

Arctic soils of  
Western Siberia

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# Stability and biodegradability of humic substances from Arctic soils of Western Siberia: insights from $^{13}\text{C}$ -NMR spectroscopy and elemental analysis

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## Abstract

Arctic soils contain large amounts of organic matter which, globally, exceed the amount of carbon stored in vegetation biomass and in the atmosphere. Recent studies emphasize the potential sensitivity for this soil organic matter (SOM) to be mineralised when faced with increasing ambient temperatures. In order to better refine the predictions about the response of SOM to climate warming, there is a need to increase the spatial coverage of empirical data on SOM quantity and quality in the Arctic area. This study provides, for the first time, a characterisation of SOM from the Gydan Peninsula in the Yamal Region, Western Siberia, Russia. On the one hand, soil humic acids and their humification state were characterised by measuring the elemental composition and diversity of functional groups using solid-state  $^{13}\text{C}$ -NMR spectroscopy. Also, the total mineralisable carbon was measured. Our results show that there is a uniformity of SOM characteristics throughout the studied region, as well as within soil profiles. Such in-depth homogeneity, together with a predominance of aliphatic carbon structures, suggests the accumulation in soil of raw and slightly decomposed organic matter. Moreover, results on total mineralisable carbon suggest a high lability of these compounds. The mineralisation rate was found to be independent of SOM quality, and to be mainly explained solely by the total carbon content. Overall, our results provide further evidence on the fundamental role that the soils of Western Siberia may have on regulating the global carbon balance when faced with increasing ambient temperatures.

## 1 Introduction

Polar soils play a key role in the global carbon balance, as they provide maximum stocks of soil organic matter (SOM) within the whole pedosphere (McGuire et al., 2009). Large amounts of organic matter have accumulated during the quaternary period (Schirrmeyer et al., 2011; Zubrzycki et al., 2013) due to continuous low temper-

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composed. In Cryosols there is often a cryogenic exchange of organic matter between different horizons which enhances additional long-term accumulation of carbon (Kaiser et al., 2007).

The general aim of this work is to characterize the humification process in soils of the Yamal region, as well as the mineralization potential of SOM. This is addressed by quantifying and characterizing SOM from different sites across the Yamal Region, in order to eventually unveil landscape-geographical trends of humification. The main methodologies used are  $^{13}\text{C}$ -NMR spectroscopy, elemental composition and total mineralisable carbon analysis.

More specifically, the objectives are:

- To quantify total soil organic carbon content, together with the total mineralisable fraction.
- To characterize the elemental composition of humic acids and observe trends in their ratios.
- To provide detailed structural characteristics of humic acids using  $^{13}\text{C}$ -NMR spectroscopy.
- To infer the humification degree of soil humic acids based on  $^{13}\text{C}$ -NMR spectroscopy and elemental composition.

## 2 Methods

### 2.1 Study sites

The sites were distributed across the Gydan Peninsula and the Belyi Island, lying within the Yamal-Nenets autonomous region of the Russian Federation (northwestern Siberian coast). The Gydan Peninsula extends into the Kara Sea, between the embayments of Ob and Yenisey rivers; whereas Belyi Island is located at the top northern part

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Landscapes here (Fig. 2d) were less differentiated in relation to the elevation and were more overmoisted than the Yavay peninsula. The depth of visible permafrost border was about 90 cm. The soil was sampled at 0–10 cm.

The landscape of the Enisey Gulf (72°22.451' N, 78°38.586' E) was predominantly flat, without any relevant relief differentiation (Fig. 2f). Therefore, soils were highly overmoisted and Histic Gleysols were dominant (Fig. 2e). The depth of permafrost in both plots of the Enisey Gulf was about 40–45 cm. The climate of the Enisey Gulf is considered as the most severe in comparison with the other investigated plots. The soil was sampled at two depths: 0–5 and 5–9 cm.

Soils of the Bely Island (73°18.421' N, 71°23.555' E) were classified as Entic Podzol (Fig. 2g). The upper part of the horizon presented weak features of podzolisation, and it was underlayed by material affected by cryoturbation in the middle profile. The lower part was a Gleyic horizon (60–170 cm) of intensive blue color, underlayed by permafrost at 200 cm depth. The soil was sampled at 0–7 cm.

In Haranasale cape (71°25.402' N, 73°03.758' E) relief forms were very different and presented hills and lowlands with relative elevation changes of about 100 m. Soils here consisted mainly of Histic Gleysols (Fig. 2h). The upper border of the permafrost layer was about 60–70 cm. Here two soil profiles were sampled: one at 0–5 and 5–11 cm depth (hereafter referred to as Haranasal-1) and another at 0–5, 5–10 and 20–30 cm depth (hereafter referred to as Haranasale-2).

## 2.3 Analytical methods

### 2.3.1 Bulk SOM characterisation

All chemical soil parameters were studied on a fine earth of soil after being passed through a 1 mm sieve. Total soil organic carbon content was determined by dichromate oxidation-titration method (Walkely, 1947). Total mineralisable carbon was determined according to Anderson (1982) aliquots (5 g) of fresh soil, adjusted to a moisture content of 60 % of the water-holding capacity were incubated at 25 °C in sealed plastic bottles

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with 1 mol L<sup>-1</sup> NaOH (Anderson, 1982) in duplicate. The amount of CO<sub>2</sub> trapped in the alkali solution was measured by titration after 7 days. Soil pH was determined according to standard procedure using soil/water or salt ratio of 1 : 2.5.

The optical index E4/E6 was measured on SOM humic substances solution as the absorbance measured at 465 nm divided by that at 690 nm (Chen et al., 1977). SOM humic substances solution was obtained by diluting soil with a 0.1 M NaOH solution at a soil/solution mass ratio of 1 : 10 followed by gravity filtration. Further, E4/E6 data were used to estimate SOM lability using Kumada's classification system (Kumada, 1987). For that, E4/E6 values were transformed into  $\Delta \log K$  using the empirical relationship  $\Delta \log K = 0.135 \times E4/E6$  (Ikeya and Watanabe, 2003).

### 2.3.2 Extraction of humic acids

Humic acids were extracted from each soil sample according to the following procedure (Schnitzer, 1982). Briefly, humic acids (HAs) were extracted with 0.1 M NaOH (soil/solution mass ratio 1 : 10) under nitrogen gas. After 24 h of shaking, the alkaline supernatant was separated from the soil residue by centrifugation at 1516 × *g* for 20 min and acidified to pH 1 with 6 M HCl to induce the precipitation of HAs. The supernatant, which contained fulvic acids (FA), was separated from the precipitate (containing HAs) by centrifugation at 1516 × *g* for 15 min. The HAs were then redissolved in 0.1 M NaOH and shaken for 4 h under N<sub>2</sub> before the suspended solids were removed by centrifugation. The HA solution was acidified again with 6 M HCl to pH 1, and the HAs were separated by centrifugation. The HAs were demineralized by shaking overnight in 0.1 M HCl/0.3 M HF (solid/solution ratio 1 : 1) and then repeatedly washed with deionized water until pH 3 was reached; they were then freeze-dried.

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### 2.3.3 Characterisation of humic acids

HAs were characterized for their elemental composition (C, N, H, and S) using a Euro EA3028-HT analyzer. Data were corrected for water and ash content. Oxygen content was calculated by difference.

<sup>13</sup>C-NMR spectra of humic acids were measured with a Bruker Avance 500 NMR spectrometer (Karlsruhe, Germany, 2003) in a 4 mm ZrO<sub>2</sub> rotor. The magic angle spinning speed was 20 kHz in all cases, and the nutation frequency of <sup>13</sup>C fields for crosspolarization was  $\omega_1/2\pi = 62.5$  kHz. Repetition delay and number of scans were 3 s. Groups of structural compounds were identified by the chemical shifts values: 190–170 ppm – carboxyl group and amidic carbonyl, 170–150 ppm – aromatic C of phenols and phenol esters, 150–135 ppm – alkylaromatic, 135–108 ppm – protonized aromatic carbon, bridgehead C, 108–100 ppm – cellulose anomeric carbon and hemiacetal carbon, 100–70 ppm – resonance region of C–H bonds, secondary alcohols, and other carbon atoms bound to oxygen, 70–50 ppm – methyl group resonance region of aliphatic and aromatic ethyl ethers, amino acid carbons, and methyl esters of carboxylic groups, 50–32 ppm – resonance region of quaternary carbon and CH carbons, 32–27 ppm – resonance region of CH<sub>2</sub> alkyl structures in transconformation, 27–10 ppm – resonance region of alkyl methyls and CH<sub>2</sub> units. Degree of aromaticity was calculated as a sum of signals intensity in regions of 100–170 + 183–190 ppm, while the sum of aliphatic compounds were calculated in intervals 0–100 + 164–183 ppm.

### 2.4 Statistical analyses

All statistical analyses were performed within the R environment (R Development Core Team, 2015). Simple relationships between variables were explored using univariate linear model correlations based on Pearson's R. For the total mineralisable carbon, a multivariate model analysis was performed to detect what SOM quantitative and qualitative variables were significantly explaining SOM mineralisation. This was achieved using a best subset selection procedure, that is, a least squares regression model was

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fit for every possible combination of explanatory variables. The best model was considered to be that with a lowest Mallows's Cp (James et al., 2013). Computations were performed using the leaps package for R (Lumley, 2009).

In order to better understand the regional distribution of carbon structures a multivariate approach was used. A non-metric multidimensional scaling (NMDS) analysis using Bray–Curtis dissimilarities was performed on the structural carbon compounds defined from <sup>13</sup>C-NMR spectra. The quality of the ordination was improved by submitting the data to Wisconsin double standardisation. Interpretation of the NMDS ordination was enhanced by overlaying information on the elemental composition of humic acids and characteristics of bulk SOM. This was achieved by performing a vector fitting analysis. Variables were centered by subtracting their mean and scaled by dividing by their standard deviation. Fittings were considered significant at  $p < 0.01$ . Computations for the multivariate analysis were performed using the vegan package for R (Oksanen et al., 2012).

### 3 Results and discussion

#### 3.1 Bulk soil organic matter characteristics

Across the studied region, soils contained an average of  $14.3 \pm 10.8$  % of total carbon (Table 1). Among the surface layers (0–5 cm), the highest amounts of carbon were found in Enisey Gulf (36.4 %), followed by Gyda Yurivey Gulf (27.4 %, 0–5 cm). More moderate amounts of carbon were found in Yavay Cape (13.7 %) and Haranasale-2 Cape (12.3 and 7.5 %). These amounts are similar to total carbon contents previously reported in tundra Gelic gleysols (Dziadowiec et al., 1994). By contrast, Belyi Island was the location with the lowest carbon content in surface soil (5.7 %).

In Enisey Gulf, the high amounts of carbon in the surface decreased substantially at subsurface layers (from 36.4 % at 0–5 cm to 18.0 % at 5–9 cm), whereas in Haranasale Cape carbon content either remained stable (7.5 % at 0–5 cm to 6.8 % at 5–11 cm,

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site1) or even increased (12.3 % at 0–5 cm to 15.2 % at 5–10 cm, site 2). Such increase of carbon content in depth may indicate cryoturbation, i.e. vertical soil mixing due to freezing and thawing cycles, a common process in Cryosols (Bockheim and Tarnocai, 1998). Further deep in the same soil profile (at 20–30 cm), carbon content was almost  
5 inexistent (0.4 %).

The optical index E4/E6 decreases with the level of carbon conjugation, and hence, has been commonly used to assess the molecular complexity of bulk organic matter (Chen et al., 1977; Peacock et al., 2014; Summers et al., 1987) and to infer the degree of humification of soil HAs (Hugelius et al., 2012; Ikeya and Watanabe, 2003; Kumada, 1987). The lowest value was found in the mineral layer of Haranasale-2 (Table 1). This is in accordance with the general finding that SOM in mineral horizons has a higher degree of humification than that in organic horizons (Hugelius et al., 2012). However, all samples exhibited E4/E6 values ranging between 0.15 and 0.86. Corresponding  $\Delta \log K$  values, derived from E4/E6 using the empirical relationship in Ikeya and Watanabe (2003), indicates that all soils correspond to the A-type, which is the highest lability class according to Kumada's (1987) classification system of HAs. Our values are also remarkably lower than those previously reported in arctic tundra soils (Dziadowiec et al., 1994).  
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### 3.2 Elemental composition of humic acids

The H/C ratio, commonly interpreted as an indicator of the aromatic degree of humic acids (Rice and McCarthy, 1991), had a very narrow range of variation (between 0.105 and 0.116, averaging  $0.109 \pm 0.004$ ,  $SD = 0.004$ ), indicating that all samples had practically the same level of molecular complexity. The highest values of these ratios were found in Enisey Gulf, Belyi Island and at the deep mineral layer of Haranasale-2 Cape  
20 (20–30 cm), indicating a slightly higher predominance of aliphatic structures in the humic acids of these soils. By contrast, the lowest H/C ratios were found in Gyda Yurivey and Yavay Cape (0.105 in both sites), hence, humics substances present a slightly more aromatic nature in these sites.  
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mainly temperature and moisture, are the main drivers of soil humic acids composition, and that chemical properties like C/N ratio and soil pH play only a secondary role (Zech et al., 1997).

In all sites,  $^{13}\text{C}$ -NMR data showed that there was a predominance of aliphatic carbons ( $68.85 \pm 3.60\%$ ) over aromatic ones ( $31.15 \pm 3.60\%$ , Table 4). This may reflect the predominance of non-ligneous vegetation as the main precursor of soil humic acids, similarly to what has been previously reported for arctic and antarctic soils (Abakumov et al., 2014; Calace et al., 1995).

Despite this eminent homogeneity among sites, some patterns of C structural diversity could be observed. As shown by the standard deviation of all measured  $^{13}\text{C}$ -NMR spectra (Figs. 4 and 5), the highest variability in the signal intensity was found in the resonance areas between 50–70 ppm and 32–27 ppm, both of which contain eminently aliphatic molecules. This indicates that aromatic compounds are more stable both regionally and along soil profiles, whereas aliphatic molecules are more variable. This may be due to the fact that this fraction is considered to be more labile and biogeochemically reactive. Among the aromatic species, the highest signal was found at 170 ppm, related to carboxyl-C.

The comparison of the surface soil samples provides an idea of the regional diversity of SOM carbon structures.  $^{13}\text{C}$ -NMR spectra (Fig. 4) show that in Enisey Gulf and Belyi Island the carbons in aromatic structures are less predominant than in the rest of sites. By contrast, their signal at 70 ppm was markedly higher, indicating an increase of CH units bound to oxygen. On the other hand, the Haranasale-1 site presents a distinctive signature, consisting in a high methoxyl C peak at 55 ppm. Finally, in the region between 0 to 50 ppm, indicating alkyl carbon structures, are higher in Enisey Gulf, Belyi Island and Haranasale-2 sites.

In Enisey Gulf and in the two Haranasale sites, some patterns could be observed along soil profiles. In the three sites it could be observed that the signal intensity for aromatic carbons (135–150 ppm) presented minor changes (Fig. 5). This has been found to be typical of initial stages of the humification processes (Zech et al., 1997),

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and hence, may indicate a low humification degree due to the low temperatures. However, some variation could be observed in the aliphatic regions. Interestingly, every site presented different patterns. In Haranasale-1 site, the subsurface soil layer exhibited a higher signal intensity at the O-alkyl C peak at 70 ppm, whereas such signal remained constant at Haranasale-2, and increased in Enisey Gulf. On the other hand, the signal at the methoxyl C peak (55 ppm) remained eminently constant in Haranasale-1 and Enisey Gulf, whereas in Haranasale 2 there was a clear decrease in depth. Finally, in the region of 0–50 ppm, the signal at the surface and subsurface layers remained stable in the three sites. However, in Haranasale-2 site, where a third depth was sampled (20–30 cm), it can be seen that in this mineral layer there was an increase of the signal intensity, indicating a higher prevalence of simple carbon bonds.

### 3.4 Total mineralisable carbon

The total carbon content of soils was found to be positively correlated with the total mineralisable carbon, explaining nearly 58 % of its variability ( $r^2 = 0.576$ ,  $p$  value = 0.01,  $df = 8$ ,  $F = 10.86$ ). Accordingly, the highest mineralization rates were found in Yavay Cape ( $2.34 \text{ mg C g soil}^{-1} \text{ day}^{-1}$ ) and in Enisey Gulf ( $2.32$  and  $2.19 \text{ mg C g soil}^{-1} \text{ day}^{-1}$  at 0–5 and 5–9 cm, respectively; Table 1). Among the surface soil samples, the minimal biodegradability was found in Belyi Island ( $1.57 \text{ mg C g soil}^{-1} \text{ day}^{-1}$ ). In the three sites where different depths were measured, located at Enisey Gulf and Haranasale Cape, the mineralization rates decreased with depth, and were minimal in the mineral deep horizon (20–30 cm) of the Hystic Gleysol in Harnasale ( $0.58 \text{ mg C g soil}^{-1} \text{ day}^{-1}$ ).

In order to better understand the relationship between SOM biodegradability and its chemical quality, a multivariate linear regression analysis was performed, introducing the variables related to bulk SOM properties (total carbon content, pH, E4/E6 ratio), as well as those related specifically with the humic acids (elemental composition and the carbon structures revealed by  $^{13}\text{C}$ -NMR spectra). Using the best subset selection procedure, it was found that these qualitative variables did not significantly improve the

predictability provided by the univariate linear regression with total carbon content (the inclusion of new variables to the model increased the  $C_p$ ).

These results emphasize that the biodegradability of SOM is not controlled by the characteristics of humic acids, most probably due to a high labile character in all sites.

- 5 This is in accordance with findings that SOM biodegradability in Cryosols is mainly limited by environmental conditions (freezing and overmoisture) rather than by chemical conditions (Weintraub and Schimel, 2003).

### 3.5 Multivariate analysis of soil organic matter properties

10 An NMDS analysis provided further insight about the spatial distribution of the C structural diversity of humic acids in the Yamal region, and its relationship with its elemental composition and bulk SOM properties. An NMDS analysis (fit-based  $R^2 = 0.999$ , Non-metric fit  $R^2 = 1$ , Fig. 6) performed on the  $^{13}\text{C}$ -NMR groups, showed that a first axis of variation defined a gradient from aromatic (Haranasale-2 and Gyda Yurivey Gulf sites) to aliphatic carbon predominance (Belyi Island, Enisey Gulf and Haranasale-1 sites).

15 An additional vector fitting analysis (variables significant at  $p < 0.01$ ) onto the NMDS ordination, revealed that the secondary axis created an oxygenation gradient between C% and H% on the positive site ( $r^2 = 0.7473$ ,  $p < 0.01$  and  $r^2 = 0.7717$ ,  $p < 0.01$ , respectively), and O% and O/C% on the negative site ( $r^2 = 0.8135$ ,  $p < 0.01$  and  $r^2 = 0.7783$ ,  $p < 0.01$ , respectively). According to that, the humic acids of Yavay Cape, 20 and upper layers of Enisey Gulf and Haranasale-2 are related to low levels of oxygenation, whereas those of Haranasale-1 and deep layer of Haranasale-2 appear with higher levels of oxygenation. Furthermore, the positive secondary axis grouped sites with higher pH ( $r^2 = 0.6074$ ,  $p < 0.05$ ) and total mineralisable carbon ( $r^2 = 0.6655$ ,  $p < 0.05$ ). Interestingly, the N elemental composition and C/N ratio of humic acids 25 where not significantly correlated within the NMDS ordination. Similarly, the optical index E4/E6 and the total carbon content were found not to be related with the distribution of carbon structures defined by  $^{13}\text{C}$ -NMR spectra.

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## 4 Conclusions

This study presents, for the first time, a detailed characterization of the humic acids of soils from western Siberia. Moreover, it expands the regional coverage of recent local scale studies of SOM lability in permafrost soils in periglacial environments (Dutta et al., 2006; Weintraub and Schimel, 2003). This may contribute to better depict the regional variability of humic acids characteristics across the arctic region.

Our results revealed little spatial variability in the characteristics of soil organic matter and humic acids across the Yamal region. This reflects a homogeneity of the climatic conditions, as well as of the precursor materials, due to a homogeneous vegetation cover across the studied region. This is in line with previous studies which have identified temperature, water regime and vegetation as the main drivers of humification (Zech et al., 1997). Also, there was little differentiation along soil profiles. The predominance of aliphatic carbon structures in depth indicates that soils remain at early stages of the humification process. This phenomenon has also been observed in other polar areas due to the effects of permafrost, where the low temperatures hinder the microbial processes responsible for the humification and decomposition of the precursor organic materials.

Our results also showed that the bioavailability of soil organic matter is mainly related to the total quantity of organic carbon, but not so much to its quality (i.e. elemental composition and carbon chemical structure). This reveals that most of the stored carbon is highly labile, and therefore the limitation for bacterial utilization is not its quality but its quantity. These results further emphasize the potential sensitivity of these soils towards increasing climatic temperatures, and the rapid mineralization of organic matter that they may undergo.

Overall, we conclude that soils of western Siberia may play a key role in the global warming. Therefore, more regionally intensive studies in these regions are required to better constrain the predictions about the consequences of global warming.

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**Table 4.** Fraction of aromatic and aliphatic carbons, and maximal signal intensity at the three ppm of maximal variability within the data set.

Site	Depth (cm)	Aromatic	Aliphatic	27 ppm	55 ppm	70 ppm
Yavay	0–5	33.22	66.78	0.10	0.07	0.10
Gyda Yuribey	0–10	33.90	66.10	0.12	0.09	0.08
Enisey Gulf	0–2	25.59	74.41	0.13	0.06	0.12
Enisey Gulf	5–9	28.55	71.45	0.12	0.08	0.10
Haranasale-1	0–5	32.44	67.56	0.14	0.07	0.08
Haranasale-1	5–12	29.11	70.89	0.13	0.08	0.10
Beliy Island	0–10	25.71	74.29	0.14	0.07	0.11
Haranasale-2	0–5	34.48	65.52	0.09	0.14	0.09
Haranasale-2	5–10	35.11	64.89	0.10	0.12	0.08
Haranasale-2	20–30	33.43	66.57	0.13	0.09	0.08



**Figure 1.** Regional distribution of study sites in the Gydan peninsula. 1 – Haranasale cape, 2 – Yavay cape, 3 – Mamont cape (Gyda Yuribey), 4 – Enisey gulf, 5 – Belyi Island.

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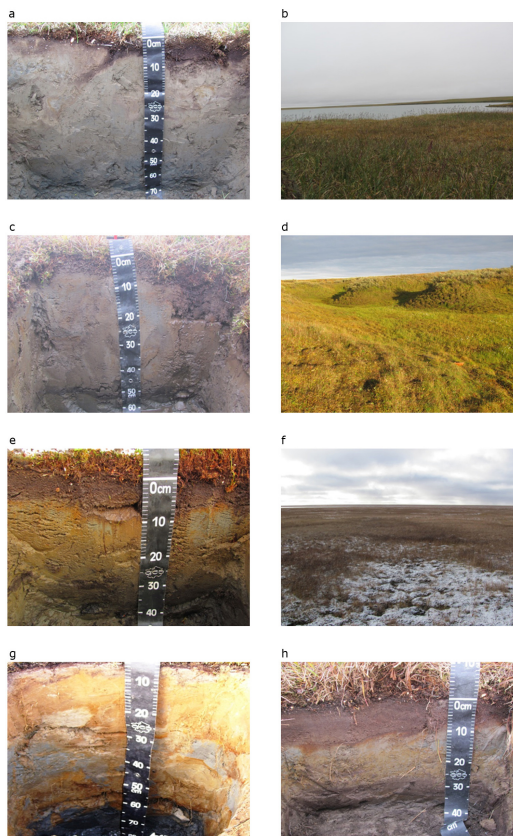
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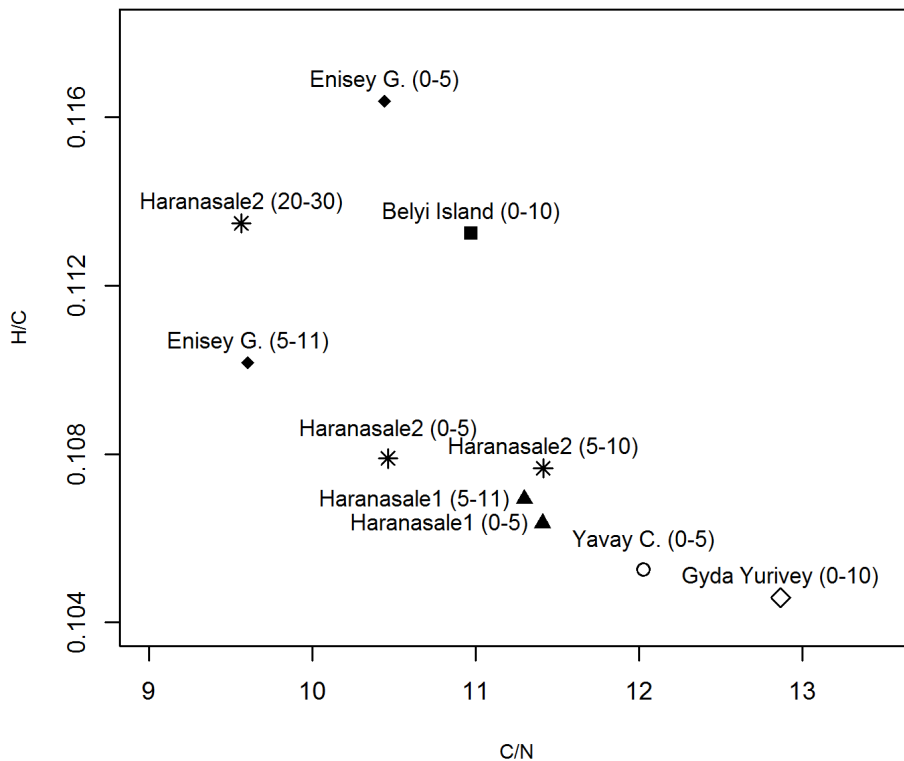
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**Figure 2.** Examples of soils and environments analyzed in this study. Yavay 1 (**a** and **b**), Gyda Yuribey (**c** and **d**), Enisey Gulf, (**e** and **f**), Belyi Island (**g**), Haranasale (**h**).



**Figure 3.** H/C vs. C/N diagram.

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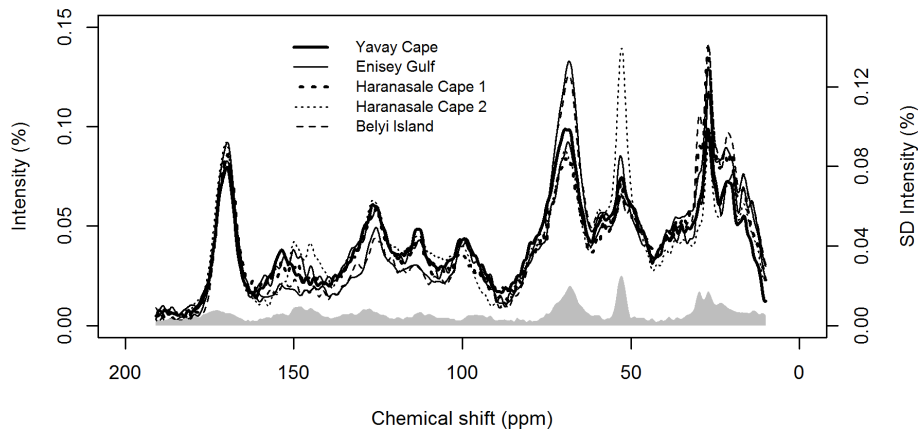
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**Figure 4.**  $^{13}\text{C}$ -NMR spectra of surface soil samples. For reference, the gray spectra corresponds to the standard deviation of all  $^{13}\text{C}$ -NMR spectra.

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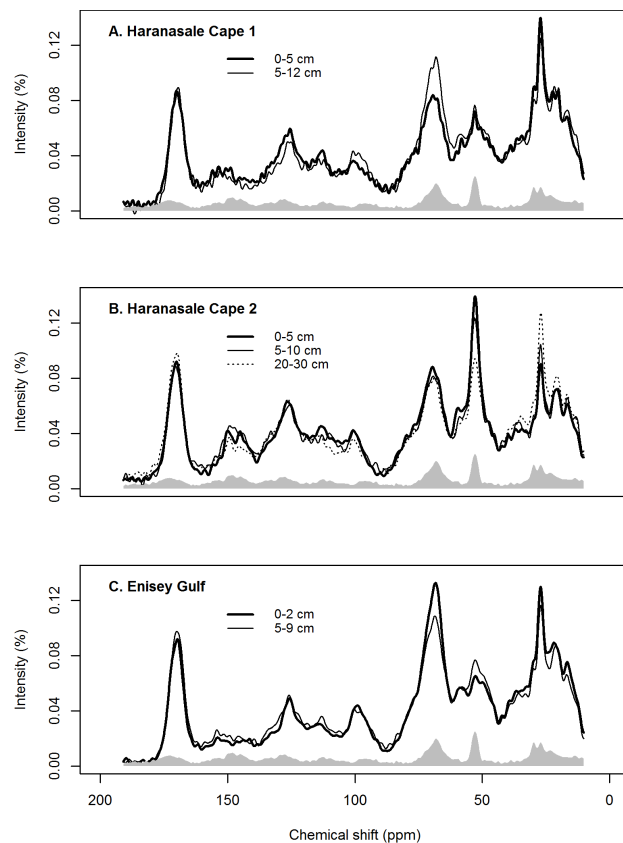
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**Figure 5.**  $^{13}\text{C}$ -NMR spectra in soil profiles. For reference, the gray spectra corresponds to the standard deviation of all  $^{13}\text{C}$ -NMR spectra.

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