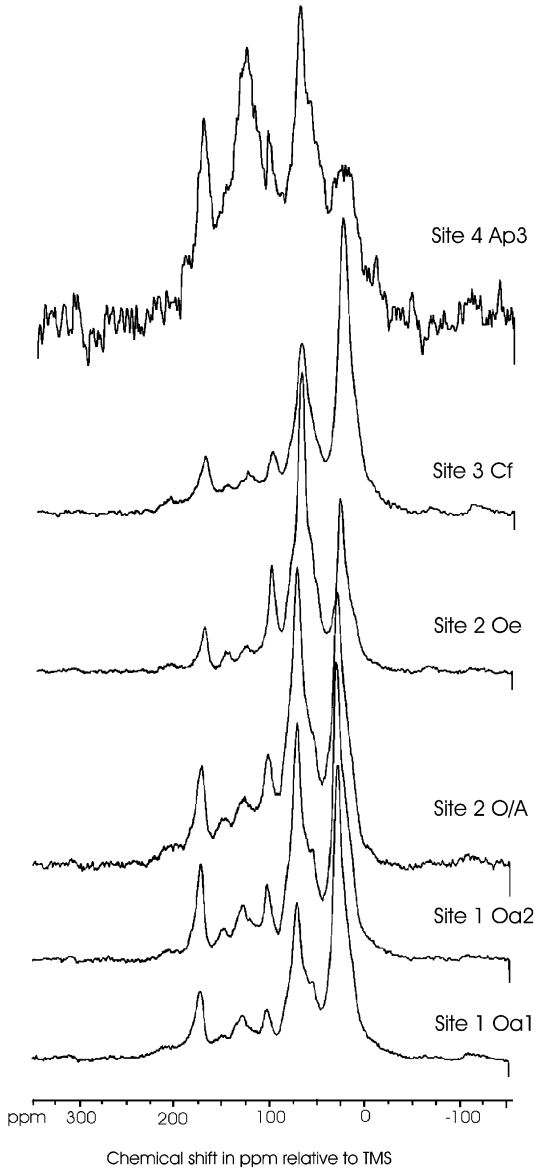

Fig. 7. CPMAS ^{13}C NMR spectra of the NEF from Sites 1, 2, 3 and 4 soils: 150917 and 61612 scans for the Site 1 soil Oa1 and Oa2 horizons, respectively; 18825 and 80914 scans for the Site 2 soil Oe and O/A horizons, respectively; 199912 scans for the Site 3 soil Cf horizon; and 261873 scans for the Site 4 soil Ap3 horizon.

Baldock et al. (1992) proposed a model of the oxidative decomposition of plant materials with three successive stages: the initial stage is the loss of carbohydrates including hemicellulose, cellulose, and protein; the second stage is the exposure and subsequent decomposition of lignin, and the last stage is the loss of the highly recalcitrant alkyl carbon including long-chain fatty acids, lipids and waxes. The stage to which plant materials are decomposed in soils is controlled by the ability of the soil to limit the plant materials through the formation of organo-mineral complexes. Based on this model, the NEF of the Mollisol had the least extent of decomposition, since it had the highest proportion of carbohydrates (including NDSC, Hcel and Cel) at 90.5% and the smallest proportion of lipid, whereas, that of the Oa1 and Oa2 showed the greatest extent of decomposition with carbohydrates at 78.2 and 80% of TOC, respectively. This result contradicts our assumption that the NEF of the Mollisol is highly humified due to the warmer climate. The result might be explained in that the Mollisol contains two groups of organic matter. One group is highly humified organic material with a high HA/FA ratio as shown in Table 2, and the other is less decomposed organic material comprised of fine roots and microbial products, which contains a high percentage of carbohydrates as shown in Fig. 6. These also explained that the higher bioavailability of SOM in the Mollisol (Table 4) was due to the higher proportion of carbohydrates in the NEF, although there is no correlation between the carbohydrates and the bioavailability among the soils.

3.2.2. Cross-polarization magic angle spinning (CP–MAS) ^{13}C NMR

Solid-state CP–MAS ^{13}CNMR spectra of NEF in the tundra soils and the Mollisol are shown in Fig. 7, and the relative C distribution is presented in Fig. 8. All NEF in the tundra soils had a very sharp intense single alkyl C peak (0–45 ppm), followed by an intense O-alkyl C peak (45–90 ppm) and a small anomeric C peak (90–110 ppm), two weak aromatic C peaks (110–140 and 140–160 ppm) as well as one small intense carboxyl C peak (160–185 ppm), whereas, NEF in the Mollisol exhibited a very different spectrum with a very large intense O-alkyl C peak dominating the other peaks, followed by small but broad multiple alkyl C peaks (due to noise), and one small and one large intense broad aromatic C peaks as well as a sharp intense carboxyl C peak (Fig. 7). The relative C distribution data also showed that NEF in the tundra soils had a greater proportion of alkyl C and a smaller proportion of aromatic and carboxyl C than those in the Mollisol. The proportion of O-alkyl C showed little difference except for the Oe horizon. Baldock et al. (1997) suggested using the ratio of alkyl/O-alkyl carbon (A/O-A) as a sensitive index for the extent of decomposition of SOM.

The Oa1 and the Cf horizons had the greatest ratios at 1.29 and 1.19, respectively. The Oe and the O/A had the smallest ratios at 0.46 and 0.67, respectively, and the Oa2 showed an intermediate ratio of 0.98. The Ap3 of the Mollisol exhibited a ratio of 0.47 that is similar to that of the Oe horizon. These results indicated that the Oe and Ap3 horizons had the least extent of decomposition of SOM, whereas, the Oa1 and the Cf horizons had the greatest extent of decomposition.

The incubation results (Dai et al., 2000) showed that the NEF of the Mollisol had the highest cumulative CO_2 respiration at the lower temperature, and the second lowest cumulative CO_2 evolution at the higher temperature (Table 4). The difference in the generation of CO_2 at the two temperatures is the enrichment of a different microbial consortium and the capability of using different substrates by different microbial communities (Dai et al., 2001b). At the lower temperature, bacteria were the dominant microorganisms, which could only use labile substrates such as carbohydrates and proteins as food and energy sources, therefore, NEF of the Mollisol had the highest availability to supply these materials for microorganisms and produced the highest CO_2 respiration; whereas at the higher temperature, fungi became dominant and the capability of decomposition increased, fungi not only use labile substrates, but also use more recalcitrant materials such as lignin and cellulose (Nadelhoffer et al., 1992; Dai et al., 2001b). The CP–MAS ^{13}C NMR data (Figs. 7 and 8) showed that the Mollisol had much higher proportion of aromatic C compared to the arctic soils, which is either attributed to lignin or highly humified organic materials. The percentage of lignin in the Mollisol obtained from the wet chemical fractionation analysis (Fig. 5) is very similar to that of the arctic soils, implying that the aromatic-C compounds in the Mollisol are mainly humified substances. This is supported by the results from SOM fractionation (Fig. 4). The HA/FA ratio of the Mollisol is double that of the arctic soils, suggesting the higher degree of humification of SOM in the Mollisol. Thus, at the higher temperature, after the labile materials in the NEF of the Mollisol were exhausted by microorganisms, there were no more bioavailable substrates for the microorganisms to continue the decomposition. Correlation coefficients between the cumulative CO_2 respiration from the NEF and the relative percentage of different C species of the NEF determined by CP–MAS ^{13}C NMR were analyzed among the arctic soils. No relationships were found. Since only five samples were used in the study, and the samples were from different horizons, we can not conclude that there are no correlations between the chemical composition of SOM determined by CP–MAS ^{13}C NMR and the bioavailability of SOM determined by laboratory incubation methods in this case.

3.2.3. Pyrolysis–gas chromatography/mass spectrometry (Py–GC/MS)

The relative proportion of the selected compounds in the NEF is shown in Fig. 9. Since the organic C content in the Mollisol is not high enough for the Py–GC/MS method, data for the NEF of the Mollisol are missing.

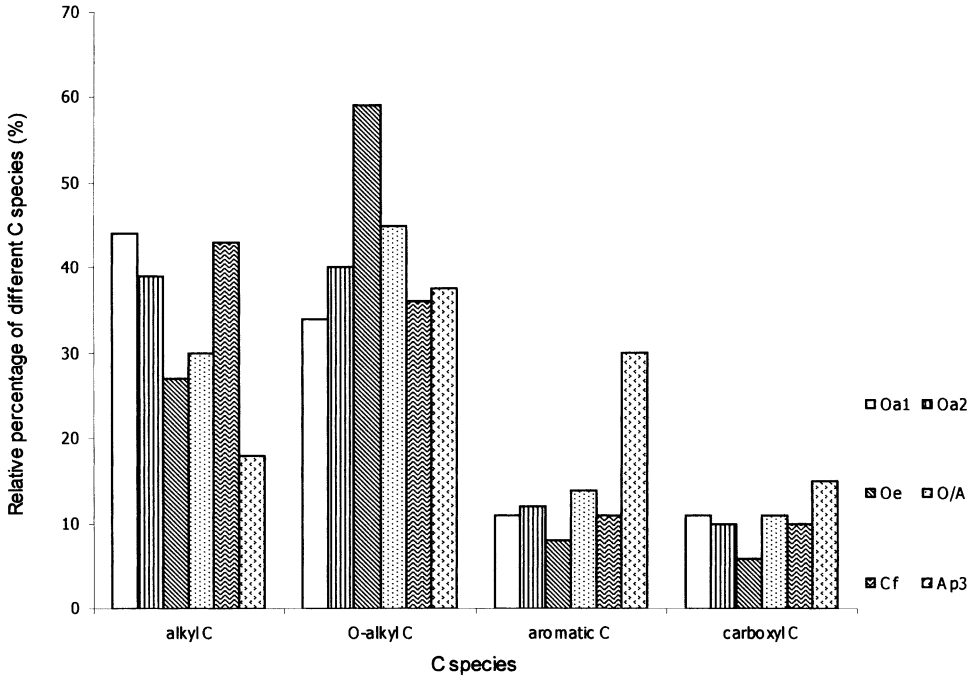

Fig. 8. Relative distribution of carbon of different organic compounds in the NEF (%) determined by CP–MAS ^{13}C NMR.

Among the tundra soils, SOM in the NEF of the Oe horizon exhibited the highest relative abundance of polysaccharides and lignin, and the lowest proportions of lipids, amino carbohydrates and phenol, suggesting the least extent of decomposition of this horizon. Since Baldock et al. (1997) used alkyl C/O-alkyl C ratio as a sensitive index for the extent of decomposition in SOM, it is assumed that the ratio of relative proportion of lipids to polysaccharides (Lip/PS) might also be used as an indicator of the extent of decomposition. Therefore, the Lip/PS ratio was calculated for the different horizons in the tundra soils. The NEF of Oa1 had the greatest Lip/PS ratio at 6.8, followed by the Cf, O/A, Oa2, and Oe horizons at 6.3, 4.6, 4.1, and 2, respectively. The trend in the Lip/PS ratio was similar to that of the A/O–A ratio except for Oa2. Both the O/O–A and Lip/PS ratios showed that the SOM in the NEF of the Oe horizon had the least extent of decomposition, and that of the Oa1 and Cf had the greatest extent of decomposition among these five tundra soil horizons. We suggest that both the A/O–A and Lip/PS ratios can be used as indices for the degree of decomposition of SOM in tundra soils.

Although the A/O–A and Lip/PS ratios could be good indicators for the decomposition degree of SOM in this study, they should be used with caution. We suggest that the A/O–A and Lip/PS ratios are better used for comparisons of the decomposition index of SOM between different horizons within the same soil profile, since these ratios are highly dependent on the input material and decomposition pathway as well as the input of microbial biomass. The ratios cannot be used as the index of bioavailability for SOM, since they showed no correlations to CO_2 evolution determined by the laboratory incubation method.

3.3. Relationships between the chemical composition of SOM in the NEF determined by different methods and the bioavailability of SOM in the NEF

One of the objectives of characterizing SOM in this study is to relate the chemical composition to the bioavailability of SOM, since the chemical composition of SOM is assumed to be a major factor influencing the bioavailability of SOM (Alperin et al., 1995; Oades, 1995; Sollins et al., 1996). Relationships between the chemical components of SOM in the NEF and the bioavailability of SOM in the NEF were not found in this study. However, in previous studies the initial proportion of HIN and the cumulative amount of CO_2 respired from whole soils incubated at 4 and 25 °C were correlated, and the correlation coefficients were 0.93 and 0.86, respectively (Dai et al., 2000); the relative proportion of carboxyl and carbonyl C in the EF was correlated to the CO_2 evolved from the different EF fractions (Dai et al., 2001a); and initial relative proportion of polysaccharides determined by Py–GC/MS was correlated to the CO_2 evolution from the whole soil incubated at 4 °C (Dai et al., 2001b). These results suggest that the chemical composition of SOM in whole soil and EF is an

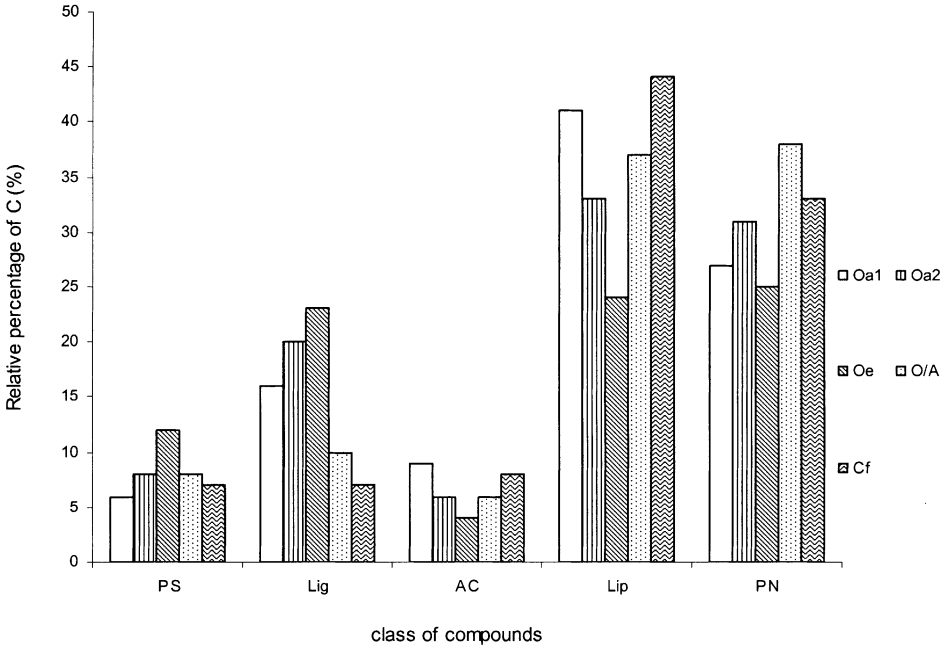

Fig. 9. Relative distribution of each class of compounds in the NEF (%) determined by Py–GC/MS. PS, polysaccharides; Lig, lignin; AC, aminocarbohydrates; Lip, lipid; PN, phenol.

important factor influencing the bioavailability of SOM. In contrast, the chemical composition of SOM in the NEF is less important to bioavailability of SOM, with other factors such as physical protection of SOM in the NEF playing more important roles in bioavailability. Components of SOM in soils, especially in the NEF can be protected from rapid degradation through adsorption, occlusion, clustering and mineral aggregate formation (Alperin et al., 1995). An organic compound that might otherwise readily decompose can persist for long periods of time due to physical protection. Physical protection of SOM in the tundra soils was not studied in this project, and we suggest this as the major focus in a future study of SOM.

4. Conclusions

The NEF in tundra soils is comprised of a large amount of SOM (70–80%). The data obtained from CP-MAS ^{13}C NMR show that 30–60% of the organic C in the NEF is O-alkyl C and 25–45% of the C is alkyl C, with the rest made of aromatic and carboxyl C. This significant proportion of O-alkyl C in the NEF of the tundra soils has a great potential to affect the C cycling in this ecosystem with climate change. The incubation study showed that the NEF of the tundra soils had a greater increase in the CO_2 evolution with an increase in temperature than that of the Mollisol (Dai et al., 2000), indicating that the NEF in the tundra soils could play an important role in C cycling and global climate change.

Generally, the chemical composition of SOM is an important factor influencing the bioavailability of SOM. However, other factors such as physical protection of SOM might play more important roles in affecting the bioavailability of SOM.

The SOM is a complex mixture of compounds with various chemistries. Each method characterizes SOM at different levels. Neither methodology can provide a complete insight into the nature and bioavailability of SOM. The wet chemical fractionation protocol is the procedural determination of chemical fractions of SOM, whereas CP–MAS ^{13}C NMR and Py–GC/MS techniques focus on chemical characteristics of SOM at a molecular level. To study bioavailability and chemical characteristic of SOM, it is better to combine the different approaches.

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