

# Soil carbon stabilization in jack pine stands along the Boreal Forest Transect Case Study

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## Abstract

Boreal forests, containing >20% of the total organic carbon (OC) present at the surface of the Earth, are expected to be highly vulnerable to global warming. The objective of this study was to compare soil OC stocks and chemistry in jack pine stands located along a latitudinal climatic transect in central Canada. Total OC stocks (0–1 m) increased with decreasing mean annual temperature (MAT). We used a combination of physical fractionation of soil OC pools,  $^{13}\text{C}$  isotopic determination and cross-polarization, magic-angle spinning  $^{13}\text{C}$  nuclear magnetic resonance (NMR) spectroscopy to further characterize OC composition at all sites. Soil OC was dominated by labile pools. As illustrated by the C/N ratios,  $\delta^{13}\text{C}$  data and results from the  $^{13}\text{C}$  NMR analysis, the light fraction showed little alteration within the soil profiles. Instead, this fraction reflected the importance of fresh litter inputs and showed an increase in root contribution with depth. As opposed to the light fraction, the clay- and silt-stabilized OC exhibited an increase in  $\delta^{13}\text{C}$  and a decrease in C/N with depth, indicating an increase in its degree of decomposition. These changes with depth were more marked at the southern than the northern sites. Results hence suggest that if the MAT were to increase in the northern boreal forest the overall jack pine soil OC stocks would decrease but the remaining OC would become more decomposed, and likely more stabilized than what is currently present within the soils.

**Keywords:** boreal soils, carbon stabilization, CPMAS  $^{13}\text{C}$  NMR, jack pine, soil fractionation, soil organic matter

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## Introduction

The boreal forest, which encircles the globe south of the arctic between the permafrost and the steppes, is the second largest forest biome on Earth with an area of  $1.37 \times 10^9$  ha (Watson *et al.*, 2000). This northern, high latitude, region is one of the most vulnerable ecosystems to global warming (Fischlin *et al.*, 2007). While global surface temperature is projected to increase on average 1.8–4.0 °C by 2100 (Meehl *et al.*, 2007), northern regions may experience up to 5 °C warming (Christensen *et al.*, 2007). The boreal forest also represents a huge reservoir of carbon on a global basis. Carbon stocks in boreal forest soils alone are estimated at 471 Gt, which is close to 25% of the global soil carbon stocks (Watson *et al.*, 2000). Yet, despite their importance in the global carbon budget and their high susceptibility to climate change, very little is known about how warming will affect carbon stocks and dynamics in these soils.

Modeling soil organic carbon (OC) dynamics in order to predict changes in carbon stocks may be accom-

plished using several pools with distinct turnover times, namely active or labile OC with a turnover from days to less than a year, passive OC (turnover of years) and recalcitrant OC with a turnover  $\geq 100$  years (e.g., Smith *et al.*, 1997; Davidson & Janssens, 2006). Soil organic matter is not composed of such finite categories, although separation into density and particle size categories yields OC fractions that are easier to categorize, more homogeneous chemically, and whose turnover rates are similar, in contrast to that of the bulk soil carbon. For instance, clay and silt-sized separates that are dominated by chemically and biochemically stabilized OC, have reported mean residence times based on  $^{14}\text{C}$  analysis of 800–1660 years for silt and 75–4400 years for clay (von Lützow *et al.*, 2007), while OC physically stabilized within fine sand-sized microaggregates show a turnover time range of 100–300 years based on  $^{13}\text{C}$  natural abundance studies (von Lützow *et al.*, 2007). In contrast, with no means of stabilization, the coarse sand-sized fraction encompasses a labile pool with rapid turnover, which shows little chemical alteration from fresh plant residues. (Christensen, 1992). A sub-component of the coarse sand-sized fraction, the low density, or light fraction, is even less degraded and

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more similar to original plant inputs (Gregorich *et al.*, 1996; Six *et al.*, 2002), although a gradient exists within the light fraction, with the lower density OC chemically more identical to the plant inputs compared with heavier free OC particles (Wagai *et al.*, 2009). Of the OC pools, the light fraction has been shown to be the most sensitive to environmental conditions (Gregorich *et al.*, 2006). It could be that the different chemistry or properties of these separates illustrates why multipool, as opposed to one-pool, modeling better represents findings from decomposition studies (Knorr *et al.*, 2005). In all cases, from an experimental point of view, quantifying soil OC distribution among density and size fractions provides better insight into OC dynamics and their response to climate than looking at bulk OC alone.

Reviews of density and particle size fractionation studies by Christensen (1992, 2001), Khanna *et al.* (2001), and von Lützow *et al.* (2007) all stated that the C/N ratio represents a good index of the degree of OC stabilization, as it is typically observed to decrease from the light fraction to the sand, silt, and clay fraction. Stable isotopic ratios of carbon and nitrogen also have been proposed as indicators of the extent of decomposition in soil organic matter. They follow a similar gradient by becoming gradually enriched in the heavier isotope with decreasing particle size (Bird *et al.*, 2002; Quideau *et al.*, 2003). An additional experimental approach, which aims to characterize OC macromolecular structure, is high-resolution solid-state cross-polarization magic-angle spinning (CPMAS)  $^{13}\text{C}$  nuclear magnetic resonance (NMR) spectroscopy (e.g., Preston, 2001; Conte *et al.*, 2004; Norris *et al.*, 2009). Compositional differences detected among soil fractions include a relative decrease in O-alkyl (carbohydrates) and an increase in aromatic (lignins, tannins) and more often alkyl (lipids) carbon with decreasing particle size. A combination of the elemental, isotopic, and NMR techniques was used previously to increase sensitivity in detecting differences among density and size fractions (Norris *et al.*, 2009); similar methods were used in this study.

Pine stands colonize the upland coarse texture fluvial outwash sites of the boreal with Scots pine (*Pinus sylvestris* L.) in the Russian taiga (Wirth *et al.*, 2002) and the Fennoscandia landscape (Skilling 1990). Jack pine (*Pinus banksiana* Lamb.) is the dominant pine species in the boreal forests of North America. These jack pine stands may be particularly sensitive to temperature and moisture variations due to the xeric conditions under which they grow (Rudolph & Laidly, 1990). In an effort to elucidate how climate may affect boreal forest OC stocks, the overall objective of this study was to investigate soil OC stabilization along a climate transect of jack pine stands located in Canada through the provinces of Saskatchewan and Manitoba.

Specific objectives included quantification of total soil OC stocks (0–1 m), distribution of OC among density and size fractions, and characterization of OC composition through elemental, isotopic and NMR analyses.

## Materials and methods

### Study sites and sampling design

The jack pine transect is part of the Boreal Forest Transect Case Study (BFTCS) project (Price & Apps, 1995). The BFTCS is one of the transects identified in the Global Change and Terrestrial Ecosystems (GCTE) Core Project of the International Geosphere–Biosphere Programme (Koch *et al.*, 1995). The BFTCS was originally initiated to study the effect of climate and disturbance on OC stocks in representative stand types of the Canadian boreal forest, i.e., trembling aspen (*Populus tremuloides* Michx.), black spruce [*Picea mariana* (Mill.) SSP] and jack pine (Bhatti *et al.*, 2002). The transect is aligned with an ecoclimatic gradient (Ecoregions Working Group, 1989) and spans several major vegetation biomes – proceeding in a northeast direction from the aspen parkland transition to the boreal forest biome and into the sparsely treed subarctic tundra.

Along the BFTCS, climatic conditions change from warm and dry in the southwest [mean annual temperature (MAT) from 0.5 °C and mean annual precipitation (MAP) from 400 mm] to cold and wet in the northeast (MAT –5.0 °C and MAP 550 mm) (Price & Apps, 1995). For this study, four sites supporting pure mature jack pine stands were sampled in 2006 along the BFTCS. These sites represented the different ecoclimatic regions with Site 1 in the parkland to low boreal transition near Prince Albert, Site 2 in the mid boreal region, Site 3 in the high boreal near Flin Flon and Site 4 further north in the high boreal near Nelson House (Table 1).

Reindeer lichen (*Cladina* spp.), with patches of bearberry (*Arctostaphylos uva-ursi* L.) and moss (*Pleurozium* spp.), dominated the ground cover at all the jack pine sites, with the occasional green alder [*Alnus crispa* (Ait.) Pursh] spread throughout the stand. All sites were located on level terrain. At each site, four random soil profiles were excavated within a 200 m radius. One profile was fully characterized, while the three others were examined for horizon sequence and depth. The soil of the northern site, Site 4, is derived from glaciolacustrine parent material deposited by Glacial Lake Agassiz while that of the other sites are of glaciofluvial origin. All soils were classified as Eluviated Dystric Brunisols according to the Canadian System of Soil Classification (Soil Classification Working Group, 1998) and Typic Dystrocrepts in the USDA system (Soil Survey Staff, 2006).

Bulk density was determined by obtaining soil samples from each of the sampling plots and from each horizon using a bulk density corer (172 cm<sup>3</sup>). The soil sample was returned to the lab, dried at 105 °C until constant weight, and weighed. Bulk density of the soil was calculated by dividing the dry weight by the soil core volume (Kalra & Maynard, 1991). Approximately 1.5 kg of sample was collected from each mineral horizon (A, Bm<sub>1</sub>, Bm<sub>2</sub>, and C) at each soil profile for

**Table 1** Site characteristics and general soil properties of a jack pine forest soil transect

Site identification	Alternate identification	Stand origin date	Region	Latitude (decimal degrees)	Longitude (decimal degrees)	MAT (°C)	MAP (mm)	Horizon	pH	% Sand	% Clay	% Silt
Site 1	PJM2	1927	Prince Albert	53.2256	-105.9436	0.50	398	Ahe	4.8	97.7	0.6	1.7
								Bm <sub>1</sub>	5.1	98.0	0.5	1.5
								Bm <sub>2</sub>	5.5	98.7	0.4	0.9
Site 2	STOJP	1915	Candle Lake	53.9147	-104.6902	0.25	440	C	4.9	98.0	0.5	1.4
								Ahe	4.9	97.3	0.4	2.2
								Bm <sub>1</sub>	5.2	97.6	0.3	2.1
Site 3	FJM3	1933	Flin Flon	54.8400	-102.5127	-0.50	474	Bm <sub>2</sub>	5.0	96.2	0.6	3.2
								C	5.1	96.0	0.6	3.1
								Ahe	3.9	97.8	0.2	2.0
Site 4	NTOJP	1936	Nelson House	55.9283	-98.6218	-3.50	544	Bm <sub>1</sub>	4.7	98.5	0.1	1.2
								Bm <sub>2</sub>	5.2	97.8	0.2	1.9
								C	5.2	98.0	0.2	1.6
								Ae	4.3	97.1	0.3	2.6
								Bm <sub>1</sub>	4.9	97.0	0.5	2.4
								Bm <sub>2</sub>	5.3	96.0	0.6	3.3
								C	5.3	97.5	0.3	2.1

MAT, mean annual temperature; MAP, mean annual precipitation.

a total of four samples per horizon from each site. Forest floor samples were also collected randomly in a 5 m radius around each pit. In addition, from Site 2 individual samples of lichen, jack pine needles from the forest floor, and a mixture of coarse and fine jack pine roots from the soil samples were collected to characterize litter inputs to these soils. Samples were transported to the laboratory, within 4 days of collection, where they were immediately air dried at room temperature. Soil samples were sieved to 2 mm and all were stored in airtight containers until further analysis.

#### Physical fractionation scheme

Each mineral soil sample collected was fractionated as described in Norris *et al.* (2009). Methodology was amended from a variety of sources (Preston *et al.*, 2000a; Christensen, 2001; Six *et al.*, 2002) to yield the following five fractions: light (<1 g cm<sup>-3</sup>) 250–2000 µm fraction; coarse sand (250–2000 µm); fine sand (53–250 µm); silt (2–53 µm); and clay (<2 µm). No standard method of physical fractionation exists in the literature, rather solutions of densities <1.6–2 g cm<sup>-3</sup> have been used to separate the light fraction (Wagai *et al.*, 2009) as has floatation in water (Preston *et al.*, 2000a; Quideau *et al.*, 2003). In an effort to minimize contamination from mineral particles and solvents or salts, water was chosen to separate the light fraction from the coarse sand-sized fraction. Specifically, fractionation of each sample began by adding 150 mL of distilled water to each of eight 100 g subsamples and placing these on a reciprocal shaker for 1 h. Samples were then wet sieved to isolate the sand-sized fractions, followed by density separation of the light fraction from the coarse sand (250–2000 µm fraction). Sedimentation was then used to separate the silt from the clay-sized particles. Clay-sized particles were flocculated with potassium chloride (KCl), with subsequent removal of KCl by dialysis tubing (Fisherbrand Nominal MWCO 12 000–14 000, Nepean, Canada) and freeze-dried using a FreeZone 6 Liter Dryer (Labconco Corporation, Kansas City, MO, USA). All >2 µm fractions were dried in a forced air oven (<40 °C). The fractions were weighed to calculate their relative distribution, as well as particle size distribution among sand (>53 µm), silt (2–53 µm), and clay (<2 µm) fractions. Texture in all samples was sandy (>95% sand) and average results are presented in Table 1. Average sample recovery was over 99%.

#### Laboratory analyses

Each horizon of the fully characterized pit at each site was analyzed for pH with an Ag/AgCl pH electrode, using a soil to 0.01 M calcium chloride solution ratio of 1:2, and a settling time of 30 min (Kalra & Maynard, 1991). Bulk soil samples from each pit as well as all five fractions were homogenized and finely ground using a Retsch MM200 ball mill grinder (Retsch Inc., Newtown, PA, USA). Samples were then analyzed for total carbon by dry combustion on a Costech ECS 4010 Elemental Analyzer equipped with a thermocouple detector (Costech Analytical Technologies Inc., Valencia, CA, USA). Some OC may be lost during the wet sieving step of the fractionation procedure as the sand fraction is repeatedly

washed with water. In order to separate sufficient material to conduct NMR analysis (>0.01 g of light fraction), up to 800 g of bulk soil and a large volume of water was required (often in excess of 15 L). This missing OC was quantified as a separate fraction, the water extractable organic carbon (WEOC), determined as the difference between bulk soil OC content and the sum of the OC content from fractionation ( $\text{kg m}^{-2}$ ). However, it is recognized that there may have been some losses in carbon content from fractionation due to the procedure used e.g., particulate matter adhering to vessel walls.

The five fractions obtained from each horizon at each pit, and the forest floor, were composited by horizon and site before carbon and nitrogen isotopic composition determination. The isotopic composition of these composite samples was measured on a Costech ECS 4010 Elemental Analyzer (Valencia, CA, USA) coupled to a Finnigan Deltaplus Advantage Isotopic Ratio Mass Spectrometer (ThermoFinnigan, Bremen, Germany). Results were expressed in the  $\delta$ -notation, the ‰ variation from the standard reference material, Pee Dee Belemnite (PDB) for carbon and atmospheric  $\text{N}_2$  for nitrogen.

Light fraction composite samples, with composite samples of the forest floor, were used for analysis by CPMAS  $^{13}\text{C}$  NMR. Preparation and analysis were identical to those described for similar jack pine forest floor and light fractions as reported in Norris *et al.* (2009). Individual samples of jack pine needles, jack pine roots and lichen collected from Site 2 were also analyzed by NMR. The spectra were acquired on a Chemagnetics CMX Infinity 200 [magnetic field ( $B_0$ ) = 4.70 T, Larmor frequency ( $\nu_L$  ( $^{13}\text{C}$ )) = 50.3 MHz] spectrometer with a 7.5 mm double-resonance MAS probe with high-power  $^1\text{H}$  decoupling (Varian, Ft. Collins, CO, USA). Samples were loaded into a 7.5 mm OD zirconium oxide rotor with Kel-F drive tips, and end caps and spacers made of Teflon (DuPont, Circleville, OH, USA). The magic angle was set to  $54.74^\circ$  by maximizing the  $^{79}\text{Br}$  NMR signal for spinning sidebands of KBr. Carbon-13 NMR spectra were referenced to tetramethylsilane (0 ppm) by setting the high-frequency adamantane peak to 38.56 ppm (Earl & VanderHart, 1982). A standard Hartmann–Hahn match CP and CW decoupling acquisition program was used (Bryce *et al.*, 2001) with a proton  $90^\circ$  pulse of 4.5  $\mu\text{s}$ . For each spectrum a total of 6000 transients were acquired, with 0.5 k data points for each FID, at a 5.0 kHz spinning rate, a 1 ms contact time, and a 2 s recycle time. The resulting free induction decay (FID) was processed with MNOVA'S MESTREC 5.2 software (Mestrelab Research SL, Santiago de Compostela, Spain). Processing the spectra included zero filling to 1 k, line broadening of 50 Hz with phase and baseline correction followed by integration of the spectra. In this study, we chose a conservative approach and divided the acquired spectra into regions based on broad carbon bonding types to allow for poor signal to noise ratios and resolution of peaks, though it is recognized that there may be some overlap of carbon types among regions. Thorough descriptions of peak identification and assignments published previously were used to classify the following four regions (Baldock *et al.*, 1997; Quideau *et al.*, 2000; Preston *et al.*, 2006): alkyl C (0–47 ppm), O-alkyl (47–112 ppm), aromatic and phenolic (112–165 ppm), and carbonyl (165–215 ppm).

It is recognized that CPMAS  $^{13}\text{C}$  NMR is a semiquantitative analytical technique. There are several instrumental and program parameters which may result in altering the spectrum e.g., spinning speed, contact time pulse, decoupling procedure (Bryce *et al.*, 2001; Conte *et al.*, 2004). Therefore peak areas reported may not be representative of absolute concentrations but rather are relative concentrations when different spectra are compared.

### Statistical analysis

Soil OC content for the forest floor and the total content of the sites were statistically compared for differences among sites. These data were normally distributed and were analyzed using a one-way analysis of variance (ANOVA) and a *post-hoc* test of Fisher's least significant difference (LSD) with an  $\alpha$  of 0.1. All analyses were run using SYSTAT 11 (Systat Software Inc., Chicago, IL, USA). In addition, differences among sites and horizons were investigated using the nonmetric multidimensional scaling (NMS) method followed by multiresponse permutation procedures (MRPP) with the PC-ORD software version 5 (MjM Software Design, Gleneden Beach, OR, USA). NMS uses numerous iterations to best organize the data within two or three dimensions based on distances between data points. A Sorenson (Bray-Curtis) distance measurement was used in the ordination. In addition to the *P*-value, MRPP results include the *T*-value, which indicates separation among groups (with larger values for stronger separation), and the *A*-value, which indicates within group homogeneity compared with random expectation. The first ordination investigated patterns in C/N and isotopic ratios ( $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ ) for the light fractions, coarse sand, fine sand, silt and clay-sized fractions among sites and horizons, while a second ordination based on the NMR integral areas and  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  isotopic ratios was performed to examine the relationships among the forest floor and light fractions of the different sites and horizons.

## Results

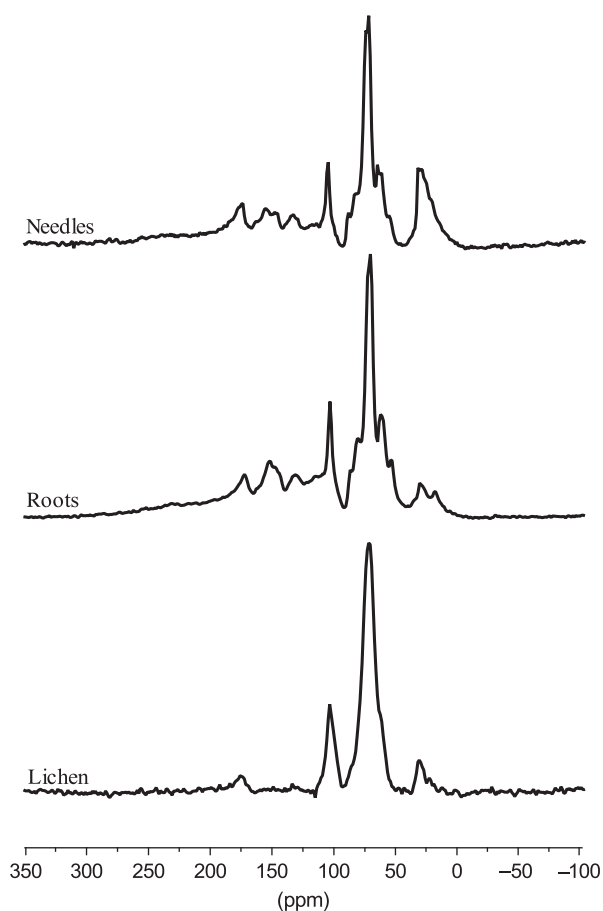
### Litter inputs to jack pine soils

To determine how litter inputs potentially influenced the light fraction and other soil fractions, the composition of jack pine needles, jack pine roots and lichen was analyzed (Fig. 1 and Table 2). CPMAS  $^{13}\text{C}$  NMR spectra were dominated by peaks in the O-alkyl C region (Fig. 1). This region, with a sharp peak at 73 ppm, was especially evident on the lichen spectrum, where it accounted for 89% of the total spectral intensity (Table 2). The remaining area of this spectrum was comprised of carbonyl or alkyl C with minimal aromatic C. In addition to a characteristically high O-alkyl signal, lichen also had the highest  $\delta^{13}\text{C}$  (–25.5‰) and C/N (101) values of the three litter types. In comparison, needles had the most negative  $\delta^{13}\text{C}$  (–27.8‰) and lowest C/N (45) values,

and a relatively important signal in the alkyl C region. Finally, for the roots, results from the NMR analysis underlined the importance of aromatic compounds, because the aromatic region made up almost a quarter of the total spectral intensity.

#### Soil OC stocks and distribution among fractions

Results from the ANOVA analysis ( $df = 3$ ,  $F = 6.887$ ,  $P = 0.006$ ) indicated significant differences in forest



**Fig. 1** Representative CPMAS  $^{13}\text{C}$  NMR spectra of jack pine needles, jack pine roots and lichen. CPMAS, cross-polarization magic-angle spinning; NMR, nuclear magnetic resonance.

**Table 2**  $^{13}\text{C}$  Isotopic composition, C/N ratios, and percent integration areas for CPMAS  $^{13}\text{C}$  NMR spectra for jack pine needles, jack pine roots, and lichen (*Cladonia* sp.)

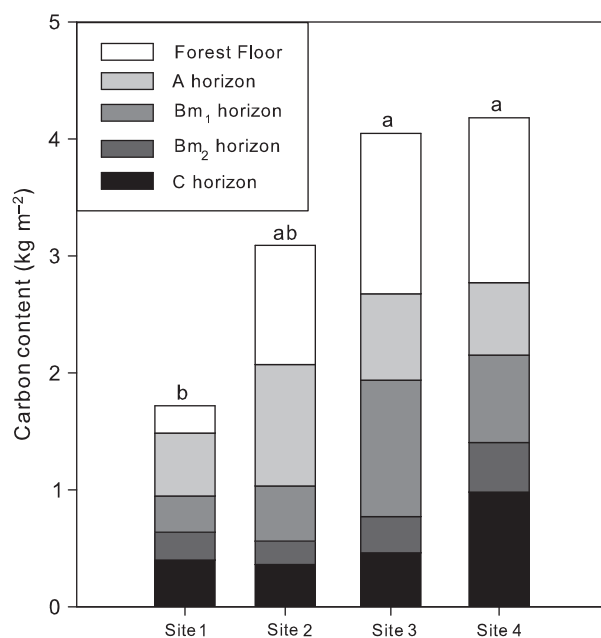
Sample	C/N	$\delta^{13}\text{C}$ (‰)	Carbonyl	Aromatic	O-alkyl	Alkyl	Alkyl/O-alkyl
Needles	44.8	-27.76	13	19	52	17	0.33
Roots	92.6	-26.8	12	24	55	8	0.15
Lichen	100.8	-25.49	4	1	89	6	0.06

Errors in integrated areas are estimated to be <10%.

CPMAS, cross-polarization magic-angle spinning; NMR, nuclear magnetic resonance.

floor OC content among sites. Specifically, forest floor OC content at the southern site, Site 1 ( $0.2 \text{ kg m}^{-2}$ ) was significantly less than at the other three sites. Forest floor OC content then tended to increase from south to north with the largest quantity present at Site 4 with  $1.4 \text{ kg m}^{-2}$  (Fig. 2), although there was no significant difference among Sites 2, 3, and 4. Statistical differences in OC content among sites were detected within the entire soil profiles (forest floor and 0–1 m of mineral soil) as indicated from the ANOVA analysis ( $df = 3$ ,  $F = 9.105$ ,  $P = 0.002$ ). Site 1, at  $1.72 \text{ kg m}^{-2}$ , contained significantly less total soil OC content, while soil profiles from Sites 2, 3, and 4 were not statistically different but, similar to the forest floor layers, showed a trend of increasing OC along the jack pine transect with increasing latitude (Fig. 2). The C horizon at Site 4 was striking in that it held more than double the OC content than that of the C horizons present at the other sites.

Results from the fractionation exercise showed that little soil OC was contained in the silt or clay-sized fractions (Fig. 3). The other four fractions accounted for 57–85% of the total soil OC stocks. Light fractions stored 9–23% of the total OC in the A horizons, and their contribution decreased with depth to 3% or less in the C horizons. In the A horizons, the coarse sand-sized fraction held almost half of the total OC at the southern sites, but <34% of the total OC at the northernmost site (Site 4). On the other hand, the WEOC pool increased with increasing latitude along the transect (Fig. 3). When estimated over the entire soil profiles (0–1 m), WEOC accounted for little OC at Site 1 ( $0.1 \text{ kg m}^{-2}$ ), but increased to  $0.24\text{--}0.29 \text{ kg m}^{-2}$  at Sites 2 and 3, and reached a maximum of  $0.87 \text{ kg m}^{-2}$  at Site 4. Differences in WEOC among sites were particularly evident in the deeper  $\text{Bm}_2$  and C horizons. For the northernmost site, the contribution of WEOC to total OC increased with increasing depth within the soil profile, and reached >40% in the  $\text{Bm}_2$  and C horizons. In contrast, for the two southern sites the clay and silt separates showed a proportional increase with increasing depth. Namely, for Sites 1 + 2, this pool increased from <20% in the A horizons to 30% or more deeper in the profiles.



**Fig. 2** Soil organic carbon content ( $\text{kg m}^{-2}$ ) in jack pine stands along the Boreal Forest Transect Case Study from Site 1 in the southwest to Site 4 in the northeast. Different letters indicate statistical differences in total carbon content among sites with  $\alpha = 0.10$ .

#### Composition of soil OC fractions

The largest C/N ratios were observed in the light fractions, particularly the light fractions of the B and C horizons (Fig. 4). In addition, ratios for the light fractions were larger than those of the forest floor samples, and increased with depth in the soil profiles. This pattern was most pronounced at Site 4, where C/N values increased from 44 in the A horizons to 60 in the C horizons. In comparison, the C/N ratios for both the silt and clay fractions were relatively small: they were  $<30$  in the A horizons and smaller than those of the forest floor samples, and further decreased with increasing depth to below 14 in the C horizons. While the rate of decrease in ratio for the fine fractions was similar across Sites 1–3, the spread of the values was smaller for the northernmost Site 4. Specifically, for Sites 1–3, the decrease in C/N values between the A and C horizons was in the range of 2–7 for the clay and 7–15 for the silt fractions. These decreases were smaller at Site 4,  $<2$  for the clay and  $<3$  for the silt. Finally, the sand fractions did not display any consistent trend as they showed no difference in spread of results among horizons of different sites or in the ratios themselves other than a general decrease with depth (data not shown).

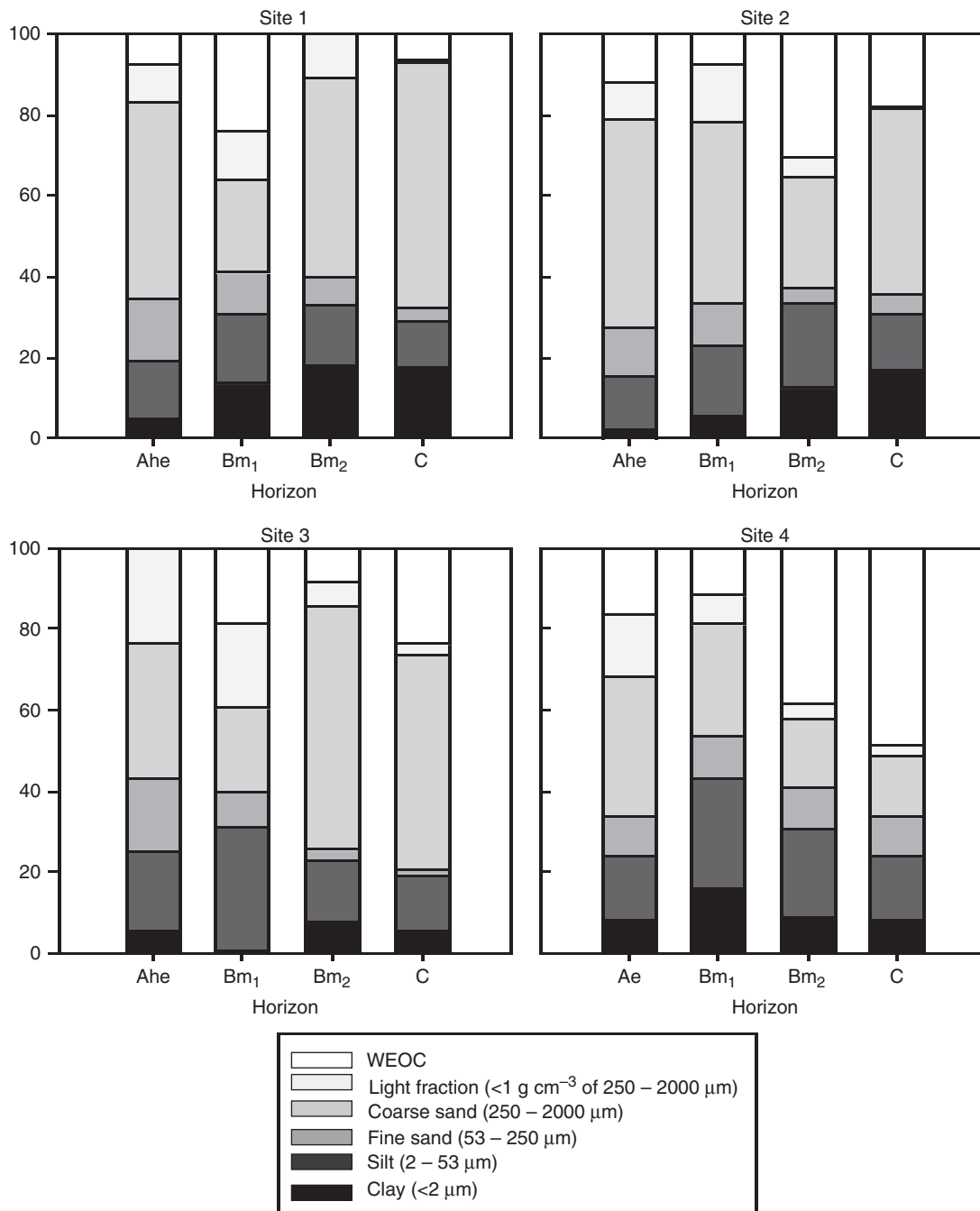
The isotopic ratios of  $^{13}\text{C}$  to  $^{12}\text{C}$  (‰) ranged from  $-28.3\text{‰}$  in the light fraction of the Site 1 C horizon to

$-21.9\text{‰}$  for the clay fraction of the Bm<sub>1</sub> horizon at Site 2 (Fig. 5). Within a given horizon, ratios were always smallest for the light fractions, followed by the silt, and then the clay fractions. Values for the fine and coarse sand separates were also enriched in the heavier isotope compared with the light fractions, but did not show any consistent trend compared with the fine ( $<53\ \mu\text{m}$ ) fractions (data not shown). A noticeable feature for the fine fractions was the steady progression of enrichment from the A to the C horizons, one that was larger at the southern end of the transect (Fig. 5). For instance, differences in silt  $\delta^{13}\text{C}$  values between these two horizons decreased along the transect from 2.8 (Site 1) to 1.0 (Site 4). On the other hand, the light fractions only showed minimal variation with increasing depth.

Results from the ordination including data on the composition of the soil OC fractions, including the isotopic ( $\delta^{13}\text{C}$ ,  $\delta^{15}\text{N}$ ) and C/N ratios of the light fractions, coarse sand, fine sand, silt- and clay-sized fractions yielded a two-dimensional solution with a final solution stress of 5.21 after 54 iterations (Fig. 6). The MRPP analysis further indicated three statistically distinct groupings ( $P = 10^{-4}$ ,  $T = -5.93$ ,  $A = 0.35$ ) corresponding to separation both by horizon and site along the climosequence. Specifically, the grouping including the A horizons from the four sites was statistically different from the B and C horizons at the two southern sites ( $P = 0.006$ ,  $T = -4.06$ ,  $A = 0.29$ ), and even more different from the B and C horizons at the northern sites ( $P = 0.002$ ,  $T = -5.14$ ,  $A = 0.38$ ). Differences were not as strong between the B and C horizon fractions of the southern sites and the northern sites ( $P = 0.01$ ,  $T = -3.10$ ,  $A = 0.14$ ). Correlation analysis showed that the A horizons were more strongly associated with the C/N ratios of the sand-sized fractions, while the northern sites were more strongly associated with the  $\delta^{13}\text{C}$  signature of these fractions.

#### NMR analysis of forest floor and light fraction OC

All of the CPMAS  $^{13}\text{C}$  NMR spectra contained six main peaks (Fig. 7) and were typically dominated by the O-alkyl C region with a relatively sharp peak at approximately 73 ppm assigned to the C2, C3, and C5 carbons of cellulose (Baldock & Preston, 1995). The O-alkyl region was greatest for the forest floor and least for the A horizon samples (Table 3). Within the O-alkyl C region, a strong peak assigned to the C1 carbon of cellulose and hemicellulose was apparent on all spectra at 105 ppm (Kögel-Knabner, 1997; Vane *et al.*, 2006), although the peak did merge with the aromatic area for the A and Bm<sub>2</sub> horizons of Site 2 and the A horizon of Site 3.

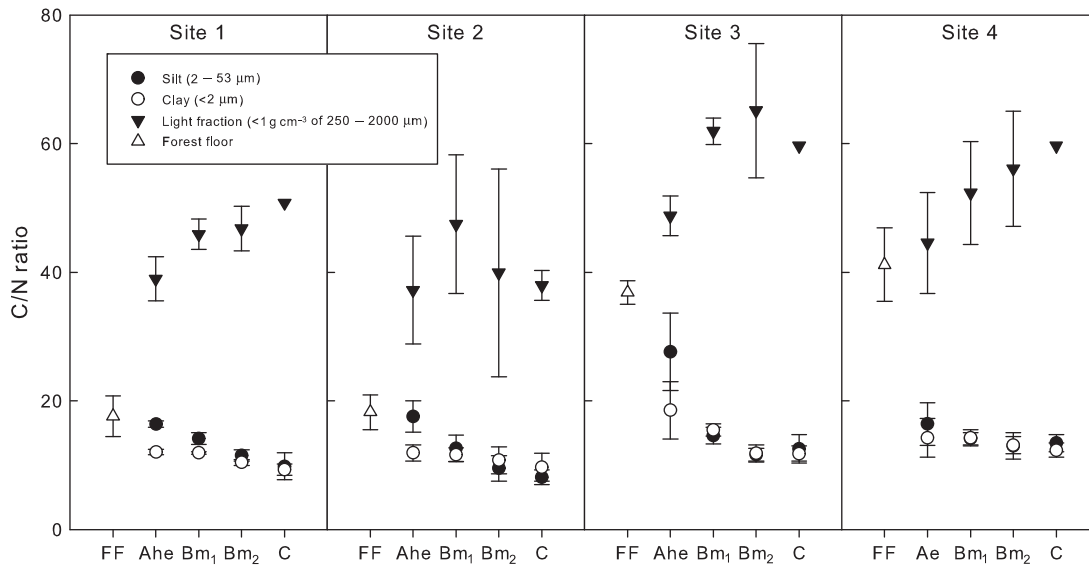


**Fig. 3** Organic C distribution (% of total soil carbon) in fractions obtained from the A (Ahe or Ae), Bm<sub>1</sub>, Bm<sub>2</sub>, and C horizons in jack pine stands along the Boreal Forest Transect Case Study.

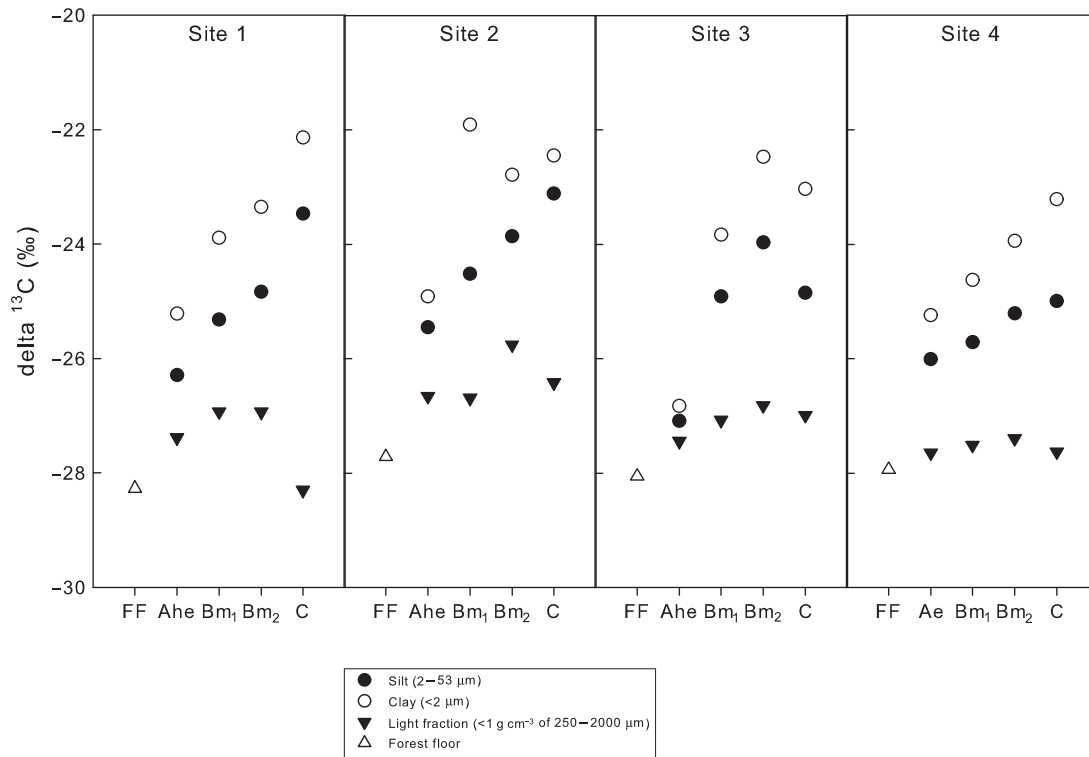
Alkyl C, at 30 ppm (Baldock & Preston, 1995), presented the second highest peak across all spectra, except for the A horizon of Site 3 where it showed the most intense peak (Fig. 7b). Other than for the A horizon at Site 3, the greatest integral area for the alkyl region was for the forest floor (Table 3). The area of peaks in the alkyl region decreased with increasing depth through the mineral soil horizons. There was little evidence of

peak splitting for methyl and ethyl carbons from the long chain alkyl C compounds (Vane *et al.*, 2006) apart from those for the northern sites in the B and C horizons.

The intensity of the aromatic region showed the greatest variation among the four regions (Fig. 7). It was least in the forest floor and fluctuated through the mineral soil horizons. Light fractions of the A and Bm<sub>2</sub>



**Fig. 4** C/N ratios of the forest floor (FF) and the silt, clay, and light fractions obtained from the A (Ahe or Ae), Bm<sub>1</sub>, Bm<sub>2</sub>, and C horizons in jack pine stands along the Boreal Forest Transect Case Study. Error bars represent 1 SD (*n* = 4).

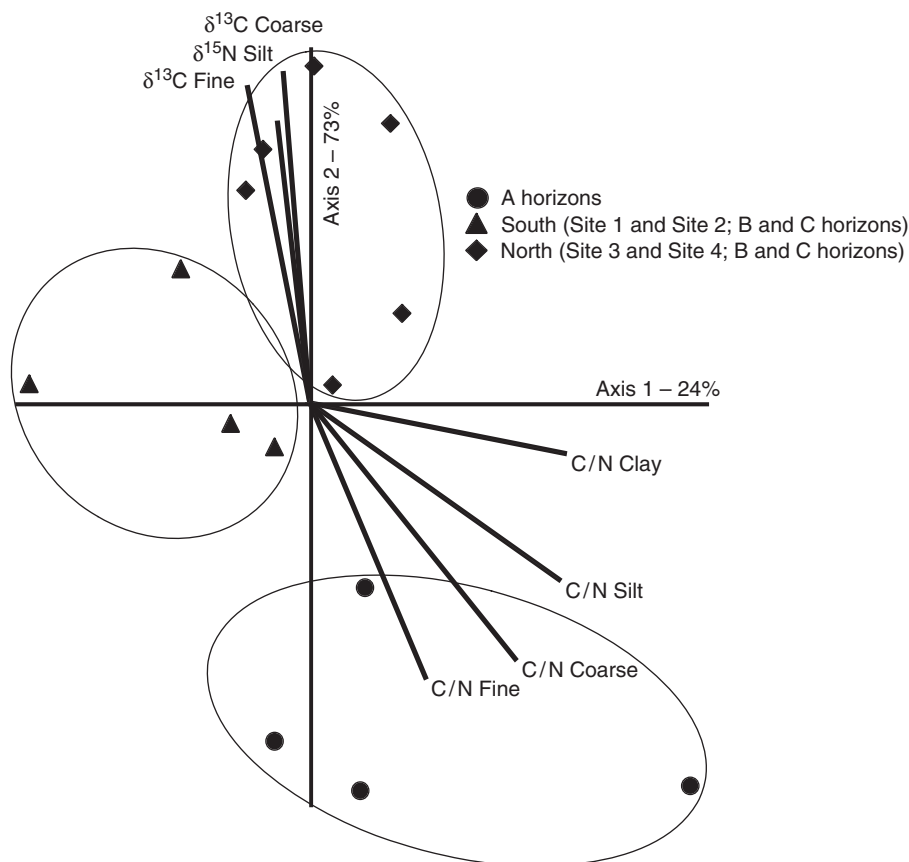


**Fig. 5** Carbon isotopic composition ( $\delta^{13}\text{C}$ ) of the forest floor (FF) and the silt, clay and light fractions obtained from the A (Ahe or Ae), Bm<sub>1</sub>, Bm<sub>2</sub>, and C horizons in jack pine stands along the Boreal Forest Transect Case Study from Site 1 in the southwest to Site 4 in the northeast.

horizons at Site 2 and of the A horizon at Site 3 were unusual among samples as the aromatic region was the largest of the four integral areas (Fig. 7, Table 3). Two defined peaks in the spectra were around 130 and 150 ppm. Lignin, from monomeric units in gymnosperms, can give rise to a peak at 131 ppm and at 148

and 151 ppm (Kögel-Knabner, 2002; Vane *et al.*, 2006). The peak at 130 ppm may also be attributed to char or black C (Preston & Schmidt, 2006). Some peak splitting was evident in the 150 ppm region of the A horizon at Site 4. Otherwise, the aromatic region was generally represented by two broad peak shapes.





**Fig. 6** NMS ordination of C/N,  $\delta^{13}\text{C}$ , and  $\delta^{15}\text{N}$  values of mineral fractions (coarse sand, fine sand, silt and clay fractions) and light fractions from four sites along the Boreal Forest Transect Case Study delineated by horizon and by location. Grouping based upon MRPP analysis is highlighted with circles. Cut off for correlation vectors was set at  $r^2 = 0.7$ . MRPP, multiresponse permutation procedures; NMS, nonmetric multidimensional scaling.

The carbonyl C region showed a sharp peak throughout all spectra, which may have derived from hemicellulose (Vane *et al.*, 2006) or cutin (Preston *et al.*, 2000b) carboxyl, amide, or ester C. The proportion of carbonyl C (% total spectral area) increased from the forest floor to the A horizons for all sites, and then stayed relatively constant within the mineral soil profiles. Finally, the alkyl/O-alkyl ratio was calculated as an index of decomposition for the forest floor and light fractions (Table 3). For all sites the greatest alkyl/O-alkyl ratio was found in either the forest floor or in the A horizon. In the deeper mineral soil horizons (i.e., Bm<sub>1</sub>, Bm<sub>2</sub>, and C), ratios varied from 0.27 to 0.43 with no apparent trend with increasing soil depth.

The ordination integrating the NMR,  $\delta^{13}\text{C}$ , and  $\delta^{15}\text{N}$  data for the forest floor and light fractions yielded a two-dimensional solution with a final stress of 5.41 after 90 iterations (Fig. 8). Four groups related to horizon and site patterns were evident from this ordination and found to be statistically different with the MRPP analysis ( $P = 0.00006$ ,  $T = -6.164$ ,  $A = 0.394$ ). Specifically,

the grouping including the forest floor horizons from the four sites was statistically different from the A horizons ( $P = 0.006$ ,  $T = -4.35$ ,  $A = 0.52$ ), the B and C horizons at the southern sites ( $P = 0.006$ ,  $T = -4.29$ ,  $A = 0.49$ ), and especially the B and C horizons at the northern sites ( $P = 0.004$ ,  $T = -4.48$ ,  $A = 0.33$ ). In addition, the A horizon group was statistically different from the B and C horizons at the northern sites ( $P = 0.003$ ,  $T = -4.716$ ,  $A = 0.257$ ). Correlation analysis showed that the forest floor samples as well as the light fractions from the A horizons at the northern sites were more strongly associated with the O-alkyl C and the  $\delta^{13}\text{C}$  signature, while the light fractions from the A horizons and the B and C horizons at the southern sites were related to higher carbonyl and aromatic C intensities.

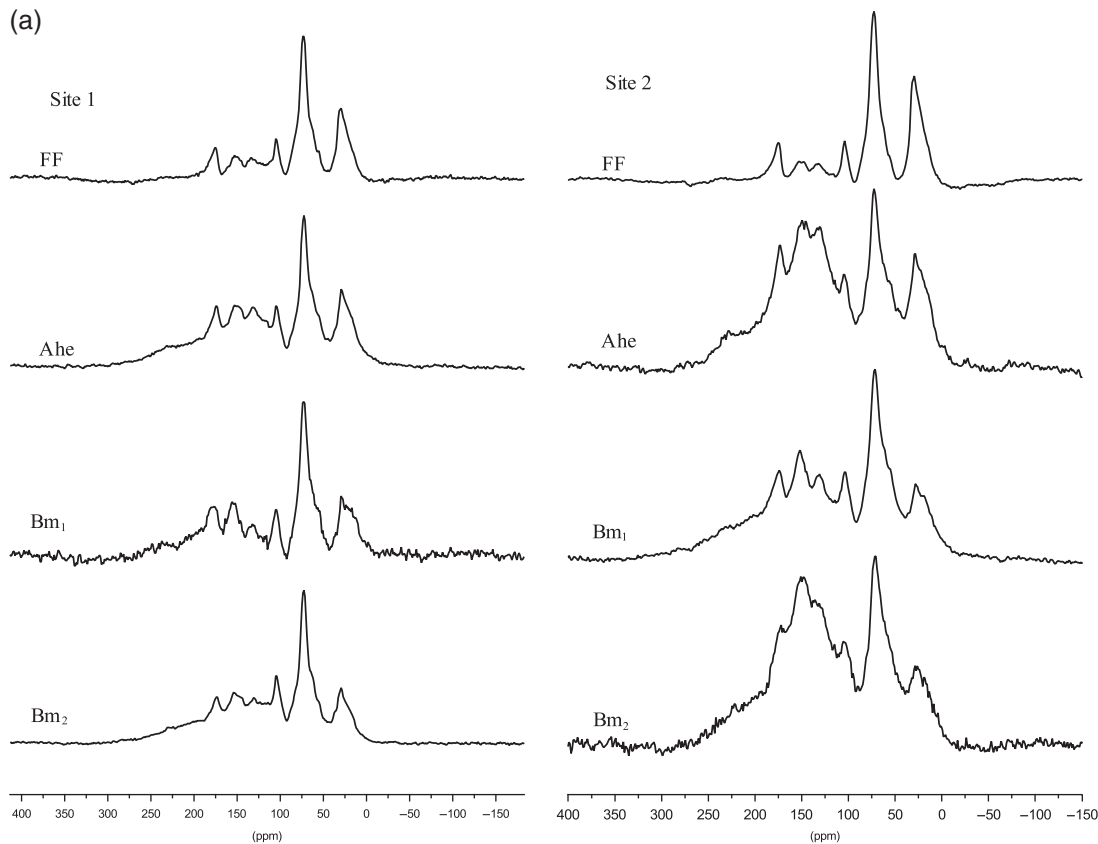
## Discussion

The soil OC content increased as MAT decreased and the MAP increased along the transect from Sites 1 to 4

(Fig. 2). Increased OC stocks, or decreased carbon mineralization, along transects of decreasing MAT and increasing MAP in the boreal forest have been noted previously (Halliwell & Apps, 1997; Preston *et al.*, 2000a; Kane *et al.*, 2005; Meyer *et al.*, 2006). Forest floor contents at Site 2 ( $1.0 \text{ kg m}^{-2}$ ) and Site 4 ( $1.4 \text{ kg m}^{-2}$ ) were similar to those reported for other jack pine stands in the region (Nalder & Wein, 1999). In a similar fashion, the soil OC content, including the forest floor, at Site 4 ( $4.2 \text{ kg m}^{-2}$ ) and Site 2 ( $3.1 \text{ kg m}^{-2}$ ) were comparable to values reported previously (Gower *et al.*, 1997; Trumbore & Harden, 1997; Howard *et al.*, 2004). Above-ground biomass carbon (including tree biomass, coarse woody debris, snags and the forest floor) for jack pine stands varied across the BFTCS from about  $6.5 \text{ kg m}^{-2}$  at Site 2 to  $3.5 \text{ kg m}^{-2}$  at Site 4 (Preston *et al.*, 2006). With the decreased aboveground biomass carbon at the northern sites, the increase in soil OC with decreasing MAT that we observed in our study hence was unlikely caused by increased organic matter input but rather by a decreased decomposition rate.

Needle littering has been estimated to average  $80 \text{ g m}^{-2} \text{ yr}^{-1}$  at Site 2 (Preston *et al.*, 2006), and lichen

and black carbon have been discussed previously as likely OC inputs to the soil at the same site (Norris *et al.*, 2009). In sandy soils with no evidence of bioturbation, root matter may represent an additional important component of soil organic matter (Nierop, 1998). Root biomass was measured at Sites 2 and 4 of the BFTCS, and roots were found to concentrate in the upper 10 cm of soil (Steele *et al.*, 1997). As low temperatures favor the production of below ground biomass (Oades, 1988), significantly more fine root biomass ( $<2 \text{ mm}$ ) was noted at Site 4 than at Site 2 (Steele *et al.*, 1997). At Site 2, 2%, or  $0.16 \text{ kg m}^{-2}$ , of the total OC biomass was comprised of fine root biomass (Kalyn & Van Rees, 2006). This is comparable to the sum of the light fractions ( $0.178 \text{ kg m}^{-2}$ ) that was measured for Site 2 in our study. In addition to a similar mass for the light fraction and roots at Site 2, the macromolecular chemistry, as seen by NMR, suggests that the light fraction from the B and C horizons results from root inputs rather than needles (Fig. 7). Specifically, NMR spectra from the B and C horizons contained relatively little alkyl C ( $\leq 18\%$ ) compared with the A and forest floor spectra, which would correspond to the low alkyl C present in



**Fig. 7** Representative CPMAS  $^{13}\text{C}$  NMR spectra of forest floor (FF) samples and light fractions from the A, Bm<sub>1</sub>, Bm<sub>2</sub>, and C horizons in jack pine stands along the Boreal Forest Transect Case Study. (a) Sites 1 and 2. (b) Sites 3 and 4. CPMAS, cross-polarization magic-angle spinning; NMR, nuclear magnetic resonance.

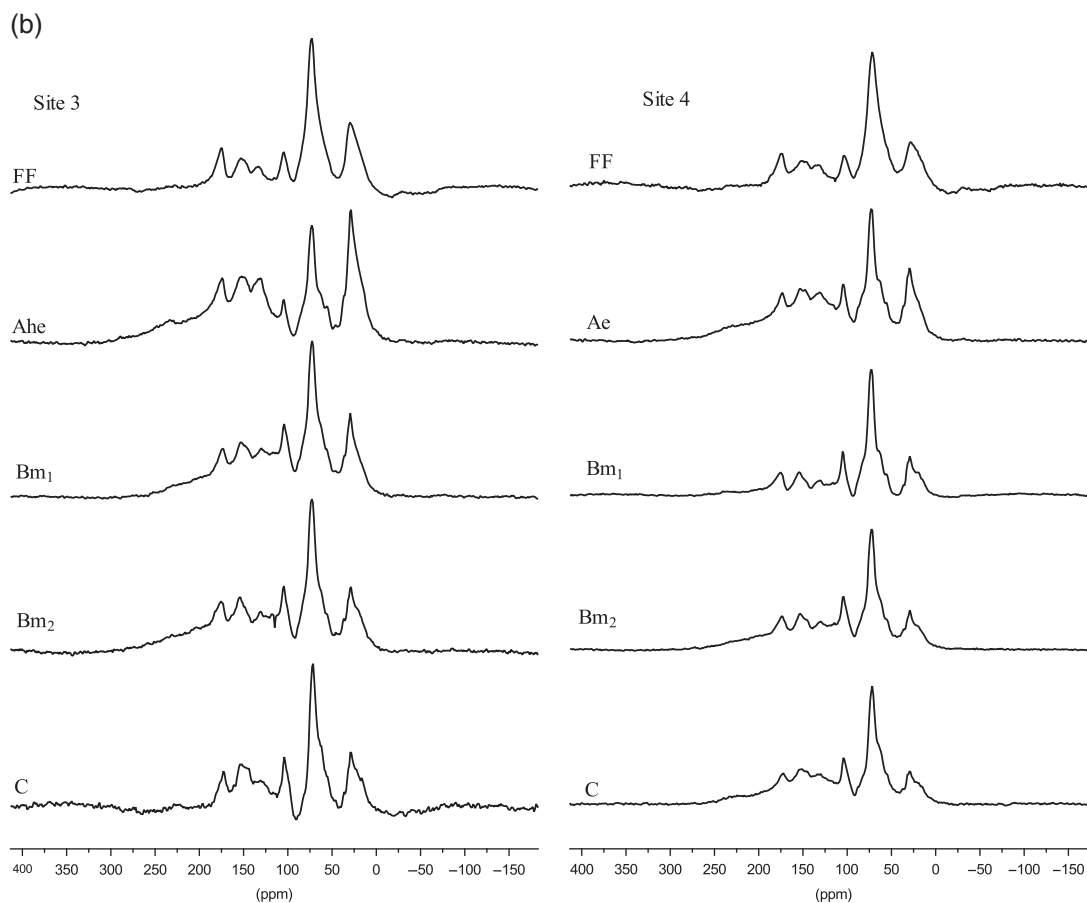


Fig. 7 Continued.

roots (8%). In addition to the NMR results, the  $\delta^{13}\text{C}$  values of the light fractions were indicative of their undecomposed nature as they stayed similar to the  $\delta^{13}\text{C}$  values of carbon inputs to the soils (Table 2), ranging from  $-28.3\text{‰}$  to  $-25.8\text{‰}$ , and showed little variation, even at depth (Fig. 5). This was in contrast to the clear increase in the C/N ratios with increasing depth (Fig. 4), which may further indicate root inputs at depth as the roots have a characteristically higher C/N than needles (Table 2). Therefore the source of the OC for the B and C horizons appeared to be due to jack pine roots, while that for the forest floor derived mostly from aboveground biomass including jack pine needles and lichen.

While OC turnover rates were not measured across the transect, the fractionation scheme used was devised in order to isolate pools of OC that have been identified as either labile or stabilized, either by chemical, biochemical, or physical means (Christensen, 2001; Six *et al.*, 2002). In addition, chemical analysis of the five fractions in our study agrees with previous works on similar size fractions, i.e.; an observed decrease in C/N and an increase in  $\delta^{13}\text{C}$  with decreasing particle size (Quideau *et al.*, 2000; von Lützow *et al.*, 2007). Therefore,

it is reasonable to classify our isolated fractions as either labile or stabilized, with the stabilized fractions including the chemically stabilized silt and biochemically stabilized clay size separates, as well as the microaggregate (physically) stabilized OC that is recovered with the fine sand-sized fraction (Six *et al.*, 2002). In our study, for the majority of the horizons, <50% of the OC content was recovered in stabilized pools (Fig. 3). Instead, the majority of soil OC was associated with either the labile coarse sand size separates, the light fraction or the WEOC fraction.

With increasing latitude along the transect, not only did the forest floor content increase but the OC content in the B and C soil horizons also tended to increase (Fig. 2). In other words, a greater proportion of the total soil OC stocks (0–1 m) was increasingly being held within the B and C horizons. Results from our fractionation procedure indicated that the greatest contribution of the WEOC fraction to bulk soil OC was for the C horizon at Site 4 (Fig. 3), suggesting that dissolved organic carbon (DOC) influx may play an important role in the observed increase in deep mineral soil OC content at this site. In a similar fashion, the contribution of leached organic matter was believed to account for part of the

**Table 3** Forest floor and light fraction CPMAS  $^{13}\text{C}$  NMR spectra percent integration areas for a jack pine forest soil transect

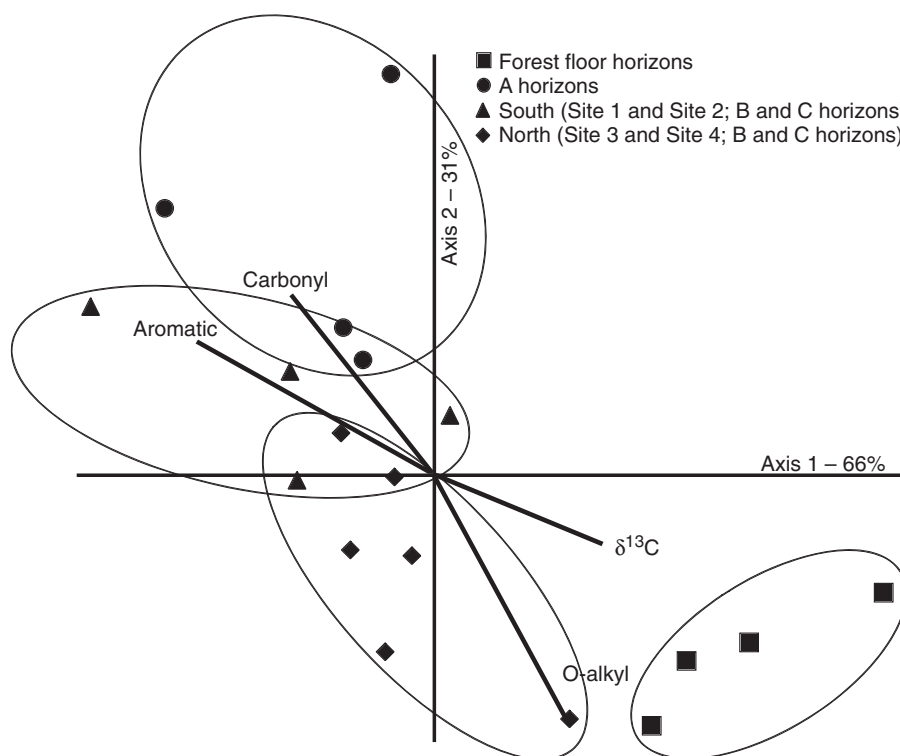
Site	Horizon	Carbonyl	Aromatic	O-alkyl	Alkyl/ O-alkyl	
					Alkyl	O-alkyl
Site 1	FF	8	15	52	24	0.47
	Ahe	17	27	38	19	0.50
	Bm <sub>1</sub>	18	20	43	18	0.43
	Bm <sub>2</sub>	18	24	44	14	0.32
Site 2	FF	9	10	50	31	0.61
	Ahe	18	34	31	17	0.55
	Bm <sub>1</sub>	18	27	39	15	0.39
	Bm <sub>2</sub>	17	36	34	13	0.39
Site 3	FF	10	15	53	22	0.42
	Ahe	19	28	27	26	0.96
	Bm <sub>1</sub>	14	25	44	17	0.40
	Bm <sub>2</sub>	18	24	42	16	0.39
	C	7	28	48	17	0.36
Site 4	FF	9	16	56	19	0.35
	Ahe	16	27	39	18	0.45
	Bm <sub>1</sub>	11	17	56	15	0.27
	Bm <sub>2</sub>	15	23	48	14	0.30
	C	14	25	47	13	0.29

Errors in integrated areas are estimated to be <10%. CPMAS, cross-polarization magic-angle spinning; NMR, nuclear magnetic resonance.

short turnover times of OC measured at depth in well drained jack pine stands near Site 4 (Trumbore & Harden, 1997). Dissolved OC may derive from fresh plant tissues as well as decomposing soil organic matter (Moore, 2003), and several studies have determined that DOC sorption occurs most strongly in the deeper mineral horizons of sandy soils (Guggenberger & Kaiser, 2003; Moore, 2003). Hence, the observed increase in deep mineral soil OC content at Site 4 might have been due to a slightly different parent material with a greater sorption ability, less favorable conditions for DOC decomposition resulting in an increased influx, or a combination of both.

Two trends were evident across the fine fractions: one with depth, and one along the climosequence (Fig. 6). Specifically, the fine fractions showed a decrease in C/N ratios and an increase in  $\delta^{13}\text{C}$  values with depth (Figs 4 and 5), indicating that the OC associated with the silt and clay fractions was more decomposed and therefore possibly more stabilized with increasing depth (Bol *et al.*, 1999; Kramer *et al.*, 2003; Quideau *et al.*, 2003). Secondly, this rate of change in C/N and  $\delta^{13}\text{C}$  with depth was greater in the south than in the two northern sites. Isotopic results hence provide further evidence, along with the C/N ratios, that the OC of the southern sites undergoes more decomposition than in the north.

The forest floor NMR spectra were low in both aromatic and carbonyl C but high in O-alkyl intensity, in agreement with lichen and needle litter inputs, while the A horizon spectra showed a larger aromatic region and almost a doubling in the carbonyl region (Tables 2 and 3). NMR spectra of jack pine needles and lichen (Fig. 1) were similar to those reported previously while the forest floor of Site 2 (Fig. 7a) showed an increase in alkyl carbon compared with a spectrum published previously (Preston *et al.*, 2006). Except where black carbon was present (Site 2 A and Bm<sub>2</sub> horizons, and Site 3 A horizon), the spectra were dominated by the O-alkyl carbon region, as is typically seen on soil organic matter NMR spectra (Mahieu *et al.*, 1999). Contrary to previous reports of increasing alkyl/O-alkyl ratio with increasing depth in soil profiles (Baldock *et al.*, 1997; Quideau *et al.*, 2000), calculated ratios in our study did not show any consistent variation with depth. Ratios were greater in either the forest floor (Site 2 soils) or the A horizon (Sites 1, 3, and 4 soils). Two proposed theories for the accumulation of alkyl C and therefore an increased alkyl/O-alkyl ratio include: (1) the hydrophobic properties of alkyl C (Baldock *et al.*, 2004) where the microbial breakdown of organic matter increases alkyl C by selective preservation compared with the preferential decomposition of more labile moieties such as carbohydrates, or 2. byproduct generation and microbial synthesis of alkyl compounds (Christensen, 2001). Both the selective preservation (Nierop *et al.*, 2001) and the microbial synthetic activities (Kögel-Knabner *et al.*, 1992) were shown to result in an accumulation of alkyl C with increasing soil depth. Yet data from our study indicated that there was neither preservation nor increase in alkyl carbon content. Instead, results from the NMS indicated that differences among sites and horizons were linked to differences in O-alkyl, aromatic, and carbonyl C (Fig. 8). Namely, a greater O-alkyl C intensity was associated with the forest floor, while aromatic and carbonyl C intensities were positively correlated with the A horizon. This may indicate that the A horizon OC was of a more decomposed form than the forest floor. Differences were also observed between the A horizons and the B and C horizons of the northern sites, further indicating that the A horizon on average was more decomposed than the light fractions of the deep horizons at the northern sites. This same separation based on horizons and sites was evident in the NMS based on the mineral soil fractions (Fig. 6). Taken together, results strongly indicate that the organic matter contained in the A horizons was different from all other horizons, including the forest floor layers, and that the OC in the deeper B and C horizons was differentiated primarily based on location along the climosequence i.e., the southern vs. northern sites. Previous work in the boreal



**Fig. 8** NMS ordination of CPMAS  $^{13}\text{C}$  NMR integral areas,  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  from the forest floor and the light fractions from four sites along the Boreal Forest Transect Case Study delineated by horizon and by location. Grouping based upon MRPP analysis is highlighted with circles. Cut off for correlation vectors was set at  $r^2 = 0.7$ . CPMAS, cross-polarization magic-angle spinning; NMR, nuclear magnetic resonance; MRPP, multiresponse permutation procedures; NMS, nonmetric multidimensional scaling.

forest showed that soil OC becomes less decomposed and more labile as latitude increases, but these studies focused on forest floor layers and surface mineral horizons (Vucetich *et al.*, 2000; Sjögersten *et al.*, 2003; Kane *et al.*, 2005; Hilli *et al.*, 2008). The present study demonstrates that this relationship is also valid at depth, as the OC present in the B and C horizons at the northern sites was less decomposed, i.e., less altered than that at the southern sites. This further confirms that the OC of the northern boreal forest is of a more labile form.

### Conclusions

Soil OC content was statistically less at Site 1, the most southerly site, and content increased going northeast with cooler and moister conditions. These results are in agreement with previous studies on the same transect and in agreement with many other transect studies. Although the OC content was greatest in the northern sites, from the chemical data, it is also evident that the OC present was of a less decomposed and more labile form than within the southern sites. The total stocks and nature of OC in these jack pine soils therefore appear to

be dependent on climatic factors, with the OC likely more undecomposed i.e., labile at the northern sites. Therefore if the projected  $5^\circ\text{C}$  warming of the northern latitudes does occur, then these data suggest that the northern soils will become a larger source of  $\text{CO}_2$  than the more southerly ones.

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