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Chemical Indicators of Cryoturbation and Microbial Processing throughout an Alaskan Permafrost Soil Depth Profile

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Although permafrost soils contain vast stores of organic C, relatively little is known about the chemical composition of their constituent soil organic matter (SOM). Mineral permafrost and organic (OAL) and mineral active layer (MAL) soils from Sagwon Hills, AK were analyzed for total C and N content and SOM chemical composition using Fourier transformed mid-infrared spectroscopy (MidIR). We also investigated techniques for proper collection of MidIR spectra on high C soils, such as permafrost. Principal Components Analysis (PCA) of the MidIR spectra revealed that the OAL was different from the MAL and permafrost based on absorbance of various organic functional groups, such as hydroxyls, alkyls, carbonyls, amines, amides, and esters. The top of the permafrost (0–15 cm below the maximum active layer thaw depth) was also differentiated from the deeper permafrost (16–40 cm below) by the same organic functional groups. Spectral data suggested that there is more chemically labile C (e.g., hydroxyl, amine groups, carbohydrates) in the OAL than the top of the permafrost, which in turn has more labile C than the MAL and deeper permafrost. The chemical similarity between the top of the permafrost and the OAL, and its differences with the MAL, suggest that organic matter (OM) is introduced into the permafrost through cryoturbation. All the soils showed evidence of microbial processing, such as organic acids and carboxylates, however the relative abundance of these compounds varied by soil depth. This study advances our understanding of permafrost C chemistry and the reactivity of constituent compounds.

Abbreviations: DOC, dissolved organic carbon; MAL, mineral active layer; MidIR, Fourier transformed mid-infrared spectroscopy; NMR, nuclear magnetic resonance; OAL, organic active layer; OM, organic matter; PCA, Principal Component Analysis; SOM, soil organic matter; TDN, total dissolved nitrogen.

Sixteen percent of the terrestrial northern hemisphere is underlain by permafrost (Kuhry et al., 2009), and permafrost-affected soils contain four times more C than global vegetation and twice as much as the atmosphere (Schuur et al., 2008; Kuhry et al., 2009; Tarnocai et al., 2009; Lee et al., 2012). Permafrost temperatures are rising (Osterkamp and Romanovsky, 1999; Hinzman et al., 2005; Romanovsky et al., 2012), and permafrost degradation and thaw have been observed

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(Osterkamp and Romanovsky, 1999; ACIA, 2004). As permafrost thaws, the previously frozen C may be decomposed and released to the atmosphere as carbon dioxide (CO_2) and methane (CH_4).

The vulnerability of SOM to decomposition is dependent on complex interactions between the physical, chemical, and biological components of the soil ecosystem (Schmidt et al., 2011). Soil organic matter can be protected from degradation by multiple mechanisms, including a molecule's inherent recalcitrance. Degradation of certain classes of compounds yields more net energy than others. In addition, the structural complexity of organic compounds affects the temperature sensitivity of their decomposition (Davidson and Janssens, 2006; Conant et al., 2008). Microbial taxa specialize on metabolizing specific substrates (Wallenstein et al., 2007; Jones et al., 2009; Goldfarb et al., 2011), so it is important that we know not only how much SOM is present, but its molecular constituents. Thus, a characterization of the OM in permafrost will deepen our understanding of this unique, but threatened, system, and can advance our ability to predict the vulnerability of C in permafrost to decomposition following thaw (White et al., 2002; Andersen and White, 2006).

Organic matter in arctic soils can be relatively non-decomposed due to frozen and waterlogged conditions (Hobbie et al., 2000; White et al., 2004; Sannel and Kuhry, 2009; Xu et al., 2009; Schirrmeister et al., 2011). In the active layer (which is the seasonally thawed soil lying atop of permafrost), there is often both an organic and mineral horizon, the latter of which contains OM that is more decomposed (White et al., 2004). Permafrost can also be mineral or organic in nature, depending on landscape position and the conditions during its formation. Incubations from mineral permafrost and the overlying, seasonally thawed active-layer soils have shown that OM from permafrost is more labile than OM from active layer (Waldrop et al., 2010). In addition, Waldrop et al. (2010) observed that OM from mineral permafrost was more labile than organic permafrost, and Lee et al. (2012) observed that mineral and organic permafrost have no differences in the lability. Further, based on nuclear magnetic resonance (NMR) and on the yield of dissolved organic carbon (DOC) and total dissolved nitrogen (TDN) during decomposition, Waldrop et al. (2010) observed that permafrost SOM was more labile than active layer SOM from both mineral and organic permafrost soils. On the other hand, permafrost C/N ratios were similar to the C/N ratios of organic-rich, active layer soils (Waldrop et al., 2010; Lee et al., 2012), which indicates a similar degree of decomposition. The contrasting results from bulk soil C/N analysis—which indicate that permafrost SOM is equally as decomposed as the active layer—and from incubations, DOC and TDN yield, and NMR studies—which all indicate that permafrost SOM is more labile—suggests that standard soil metrics, such as C/N, are not sufficient indicators of permafrost SOM chemistry or that decomposition products are not inherently more recalcitrant. Insight into the chemical complexity of these soils may resolve these questions. Studies of the chemistry of SOM in the active layer have revealed that SOM quality declines with depth (Michaelson et al., 1996; Dai et al., 2002; White et al., 2002; Andersen and White, 2006), but fewer studies have directly investigated the chemistry of the permafrost SOM (Xu et al., 2009; Waldrop et al., 2010).

The objective of this study was to catalog the functional groups that comprise the SOM in mineral permafrost and compare them to mineral and organic active layer soils. Fourier transformed mid-infrared spectroscopy (MidIR), also known as DRIFTS and FTIR, enables the analysis of the functional groups that make up SOM and soil minerals without chemical extraction (Baes and Bloom, 1989; Nguyen et al., 1991; Haberhauer and Gerzabek, 1999; Janik et al., 2007; Bornemann et al., 2010; Calderón et al., 2013). Inferences can also be made about the lability of the constituents of SOM and the relative degrees of decomposition the compound has undergone (e.g., Artz et al., 2006; Calderón et al., 2011b; Haberhauer et al., 1998). We hypothesized that the permafrost contains an organic C molecular signature consistent with chemical preservation due to continuously frozen temperatures and likely anoxic conditions. Additionally, we hypothesized that the organic horizon of the active layer contains a mixture of SOM from recently added plant residue and more decomposed SOM. Thus we might expect the permafrost and the OAL to contain carbohydrates, cellulose, and proteins, and the OAL to contain carboxylates and organic acids. In addition to MidIR analysis of bulk permafrost and active layer soils (henceforth called "whole-soil"), we also evaluated methods for analyzing the organic component of these high OM soils. Because chemical extraction of OM can alter its composition (Kögel-Knabner, 2002; Schmidt et al., 2011), we evaluated two OM removal methods—ashing at high temperature or chemical oxidation with hypochlorite—after which we collected mineral spectra and then characterized the organic component through spectral subtraction.

MATERIALS AND METHODS

Description of the Study Site and Soil Sampling

Organic active layer, MAL, and permafrost soils were collected from Sagwon Hills, AK (N 69° 25′ 32.190″ W 148° 41′ 38.731″, 288 m above sea level), which is in the foothills north of the Brooks Range. Sagwon Hills, AK is within the continuous permafrost zone and is 65 miles south of the Prudhoe Bay, AK on the Dalton Highway. Borden et al. (2010) previously described the site. The soils were collected from under moist acidic tundra vegetation, which are classified as Ruptic Histic Aquiturbels (Borden et al., 2010). The permafrost at Sagwon Hills is of loess origin over gravel deposits (Borden et al., 2010). Cores were collected from 15 plots representative of the site on a grid covering 150 m^2. The depth of the seasonally thawed active layer was 26.8 ± 1.3 cm in August of 2009, and consisted of an organic and mineral horizon with evidence of cryoturbation (Fig. 1a). The organic horizon of mildly decomposed plant material (peat) with many fine roots was between 5 and 14 cm in depth. The remainder of the active layer was visibly gleyed mineral soil with no additional horizonation. In two plots, a buried organic horizon was visible. In these cases, samples were taken

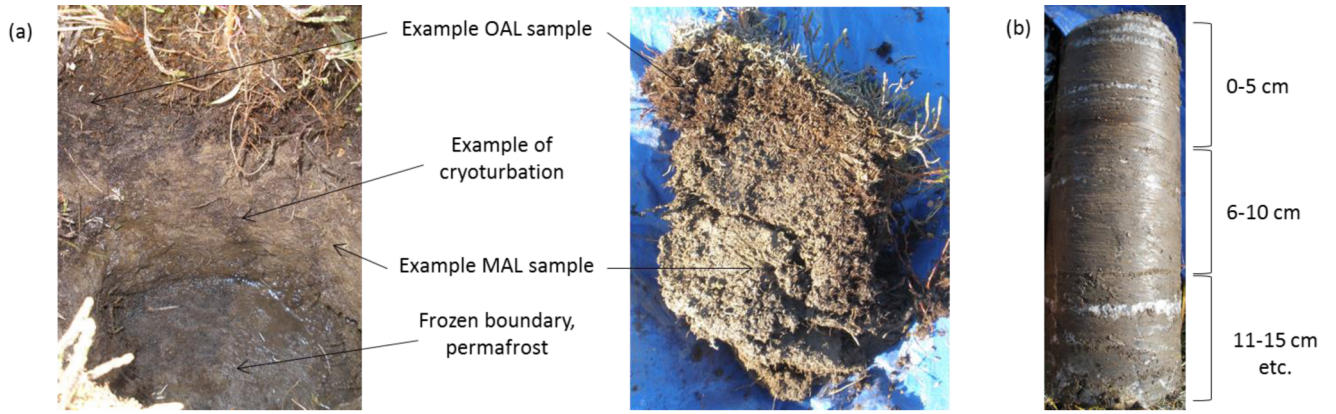

Fig. 1. (a) Example soil pit from Sagwon Hills, Alaska showing soil sampling depths and portraying cryoturbation. (b) Permafrost core showing how samples were divided.

from the mineral soil and not from within the buried organic horizon. At each plot, the active layer was removed and placed on a tarp as a monolith (Fig. 1a). The OAL and MAL soils were sampled from the monolith from the center of their respective depths (OAL: ~2 cm, MAL: ~10 cm), and live plant material was removed from the OAL. Permafrost soils were obtained as cores using a Tanaka auger fitted with a SIPRE-style (Snow, Ice, and Permafrost Research Establishment, (Tarnocai, 1993) soil corer with carbide bits (Jon's Machine Shop, Fairbanks, AK; Fig. 1b). Permafrost cores were collected as deep as possible before encountering glacial till (30–47 cm below the frozen boundary).

The samples were kept frozen through sampling, transportation, and storage. Permafrost cores were processed frozen in a walk-in −10°C freezer. First, they were scraped under aseptic conditions to remove outer layers of soil. Then they were separated into 5-cm increments (Fig. 1b). If there was a natural fracture point at an ice lens within 2 cm of the 5-cm fracture point, that point was chosen; Permafrost, OAL, and MAL soils were homogenized as frozen, unthawed soil by crushing them with a hammer to an approximate 2- to 4-mm sieve size in sterile bags. Because these samples remained unthawed until analysis, dead roots were not removed. This choice is justified by our intention to study the potential dynamics of SOM after thaw, during which dead roots will be an important part of SOM decomposition. In addition, root material is not visible to the naked eye. Subsamples (approximately 10 g) were air dried at 55°C for 36 h, ground with a mortar and pestle, and stored in 20-mL glass scintillation vials until analysis. The number of replicates varied as a function of samples lost during processing and inability to collect deep cores at some sites. For OAL, n = 12; MAL, n = 9; permafrost 0 to 5 cm (below the maximum active layer thaw depth), n = 15; 6 to 10 cm, n = 15; 11 to 15 cm, n = 15; 16 to 20 cm, n = 14; 21 to 25 cm, n = 12; 26 to 30 cm, n = 8; 31 to 35 cm, n = 4; 36 to 40 cm, n = 2.

Soil Carbon and Nitrogen Analysis

The dried and ground soils (0.10–0.21 g, depending on C content) were analyzed for total C and N content using a LECO Tru-SPEC elemental analyzer (Leco Corp., St. Joseph, MI). Due to the high SOM content of the OAL soils, a C and N standard of mixed grass was used. For mineral soils with relatively less OM, an agricultural soil standard from Sidney, NE was used. Measured values for the standards provided values within 0.94% for C and 0.046% for N, with a coefficient of variation <2.4% of the mean for C and <2% for N.

Differences between the means of the C content, N content, and C/N ratio of each depth increment was determined using an analysis of variance (ANOVA; PROC MIXED, SAS version 9.2, SAS Institute, Inc., Cary, NC) with a Tukey Honestly Significant Difference (HSD) multiple comparison adjustment. We chose a mixed model because we wanted to explore depth trends while accounting for the fact that multiple depths were taken from a core, which we included as a random effect. The data were log transformed to satisfy the ANOVA model assumptions of equal variance.

Collection and Analysis of MidIR Spectra

The objective of this study was to evaluate the chemistry of the organic component of the soils, for which MidIR is a powerful tool (Haberhauer et al., 1998; Artz et al., 2006; Calderón et al., 2011b), but mineral interference in the MidIR region should be considered. Analysis of the organic components in absence of soil minerals requires harsh extractions that can alter the chemistry of the OM (Kögel-Knabner, 2002; Schmidt et al., 2011). The MidIR provides an opportunity to remove the OM from the mineral matrix, collect a spectrum of the minerals, and subtract the spectrum of the mineral matrix from the spectrum of the whole soil to resolve the spectral features of the organic functional groups in the absence of spectral interference from minerals (Cox et al., 2000; Sarkhot et al., 2007). We investigated the efficacy of OM removal during ashing and chemical oxidation to evaluate the use of spectral subtraction as a tool for Arctic soils and other C rich soils. Therefore, MidIR spectra were collected on both whole-soil samples and samples after removal of the OM by ashing and hypochlorite treatment. To remove the OM with ashing, 4-g samples were placed in crucibles in a furnace at 550°C for 3 h. Removal of SOM with hypochlorite was based on the method first described by Anderson (1961). Briefly, 4 g of soil were thoroughly

mixed with sodium hypochlorite (25 mL 6% w/w, pH 9.5) and incubated in a hot-water bath to allow oxidation (15 min, 80°C). Solutions were centrifuged (15 min at 1081 × *g*) and the supernatant discarded. This procedure was done three times per sample. Soils were then washed twice with double deionized H_2O (20 mL, 15 min at 1081 × *g*), allowed to air dry, and briefly re-ground to homogenize air-dried crusting. Total C loss was measured with a C/N Analyzer (ECS 4010 Costech Analyzer, Costech Analytical Technologies, Valencia, CA).

The sample set of whole-soils consisted of 106 samples, which included the OAL, MAL, and all permafrost depths. There were also 106 ashed samples to correspond with each of the whole-soil samples, as well as a subset of hypochlorite-oxidized samples. All dried and ground soil samples were scanned undiluted (neat) in the mid-infrared region on a Digilab FTS 7000 Fourier transform spectrometer (Varian, Inc., Palo Alto, CA) with a deuterated, Peltier-cooled, triglycine sulfate detector and potassium bromide beam splitter. The spectrometer was fitted with a Pike AutoDIFF diffuse reflectance accessory (Pike Technologies, Madison, WI) and potassium bromide was used as background. Data was obtained as pseudo-absorbance (log [1/Reflectance]). Spectra were collected at 4 cm^{-1} resolution, with 64 co-added scans per spectrum from 4000 to 400 cm^{-1}. Duplicate scans of each sample were performed and the duplicate spectra were averaged. All spectra were mean-centered and pretreated with multiplicative scatter correction before further analysis (spectral averages, spectral subtraction, and PCA analyses).

Spectral differences between the depths were determined by calculating the average spectra for each depth and also by PCA using the *PLS Plus*/IQ software in GRAMS/AI Ver. 9 for both analyses (Thermo Galactic, Salem, NH). Correlations of whole-soil with soil C and N were performed in GRAMS/AI. Correlations with an $R > 0.8$ to C or N data were considered positively correlated, and those with an $R < -0.8$ were considered negatively correlated. Principal Component Analysis was performed on the whole spectra across 4000 to 400 cm^{-1} to reduce the dimensionality of the data. Rather than presenting the component loadings as a PCA biplot, we plotted them across the wavelengths scanned to provide visual evidence of which spectral bands explained the distribution of the sample scores along the principal components. A PCA of the permafrost and MAL samples in absence of the OAL was also performed to increase the resolution between the MAL and permafrost soils. The spectral subtractions of ashed or oxidized spectra from whole-soil sample spectra were performed with GRAMS/AI software.

The ratio of carbohydrates (1030 cm^{-1}) to carboxylates (1600 cm^{-1}; Table 1) was calculated as a proxy for the degree of decomposition in these soils (as suggested by Artz et al., 2006; Haberhauer et al., 1998; and Calderón et al., 2006). Differences by depth were determined with ANOVA with a mixed model (PROC MIXED, SAS version 9.2, SAS Institute, Inc.Cary, NC) with a Tukey Honestly Significant Difference (HSD) multiple comparison adjustment.

Table 1. Putative assignments for the bands relevant to this study. Note that mid infrared absorption bands occur over a range, and that there are overtone and combination bands from several different functional groups that may overlap with these frequencies. δ is bending, and ν is stretching.

wn (cm^{-1})	Assignment
3660–3620	ν O-H in clays†
3400	ν O-H and ν N-H‡
2930–2845	ν C-H‡
2515	Carbonates, O-H of H-bonded carboxylic acids§
2200–2000	Overtones of ν–COH¶
2000–1770	Quartz overtones†
1740–1700	ν C=O bond stretching in carboxylic acids and/or esters §, ring ν C=C
1670–1600	Amide I, or phenyl ring ν C=C ‡§
1630–1600	Carboxylate §
1590–1570	Ring ν C=C of phenyl‡
1560–1480	Amide II band ν C-N and δC-N-H‡#. Also δCH in phenyl rings
1530	ν C=N, or ν C=C‡
1450–1400	C-O single bond absorbance, δCH#
1450–1370	δ(CH_2) in polysaccharides and proteins‡. Also, N-H, and ν C-N#
1330	Carboxylate C-O††, ν C-N in amides, δ(CH) in phenyls and polysaccharides‡
1320–1220	Amide III band‡
1170–1060	ν C-O in carbohydrates, nucleic acids, proteins‡
1050	δ C-O in carbohydrates‡
810	silica‡‡

† Nguyen et al., 1991.
‡ Movasaghi et al., 2008.
§ Piccolo et al., 1992.
¶ Janik et al., 2007.
# Haberhauer and Gerzabek, 1999.
‡‡Calderón et al., 2011b.
††Tatzber et al., 2007.

RESULTS

Soil Carbon and Nitrogen Content

The OAL soils had nearly three times the amount of C compared with the MAL soils and soils collected from the top of the permafrost (Fig. 2). Carbon content in the permafrost soils decreased with increased distance from the active layer boundary. The permafrost layers within the top 15 cm had greater C content than the deeper permafrost layers (Fig. 2). The OAL soil had considerably more N than any of the mineral layers, including the MAL soil and all the permafrost layers (Fig. 2). There was a gradual decline in soil N content in the permafrost soil profile starting at the 16- to 20-cm core, which became significant at the deepest depth (36–40 cm). The C/N ratio of the OAL soil (27.4 ± 0.8) was greater than the MAL and permafrost layers (Fig. 2).

MidIR Spectral Properties of Active Layer and Permafrost Soils as Determined by Whole Soil Spectra

Given the statistical difference in soil C between the 0- to 15-cm permafrost (which is henceforth called the "top of the permafrost") and 16- to 40-cm permafrost (which is called "deeper permafrost"; Fig. 2), spectral averages for

the whole-soil spectra were calculated for these layers, as well as for the OAL and MAL (Fig. 3a). Various spectral features show similarities and differences of the chemistries through the depth profile, which we describe from the greatest to smallest wavelength in the MidIR region. Greater clay content in the permafrost and MAL than in the OAL soils was indicated by absorbance at 3620 cm^{-1} (Table 1). The presence of OH and NH functional groups was indicated by absorbance at 3400 cm^{-1} (Table 1) in all soils, but it was greater in the OAL than other soil layers. Greater absorbance at 2930 to 2850 cm^{-1} and 1470 to 1370 cm^{-1} in the OAL samples indicated higher amounts of aliphatic CH bonds in this soil relative to other compounds (Table 1), however all soils showed evidence of aliphatic compounds. The permafrost soils had greater quartz absorbance than the OAL soils, as expected by their mineral nature, which was indicated by spectral features between 2000 and 1770 cm^{-1} and the inversion band between 1226 and 1070 cm^{-1}. All soils had a feature at 1730 cm^{-1}, assigned to C=O absorbance from a variety of functional groups including lipids, hemicelluloses, and nitrogenous bases (Movasaghi et al., 2008), that could also have influence from aromatic C=C (Cox et al., 2000; Calderón et al., 2011b; Table 1).

The OAL had a peak at 1655 to 1615 cm^{-1}, whereas the rest of the layers had a peak at 1610 cm^{-1} (Fig. 3a). This difference suggested that the OAL had increased influence of amide I (1655 cm^{-1}) absorbance, but the rest of the layers had relatively more aromatic C=C absorbance (Table 1). This assertion was supported by the greater absorbance of amide III (shoulder at 1320 to 1220 cm^{-1}) in the OAL and greater absorbance in the aromatic C-H bending region (from 950 to 750 cm^{-1}) for the MAL and permafrost soils. Carboxylates were indicated by absorbance between 1630 and 1600 cm^{-1} for all the soils (Piccolo et al., 1992). The presence of carboxylates can be corroborated by absorbance at 1730 cm^{-1}, which also indicates carboxylic acids (Table 1). The deeper permafrost had a peak at 1420 cm^{-1} that was not as pronounced in the top of the permafrost, suggestive of polysaccharide CH_2, protein CH_2, C-H, or C-O (Table 1). Due to the relative abundance of other peaks representing these compounds, 1420 cm^{-1} is likely due to aliphatics or carbohydrates. The MAL and the shallower permafrost had a shoulder at 1370 cm^{-1} that is absent in the 16- to 40-cm permafrost (Fig. 3a). This region contains bands for phenolic and carboxylate C-O stretching, C-H, N-H, C-N, and O-H deformation. Ester C-O bonds, as shown by the rounded shoulder at 1350 cm^{-1}, were observed in the OAL, MAL, and top of the permafrost, but were not evident in the deeper permafrost.

Principal components analysis of the whole-soil spectra showed separation of the OAL soils from the permafrost and MAL along the first component, which explained nearly 85% of the variation in the data (Fig. 3b). Permafrost and MAL soils had low Component 1 scores, while OAL soils had larger Component 1 scores (Fig. 3c). Component loadings indicate the absorbance values that are responsible for the separation of sam-

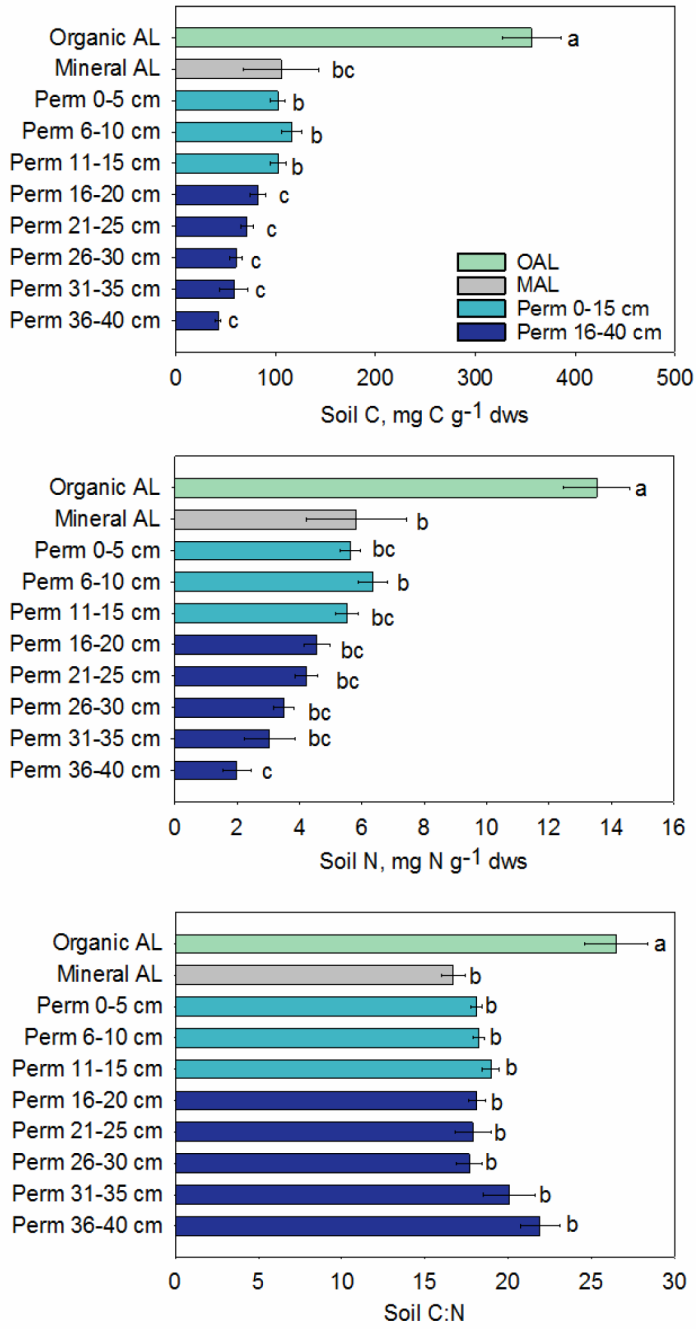

Fig. 2. Soil C and N content and C/N ratios through the depth profile. Different letters indicate significant differences in the means of the log-transformed data after using the Tukey HSD multiple comparison adjustment ($p < 0.05$). Colors reflect these differences in the means for C content.

ple scores, such that samples with high scores for Component 1 have higher absorbance at the positive peaks in the loadings graph (e.g., 3400, 2930, 1740, and 1220–1090 cm^{-1}). Component 1 loadings showed that the samples from the permafrost and MAL soils absorbed at the sand and clay bands more than the OAL soils (Fig. 3c), which was consistent with the average spectral data (Fig. 3a). Component 1 loadings also showed that the OAL soils are distinguished by absorbance of organic bands indicating the greater concentrations of OH/NH (3400 cm^{-1}), aliphatics (2850–2930 cm^{-1}), carbonyls (1740 cm^{-1}), Amide III (1225 cm^{-1}), and carbohydrates or esters (1093 cm^{-1}; Table 1),

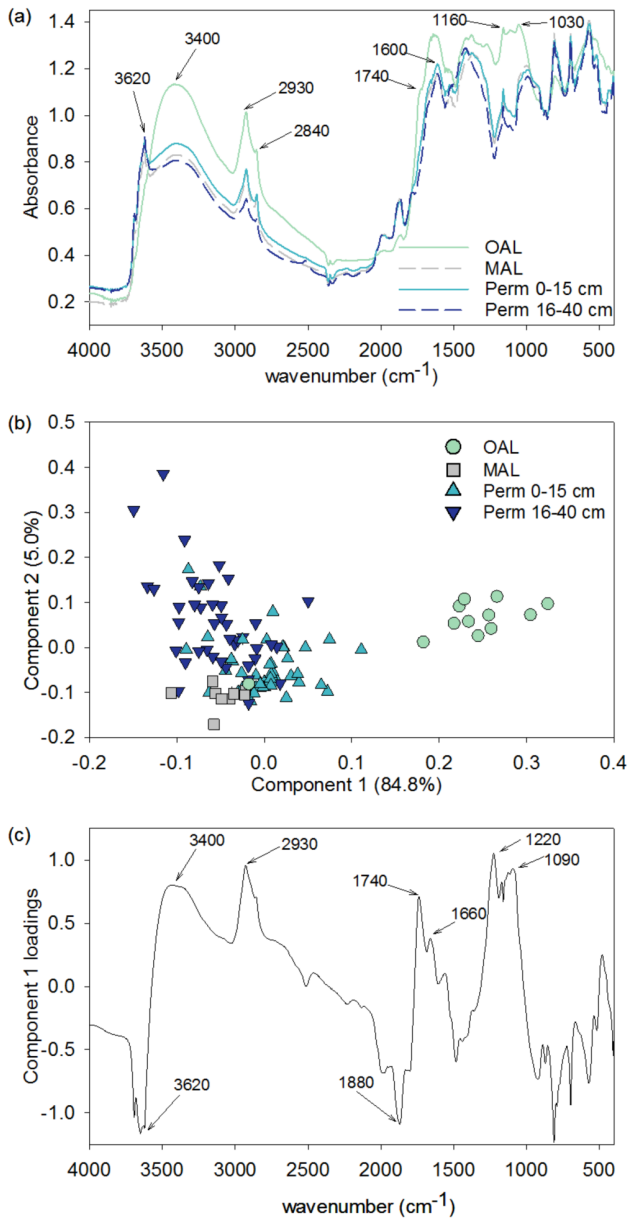

Fig. 3. Analysis of whole-soil (not oxidized) MidIR spectra showing (a) average MidIR spectra of the different soil depths, (b) PCA of MidIR spectra, and (c) loadings of PCA Component 1. Component 1 identified the banding regions important in separating OAL from MAL and permafrost soils. Loadings can be interpreted by considering the direction of the separation along the component. For example, OAL soils have positive Component 1 scores in the PCA (b); therefore, the wavelengths with positive Component 1 scores in (c) contributed to the separation of the OAL.

1740 cm^{-1} is due to the absorbance of the C=O bond in carboxylic acids and esters (Haberhauer and Gerzabek, 1999; Cox et al., 2000; Janik et al., 2007; Sarkhot et al., 2007) and is sometimes assigned to humic substances (Cox et al., 2000), however the prevalence of humification as a preservation mechanism in soils is no longer widely accepted (Kleber et al., 2011). Peaks in the 1730 and 1740 region can also be attributed to the presence of organic acids produced during decomposition (Hodgkins et al., 2014). The 1093 cm^{-1} represents carbohydrates (Movasaghi et al., 2008). The permafrost and the MAL soils were partially

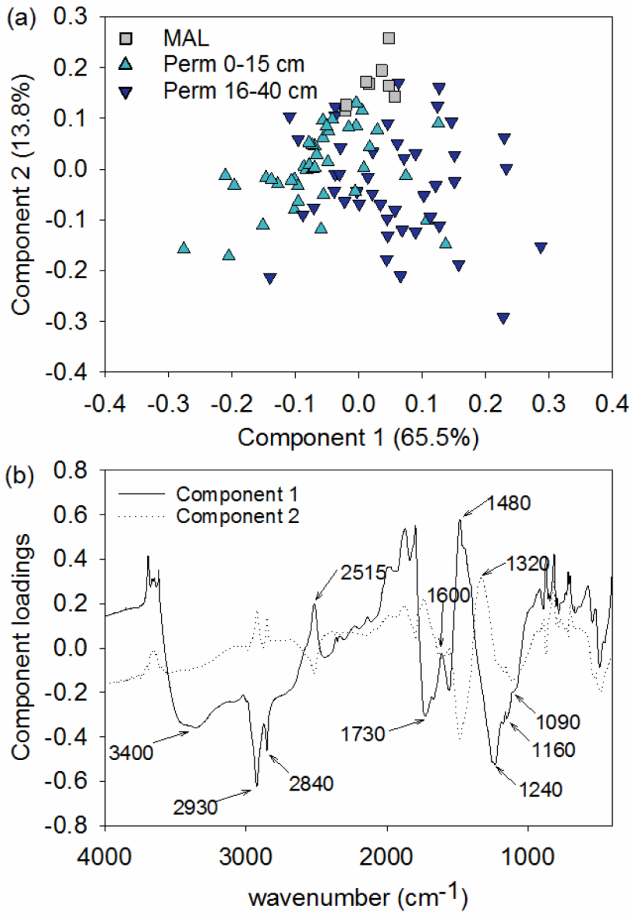

Fig. 4. (a) PCA of whole-soil MidIR spectra of the MAL, top of the permafrost and deeper permafrost only. (b) Loadings of PCA components. Component 1 identifies the banding regions important in separating the two permafrost depths, and Component 2 explains differences between the mineral active layer and the permafrost soils.

separated along the second and third components in this PCA, but to gain greater resolution into the differences between the mineral layers, we removed the OAL (Fig. 4).

The MidIR spectral properties of the permafrost samples were different from the MAL samples (Fig. 4). The top of the permafrost (0–15 cm) and deeper permafrost (16–40 cm) layers were separated along Component 1. Loadings indicate that the soils from the top of the permafrost had more absorbance at O-H/N-H (3400 cm^{-1}), aliphatic C-H bonds (2930–2850 cm^{-1}), C=O bonds (1740 cm^{-1}), and Amide III (1240 cm^{-1}), and the deeper permafrost had more absorbance of O-H bonds in clays (3620 cm^{-1}), carbonates (2515 cm^{-1}), quartz (1795 cm^{-1}), Amide II (1487 cm^{-1}), and silica (810 cm^{-1}). The co-occurrence of 1795 and 810 cm^{-1} indicated that the deeper permafrost has greater amounts of silicates, which was confirmed by the high loadings for clay at 3620. Component 2 loadings showed that the MAL samples were characterized by Amide II (1320 cm^{-1}) and aliphatic C-H (2930–2850 cm^{-1}) groups, and had less absorbance due to carbonates (2515 cm^{-1}) and Amide II (1480 cm^{-1}) than the permafrost soils (Fig. 4b).

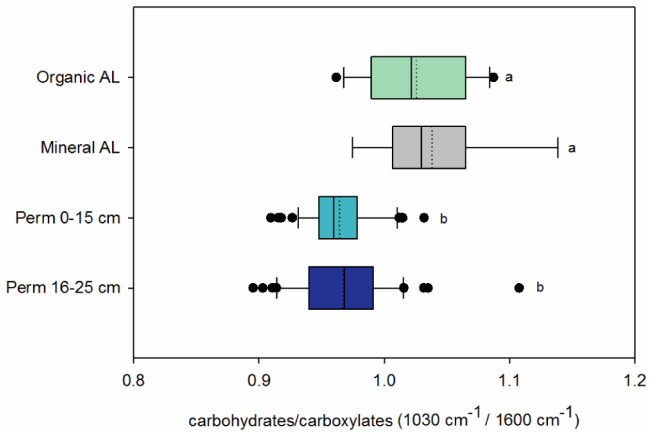

Fig. 5. Boxplots of the ratio carbohydrates (1030 cm^{-1}) to carboxylates (1600 cm^{-1}). Solid lines show the medians, and box limits indicate the 25th and 75th percentiles. The whiskers extend 1.5 times the interquartile range from the 25th and 75th percentiles, and outliers are represented by dots. Dashed lines represent sample means. Different letters indicate statistically significant differences (α = 0.05) using a Tukey HSD multiple testing adjustment. *n* = 12, 9, 45, 40 sample points.

Spectral Band Ratios

Previous studies have explored band ratios as 'indices of decomposition' (Haberhauer et al., 1998; Calderón et al., 2006; Artz et al., 2006) to identify which bands decrease or accumulate over the course of incubation or with depth. Band ratios may show small changes in the OM pool that cannot be detected by PCA or spectra alone. We explored the ratios of a putatively labile compound, carbohydrates (1030 cm^{-1}; White et al., 2002; Andersen and White, 2006; Calderón et al., 2011b), to carboxylates (1600 cm^{-1}), a region of the MidIR spectrum that accumulates during decomposition (Haberhauer et al., 1998; Artz et al., 2006; Calderón et al., 2011b). The ratio of carbohydrates/carboxylates (1030/1600 cm^{-1}) was higher in the active layer soils (both OAL and MAL) than the two permafrost depths, which were the same (Fig. 5). Active layer soils had a ratio slightly over one (OAL = 1.03 ± 0.01; MAL = 1.04 ± 0.02), and permafrost soils had a ratio just below 1 (Perm 0–15cm = 0.96 ± 0.004; Perm 16–40 cm = 0.97 ± 0.007).

Methods Analysis of Spectral Subtraction as a Tool for Analysis of the Organic Component of High Carbon Content Soils

To identify organic features of the spectra in the permafrost and active layer samples in the absence of the mineral background, we assessed two OM removal techniques—oxidation with heat (ashing) and chemical oxidation with hypochlorite (Fig. 6). Proportionally more C was left unoxidized in the OAL relative to the other layers due to the large amount of initial C content, however C removal was greater than other studies (Siregar et al., 2005). The C removal using hypochlorite oxidation ranged from 78.9% in the OAL samples to 91% in the MAL samples. The hypochlorite-treated OAL soils retained the aliphatic C-H band at 2630–2850, the Amide I band at 1655, and the band at 1330 cm^{-1} for carboxylate C-O (Table 1).

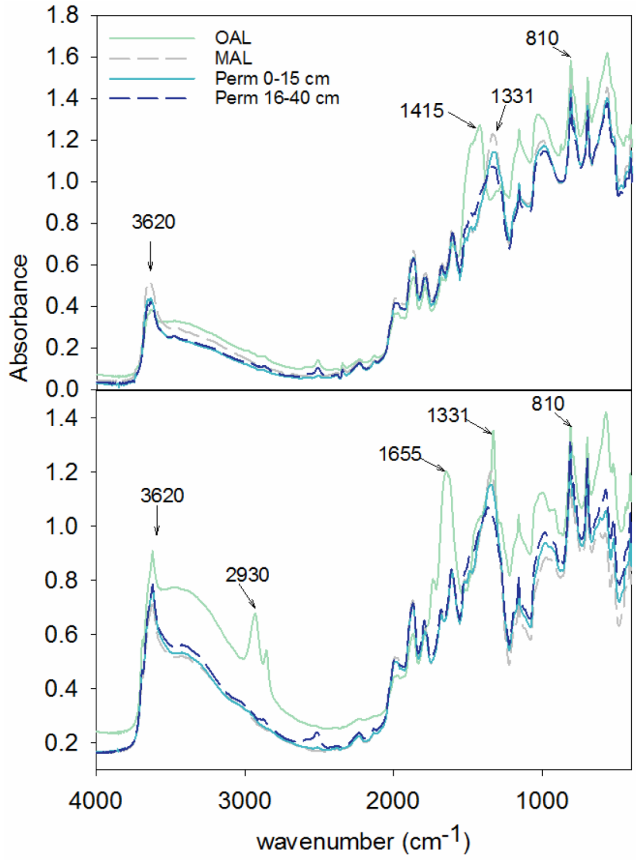

Fig. 6. Average MidIR spectra of material after (top) ashing and (bottom) chemical oxidation.

In the ashing procedure, the high temperature caused clay degradation, as observed by a rounding of the clay peak at 3620 cm^{-1}, which was not observed with the hypochlorite oxidation (Fig. 6). Both ashing and chemical oxidation enhanced the band generally representative of carbonates (2515 cm^{-1}) in the deeper permafrost (Fig. 6). However, due to the low pH of these soils, this diffuse "shoulder" could alternately be due to O-H of H-bonded carboxylic acids (Piccolo et al., 1992) that remained after oxidation. The silicate inversion band at 1225 to 1075 cm^{-1} was enhanced with both types of OM removal, but especially in the ashing treatment. A peak representative of silica (810 cm^{-1}; Calderón et al., 2011a) was apparent in the soils after both oxidation treatments. After spectral subtraction, the hypochlorite treated OAL showed a negative peak at 3620 cm^{-1}, possibly due to washing off of clays (Fig. 6).

The spectral subtractions of ashed soils from whole-soils showed that active layer and permafrost soils differed in the amount and type of OM (Fig. S1b). The OAL soil had greater absorbance due to O-H/N-H (3400 cm^{-1}), aliphatic C-H (2930–2850 cm^{-1}), C=O (1740 cm^{-1}), Amide I (1655 cm^{-1}), carboxylic acids (1600), esters (1350 cm^{-1}), Amide III (1240 cm^{-1}), and C-O (1090 cm^{-1}) functional groups than the MAL soil or permafrost, which represent organic functional groups with varying reactivities (Table 1). The spectra produced from spectral subtraction also revealed differences between the OAL and the permafrost and MAL soils. For example, samples from the perma-

frost and MAL had absorbance due to amide II (1560 cm^{-1}) and C-O (1450 cm^{-1}; Fig. S1). However, incomplete removal of the OM at 1450 cm^{-1} in the OAL may bias this finding (Fig. 6). In regions where OM removal was similar between methods (e.g., O-H/N-H groups, aliphatics), the results were not different than those garnered from the whole-soil spectra.

To determine which regions of the whole-soil spectra were representative of OM, we correlated absorbance across the MidIR spectrum with C and N content. Correlations between C and N content and the absorbance at wavenumbers between 3520 and 3240, 2850 and 2930, 1760 and 1650, and 1270 and 1047 cm^{-1} were highly positive ($R > 0.8$) (Fig. S2); these bands represent organic regions including the OH/NH stretch, aliphatics, proteins and amides, and carbohydrates (Table 1). The band at 1230 cm^{-1} can be either highly processed, clay-associated OM (Calderón et al., 2011b) or a clay mineral band. Because a high correlation to C or N content was not observed with the 3620 cm^{-1} clay band, the 1230 cm^{-1} region was likely due to the absorbance of organic material rather than clay. Negative correlations were found with mineral bands near 3620, 1970–1870, and 805 cm^{-1}, representative of clays, quartz, and silica. Bands associated with proteins at 1650 and 1550 cm^{-1} had slightly higher positive correlations (R = 0.68 and 0.81, respectively) with percentage of N compared with the correlation with percentage of C. Both of these bands can be assigned to phenyl groups or N containing compounds, such as amines, but their higher correlation with N than C suggests that in these soils, they are due to the presence amines. The region 1390 to 1545 cm^{-1}, traditionally thought to be within the MidIR organic fingerprint region, was less correlated to total soil C than would be expected from the various organic assignments in this region (Table 1).

DISCUSSION

Using MidIR spectroscopy, we observed fine scale differences in chemistry, which may have implications for the decomposability of permafrost OM. The chemistry of the OAL differed from the MAL and permafrost samples (Fig. 2), due to the absorbance of bands representing organics of varying reactivity (Fig. 3c; Table 1). Our results are consistent with OM movement from the active layer to the top of the permafrost. Previous studies have shown that the OM in the active layer is very sensitive to decomposition (Mikan et al., 2002; Hartley et al., 2008; Waldrop et al., 2010), and by extension suggests that the OM in the top of the permafrost could be as well.

Analysis of Chemical Make-Up of Permafrost and Active Layer Soils

The OAL has a chemical signature consistent with relatively non-decomposed OM, such as root exudates and non-decomposed organic materials (Dai et al., 2002; White et al., 2002; 2004; Andersen and White, 2006; Xu et al., 2009). The OAL is characterized by bands of functionalities that should be readily utilized during decomposition, such as O-H/N-H groups (3400 cm^{-1}) and carbohydrates (1160 and 1090–1000 cm^{-1}; White et al., 2002, 2004; Calderón et al., 2011b), which likely due to root growth and exudation, as well as recent addition of light fraction plant material (Calderón et al., 2011a). Aliphatics (2870–2930 cm^{-1}) and amides (1320–1220 cm^{-1}) were also important in distinguishing the OAL from the other soils (Fig. 3c). Calderón et al. (2011b) found that bands with absorbance at 2870 to 2930 and 1223 cm^{-1} were indicative of low mean residence time OM in agricultural soils, decreasing rapidly from the light fraction on incubation. But, assigning whether a molecular class denotes a chemically labile compound can be challenging, as mechanisms for protection can vary between soil types and fractions. Whether aliphatics (2930–2870 cm^{-1}) are depleted or accumulate during decomposition may be dependent on the mineral character of the soil, and whether the aliphatics are associated to soluble versus insoluble organic molecules.

Chemical similarity between the top of the permafrost and the OAL, and differences with the deeper permafrost, suggests that the top of the permafrost receives fresh OM (Fig. 3b). For example, O-H/N-H groups (3400 cm^{-1}), carbohydrates (1160 and 1090–1000 cm^{-1}), aliphatics (2930–2870 cm^{-1}), and amides (1320–1220 cm^{-1}) were important in distinguishing the chemistry of the top of the permafrost from the deeper permafrost, just as they were for distinguishing the OAL from the other soils. We expected to observe a chemical signature of fresh material in the permafrost due to preservation under frozen conditions, but the differentiation between the top of the permafrost and deeper permafrost is inconsistent with preservation of OM.

Introduction of OM into the permafrost can occur through a variety of mechanisms, including cryoturbation, when OM is transported through the soil profile, or via DOC incorporation. Active layer DOC could be introduced into the permafrost through either syngenetic permafrost formation, where the bottom of the active layer is incorporated into the permafrost during cold years, or in warm years when the top of the permafrost is thawed or at near-freezing temperatures. Dissolved organic C incorporation into permafrost is an unlikely mechanism to explain the existence of these chemical constituents, because the isotopic signature of the DOC at Sagwon Hills indicates it is heavily microbially processed (Xu et al., 2009). Further, various organic bands in the spectra separated the MAL from the permafrost, and the observation that the MAL and the permafrost were chemically different provides further support that OM is incorporated into the permafrost through cryoturbation of materials, which can introduce non-decomposed material to deeper soils (Ping et al., 1998; Xu et al., 2009). The chemical similarity between the OAL and the top of the permafrost (Fig. 3c) and the top of the permafrost's differences from the MAL (Fig. 4b) supports the hypothesis that fresh OM is incorporated into the top of the permafrost through cryoturbation.

Decomposition Products in Permafrost Soils

Soils from all of the depths contained compounds that are putative products of decomposition, such as organic acids and carboxylic acids (1730 cm^{-1}; Hodgkins et al., 2014) and carbox-

ylates (1600 cm^{-1}; Haberhauer et al., 1998; Artz et al., 2006). These regions were found to increase during decomposition (Haberhauer et al., 1998; Calderón et al., 2006, 2011b). The presence of organic acids (1730 cm^{-1}) was particularly important in distinguishing the OAL from the other layers (Fig. 3c), but also the MAL from the permafrost (Fig. 4b). The presence of organic acids also distinguished the top of the permafrost from deeper permafrost. The presence of carboxylates (1600 cm^{-1}) in all the samples suggests that decomposition has occurred. Carboxylic acids and carboxylates are products of the incomplete oxidation of organic molecules during decomposition (Pérez et al., 2002). Their presence further supports our hypothesis that OM with a chemical composition similar to the OAL has been introduced to the permafrost, or that new OM has been incorporated into the permafrost and subsequently decomposed.

Heterotrophic microbes are capable of metabolic activity well below freezing (McMahon et al., 2009; Drotz et al., 2010), and previous studies have found an active microbial community in permafrost (Uhlířová et al., 2007; Coolen et al., 2011). Observations of CO_2 and CH_4 trapped in permafrost confirm that OM is oxidized in situ (Rivkina et al., 1998, 2004; Michaelson et al., 2011). Also, OM can be oxidized through abiotic mechanisms, such as oxidation of phenols to quinones by metal oxide catalysis (Sollins et al., 1996). The C/N ratios (Fig. 2) and ratios of non-decomposed material to decomposition products, such as carbohydrates/carboxylates (Fig. 5), were lower in the permafrost than the OAL, suggesting that these soils have had more decomposition than fresh OM inputs.

Despite the low C/N and lower ratio of carbohydrates/carboxylates, our inventory of the chemistry of the permafrost suggests that permafrost SOM is potentially decomposable. The permafrost layers contained carbohydrates in equal, or near equal, proportions to carboxylates (Fig. 5), suggesting that there is a large pool of readily decomposable substrate for microbial decay. Previous reports also found contrasting results between potential permafrost lability, as indicated by NMR and incubation studies, and C/N ratios (Waldrop et al., 2010; Lee et al., 2012). The C/N ratios may not be an adequate index of SOM decomposition in high C content soils, and more detailed chemical analysis, such as MidIR spectroscopy, can help to elucidate the chemical constituents of interest.

MidIR as a Tool to Investigate the Properties of Organic Matter in High Carbon Content Soils

Soils contain both organic and mineral components, both of which absorb in the MidIR region. The organic component of soils is the biologically active portion, so MidIR analysis of the organic component can inform our understanding of the relative reactivities of the SOM. To circumvent challenges posed by extractions of OM (Kögel-Knabner, 2002; Schmidt et al., 2011), we used spectral subtraction to analyze the organic component of these soils (Calderón et al., 2011b). Subtracting a MidIR spectrum of the mineral component from the whole-soil spectrum produces a spectrum representative of the organic component of each soil (Calderón et al., 2011b; Parikh et al., 2013). We obtained mineral spectra through two methods for removing OM—oxidation with heat (ashing) and chemical oxidation with hypochlorite (Fig. 6 and S1). Despite the potential for artifacts due to clay heating in the ashing procedure (Reeves, 2012), the inadequate removal of OM from these soils in the hypochlorite oxidation indicated that the ashing technique was the better tool in these OM-rich soils. However, comparison of the two methods showed regions in the ashed spectra that posed additional challenges to interpretation (e.g., 1415, 1331, and 1225–1075 cm^{-1}; Fig. 6) in addition to those observed by Reeves (2012).

We found that our interpretation of the SOM chemistry of these soils did not vary, regardless of whether we analyzed spectra obtained on the whole soil or after spectral subtraction with either method (Fig. S1). This was likely because the high OM content of the soils minimized mineral interference. Therefore, we chose to present the spectra with the least manipulation, those of the whole-soils, and analyzed the organic component by generally drawing on MidIR regions with minimal overlap of organic and mineral absorbance (Reeves, 2012). We focused our analysis on the regions where the C and N content correlated positively and strongly ($R > 0.8$) with the organic bands (i.e., 3400, 2930–2850, 1740–1700, 1650–1600, 1550, 1230, and 1100–1030 cm^{-1}; Fig. S2).

CONCLUSIONS

The chemical signatures of the OAL and the top of the permafrost were similar, whereas the top of the permafrost and deeper permafrost had MidIR regions that differentiated them. We found that the ashing and oxidation approaches did not resolve the soil layer differences beyond what could be learned from the multivariate analysis of whole-soil spectral data. This is likely because the high OM content in these soils resulted in less mineral interference in the MidIR region. In addition, the high OM content of these soils resulted in the incomplete oxidation of OM during ashing the chemical oxidation procedures.

Both the OAL and the top of permafrost contained bands considered to be chemically labile, but also showed evidence of decomposition. The top of the permafrost often was more similar to the OAL than the MAL or deeper permafrost, and relative to the deeper permafrost, the top of the permafrost contained a signature indicative of fresh OM (i.e., O-H/N-H absorbance) and of other labile organic functional groups (esters, amides). Together this suggests that compounds have been introduced to the top of the permafrost, most likely through cryoturbation. Relative to the MAL, permafrost had less accumulation of compounds indicative of previous decomposition (i.e., carboxylates, organic acids). These results are in congruence with previous findings showing that OM at the top of the frozen boundary of permafrost is a repository for chemically labile, easy to decompose OM (Xu et al., 2009; Waldrop et al., 2010; Coolen et al., 2011; Lee et al., 2012; Gillespie et al., 2014). These results add to our growing understanding of the chemical composition of OM in permafrost. Future incubation studies on these soils will

shed further light on the reactivity and decomposability of these infrared bands in soils.

SUPPLEMENTAL INFORMATION

Supplementary information provides additional figures depicting methodological differences in the analysis of the organic component of high C soils.

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