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Title: Soil organic carbon depletion and degradation in surface soil after long-term non-growing season warming in High Arctic Svalbard

Article Type: Research Paper

Keywords: snow fence; NMR; carbon loss; decomposition; anthropogenic C emission feedback; tundra

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Abstract: Arctic tundra active-layer soils are at risk of soil organic carbon (SOC) depletion and degradation upon global climate warming because they are in a stage of relatively early decomposition. Non-growing season (NGS) warming is particularly pronounced, and observed increases of CO2 emissions during experimentally warmed NGSs give concern for great SOC losses to the atmosphere. Here, we used snow fences in Arctic Spitsbergen dwarf shrub tundra to simulate 1.86 °C NGS warming for 9 consecutive years, while growing season temperatures remained unchanged. In the snow fence treatment, the 4-11cm thick A-horizon had a 2% lower SOC concentration and a 0.48 kg C m-2 smaller pool size than the controls, indicating SOC pool depletion. The snow fence treatment's A-horizon's alkyl/ O-alkyl ratio was also significantly increased, indicating an advance of SOC degradation. The underlying 5cm of B/C-horizon did not show these effects. Our results support the hypothesis that SOC depletion and degradation are connected to the long-term transience of observed ecosystem respiration (ER) increases upon soil warming. We suggest that the bulk of warming induced ER increases may originate from surface and not deep active layer or permafrost horizons. The observed losses of SOC might be significant for the ecosystem in question, but are in magnitude comparatively small relative to anthropogenic greenhouse gas enrichment of the atmosphere. We conclude that a positive feedback of carbon losses from surface soils of Arctic dwarf shrub tundra to anthropogenic forcing will be minor, but not negligible.

Response to Reviewers: Dear Dr. Jay Gan, Reviewer #2 and Reviewer #5,
We are very thankful that you took your time to review our manuscript "Soil organic carbon depletion and degradation in surface soil after long-term non-growing season warming in High Arctic Svalbard" and happy that you only have minor revision suggestions. Please see below our point-by-point replies (in red italics) to all issues that you raised. We think that all comments are warranted and tried to incorporate them into

the manuscript as good as we could. For any further comments or questions, please feel free to contact us anytime.
All the best,
On behalf of my colleagues,
Philipp Semenchuk

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We are glad that we managed to write a clear and easy to understand story. Thanks for acknowledging that!
We are aware that longer term (e.g. after 20 or more years of experimental warming) and spatially more spread studies (e.g. samples from snow fence sites across the Arctic) would be beneficial to validate our findings, as it would be beneficial for every study based on single field experiments. This is already mentioned in the fourth paragraph of the discussion. It's a question of money and time, since the chemical analyses done here are expensive and time consuming. We neither have the data nor the budget for such an enhancement of this study and need to keep it as it is now.
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As a slight critic, the presented results actually originate from an experiment already described in Semenchuk (2016): "Long term experimentally deepened snow decreases growing season respiration in low and high arctic tundra ecosystem". As a consequence some relevant figures and results about ecosystem respiration are located there and it lessens a bit the experience not to get them directly.
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Title page

Title

Soil organic carbon depletion and degradation in surface soil after long-term non-growing season warming in High Arctic Svalbard

Author names

Philipp R. Semenchuk[1, 2], Eveline J. Krab[2, 3], Mattias Hedenström[4], Carly A. Phillips[5], Francisco J. Ancin-Murguzur[1], Elisabeth J. Cooper[1]

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Highlights

- Soil was warmed *in situ* for nine consecutive non-growing seasons (NGS) in Svalbard
- NGS warming depleted soil organic carbon (SOC) pool of the soil's shallow A-horizon
- NGS warming transitioned the A-horizon SOC to an advanced state of decomposition
- The underlying B/C-horizon's SOC pool and state was not affected
- NGS warming mineralizes more C in shallow than in deep soil

Abstract

Arctic tundra active-layer soils are at risk of soil organic carbon (SOC) depletion and degradation upon global climate warming because they are in a stage of relatively early decomposition. Non-growing season (NGS) warming is particularly pronounced, and observed increases of CO_2 emissions during experimentally warmed NGSs give concern for great SOC losses to the atmosphere. Here, we used snow fences in Arctic Spitsbergen dwarf shrub tundra to simulate 1.86 °C NGS warming for 9 consecutive years, while growing season temperatures remained unchanged. In the snow fence treatment, the 4-11cm thick A-horizon had a 2% lower SOC concentration and a 0.48 kg C m^{-2} smaller pool size than the controls, indicating SOC pool depletion. The snow fence treatment's A-horizon's alkyl/ O-alkyl ratio was also significantly increased, indicating an advance of SOC degradation. The underlying 5 cm of B/C-horizon did not show these effects. Our results support the hypothesis that SOC depletion and degradation are connected to the long-term transience of observed ecosystem respiration (ER) increases upon soil warming. We suggest that the bulk of warming induced ER increases may originate from surface and not deep active layer or permafrost horizons. The observed losses of SOC might be significant for the ecosystem in question, but are in magnitude comparatively small relative to anthropogenic greenhouse gas enrichment of the atmosphere. We conclude that a positive feedback of carbon losses from surface soils of Arctic dwarf shrub tundra to anthropogenic forcing will be minor, but not negligible.

Key words

snow fence; NMR; carbon loss; decomposition; anthropogenic C emission feedback; tundra

1. Introduction

Temperature is one of the main limiting factors for decomposition in Arctic soils (Wallenstein et al., 2009), leading to vast soil organic carbon (SOC) pools exceeding Earth's atmosphere's C stock (Hugelius et al., 2014; Tarnocai et al., 2009). In the face of climate warming, temperature limitations on decomposition processes might be alleviated, putting the biologically degradable part of this SOC pool at risk of being released to the atmosphere (Kleber, 2010; Schmidt et al., 2011). In Arctic regions, climate warming is especially pronounced during the non-growing season (NGS) (Stocker et al., 2014). As the NGS is the predominant part of the year, changes in its climate can have a disproportionally large effect on decomposition processes: relatively low decomposer activities at low temperatures can be offset by the long duration of the NGS and lead to long-term SOC loss. Soil organic C in the Arctic dwarf shrub tundra's active layer consists of a large proportion of readily decomposable compounds (Pautler et al., 2010; Pedersen et al., 2011; Sjögersten et al., 2003), and here we test if long-term *in situ* NGS warming could not only have an effect on its SOC pool size, but also on its bulk chemical composition.

Soils from cold dominated ecosystems appear to be in early stages of decomposition and at risk for rapid SOC loss with increasing temperature. Warming may specifically accelerate the degradation of readily decomposable compounds and thereby progress its decomposition stage. As an indicator for the relative degree of a given soil's SOC decomposition stage, the alkyl/O-alkyl ratio has been used (Feng and Simpson, 2008; Pautler et al., 2010; Pedersen et al., 2011; Simpson et al., 2008; Sjögersten et al., 2003). For instance, Sjögersten *et al.* (2003) observed higher alkyl/ O-alkyl ratios in more decomposed forest soils as compared to soils in less decomposed stages from nearby tundra soils in northern Scandinavia~~,~~for which they attributed to higher decomposer activities in forest soils due to higher soil temperatures in the more sheltered microclimate. Further, an extreme-event induced active layer detachment in northern Canada where sub-surface active layer soil was exposed to air temperatures via soil movements after extensive rain-fall accelerated SOC decomposition and increased its alkyl/ O-alkyl ratio (Pautler et al., 2010). Similar effects can be expected in response to NGS warming through climate change of Arctic dwarf shrub tundra ecosystems.

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The effects of warming on SOC pool depletion and its alkyl/O-alkyl ratio can be expected to be strongest in surface soil horizons of the active layer for several reasons. Arctic and alpine surface soils are reported to be richer in O-alkyl carbon (C) than deeper soil horizons, i.e. the alkyl/O-alkyl ratio increases with depth, indicating that the stage of decomposition advances with depth, an effect already visible in the upper few cm of soil profiles (Pedersen et al., 2011; Sjögersten et al., 2003). Further, environmental controls in deeper horizons may stabilize otherwise chemically readily decomposable compounds from microbial decomposition by e.g. sorption to the mineral phase (Kawahigashi et al., 2006; Kleber, 2010; Schmidt et al., 2011; Trumbore, 2009), which becomes more prominent in deeper horizons with a higher mineral proportion. Hence, total loss of SOC in deeper horizons upon warming could be lower than in surface near horizons. Increases in ecosystem respiration (ER) by experimental NGS warming in the Arctic (Björkman et al., 2010; Morgner et al., 2010; Nobrega and Grogan, 2007; Schimel et al., 2004; Semenchuk et al., 2016a; Webb et al., 2016) may thus be primarily (but not exclusively) driven by degradation and depletion of SOC substrates from surface rather than deep soil or thawed permafrost (cf. Schuur *et al.*, 2009; Natali *et al.*, 2014). This is supported by recent studies finding significantly higher soil CO_2 fluxes and stronger responses to warming in surface horizons up to 10 cm depth than in deeper soil horizons (Hicks Pries et al., 2017; Lee et al., 2010).

In the Arctic, significant ER increases have been shown to respond directly to *in situ* NGS warming in a variety of tundra ecosystems (Björkman et al., 2010; Morgner et al., 2010; Natali et al., 2014; Nobrega and Grogan, 2007; Schimel et al., 2004; Semenchuk et al., 2016a; Webb et al., 2016). ~~(~~Semenchuk et al.~~,~~ (2016a) demonstrated that these effects were followed by decreased growing season ER after eight years of NGS warming in Svalbard dwarf shrub tundra. Similarly, ER responses to experimental continuous *in situ* soil warming are shown to be transient and decrease after a few years of continuous warming in a mid-latitude forest site (Melillo et al., 2002, 2017). One explanation for these effects suggests that soil warming accelerates decomposition processes and alters SOC pool size and composition; soil OC is transformed from a relatively early stage to a later stage of decomposition, and thus provides a less favourable substrate for respiring decomposing organisms

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(Kirschbaum, 2004; Eliasson et al., 2005; Bradford et al., 2008). Here, we use the opportunity to collect soil and verify that hypothesis within the experiment used by Semenchuk *et al.* (2016a).

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We test whether nine years of continuous *in situ* NGS warming (average 2 degrees warming within a snow manipulation treatment) of relatively C poor (3-25 % C) High Arctic dwarf shrub tundra surface soil (16 cm depth) (i) reduced SOC pool size and (ii) changed the SOC chemical composition towards a more advanced decomposition stage in a snow fence experiment in Adventdalen, Svalbard. In the same experiment, Semenchuk *et al.* (2016a) found that 8 years of continuous NGS warming and connected increased ER during the NGS lead to decreased growing season ER and suggested that changes in SOC pool size and composition could account for this. Based on these results, we test the following:

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1.1 Hypotheses

(1) Carbon content is depleted in NGS warmed surface soils, i.e. long-term increased NGS ER decreased the C pool.

(2) Carbon compound composition is altered in NGS warmed soils. More specifically, we expect the alkyl/ O-alkyl ratio to be higher in NGS warmed soils, i.e. that the relative degree of SOC decomposition is advanced.

(3) The effects from Hypotheses 1 and 2 are larger in the A-horizon than in the top 5 cm of the underlying, C poorer and mineral richer B/C-horizon, i.e. the combination of environmental conditions and initial SOC composition in the B/C-horizon render the bulk SOC there more resistant to warming.

2. Material and Methods

2.1 Site description (location, soil, vegetation, climate, seasonality)

The study site is on the southern (left) riverbank in Adventdalen, a large valley about 12 km east of Longyearbyen on Spitsbergen, Svalbard (78°10′N, 16°04′E) with continuous permafrost with an active layer thickness/ maximum thaw depth of about 75 to 90 cm at the study site (own data, Figure S2). The cryoturbated gelisol soils at the study site (Semenchuk et al., 2016a) are dominated by fluvial and aeolian sedimentation and consist of a relatively thin and C poor, dark brown A-horizon of about 2-11 cm thickness with about 15-25% C content and an underlying, grey, silty B/C-horizon with about 3-9% C content, which extends to the permafrost table (see Strebel *et al.*, 2010 and own data below).

Situated in the bioclimatic subzone C, the vegetation type is classified as Prostrate/ hemiprostrate dwarf-shrub tundra (CAVM Team, 2003), dominated by the dwarf shrubs *Cassiope tetragona*, *Dryas octopetala* and *Salix polaris*.

Average air temperature 2000 to 2011 at Longyearbyen airport, about 20 km west of the study site, during the approximate NGS months October to April were -9 °C and during the approximate growing season months 3.4 °C (www.eklima.no). Average snow depth in the control area was about 40 cm (own data, Figure S1).

Non-growing season (NGS) in this study is defined as the time of year when the soil surface is frozen, i.e. has a temperature below 0 °C as measured by temperature loggers employed at around 2 cm depth (see S3), and ranges in extreme cases from about early October until early May, depending on year, replicate plot and snow fence treatment (see below).

2.2 Experimental design

Eleven 1.5m high snow fences were erected in autumn 2006, i.e. 9 years before the present study was conducted. These created winter snow drifts of maximum 1.5 m depth, i.e. about 1.1 m deeper than average ambient snow depth of about 0.4 m (see S1), which declined in depth with distance from the fence. Foci areas behind the fences receiving about 0.7 m snow depth (see S1) were identified for soil sampling, here referred to as “snow fence treatment”. Each snow fence was associated with an unmanipulated control area with ambient snow conditions about 10-15 m away from each fence. The snow drifts’ shapes and extents were very similar each year (own observations) due to the prevailing easterly wind direction along the valley.

The snow fence treatment chosen for this study increased the average NGS surface soil temperature (i.e. when soil surface is frozen) by 1.86 °C as determined by continuous hourly temperature measurements (see S3). There was no diurnal variability within the snow fence treatment’s temperature effects, and the warming started a few weeks after the start of the NGS when the snowpack established (see S3). Soil moisture in the snow fence treatment was observed to be slightly increased at the beginning of the growing season only (Semenchuk et al., 2016a), but was neither changed in the A- nor B/C-Horizon at time of soil sampling for this study (see S4).

Please note that for the present study we sampled a part of a larger experimental setup. Samples for this study were taken from areas behind snow fences that in other studies from the same site are referred to as “medium” snow or similar, in which snow depth was about 0.7 m, as opposed to “deep” snow, in which snow depth was about 1.5 m (Cooper et al., 2011; Rumpf et al., 2014; Semenchuk et al., 2016a, 2015, 2013). The reason why we did not use the area with the maximum or deep snow increase was to preserve that relatively small area for future research and not disturb it with invasive sampling.

2.3 Soil sampling procedure

On July 23rd 2015, 3 soil cores between 0.5 ~~to~~ and 1 m apart were sampled with a soil corer with 2 cm diameter (3.14 cm^{-2} area) in each plot and treatment (snow fence and control). To exclude potentially confounding vegetation effects on soil parameters in question for this study, sampling locations were chosen where *Salix polaris*, a dominant dwarf shrub across the study site, was the dominant species.

The brown A-horizon of each core was separated visually from the underlying grey B/C-horizon in the field, its thickness/ length measured, and each horizon of the core triplets combined in one plastic bag. Before bagging, above ground litter (O-horizon) and plants were coarsely removed from above the A-horizon. The upper 5 cm of the B/C-horizon directly under the A-horizon of each core were sampled and also combined. The maximum sampled A-horizon thickness was 11 cm, i.e. the deepest B/C-horizon sample was between 11 and 16 cm depth, far above the permafrost table which in our study site is at about 75 to 90 cm depth (see S2).

2.4 Soil treatment prior to chemical analyses

After collection in the field, soil samples were kept at 4 °C for five days in Longyearbyen, Svalbard, then transported to Abisko, Sweden, and processed within two days while being kept at 4 °C. Remaining above ground plant material, roots and stones of each sample were removed during 3 minutes per sample, the remaining soil mass homogenized, weighed and then directly oven dried at 70 °C for 48 h. The dried samples were then weighed again and ground in a ball mill to a fine powder and transported to Umea, Sweden, for NMR analyses and aliquots sent to Copenhagen, Denmark, for elemental analysis (see below).

Based on previously published material, we expected the total Fe (iron) content of the soil to be relatively high (Ottesen et al., 2010) with simultaneously low C content (Moni et al., 2015; Strebel et al., 2010) leading to a C:Fe ratio < 6 . Such high concentration of Fe-associated paramagnetic compounds has been shown to have a strong adverse effect on the quality of NMR spectra (Schilling and Cooper, 2004; Schmidt et al., 1997). After initial trials, this turned out to be only the case for the B/C-horizon samples, since NMR spectra of the A-horizon were of sufficiently good quality.

Therefore, we demineralized the B/C-horizon following the procedure in Baldock *et al.* (2001). We washed the soil with hydrofluoric acid (HF treatment) to remove paramagnetic and mineral compounds and thus increase the C content of the remaining soil leading to improved NMR spectra (Gélinas et al., 2001; Schilling and Cooper, 2004; Schmidt et al., 1997). In short we exposed 1 g of each sample for 12 h in 30 mL of 10% HF in 1N HCl solution, removed the supernatant and exposed the remaining pellet to fresh HF/ HCl solution twice more, then washed 3 times with water and freeze dried the pellet and used it for further analyses.

In the following, data based on NMR spectroscopy (spectra, integrals, and alkyl/O-alkyl ratio) are from HF-treated B/C-horizon samples, while data based on bulk soil parameters (bulk density, C concentration and pool size) are from untreated B/C-horizons. The A-horizon was always untreated.

2.5 Soil ~~carbon~~ C concentrations

To measure soil ~~carbon~~ C concentrations (%~~C~~), we weighed soils into tin capsules and quantified total C for each sample on an Isoprime isotope ratio mass spectrometer coupled to a Eurovector CN elemental analyser.

2.6 Bulk density

Bulk density of dry soil without roots or stones was calculated by dividing the dry weight of each full sample (which consisted~~s~~ of three combined, individual soil cores) with its volume. The samples' volumes were calculated by multiplying the average depth of all three cores with three times the area of the soil corer (9.42 cm^{-2}).

2.7 Soil ~~carbon~~ C pool size

Soil ~~carbon~~ C pool size per area was calculated by multiplying each full sample's C concentration/ fraction with its dry weight, divided by three times the area of the soil corer (9.42 cm^{-2}), in unit kg C m^{-2}.

2.8 Solid state ^{13}C CP/MAS NMR spectroscopy

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Approximately 100 mg of each soil sample was loaded into a 4 mm ZrO_2 rotor with a KEL-F cap. ^{13}C Cross-Polarization Magic Angle Spinning (CP-MAS) experiments were performed on a Bruker 500 MHz Avance III spectrometer operating at a ^{13}C frequency of 125.75 MHz (Bruker Biospin, Germany). Spinning rate was set to 7 kHz, the contact-time to 1 ms and the sweep-width to 250 ppm. 4096 scans were recorded with a relaxation delay of 2 s resulting in an experimental time of approximately 2.3 hours per sample. The FIDs were multiplied with a Gaussian function with LB = -10 Hz and GB = 0.01 prior to fourier transform. All spectra were calibrated using adamantane as an external reference. Processing was performed in Topspin 3.2 (Bruker Biospin, Germany).

2.9 Processing of raw NMR spectra/ separation into integrals

The processed spectra were transferred to a matrix with each spectrum as a row and the columns representing the intensity in each of the 4096 data-points that constitutes a CP-MAS spectrum using an in-house Matlab script. Each spectrum was normalized to a constant sum before statistical analysis.

Different chemical shift regions of the spectra, containing information about different functional groups (Preston et al., 1997) were also integrated and analysed as a separate data set. The regions were defined as follows: 0-50 ppm (alkyl); 50-60 ppm (methoxy/N-alkyl); 60-93 ppm (O-alkyl); 93-112 ppm (di-O-alkyl); 112-140 ppm (aromatic); 140-165 ppm (O-aromatic) and 165-190 ppm (carbonyl). The integrals were also normalized to a constant sum before statistical analysis.

2.10 Statistical analyses

All analyses were performed with R version 3.4.3 (R Core Team, 2017) and all packages mentioned below are R packages.

The effects of the snow fence treatment on A-horizon thickness, bulk density, ~~carbon~~ C concentration and pool size, integrated regions of the NMR spectra, and alkyl/ O-alkyl ratio were analysed with linear mixed effects models with plots as random effects using the “lme” package (Bates et al., 2015). The significance of the snow fence treatment was tested with a likelihood ratio test between the full model including treatment as predictor variable and the Null model, and a p-value of lower than 0.05 was considered to be significant. We then refitted the full models with restricted maximum likelihood estimation and extracted the 95% confidence intervals of the effect sizes and model term estimates with the “multcomp” package (Hothorn et al., 2008), which we present here together with the p-value from the likelihood ratio test. All model fits had no trend in Pearson residuals plotted against fitted values, and residuals were normally distributed (visual examination).

To test whether NMR spectra from the control and snow fence treated soils differ, partial least squares discriminant analysis (PLS-DA) was performed with the “caret” package (Kuhn, 2008). Classification was carried out based on the probability of the normalized NMR spectra belonging to either group (snow fence or control), having each spectrum assigned to the class with the highest associated probability. The smallest number of latent variables needed to reach 100% discrimination between snow fence treatment and controls were chosen. The first two loading variables from each model were taken to assess which shift regions were responsible for discrimination.

Data from A- and B/C-horizons were analysed separately, because the demineralization procedure of the B/C-horizon could potentially change a number of factors independent of horizon or the experimental *in situ* warming treatment (Dai & Johnson, 1999; Gélinas *et al.*, 2001; Keeler *et al.*, 2003; Schilling & Cooper, 2004; Rumpel *et al.*, 2006), making the two horizons incomparable, while qualitative treatment effect comparisons are valid (e.g. effect present or not).

3. Results

3.1 A-horizon thickness

Average A-horizon thickness across the study site's control area was 4.7 cm (95% CI: 3.7 – 5.7 cm) and 3.8 cm (95% CI: 2.8 – 4.8 cm) in the snow fence treatment, i.e. 0.9 cm lower than in the control area (95% CI: -1.7 - -0.03 cm; p = 0.0433).

3.2 Bulk density

Average bulk density across the study site's control area was 0.33 g cm^{-3} (95% CI: 0.24 – 0.41 g cm^{-3}) in the A-horizon, and 1.09 g cm^{-3} (95% CI: 0.94 – 1.24 g cm^{-3}) in the upper five cm of the B/C-horizon. The snow fence treatment influenced bulk density of neither A- nor B/C-horizon, i.e. no significant effects were found.

3.3 Soil ~~carbon~~ C concentrations (Figure 1 and Table 1)

Average C concentrations across the study site's control area were 16.6% (95% CI: 13.6 - 19.7%) in the A-horizon and 5.4% (95% CI: 4.3 - 6.6%) in the upper 5 cm of the untreated B/C-horizon (i.e. the soil aliquot which was not HF treated; Figure 1). Carbon concentrations in the HF treated B/C-horizon soils (i.e. the HF treated soil samples that we will refer to when describing and discussing data from the NMR analysis) were 14.2% (95% CI: 12.1 - 16.3%; data not shown).

The snow fence treatment influenced C concentrations of the A-, but not of the B/C-horizon. In the A-horizon, samples from the snow fence treatment had 2% lower (95% CI: -3.7 - -0.5%; p = 0.021; Table 1) C concentrations than samples from controls. In the untreated B/C-horizon, samples from the snow fence plots had no significantly different C concentration (95% CI: -0.1 to 2%; p = 0.111; Table 1). In the HF-treated B/C-horizon, samples from the snow fence plots also had no significantly different C concentration (95% CI: -0.4 to 4.1%; p = 0.073; Table 1).

3.4 Soil ~~carbon~~ C pools (Figure 1 and Table 1)

Average C pool sizes across the study site’s control area were 2.34 kg C m^{-2} (95% CI: 1.96 – 2.73 kg C m^{-2}) in the A-horizon and 2.92 kg C m^{-2} (95% CI: 2.54 – 3.3 kg C m^{-2}) in the upper five cm of the untreated B/C-horizon (i.e. the soil aliquot which was not HF treated; Figure 1).

The snow fence treatment influenced C pools of the A-, but not of the B/C-horizon. In the A-horizon, samples from the snow fence treatment had 0.48 kg C m^{-2} lower (95% CI: -0.9 - -0.07 kg C m^{-2}; p = 0.032; Table 1) C pool sizes than samples from controls. In the B/C-horizon, samples from the snow fence plots had no significantly different C pool sizes (95% CI: -0.42 to 0.53 kg C m^{-2}; p = 0.82; Table 1).

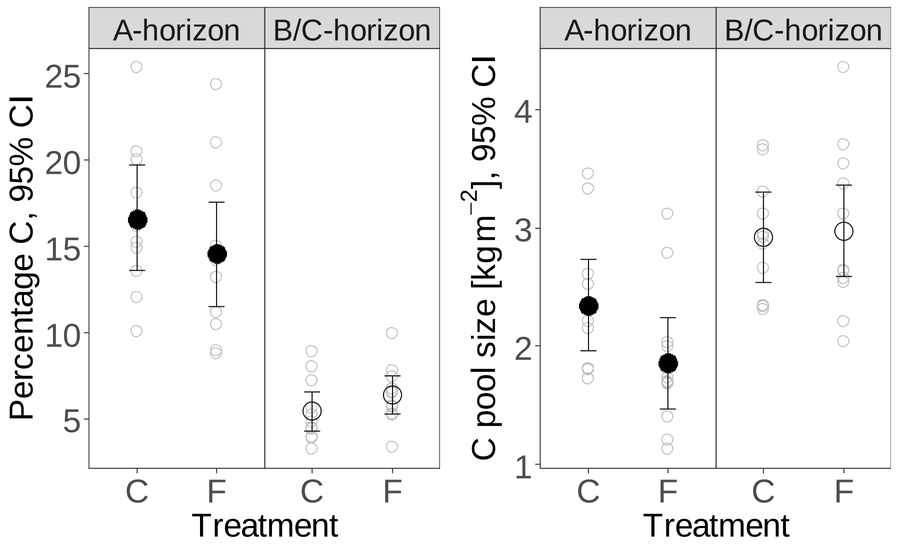


Figure 1: Carbon concentration (percentage C, left) and pool size (kg C m^{-2}, right) in A- and untreated (i.e. non-HF treated) B/C-horizons for each soil sample (raw data, grey open circles) and modelled with 95% confidence intervals (black circles). Solid black circles in the A-horizon data denote statistically significant differences between treatments (see Table 1); open black circles in the B/C-horizon data denote no statistically significant difference between treatments. Treatment C =

Control (ambient conditions, ca. 40 cm snow depth), F = Snow Fence (enhanced snow, ca. 70 cm snow depth). See Table 1 for statistical tests between treatments, also including the C concentration model for the HF-treated B/C-horizon.

Table 1: *Modelled mean effect sizes and 95% confidence intervals (CI) of the snow fence treatment on carbon concentration (%C) and carbon pool size (kg C m^{-2}) of A-, untreated B/C-, and HF-treated B/C-horizons (only for %C). The values show estimated differences of samples from the snow fence treatment compared to controls based on linear mixed effects models. P-values are based on a likelihood ratio test between the full and the Null model. Significant effect sizes are in bold ($p<0.05$ and CI not overlapping zero). Each line shows results of a separate model.*

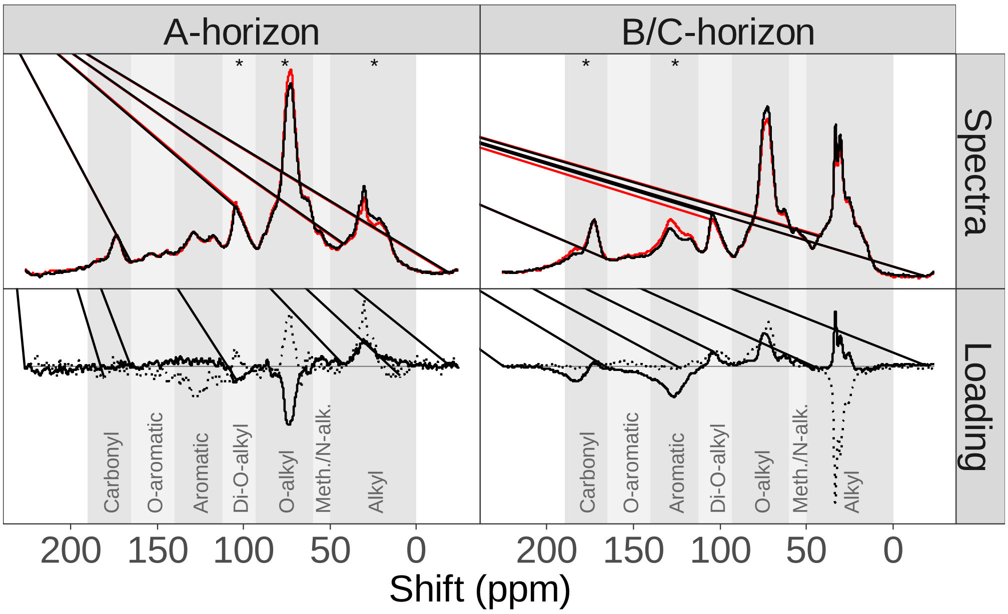
Response	*Horizon*	*Effect size*	*Lower 95% CI*	*Upper 95% CI*	*p-value*
%C	***A***	**-2.0563**	**-3.6623**	**-0.4503**	**0.0205**
%C	*B/C*	0.9525	-0.06051	1.96552	0.1113
%C	*B/C (HF-treated)*	1.8646	-0.4052	4.1344	0.0729
kg C m^{-2}	***A***	**-0.48285**	**-0.89685**	**-0.06886**	**0.03152**
kg C m^{-2}	*B/C*	0.05238	-0.42044	0.52520	0.8201

3.5 Spectral analysis (Figure 2)

PLS-DA models on samples from the snow fence treatment and controls showed a 100% discrimination using 3 and 5 latent variables for the A- and HF-treated B/C-horizons, respectively, indicating a significant structural difference between treatments in both horizons. The chemical shift regions causing the differences were visible in the spectra and the models’ first and second loading components (Figure 2). In other words, regions with the highest model loadings are the regions with the highest importance to distinguish between the treatments.

In the A-horizon, clear differences between snow fence and control soil samples were visible in the O-alkyl and alkyl regions, as well as subtle differences in the aromatic region.

In the B/C-horizon, the effect of snow fence treatment differed compared to the A-horizon. Here we observed a decrease in the signal in the carbonyl and aromatic regions as well as an increase in O-alkyl and alkyl signals.



<u>Figure 2:</u> Top row: Mean NMR spectra for soil samples from snow fence plots (black line) and controls (red line) in A- (left column) and HF-treated B/C-horizons (right column). Bottom row: first (solid line) and second (dotted line) loading components of the associated PLS-DA models. Vertical grey bars denote integrated chemical shift regions as defined in Table 2, from left to right (with region boundaries): carbonyl (190-165), O-aromatic (165-140), aromatic (140-112), di-O-alkyl (112-93), O-alkyl (93-60), methoxy/ N-alkyl (60-50), alkyl (50-0). Small stars on top of the top row denote where statistically significant differences between treatments were found on individual integrated chemical shift regions (Table 2).

3.6 Integrated shift regions (Figure 3 and Table 2)

In the A-horizon, samples from the snow fence plots had a 2% higher (95% CI: 0.9 – 2.9%; p = 0.002) concentration of alkyl signals than samples from controls, while the concentrations of di-O-alkyl and O-alkyl signals were significantly lowered by 0.6% (95% CI: -0.9 - -0.2%; p = 0.007) and 2% (95% CI: -3.3 to -0.7%; p = 0.009), respectively. The other integral regions' contributions to the A-horizon spectra were not significantly different between the treatments. See Figure 3 and Table 2.

In the HF-treated B/C-horizon, samples from the snow fence plots had 2% (95% CI: -3.7 - -0.2; p = 0.035) and 0.7% (95% CI: -1.3 - -0.1%; p = 0.027) lower concentrations of aromatic and carbonyl signals, respectively, than samples from controls, while the O-alkyl signals had a non-significant trend towards 1.8% (95% CI: -0.9 – 4.6%; p = 0.186) concentrations. The other integral regions' contributions to the B/C-horizon spectra were not significantly different between the treatments. See Figure 3 and Table 2.

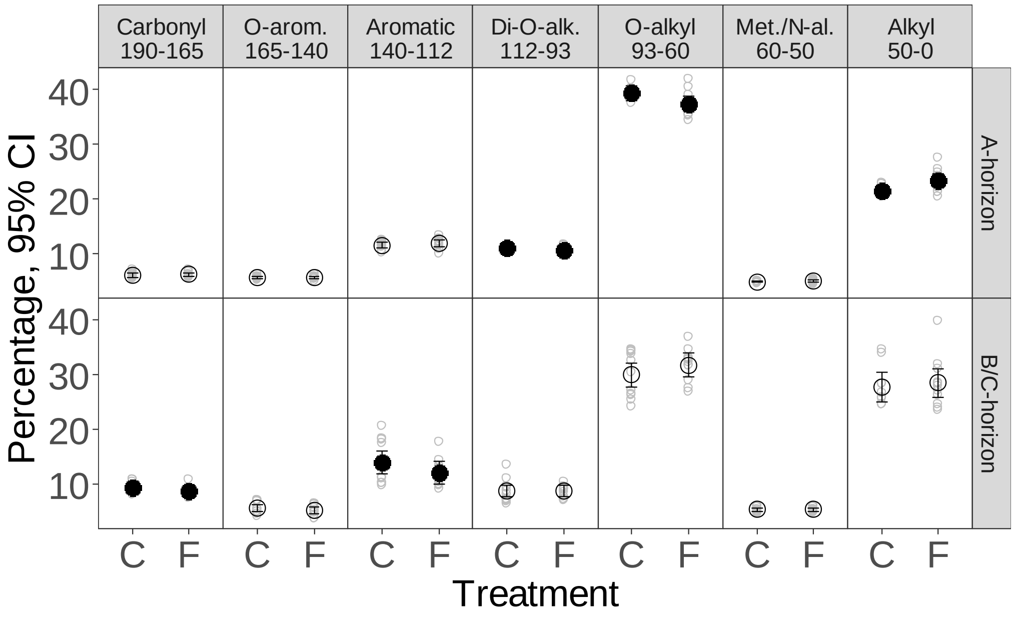


Figure 3: Relative contribution of integrated NMR spectra signal regions in A- and HF-treated B/C-horizons for each soil sample (raw data, grey open circles) and modelled with 95%

confidence intervals (black circles). Solid black circles denote statistically significant differences between treatments; open black circles denote no statistically significant difference between treatments. Treatment C = Control, F = Snow Fence. See Table 2 for statistical tests between treatments.

Table 2: *Modelled mean effect sizes and 95% confidence intervals (CI) of the snow fence treatment on relative contribution of integrated NMR spectra signal regions and alkyl/O-alkyl ratio of A- and HF-treated B/C-horizon samples. The values show estimated differences of samples from the snow fence plots compared to controls based on linear mixed effects models. Region denotes the ppm range chosen to define each integral. P-values are based on a likelihood ratio test between the full and the Null model, and cases where the full model is statistically significantly better than the Null model with* $p<0.05$ *are in bold. Each line shows results of a separate model.*

Horizon	*Integral*	*Region*	*Effect size*	*Lower 95 %CI*	*Upper 95% CI*	*p-value*
A	*Carbonyl*	*190-165*	0.000683	-0.0009593	0.0023253	0.3987
A	*O-aromatic*	*165-140*	0.0001435	-0.00281	0.003097	0.92
A	*Aromatic*	*140-112*	0.003705	-0.001253	0.008663	0.1437
A	***Di-O-alkyl***	***112-93***	**-0.005542**	**-0.009067**	**-0.002018**	**0.007272**
A	***O-alkyl***	***93-60***	**-0.019813**	**-0.032911**	**-0.006714**	**0.009047**
A	*Methoxy/ N-alkyl*	*60-50*	0.001589	-0.0006453	0.0038233	0.1522
A	***Alkyl***	***50-0***	**0.019235**	**0.009103**	**0.029367**	**0.002273**
A	***alkyl/ O-alkyl***	***ratio***	**0.085**	**0.03685**	**0.13316**	**0.003633**
B/C	***Carbonyl***	***190-165***	**-0.006956**	**-0.012728**	**-0.001184**	**0.02721**
B/C	*O-aromatic*	*165-140*	-0.0050147	-0.0104986	0.0004692	0.08004
B/C	***Aromatic***	***140-112***	**-0.019838**	**-0.037232**	**-0.002445**	**0.03473**
B/C	*Di-O-alkyl*	*112-93*	0.0006397	-0.0134508	0.0147301	0.9257
B/C	*O-alkyl*	*93-60*	0.018268	-0.009001	0.045538	0.1859

B/C	*Methoxy/ N-alkyl*	*60-50*	0.0007683	-0.0028917	0.0044283	0.6674
B/C	*Alkyl*	*50-0*	0.006997	-0.011176	0.025171	0.4351
B/C	*alkyl/ O-alkyl*	*ratio*	-0.03617	-0.17731	0.10497	0.6006

3.7 Alkyl/O-alkyl ratio (Figure 4 and Table 2)

Average alkyl/O-alkyl ratios across the study site's control area were 0.55 (95% CI: 0.5 - 0.59) in the A-horizon and 0.95 (95% CI: 0.82 - 1.1) in the HF-treated B/C-horizon. See Figure 4.

The snow fence treatment influenced the alkyl/O-alkyl ratio of the A-, but not of the B/C-horizon. In the A-horizon, samples from the snow fence plots had a 0.085 higher (95% CI: 0.037 - 0.133; p = 0.004) alkyl/O-alkyl ratio than controls. In the B/C-horizon, samples from the snow fence plots had no significantly different alkyl/O-alkyl ratio compared to controls (-0.036; 95% CI: -0.18 - 0.1; p = 0.6).

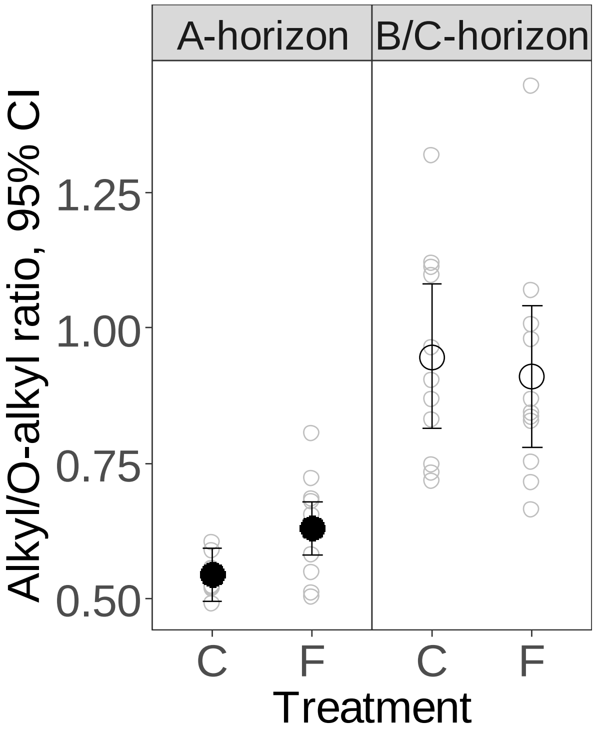


Figure 4: Alkyl/O-alkyl ratios in A- and HF-treated B/C-horizons for each soil sample (raw data, grey open circles) and modelled with 95% confidence intervals (black circles). Solid black circles denote statistically significant differences between treatments (only A-horizon); open black circles denote no statistically significant difference between treatments (only B/C-horizon). Treatment C = Control, F = Snow Fence. See Table 2 for statistical tests between treatments.

4. Discussion

We found that the A-horizon, i.e. the study site's C-richer upper soil layer (average 16.6% C and 4.7 cm thickness), of high Arctic tundra plots which were exposed to 9 years of experimental *in situ* NGS warming via snow fences, on average had a 2% lower C concentration, contained 0.48 kg less C m^{-2} (hypothesis 1) and was in a more advanced stage of decomposition (as indicated by a 0.09 higher alkyl/ O-alkyl ratio, hypothesis 2) compared to unmanipulated control plots. These effects were not observed in the first 5 cm of the underlying, C poorer B/C-horizon (average 5.4% C, hypothesis 3). Below we will discuss potential causes, effects and implications of our findings.

4.1 SOC loss through NGS warming only in A-horizon, not B/C-horizon (hypotheses 1 & 3)

The smaller SOC pool in the snow fence plots' A-Horizon compared to the controls is possibly caused by a temperature induced increase of decomposition and subsequent loss of SOC during the nine preceding warmed NGSs. Non-growing season ER was higher in snow fence treatments in this (Morgner et al., 2010; Semenchuk et al., 2016a) and other Arctic sites (Natali et al., 2014; Nobrega and Grogan, 2007; Schimel et al., 2004; Webb et al., 2016). Further, soil nutrient concentrations were higher in a deeper snow fence treatment with stronger temperature increase in this (Semenchuk *et al.*, 2015; Mörsdorf *et al.*, submitted) and another Arctic site (Schimel et al., 2004), and *Salix polaris'* (and other species') leaf nitrogen content was higher in this (Mörsdorf *et al.*, submitted) and another Arctic site (Welker et al., 2005), suggesting a stabilization of surplus nutrients from the soil. In sum, these findings strongly indicate increased activities of decomposing organisms during warmed NGSs in a variety of Arctic tundra ecosystems, which in the long run possibly lead to more mineralization of SOC than could be replenished by plants during the growing season through e.g. plant litter or root exudate inputs (Bradford et al., 2008; Eliasson et al., 2005; Kirschbaum, 2004).

Whether this SOC depletion is a transient phenomenon driven by fast cycling SOC pools as observed in e.g. permafrost soil incubations (e.g. Moni *et al.*, 2015) or plant litter mass loss studies (e.g. Demarco *et al.*, 2014) is uncertain. The observed decrease of growing season ER (Semenchuk et al., 2016a) and degradation of SOC to advanced stages of decomposition indicate that, under current conditions, further depletion might come to a halt. However, as suggested by ~~(~~Melillo et al.~~,~~ (2017), it is possible that the microbial communities in the snow fence plots are undergoing a phase of reorganization as response to the altered SOC pool size and quality, soil nutrient status, and temperature conditions. Once acclimated, further depletion and degradation of the remaining SOC pool could continue. Since these non-linear and possibly interactive responses cannot necessarily be generalized across spatial and temporal boundaries, further large-scale and long-term studies (e.g. time series) are warranted to enable us to project the presented findings on possible future climate scenarios.

Whether the findings of SOC depletion from this study are context specific can only be speculated without further long-term and orchestrated biome-wide studies, but a few available long-term studies (>8 years warming) do indicate context specificity. The growing season ER reduction after long-term NGS warming is also reported from Daring Lake, a low-Arctic site (Semenchuk et al., 2016a), and both reduction of long-term effects on ER and depletion of easily degradable SOC after long-term warming are reported from the Harvard Forest, a deciduous hard-wood forest (Bradford et al., 2008; Melillo et al., 2017). However, long-term warming did not result in reductions of growing season ER nor decline of NGS ER effects from a sub-Arctic peat site in Abisko (Dorrepaal et al., 2009). All mentioned studies are from different ecosystems, and taken together it seems that context specificity is given. Which factors are responsible for the contrasting results between these studies is unclear and motivates for further studies. However, the high C and low mineral content in the Abisko peat site might play important roles in determining long-term effects of warming on SOC pool size.

Vegetation related variables can partly be ruled out as explanatory factors for SOC pool size in this study. Firstly, soil was exclusively sampled under *Salix polaris*, which excludes potential confounding effects on SOC properties by sampling under different species. Second, neither species composition (Cooper *et al.*, submitted) nor *Salix polaris'* growth (Rumpf et al., 2014) in the snow fence treatment used here were significantly different from the controls. However, the aforementioned higher leaf nitrogen content in the snow fence plots (Mörsdorf *et al.*, submitted) might be forewarning increased performance of *Salix polaris* in the long run. Conversely, results from the same experiment but from a two times deeper snow fence treatment than used here (150 cm vs. 70 cm snow depth) found significantly less seasonal growth (Rumpf et al., 2014) and lower abundance of *Salix polaris* and most other vascular plant species (Cooper *et al.*, submitted). Whether these changes, caused by a more extreme snow depth increase, are representing the future state of the snow fence treatment used here is questionable. While the NGS warming and connected biogeochemical effects are stronger in the deeper snow fence treatment (Semenchuk et al., 2015; Mörsdorf et al., submitted), the negative effects on the vegetation there are possibly caused by an average 7 days later snow melt compared to the snow fence treatment used here (Semenchuk et al., 2016b, 2013) rather than by biogeochemical cascading effects.

NGS warming significantly lowered SOC concentrations in the A-horizon but not in the directly underlying 5 cm of B/C-horizon, a result similar to the findings of Melillo *et al.* (2017). We assume that B/C-horizon properties stabilize SOC, such that it cannot be fully mineralized or accessed by decomposing organisms. In our case especially sorption to mineral particles (Kawahigashi et al., 2006; Kleber, 2010; Schmidt et al., 2011; Trumbore, 2009), but also other environmental controls such as smaller soil pore size (as indicated by the B/C-horizon's higher bulk density) and particle aggregation might have rendered parts of its SOC pool inaccessible to decomposing organisms (Conant et al., 2011; Ekschmitt et al., 2008). Additionally, lower root density (own observation and Strebel *et al.* (2010)) and possibly different species composition of the B/C-horizon's rhizosphere (Iversen et al., 2015) could be responsible for the initially low SOC concentrations found in the B/C-horizon. This low SOC concentration in the deeper horizons might create microhabitats unsuitable for a bulk population of decomposing organisms capable of mineralizing significant fractions of the existing SOC pool (Ekschmitt et al., 2008; Schmidt et al., 2011).

Leaching of dissolved ~~carbon~~ C from the A-horizon by additional melt water production from the extra snow pack is conceivable. However, three qualitative observations lead to the assumption that this effect may be minor. First, the total melt water runoff from the large area above the experimental site by far outweighs the amounts produced by the additional snowpack. This runoff is unchanged by the experimental snow addition and hence the additional melt water might not have a significant impact. Second, a large part of the melt water produced by the deepened snow packs after the surrounding snow is gone seems to get diverted by the ice layer at the bottom of the snow pack (Semenchuk et al., 2013) and may not reach the soil until it reaches the edges of the plots. Third, the soil in this study site is frozen solid until a few days after melt out and any melt water flowing over it may only touch the vegetation and soil surface. All three points are based on own qualitative observations only and yield opportunities for further studies on potential artefacts of snow fences as experimental treatments in ecological studies.

4.2 Implications of SOC loss on ER and atmosphere

The loss of SOC from the A-horizon possibly explains the decline of growing season ER as reported by Semenchuk *et al.* (2016a) in the same experimental setup. Soil OC availability as substrate for heterotrophic organisms may be depleted by increased consumption below a threshold where steady state respiration can be maintained (Bradford et al., 2008; Eliasson et al., 2005; Kirschbaum, 2004). This phenomenon could be partly confounded with the aforementioned higher soil nutrient availability in NGS warmed plots (Schimel *et al.*, 2004; Semenchuk *et al.*, 2015; Mörsdorf *et al.*, submitted) resulting in ER responses independent of SOC loss. For instance, reduced nutrient limitations could reduce plant roots' foraging for nutrients and thereby reduce root exudate production and connected decomposer stimulation leading to some kind of "negative priming" effects (cf. Fontaine *et al.*, 2004; Hartley *et al.*, 2012). More studies to disentangle the possible mechanisms behind this are needed.

Our observation that SOC was only lost from the A-horizon (which with an average thickness of 4.7 cm lies well above the permafrost table at about 90 cm depth) allows the speculation that the increase of ER during warmed NGSs in this study site (Morgner et al., 2010; Semenchuk et al., 2016a) might primarily originate from the A-horizon, too. If this holds true, then the fact that growing season ER was reduced as a response to NGS warming (Semenchuk et al., 2016a) also suggests that

~~also~~ the growing season bulk ER originates from surface horizons, as has been shown in studies measuring CO_2 fluxes in different soil depths (Hicks Pries et al., 2017; Lee et al., 2010). While understudied to date, the implications of these thoughts are of importance to determine the relative contribution of CO_2 emissions from surface and deep soils, such as thawing permafrost. With surface horizons potentially being the primary source of ER derived CO_2 emissions from tundra ecosystems, then warming induced increases of C loss from thawing permafrost (Moni et al., 2015; Schuur et al., 2009) might be relatively minor compared to CO_2 emissions from surface soils during timescales relevant for the ongoing anthropogenic forcing (Stocker et al., 2014). In fact, Hicks Pries *et al.* (2017) found that about 80% of all soil respiration and about 90% of respiration response to 4 °C warming occurred in the upper 30 cm of temperate forest soil, and Lee *et al.* (2010) found that the upper 10 cm of upland tundra soil had ten times higher CO_2 fluxes that the underlying 20 cm horizon. In sum, these findings point towards a predominance of the more exposed surface soils as C sources to the atmosphere and warrant further studies.

A back of the envelope calculation scaling up the effect size from the C pool model to the total global area of Prostrate/hemiprostrate dwarf-shrub tundra (i.e. the vegetation type studied here, worldwide covering 140000 km^2, CAVM Team, (2003)) estimates a potential total loss of 67.6 Mt C or 248.1 Mt CO_2 equivalent upon global NGS warming from the 4.7 cm thick A-horizon only. This is in magnitude comparable with the annual CO_2 emissions of, for instance, Florida (Desai et al., 2017) or Egypt (EDGARv4.3.2, 2016), but with 0.69% an insignificant contribution to the still rising annual global anthropogenic CO_2 emissions of 36062 Mt in 2015 (EDGARv4.3.2, 2016).

4.3 Shifts in relative abundance of carbon compounds (hypothesis 2)

Carbon compound composition was clearly different in the snow fence treatment from the controls in both A- and B/C-horizons. In the A-horizon, the differences were mainly in the alkyl and O-alkyl groups leading to a higher alkyl/ O-alkyl ratio in the snow fence treatment. This indicates that NGS warming indeed transformed the A-horizon to a more advanced stage of decomposition (Feng

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and Simpson, 2008; Pautler et al., 2010; Pedersen et al., 2011; Simpson et al., 2008; Sjögersten et al., 2003). Together with the above discussed phenomenon of SOC loss, this supports the idea that the balance between SOC consumption and replenishment was significantly disturbed and could explain the observed decrease of growing-season ER (Semenchuk et al., 2016a).

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In the B/C-horizon, the main changes were observed in the aromatic regions, indicating a decrease of amino-acids (Simpson and Simpson, 2012). These compounds were possibly transformed to O-alkyl compounds, even though the increase of that group of compounds was not statistically significant (compare with Table 2). The reason for this discrepancy between A- and B/C-horizons is out of the scope of this study, however, may be rooted in selective sorption of O-alkyl compounds to mineral particles, forcing decomposing organisms to use aromatic compounds as substrates.

4.4 Conclusions

We found that 9 years of ca. 2 °C NGS warming reduced the A-horizon's C pool and degraded it to a more advanced stage of decomposition. Our results support the hypothesis that the transient nature of increased ER by soil warming as observed elsewhere is connected to excessive and selective consumption of SOC leading to a depletion of favourable substrates for decomposers. We further suggest that the NGS warming induced increases of ER in our study site primarily originate in the relatively shallow A-horizon and dominate total CO_2 emissions compared to deep soil or thawing permafrost. The estimated absolute expected loss over the whole global area of this ecosystem, however, is dwarfed by comparison with still rising global anthropogenic greenhouse gas emissions. We conclude that a positive feedback from surface soils of circumarctic dwarf shrub tundra communities to anthropogenic forcing might be minor during the timescales covered in this study, but due to their dynamic nature deserve a place in modelling studies.

5. Acknowledgements

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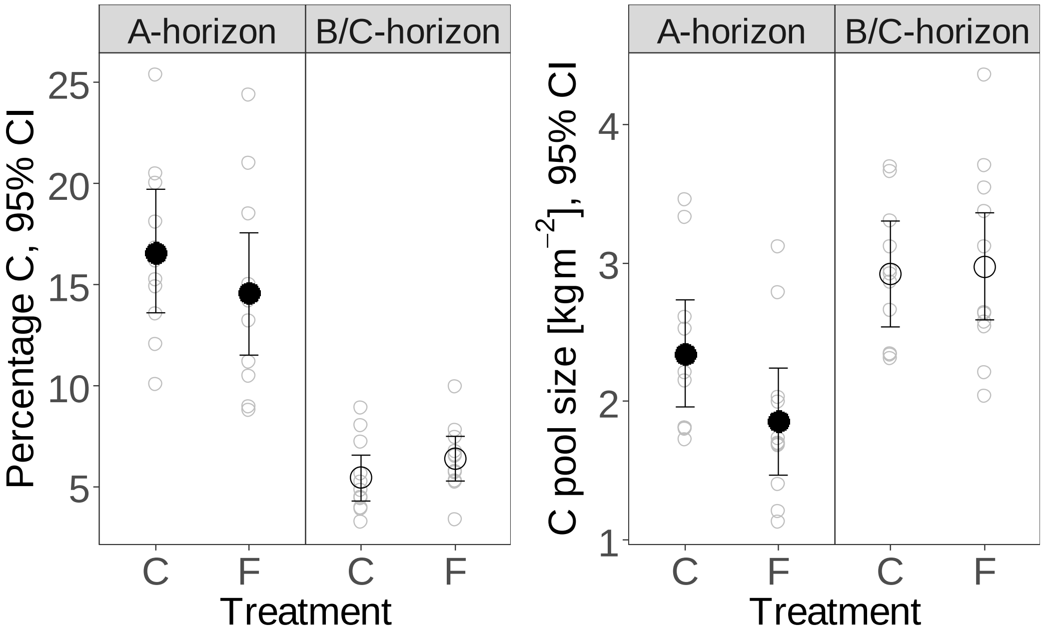
***Graphical Abstract**



***Highlights (for review : 3 to 5 bullet points (maximum 85 characters including spaces per bullet point)**

Highlights

- Soil was warmed *in situ* for nine consecutive non-growing seasons (NGS) in Svalbard
- NGS warming depleted soil organic carbon (SOC) pool of the soil's shallow A-horizon
- NGS warming transitioned the A-horizon SOC to an advanced state of decomposition
- The underlying B/C-horizon's SOC pool and state was not affected
- NGS warming mineralizes more C in shallow than in deep soil

Title page

Title

Soil organic carbon depletion and degradation in surface soil after long-term non-growing season warming in High Arctic Svalbard

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Abstract

Arctic tundra active-layer soils are at risk of soil organic carbon (SOC) depletion and degradation upon global climate warming because they are in a stage of relatively early decomposition. Non-growing season (NGS) warming is particularly pronounced, and observed increases of CO_2 emissions during experimentally warmed NGSs give concern for great SOC losses to the atmosphere. Here, we used snow fences in Arctic Spitsbergen dwarf shrub tundra to simulate 1.86 °C NGS warming for 9 consecutive years, while growing season temperatures remained unchanged. In the snow fence treatment, the 4-11cm thick A-horizon had a 2% lower SOC concentration and a 0.48 kg C m^{-2} smaller pool size than the controls, indicating SOC pool depletion. The snow fence treatment's A-horizon's alkyl/ O-alkyl ratio was also significantly increased, indicating an advance of SOC degradation. The underlying 5 cm of B/C-horizon did not show these effects. Our results support the hypothesis that SOC depletion and degradation are connected to the long-term transience of observed ecosystem respiration (ER) increases upon soil warming. We suggest that the bulk of warming induced ER increases may originate from surface and not deep active layer or permafrost horizons. The observed losses of SOC might be significant for the ecosystem in question, but are in magnitude comparatively small relative to anthropogenic greenhouse gas enrichment of the atmosphere. We conclude that a positive feedback of carbon losses from surface soils of Arctic dwarf shrub tundra to anthropogenic forcing will be minor, but not negligible.

Key words

snow fence; NMR; carbon loss; decomposition; anthropogenic C emission feedback; tundra

1. Introduction

Temperature is one of the main limiting factors for decomposition in Arctic soils (Wallenstein et al., 2009), leading to vast soil organic carbon (SOC) pools exceeding Earth's atmosphere's C stock (Hugelius et al., 2014; Tarnocai et al., 2009). In the face of climate warming, temperature limitations on decomposition processes might be alleviated, putting the biologically degradable part of this SOC pool at risk of being released to the atmosphere (Kleber, 2010; Schmidt et al., 2011). In Arctic regions, climate warming is especially pronounced during the non-growing season (NGS) (Stocker et al., 2014). As the NGS is the predominant part of the year, changes in its climate can have a disproportionally large effect on decomposition processes: relatively low decomposer activities at low temperatures can be offset by the long duration of the NGS and lead to long-term SOC loss. Soil organic C in the Arctic dwarf shrub tundra's active layer consists of a large proportion of readily decomposable compounds (Pautler et al., 2010; Pedersen et al., 2011; Sjögersten et al., 2003), and here we test if long-term *in situ* NGS warming could not only have an effect on its SOC pool size, but also on its bulk chemical composition.

Soils from cold dominated ecosystems appear to be in early stages of decomposition and at risk for rapid SOC loss with increasing temperature. Warming may specifically accelerate the degradation of readily decomposable compounds and thereby progress its decomposition stage. As an indicator for the relative degree of a given soil's SOC decomposition stage, the alkyl/O-alkyl ratio has been used (Feng and Simpson, 2008; Pautler et al., 2010; Pedersen et al., 2011; Simpson et al., 2008; Sjögersten et al., 2003). For instance, Sjögersten *et al.* (2003) observed higher alkyl/ O-alkyl ratios in more decomposed forest soils as compared to soils in less decomposed stages from nearby tundra soils in northern Scandinaviafor which they attributed to higher decomposer activities in forest soils due to higher soil temperatures in the more sheltered microclimate. Further, an extreme-event induced active layer detachment in northern Canada where sub-surface active layer soil was exposed to air temperatures via soil movements after extensive rain-fall accelerated SOC decomposition and increased its alkyl/ O-alkyl ratio (Pautler et al., 2010). Similar effects can be expected in response to NGS warming through climate change of Arctic dwarf shrub tundra ecosystems.

The effects of warming on SOC pool depletion and its alkyl/O-alkyl ratio can be expected to be strongest in surface soil horizons of the active layer for several reasons. Arctic and alpine surface soils are reported to be richer in O-alkyl carbon (C) than deeper soil horizons, i.e. the alkyl/O-alkyl ratio increases with depth, indicating that the stage of decomposition advances with depth, an effect already visible in the upper few cm of soil profiles (Pedersen et al., 2011; Sjögersten et al., 2003). Further, environmental controls in deeper horizons may stabilize otherwise chemically readily decomposable compounds from microbial decomposition by e.g. sorption to the mineral phase (Kawahigashi et al., 2006; Kleber, 2010; Schmidt et al., 2011; Trumbore, 2009), which becomes more prominent in deeper horizons with a higher mineral proportion. Hence, total loss of SOC in deeper horizons upon warming could be lower than in surface near horizons. Increases in ecosystem respiration (ER) by experimental NGS warming in the Arctic (Björkman et al., 2010; Morgner et al., 2010; Nobrega and Grogan, 2007; Schimel et al., 2004; Semenchuk et al., 2016a; Webb et al., 2016) may thus be primarily (but not exclusively) driven by degradation and depletion of SOC substrates from surface rather than deep soil or thawed permafrost (cf. Schuur *et al.*, 2009; Natali *et al.*, 2014). This is supported by recent studies finding significantly higher soil CO_2 fluxes and stronger responses to warming in surface horizons up to 10 cm depth than in deeper soil horizons (Hicks Pries et al., 2017; Lee et al., 2010).

In the Arctic, significant ER increases have been shown to respond directly to *in situ* NGS warming in a variety of tundra ecosystems (Björkman et al., 2010; Morgner et al., 2010; Natali et al., 2014; Nobrega and Grogan, 2007; Schimel et al., 2004; Semenchuk et al., 2016a; Webb et al., 2016). Semenchuk et al. (2016a) demonstrated that these effects were followed by decreased growing season ER after eight years of NGS warming in Svalbard dwarf shrub tundra. Similarly, ER responses to experimental continuous *in situ* soil warming are shown to be transient and decrease after a few years of continuous warming in a mid-latitude forest site (Melillo et al., 2002, 2017). One explanation for these effects suggests that soil warming accelerates decomposition processes and alters SOC pool size and composition; soil OC is transformed from a relatively early stage to a later stage of decomposition, and thus provides a less favourable substrate for respiring decomposing organisms

(Kirschbaum, 2004; Eliasson et al., 2005; Bradford et al., 2008). Here, we use the opportunity to collect soil and verify that hypothesis within the experiment used by Semenchuk *et al.* (2016a).

We test whether nine years of continuous *in situ* NGS warming (average 2 degrees warming within a snow manipulation treatment) of relatively C poor (3-25 % C) High Arctic dwarf shrub tundra surface soil (16 cm depth) (i) reduced SOC pool size and (ii) changed the SOC chemical composition towards a more advanced decomposition stage in a snow fence experiment in Adventdalen, Svalbard. In the same experiment, Semenchuk *et al.* (2016a) found that 8 years of continuous NGS warming and connected increased ER during the NGS lead to decreased growing season ER and suggested that changes in SOC pool size and composition could account for this. Based on these results, we test the following:

1.1 Hypotheses

(1) Carbon content is depleted in NGS warmed surface soils, i.e. long-term increased NGS ER decreased the C pool.

(2) Carbon compound composition is altered in NGS warmed soils. More specifically, we expect the alkyl/ O-alkyl ratio to be higher in NGS warmed soils, i.e. that the relative degree of SOC decomposition is advanced.

(3) The effects from Hypotheses 1 and 2 are larger in the A-horizon than in the top 5 cm of the underlying, C poorer and mineral richer B/C-horizon, i.e. the combination of environmental conditions and initial SOC composition in the B/C-horizon render the bulk SOC there more resistant to warming.

2. Material and Methods

2.1 Site description (location, soil, vegetation, climate, seasonality)

The study site is on the southern (left) riverbank in Adventdalen, a large valley about 12 km east of Longyearbyen on Spitsbergen, Svalbard (78°10′N, 16°04′E) with continuous permafrost with an active layer thickness/ maximum thaw depth of about 75 to 90 cm at the study site (own data, Figure S2). The cryoturbated gelisol soils at the study site (Semenchuk et al., 2016a) are dominated by fluvial and aeolian sedimentation and consist of a relatively thin and C poor, dark brown A-horizon of about 2-11 cm thickness with about 15-25% C content and an underlying, grey, silty B/C-horizon with about 3-9% C content, which extends to the permafrost table (see Strebel *et al.*, 2010 and own data below).

Situated in the bioclimatic subzone C, the vegetation type is classified as Prostrate/ hemiprostrate dwarf-shrub tundra (CAVM Team, 2003), dominated by the dwarf shrubs *Cassiope tetragona*, *Dryas octopetala* and *Salix polaris*.

Average air temperature 2000 to 2011 at Longyearbyen airport, about 20 km west of the study site, during the approximate NGS months October to April were -9 °C and during the approximate growing season months 3.4 °C (www.eklima.no). Average snow depth in the control area was about 40 cm (own data, Figure S1).

Non-growing season (NGS) in this study is defined as the time of year when the soil surface is frozen, i.e. has a temperature below 0 °C as measured by temperature loggers employed at around 2 cm depth (see S3), and ranges in extreme cases from about early October until early May, depending on year, replicate plot and snow fence treatment (see below).

2.2 Experimental design

Eleven 1.5m high snow fences were erected in autumn 2006, i.e. 9 years before the present study was conducted. These created winter snow drifts of maximum 1.5 m depth, i.e. about 1.1 m deeper than average ambient snow depth of about 0.4 m (see S1), which declined in depth with distance from the fence. Foci areas behind the fences receiving about 0.7 m snow depth (see S1) were identified for soil sampling, here referred to as “snow fence treatment”. Each snow fence was associated with an unmanipulated control area with ambient snow conditions about 10-15 m away from each fence. The snow drifts’ shapes and extents were very similar each year (own observations) due to the prevailing easterly wind direction along the valley.

The snow fence treatment chosen for this study increased the average NGS surface soil temperature (i.e. when soil surface is frozen) by 1.86 °C as determined by continuous hourly temperature measurements (see S3). There was no diurnal variability within the snow fence treatment’s temperature effects, and the warming started a few weeks after the start of the NGS when the snowpack established (see S3). Soil moisture in the snow fence treatment was observed to be slightly increased at the beginning of the growing season only (Semenchuk et al., 2016a), but was neither changed in the A- nor B/C-Horizon at time of soil sampling for this study (see S4).

Please note that for the present study we sampled a part of a larger experimental setup. Samples for this study were taken from areas behind snow fences that in other studies from the same site are referred to as “medium” snow or similar, in which snow depth was about 0.7 m, as opposed to “deep” snow, in which snow depth was about 1.5 m (Cooper et al., 2011; Rumpf et al., 2014; Semenchuk et al., 2016a, 2015, 2013). The reason why we did not use the area with the maximum or deep snow increase was to preserve that relatively small area for future research and not disturb it with invasive sampling.

2.3 Soil sampling procedure

On July 23rd 2015, 3 soil cores between 0.5 and 1 m apart were sampled with a soil corer with 2 cm diameter (3.14 cm^{-2} area) in each plot and treatment (snow fence and control). To exclude potentially confounding vegetation effects on soil parameters in question for this study, sampling locations were chosen where *Salix polaris*, a dominant dwarf shrub across the study site, was the dominant species.

The brown A-horizon of each core was separated visually from the underlying grey B/C-horizon in the field, its thickness/ length measured, and each horizon of the core triplets combined in one plastic bag. Before bagging, above ground litter (O-horizon) and plants were coarsely removed from above the A-horizon. The upper 5 cm of the B/C-horizon directly under the A-horizon of each core were sampled and also combined. The maximum sampled A-horizon thickness was 11 cm, i.e. the deepest B/C-horizon sample was between 11 and 16 cm depth, far above the permafrost table which in our study site is at about 75 to 90 cm depth (see S2).

2.4 Soil treatment prior to chemical analyses

After collection in the field, soil samples were kept at 4 °C for five days in Longyearbyen, Svalbard, then transported to Abisko, Sweden, and processed within two days while being kept at 4 °C. Remaining above ground plant material, roots and stones of each sample were removed during 3 minutes per sample, the remaining soil mass homogenized, weighed and then directly oven dried at 70 °C for 48 h. The dried samples were then weighed again and ground in a ball mill to a fine powder and transported to Umea, Sweden, for NMR analyses and aliquots sent to Copenhagen, Denmark, for elemental analysis (see below).

Based on previously published material, we expected the total Fe (iron) content of the soil to be relatively high (Ottesen et al., 2010) with simultaneously low C content (Moni et al., 2015; Strebel et al., 2010) leading to a C:Fe ratio < 6 . Such high concentration of Fe-associated paramagnetic compounds has been shown to have a strong adverse effect on the quality of NMR spectra (Schilling and Cooper, 2004; Schmidt et al., 1997). After initial trials, this turned out to be only the case for the B/C-horizon samples, since NMR spectra of the A-horizon were of sufficiently good quality.

Therefore, we demineralized the B/C-horizon following the procedure in Baldock *et al.* (2001). We washed the soil with hydrofluoric acid (HF treatment) to remove paramagnetic and mineral compounds and thus increase the C content of the remaining soil leading to improved NMR spectra (Gélinas et al., 2001; Schilling and Cooper, 2004; Schmidt et al., 1997). In short we exposed 1 g of each sample for 12 h in 30 mL of 10% HF in 1N HCl solution, removed the supernatant and exposed the remaining pellet to fresh HF/ HCl solution twice more, then washed 3 times with water and freeze dried the pellet and used it for further analyses.

In the following, data based on NMR spectroscopy (spectra, integrals, and alkyl/O-alkyl ratio) are from HF-treated B/C-horizon samples, while data based on bulk soil parameters (bulk density, C concentration and pool size) are from untreated B/C-horizons. The A-horizon was always untreated.

2.5 Soil C concentrations

To measure soil C concentrations (%), we weighed soils into tin capsules and quantified total C for each sample on an Isoprime isotope ratio mass spectrometer coupled to a Eurovector CN elemental analyser.

2.6 Bulk density

Bulk density of dry soil without roots or stones was calculated by dividing the dry weight of each full sample (which consisted of three combined, individual soil cores) with its volume. The samples' volumes were calculated by multiplying the average depth of all three cores with three times the area of the soil corer (9.42 cm^{-2}).

2.7 Soil C pool size

Soil C pool size per area was calculated by multiplying each full sample's C concentration/ fraction with its dry weight, divided by three times the area of the soil corer (9.42 cm^{-2}), in unit kg C m^{-2}.

2.8 Solid state ^{13}C CP/MAS NMR spectroscopy

Approximately 100 mg of each soil sample was loaded into a 4 mm ZrO_2 rotor with a KEL-F cap. ^{13}C Cross-Polarization Magic Angle Spinning (CP-MAS) experiments were performed on a Bruker 500 MHz Avance III spectrometer operating at a ^{13}C frequency of 125.75 MHz (Bruker Biospin, Germany). Spinning rate was set to 7 kHz, the contact-time to 1 ms and the sweep-width to 250 ppm. 4096 scans were recorded with a relaxation delay of 2 s resulting in an experimental time of approximately 2.3 hours per sample. The FIDs were multiplied with a Gaussian function with LB = -10 Hz and GB = 0.01 prior to fourier transform. All spectra were calibrated using adamantane as an external reference. Processing was performed in Topspin 3.2 (Bruker Biospin, Germany).

2.9 Processing of raw NMR spectra/ separation into integrals

The processed spectra were transferred to a matrix with each spectrum as a row and the columns representing the intensity in each of the 4096 data-points that constitutes a CP-MAS spectrum using an in-house Matlab script. Each spectrum was normalized to a constant sum before statistical analysis.

Different chemical shift regions of the spectra, containing information about different functional groups (Preston et al., 1997) were also integrated and analysed as a separate data set. The regions were defined as follows: 0-50 ppm (alkyl); 50-60 ppm (methoxy/N-alkyl); 60-93 ppm (O-alkyl); 93-112 ppm (di-O-alkyl); 112-140 ppm (aromatic); 140-165 ppm (O-aromatic) and 165-190 ppm (carbonyl). The integrals were also normalized to a constant sum before statistical analysis.

2.10 Statistical analyses

All analyses were performed with R version 3.4.3 (R Core Team, 2017) and all packages mentioned below are R packages.

The effects of the snow fence treatment on A-horizon thickness, bulk density, C concentration and pool size, integrated regions of the NMR spectra, and alkyl/ O-alkyl ratio were analysed with linear mixed effects models with plots as random effects using the “lme” package (Bates et al., 2015). The significance of the snow fence treatment was tested with a likelihood ratio test between the full model including treatment as predictor variable and the Null model, and a p-value of lower than 0.05 was considered to be significant. We then refitted the full models with restricted maximum likelihood estimation and extracted the 95% confidence intervals of the effect sizes and model term estimates with the “multcomp” package (Hothorn et al., 2008), which we present here together with the p-value from the likelihood ratio test. All model fits had no trend in Pearson residuals plotted against fitted values, and residuals were normally distributed (visual examination).

To test whether NMR spectra from the control and snow fence treated soils differ, partial least squares discriminant analysis (PLS-DA) was performed with the “caret” package (Kuhn, 2008). Classification was carried out based on the probability of the normalized NMR spectra belonging to either group (snow fence or control), having each spectrum assigned to the class with the highest associated probability. The smallest number of latent variables needed to reach 100% discrimination between snow fence treatment and controls were chosen. The first two loading variables from each model were taken to assess which shift regions were responsible for discrimination.

Data from A- and B/C-horizons were analysed separately, because the demineralization procedure of the B/C-horizon could potentially change a number of factors independent of horizon or the experimental *in situ* warming treatment (Dai & Johnson, 1999; Gélinas *et al.*, 2001; Keeler *et al.*, 2003; Schilling & Cooper, 2004; Rumpel *et al.*, 2006), making the two horizons incomparable, while qualitative treatment effect comparisons are valid (e.g. effect present or not).

3. Results

3.1 A-horizon thickness

Average A-horizon thickness across the study site's control area was 4.7 cm (95% CI: 3.7 – 5.7 cm) and 3.8 cm (95% CI: 2.8 – 4.8 cm) in the snow fence treatment, i.e. 0.9 cm lower than in the control area (95% CI: -1.7 - -0.03 cm; p = 0.0433).

3.2 Bulk density

Average bulk density across the study site's control area was 0.33 g cm^{-3} (95% CI: 0.24 – 0.41 g cm^{-3}) in the A-horizon, and 1.09 g cm^{-3} (95% CI: 0.94 – 1.24 g cm^{-3}) in the upper five cm of the B/C-horizon. The snow fence treatment influenced bulk density of neither A- nor B/C-horizon, i.e. no significant effects were found.

3.3 Soil C concentrations (Figure 1 and Table 1)

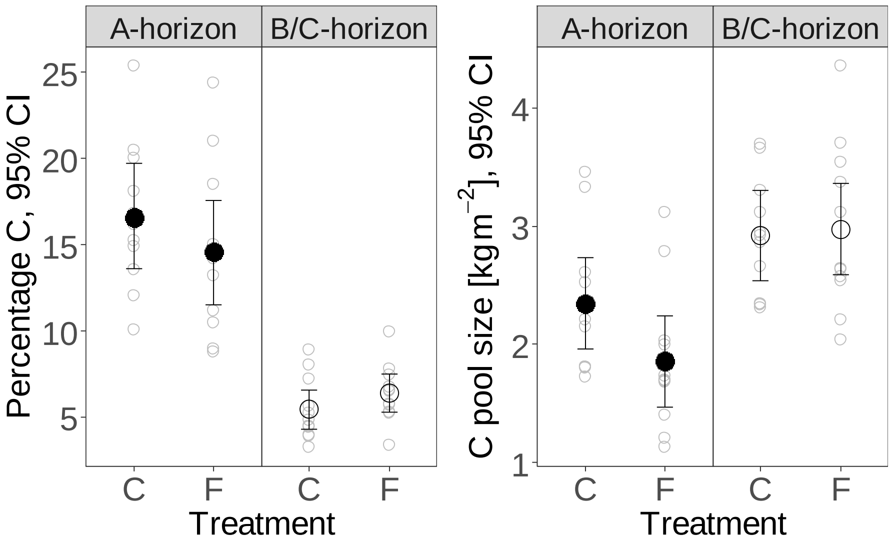
Average C concentrations across the study site's control area were 16.6% (95% CI: 13.6 - 19.7%) in the A-horizon and 5.4% (95% CI: 4.3 - 6.6%) in the upper 5 cm of the untreated B/C-horizon (i.e. the soil aliquot which was not HF treated; Figure 1). Carbon concentrations in the HF treated B/C-horizon soils (i.e. the HF treated soil samples that we will refer to when describing and discussing data from the NMR analysis) were 14.2% (95% CI: 12.1 - 16.3%; data not shown).

The snow fence treatment influenced C concentrations of the A-, but not of the B/C-horizon. In the A-horizon, samples from the snow fence treatment had 2% lower (95% CI: -3.7 - -0.5%; p = 0.021; Table 1) C concentrations than samples from controls. In the untreated B/C-horizon, samples from the snow fence plots had no significantly different C concentration (95% CI: -0.1 to 2%; p = 0.111; Table 1). In the HF-treated B/C-horizon, samples from the snow fence plots also had no significantly different C concentration (95% CI: -0.4 to 4.1%; p = 0.073; Table 1).

3.4 Soil C pools (Figure 1 and Table 1)

Average C pool sizes across the study site's control area were 2.34 kg C m^{-2} (95% CI: 1.96 – 2.73 kg C m^{-2}) in the A-horizon and 2.92 kg C m^{-2} (95% CI: 2.54 – 3.3 kg C m^{-2}) in the upper five cm of the untreated B/C-horizon (i.e. the soil aliquot which was not HF treated; Figure 1).

The snow fence treatment influenced C pools of the A-, but not of the B/C-horizon. In the A-horizon, samples from the snow fence treatment had 0.48 kg C m^{-2} lower (95% CI: -0.9 - -0.07 kg C m^{-2}; p = 0.032; Table 1) C pool sizes than samples from controls. In the B/C-horizon, samples from the snow fence plots had no significantly different C pool sizes (95% CI: -0.42 to 0.53 kg C m^{-2}; p = 0.82; Table 1).



<u>Figure 1:</u> Carbon concentration (percentage C, left) and pool size (kg C m^{-2}, right) in A- and untreated (i.e. non-HF treated) B/C-horizons for each soil sample (raw data, grey open circles) and modelled with 95% confidence intervals (black circles). Solid black circles in the A-horizon data denote statistically significant differences between treatments (see Table 1); open black circles in the B/C-horizon data denote no statistically significant difference between treatments. Treatment C =

Control (ambient conditions, ca. 40 cm snow depth), F = Snow Fence (enhanced snow, ca. 70 cm snow depth). See Table 1 for statistical tests between treatments, also including the C concentration model for the HF-treated B/C-horizon.

Table 1: *Modelled mean effect sizes and 95% confidence intervals (CI) of the snow fence treatment on carbon concentration (%C) and carbon pool size (kg C m^{-2}) of A-, untreated B/C-, and HF-treated B/C-horizons (only for %C). The values show estimated differences of samples from the snow fence treatment compared to controls based on linear mixed effects models. P-values are based on a likelihood ratio test between the full and the Null model. Significant effect sizes are in bold ($p<0.05$ and CI not overlapping zero). Each line shows results of a separate model.*

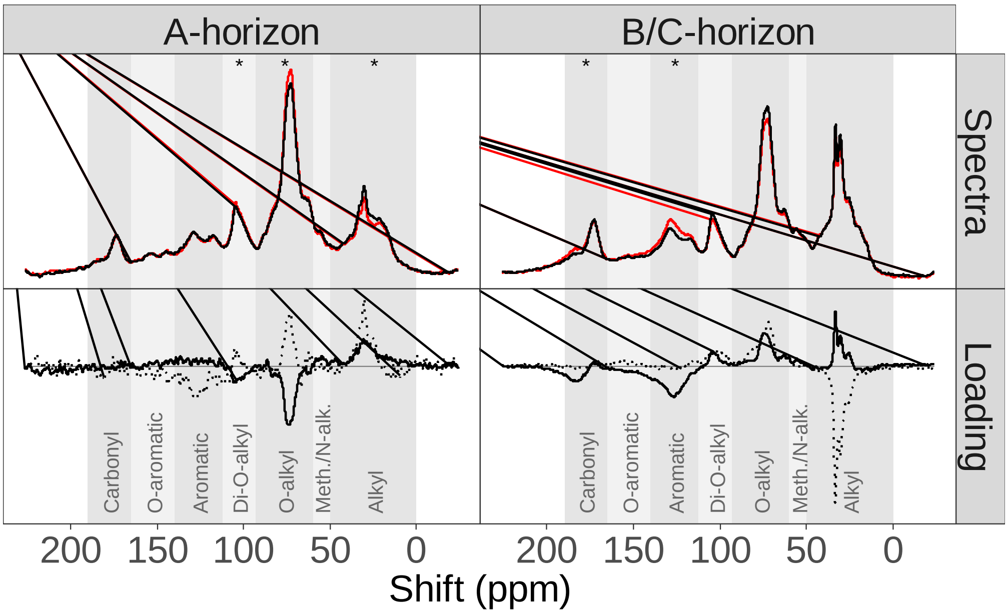
Response	*Horizon*	*Effect size*	*Lower 95% CI*	*Upper 95% CI*	*p-value*
%C	***A***	**-2.0563**	**-3.6623**	**-0.4503**	**0.0205**
%C	*B/C*	0.9525	-0.06051	1.96552	0.1113
%C	*B/C (HF-treated)*	1.8646	-0.4052	4.1344	0.0729
kg C m^{-2}	***A***	-0.48285	**-0.89685**	**-0.06886**	**0.03152**
kg C m^{-2}	*B/C*	0.05238	-0.42044	0.52520	0.8201

3.5 Spectral analysis (Figure 2)

PLS-DA models on samples from the snow fence treatment and controls showed a 100% discrimination using 3 and 5 latent variables for the A- and HF-treated B/C-horizons, respectively, indicating a significant structural difference between treatments in both horizons. The chemical shift regions causing the differences were visible in the spectra and the models' first and second loading components (Figure 2). In other words, regions with the highest model loadings are the regions with the highest importance to distinguish between the treatments.

In the A-horizon, clear differences between snow fence and control soil samples were visible in the O-alkyl and alkyl regions, as well as subtle differences in the aromatic region.

In the B/C-horizon, the effect of snow fence treatment differed compared to the A-horizon. Here we observed a decrease in the signal in the carbonyl and aromatic regions as well as an increase in O-alkyl and alkyl signals.



<u>Figure 2:</u> Top row: Mean NMR spectra for soil samples from snow fence plots (black line) and controls (red line) in A- (left column) and HF-treated B/C-horizons (right column). Bottom row: first (solid line) and second (dotted line) loading components of the associated PLS-DA models. Vertical grey bars denote integrated chemical shift regions as defined in Table 2, from left to right (with region boundaries): carbonyl (190-165), O-aromatic (165-140), aromatic (140-112), di-O-alkyl (112-93), O-alkyl (93-60), methoxy/ N-alkyl (60-50), alkyl (50-0). Small stars on top of the top row denote where statistically significant differences between treatments were found on individual integrated chemical shift regions (Table 2).

3.6 Integrated shift regions (Figure 3 and Table 2)

In the A-horizon, samples from the snow fence plots had a 2% higher (95% CI: 0.9 – 2.9%; p = 0.002) concentration of alkyl signals than samples from controls, while the concentrations of di-O-alkyl and O-alkyl signals were significantly lowered by 0.6% (95% CI: -0.9 - -0.2%; p = 0.007) and 2% (95% CI: -3.3 to -0.7%; p = 0.009), respectively. The other integral regions' contributions to the A-horizon spectra were not significantly different between the treatments. See Figure 3 and Table 2.

In the HF-treated B/C-horizon, samples from the snow fence plots had 2% (95% CI: -3.7 - -0.2; p = 0.035) and 0.7% (95% CI: -1.3 - -0.1%; p = 0.027) lower concentrations of aromatic and carbonyl signals, respectively, than samples from controls, while the O-alkyl signals had a non-significant trend towards 1.8% (95% CI: -0.9 – 4.6%; p = 0.186) concentrations. The other integral regions' contributions to the B/C-horizon spectra were not significantly different between the treatments. See Figure 3 and Table 2.

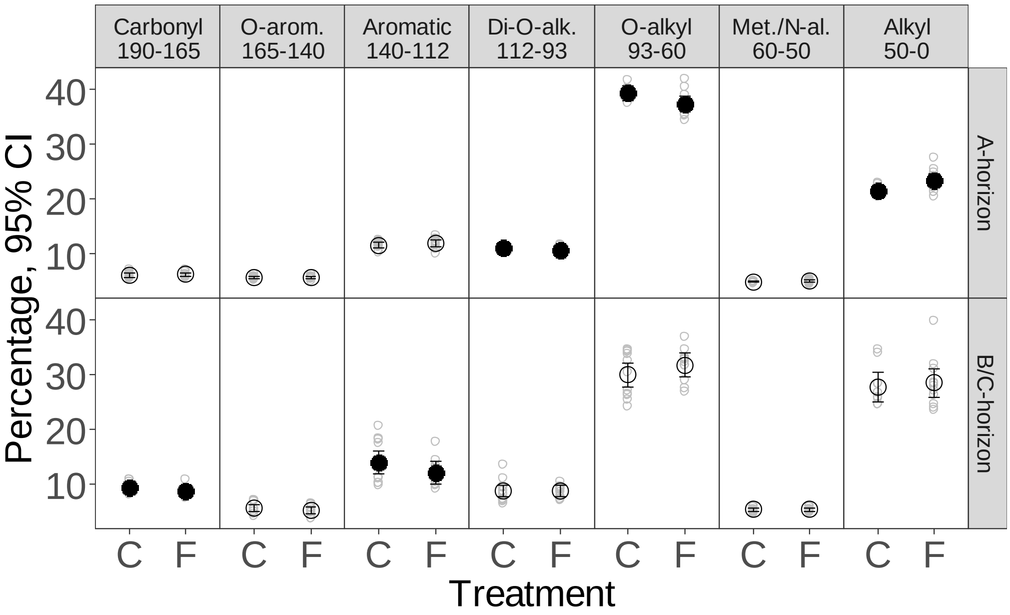


Figure 3: Relative contribution of integrated NMR spectra signal regions in A- and HF-treated B/C-horizons for each soil sample (raw data, grey open circles) and modelled with 95%

confidence intervals (black circles). Solid black circles denote statistically significant differences between treatments; open black circles denote no statistically significant difference between treatments. Treatment C = Control, F = Snow Fence. See Table 2 for statistical tests between treatments.

<u>Table 2:</u> *Modelled mean effect sizes and 95% confidence intervals (CI) of the snow fence treatment on relative contribution of integrated NMR spectra signal regions and alkyl/O-alkyl ratio of A- and HF-treated B/C-horizon samples. The values show estimated differences of samples from the snow fence plots compared to controls based on linear mixed effects models. Region denotes the ppm range chosen to define each integral. P-values are based on a likelihood ratio test between the full and the Null model, and cases where the full model is statistically significantly better than the Null model with p<0.05 are in bold. Each line shows results of a separate model.*

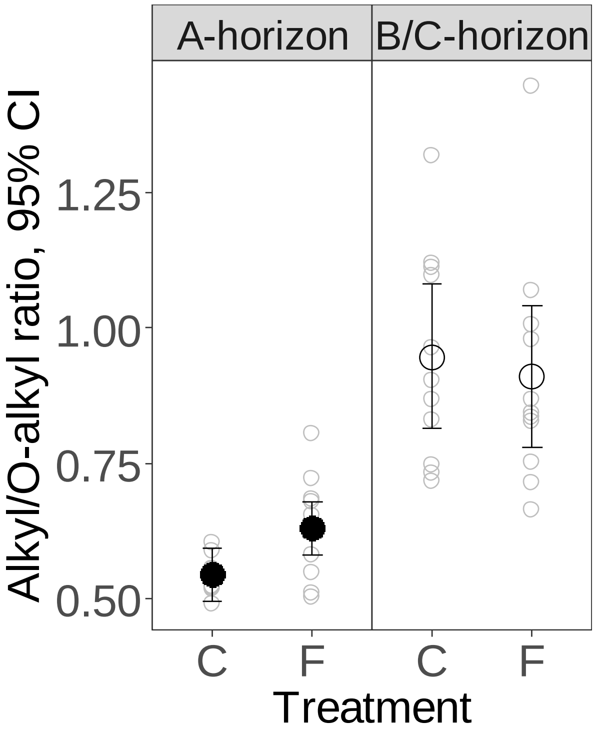
Horizon	*Integral*	*Region*	*Effect size*	*Lower 95 %CI*	*Upper 95% CI*	*p-value*
A	*Carbonyl*	*190-165*	0.000683	-0.0009593	0.0023253	0.3987
A	*O-aromatic*	*165-140*	0.0001435	-0.00281	0.003097	0.92
A	*Aromatic*	*140-112*	0.003705	-0.001253	0.008663	0.1437
A	***Di-O-alkyl***	***112-93***	**-0.005542**	**-0.009067**	**-0.002018**	**0.007272**
A	***O-alkyl***	***93-60***	**-0.019813**	**-0.032911**	**-0.006714**	**0.009047**
A	*Methoxy/ N-alkyl*	*60-50*	0.001589	-0.0006453	0.0038233	0.1522
A	***Alkyl***	***50-0***	**0.019235**	**0.009103**	**0.029367**	**0.002273**
A	***alkyl/ O-alkyl***	***ratio***	**0.085**	**0.03685**	**0.13316**	**0.003633**
B/C	***Carbonyl***	***190-165***	**-0.006956**	**-0.012728**	**-0.001184**	**0.02721**
B/C	*O-aromatic*	*165-140*	-0.0050147	-0.0104986	0.0004692	0.08004
B/C	***Aromatic***	***140-112***	**-0.019838**	**-0.037232**	**-0.002445**	**0.03473**
B/C	*Di-O-alkyl*	*112-93*	0.0006397	-0.0134508	0.0147301	0.9257
B/C	*O-alkyl*	*93-60*	0.018268	-0.009001	0.045538	0.1859

B/C	*Methoxy/ N-alkyl*	*60-50*	0.0007683	-0.0028917	0.0044283	0.6674
B/C	*Alkyl*	*50-0*	0.006997	-0.011176	0.025171	0.4351
B/C	*alkyl/ O-alkyl*	*ratio*	-0.03617	-0.17731	0.10497	0.6006

3.7 Alkyl/O-alkyl ratio (Figure 4 and Table 2)

Average alkyl/O-alkyl ratios across the study site's control area were 0.55 (95% CI: 0.5 - 0.59) in the A-horizon and 0.95 (95% CI: 0.82 - 1.1) in the HF-treated B/C-horizon. See Figure 4.

The snow fence treatment influenced the alkyl/O-alkyl ratio of the A-, but not of the B/C-horizon. In the A-horizon, samples from the snow fence plots had a 0.085 higher (95% CI: 0.037 - 0.133; p = 0.004) alkyl/O-alkyl ratio than controls. In the B/C-horizon, samples from the snow fence plots had no significantly different alkyl/O-alkyl ratio compared to controls (-0.036; 95% CI: -0.18 - 0.1; p = 0.6).



<u>Figure 4:</u> Alkyl/O-alkyl ratios in A- and HF-treated B/C-horizons for each soil sample (raw data, grey open circles) and modelled with 95% confidence intervals (black circles). Solid black circles denote statistically significant differences between treatments (only A-horizon); open black circles denote no statistically significant difference between treatments (only B/C-horizon). Treatment C = Control, F = Snow Fence. See Table 2 for statistical tests between treatments.

4. Discussion

We found that the A-horizon, i.e. the study site's C-richer upper soil layer (average 16.6% C and 4.7 cm thickness), of high Arctic tundra plots which were exposed to 9 years of experimental *in situ* NGS warming via snow fences, on average had a 2% lower C concentration, contained 0.48 kg less C m^{-2} (hypothesis 1) and was in a more advanced stage of decomposition (as indicated by a 0.09 higher alkyl/ O-alkyl ratio, hypothesis 2) compared to unmanipulated control plots. These effects were not observed in the first 5 cm of the underlying, C poorer B/C-horizon (average 5.4% C, hypothesis 3). Below we will discuss potential causes, effects and implications of our findings.

4.1 SOC loss through NGS warming only in A-horizon, not B/C-horizon (hypotheses 1 & 3)

The smaller SOC pool in the snow fence plots' A-Horizon compared to the controls is possibly caused by a temperature induced increase of decomposition and subsequent loss of SOC during the nine preceding warmed NGSs. Non-growing season ER was higher in snow fence treatments in this (Morgner et al., 2010; Semenchuk et al., 2016a) and other Arctic sites (Natali et al., 2014; Nobrega and Grogan, 2007; Schimel et al., 2004; Webb et al., 2016). Further, soil nutrient concentrations were higher in a deeper snow fence treatment with stronger temperature increase in this (Semenchuk *et al.*, 2015; Mörsdorf *et al.*, submitted) and another Arctic site (Schimel et al., 2004), and *Salix polaris'* (and other species') leaf nitrogen content was higher in this (Mörsdorf *et al.*, submitted) and another Arctic site (Welker et al., 2005), suggesting a stabilization of surplus nutrients from the soil. In sum, these findings strongly indicate increased activities of decomposing organisms during warmed NGSs in a variety of Arctic tundra ecosystems, which in the long run possibly lead to more mineralization of SOC than could be replenished by plants during the growing season through e.g. plant litter or root exudate inputs (Bradford et al., 2008; Eliasson et al., 2005; Kirschbaum, 2004).

Whether this SOC depletion is a transient phenomenon driven by fast cycling SOC pools as observed in e.g. permafrost soil incubations (e.g. Moni *et al.*, 2015) or plant litter mass loss studies (e.g. Demarco *et al.*, 2014) is uncertain. The observed decrease of growing season ER (Semenchuk et al., 2016a) and degradation of SOC to advanced stages of decomposition indicate that, under current conditions, further depletion might come to a halt. However, as suggested by Melillo et al. (2017), it is possible that the microbial communities in the snow fence plots are undergoing a phase of reorganization as response to the altered SOC pool size and quality, soil nutrient status, and temperature conditions. Once acclimated, further depletion and degradation of the remaining SOC pool could continue. Since these non-linear and possibly interactive responses cannot necessarily be generalized across spatial and temporal boundaries, further large-scale and long-term studies (e.g. time series) are warranted to enable us to project the presented findings on possible future climate scenarios.

Whether the findings of SOC depletion from this study are context specific can only be speculated without further long-term and orchestrated biome-wide studies, but a few available long-term studies (>8 years warming) do indicate context specificity. The growing season ER reduction after long-term NGS warming is also reported from Daring Lake, a low-Arctic site (Semenchuk et al., 2016a), and both reduction of long-term effects on ER and depletion of easily degradable SOC after long-term warming are reported from the Harvard Forest, a deciduous hard-wood forest (Bradford et al., 2008; Melillo et al., 2017). However, long-term warming did not result in reductions of growing season ER nor decline of NGS ER effects from a sub-Arctic peat site in Abisko (Dorrepaal et al., 2009). All mentioned studies are from different ecosystems, and taken together it seems that context specificity is given. Which factors are responsible for the contrasting results between these studies is unclear and motivates for further studies. However, the high C and low mineral content in the Abisko peat site might play important roles in determining long-term effects of warming on SOC pool size.

Vegetation related variables can partly be ruled out as explanatory factors for SOC pool size in this study. Firstly, soil was exclusively sampled under *Salix polaris*, which excludes potential confounding effects on SOC properties by sampling under different species. Second, neither species composition (Cooper *et al.*, submitted) nor *Salix polaris'* growth (Rumpf et al., 2014) in the snow fence treatment used here were significantly different from the controls. However, the aforementioned higher leaf nitrogen content in the snow fence plots (Mörsdorf *et al.*, submitted) might be forewarning increased performance of *Salix polaris* in the long run. Conversely, results from the same experiment but from a two times deeper snow fence treatment than used here (150 cm vs. 70 cm snow depth) found significantly less seasonal growth (Rumpf et al., 2014) and lower abundance of *Salix polaris* and most other vascular plant species (Cooper *et al.*, submitted). Whether these changes, caused by a more extreme snow depth increase, are representing the future state of the snow fence treatment used here is questionable. While the NGS warming and connected biogeochemical effects are stronger in the deeper snow fence treatment (Semenchuk et al., 2015; Mörsdorf et al., submitted), the negative effects on the vegetation there are possibly caused by an average 7 days later snow melt compared to the snow fence treatment used here (Semenchuk et al., 2016b, 2013) rather than by biogeochemical cascading effects.

NGS warming significantly lowered SOC concentrations in the A-horizon but not in the directly underlying 5 cm of B/C-horizon, a result similar to the findings of Melillo *et al.* (2017). We assume that B/C-horizon properties stabilize SOC, such that it cannot be fully mineralized or accessed by decomposing organisms. In our case especially sorption to mineral particles (Kawahigashi et al., 2006; Kleber, 2010; Schmidt et al., 2011; Trumbore, 2009), but also other environmental controls such as smaller soil pore size (as indicated by the B/C-horizon's higher bulk density) and particle aggregation might have rendered parts of its SOC pool inaccessible to decomposing organisms (Conant et al., 2011; Ekschmitt et al., 2008). Additionally, lower root density (own observation and Strebel *et al.* (2010)) and possibly different species composition of the B/C-horizon's rhizosphere (Iversen et al., 2015) could be responsible for the initially low SOC concentrations found in the B/C-horizon. This low SOC concentration in the deeper horizons might create microhabitats unsuitable for a bulk population of decomposing organisms capable of mineralizing significant fractions of the existing SOC pool (Ekschmitt et al., 2008; Schmidt et al., 2011).

Leaching of dissolved C from the A-horizon by additional melt water production from the extra snow pack is conceivable. However, three qualitative observations lead to the assumption that this effect may be minor. First, the total melt water runoff from the large area above the experimental site by far outweighs the amounts produced by the additional snowpack. This runoff is unchanged by the experimental snow addition and hence the additional melt water might not have a significant impact. Second, a large part of the melt water produced by the deepened snow packs after the surrounding snow is gone seems to get diverted by the ice layer at the bottom of the snow pack (Semenchuk et al., 2013) and may not reach the soil until it reaches the edges of the plots. Third, the soil in this study site is frozen solid until a few days after melt out and any melt water flowing over it may only touch the vegetation and soil surface. All three points are based on own qualitative observations only and yield opportunities for further studies on potential artefacts of snow fences as experimental treatments in ecological studies.

4.2 Implications of SOC loss on ER and atmosphere

The loss of SOC from the A-horizon possibly explains the decline of growing season ER as reported by Semenchuk *et al.* (2016a) in the same experimental setup. Soil OC availability as substrate for heterotrophic organisms may be depleted by increased consumption below a threshold where steady state respiration can be maintained (Bradford et al., 2008; Eliasson et al., 2005; Kirschbaum, 2004). This phenomenon could be partly confounded with the aforementioned higher soil nutrient availability in NGS warmed plots (Schimel *et al.*, 2004; Semenchuk *et al.*, 2015; Mörsdorf *et al.*, submitted) resulting in ER responses independent of SOC loss. For instance, reduced nutrient limitations could reduce plant roots' foraging for nutrients and thereby reduce root exudate production and connected decomposer stimulation leading to some kind of "negative priming" effects (cf. Fontaine *et al.*, 2004; Hartley *et al.*, 2012). More studies to disentangle the possible mechanisms behind this are needed.

Our observation that SOC was only lost from the A-horizon (which with an average thickness of 4.7 cm lies well above the permafrost table at about 90 cm depth) allows the speculation that the increase of ER during warmed NGSs in this study site (Morgner et al., 2010; Semenchuk et al., 2016a) might primarily originate from the A-horizon, too. If this holds true, then the fact that growing season ER was reduced as a response to NGS warming (Semenchuk et al., 2016a) also suggests that the growing season bulk ER originates from surface horizons, as has been shown in studies measuring CO_2 fluxes in different soil depths (Hicks Pries et al., 2017; Lee et al., 2010). While understudied to date, the implications of these thoughts are of importance to determine the relative contribution of CO_2 emissions from surface and deep soils, such as thawing permafrost. With surface horizons potentially being the primary source of ER derived CO_2 emissions from tundra ecosystems, then warming induced increases of C loss from thawing permafrost (Moni et al., 2015; Schuur et al., 2009) might be relatively minor compared to CO_2 emissions from surface soils during timescales relevant for the ongoing anthropogenic forcing (Stocker et al., 2014). In fact, Hicks Pries *et al.* (2017) found that about 80% of all soil respiration and about 90% of respiration response to 4 ºC warming occurred in the upper 30 cm of temperate forest soil, and Lee *et al.* (2010) found that the upper 10 cm of upland tundra soil had ten times higher CO_2 fluxes that the underlying 20 cm horizon. In sum, these findings point towards a predominance of the more exposed surface soils as C sources to the atmosphere and warrant further studies.

A back of the envelope calculation scaling up the effect size from the C pool model to the total global area of Prostrate/hemiprostrate dwarf-shrub tundra (i.e. the vegetation type studied here, worldwide covering 140000 km^2, CAVM Team, (2003)) estimates a potential total loss of 67.6 Mt C or 248.1 Mt CO_2 equivalent upon global NGS warming from the 4.7 cm thick A-horizon only. This is in magnitude comparable with the annual CO_2 emissions of, for instance, Florida (Desai et al., 2017) or Egypt (EDGARv4.3.2, 2016), but with 0.69% an insignificant contribution to the still rising annual global anthropogenic CO_2 emissions of 36062 Mt in 2015 (EDGARv4.3.2, 2016).

4.3 Shifts in relative abundance of carbon compounds (hypothesis 2)

Carbon compound composition was clearly different in the snow fence treatment from the controls in both A- and B/C-horizons. In the A-horizon, the differences were mainly in the alkyl and O-alkyl groups leading to a higher alkyl/ O-alkyl ratio in the snow fence treatment. This indicates that NGS warming indeed transformed the A-horizon to a more advanced stage of decomposition (Feng and Simpson, 2008; Pautler et al., 2010; Pedersen et al., 2011; Simpson et al., 2008; Sjögersten et al., 2003). Together with the above discussed phenomenon of SOC loss, this supports the idea that the balance between SOC consumption and replenishment was significantly disturbed and could explain the observed decrease of growing-season ER (Semenchuk et al., 2016a).

In the B/C-horizon, the main changes were observed in the aromatic regions, indicating a decrease of amino-acids (Simpson and Simpson, 2012). These compounds were possibly transformed to O-alkyl compounds, even though the increase of that group of compounds was not statistically significant (compare with Table 2). The reason for this discrepancy between A- and B/C-horizons is out of the scope of this study, however, may be rooted in selective sorption of O-alkyl compounds to mineral particles, forcing decomposing organisms to use aromatic compounds as substrates.

4.4 Conclusions

We found that 9 years of ca. 2 °C NGS warming reduced the A-horizon’s C pool and degraded it to a more advanced stage of decomposition. Our results support the hypothesis that the transient nature of increased ER by soil warming as observed elsewhere is connected to excessive and selective consumption of SOC leading to a depletion of favourable substrates for decomposers. We further suggest that the NGS warming induced increases of ER in our study site primarily originate in the relatively shallow A-horizon and dominate total CO_2 emissions compared to deep soil or thawing permafrost. The estimated absolute expected loss over the whole global area of this ecosystem, however, is dwarfed by comparison with still rising global anthropogenic greenhouse gas emissions. We conclude that a positive feedback from surface soils of circumarctic dwarf shrub tundra communities to anthropogenic forcing might be minor during the timescales covered in this study, but due to their dynamic nature deserve a place in modelling studies.

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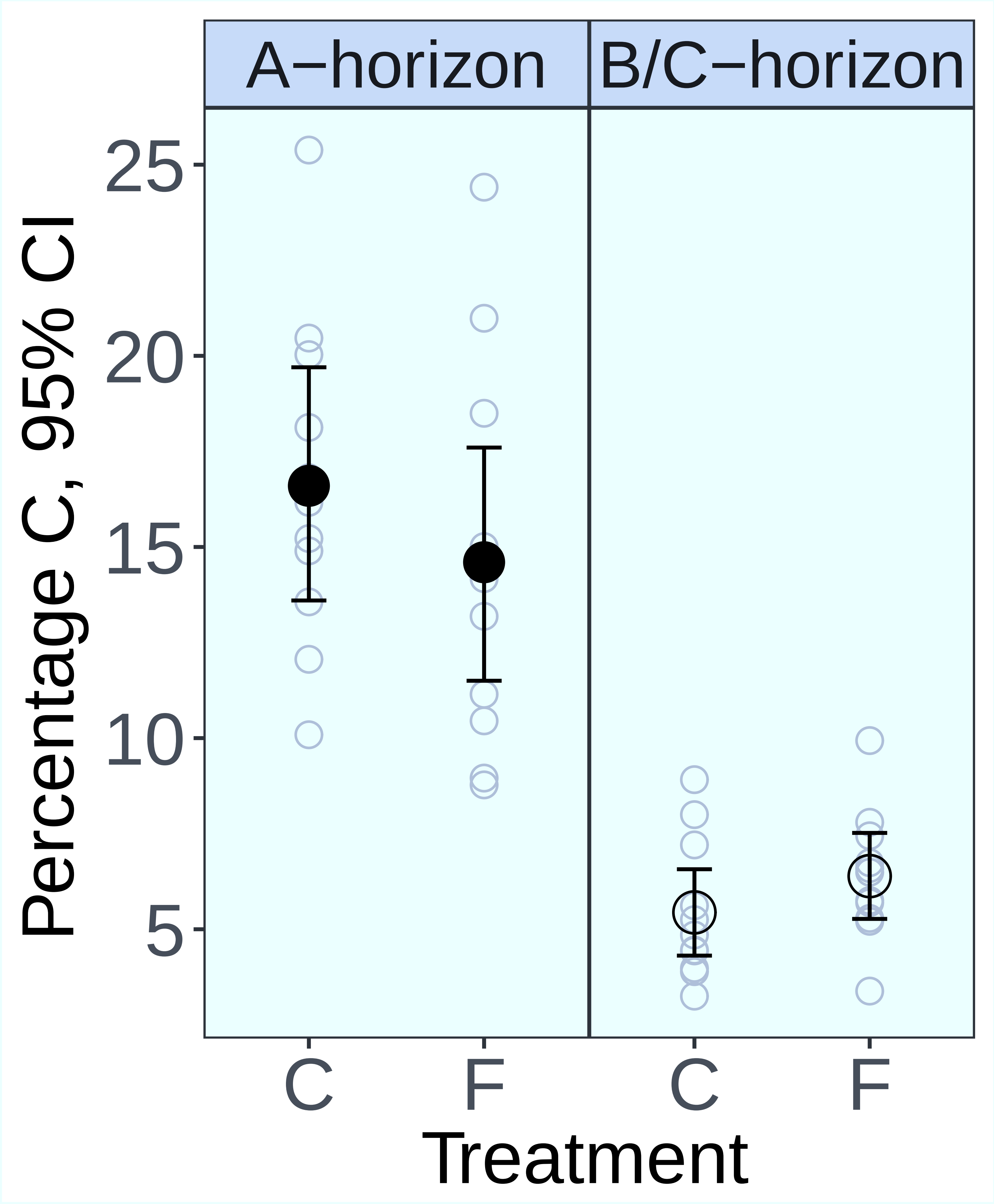
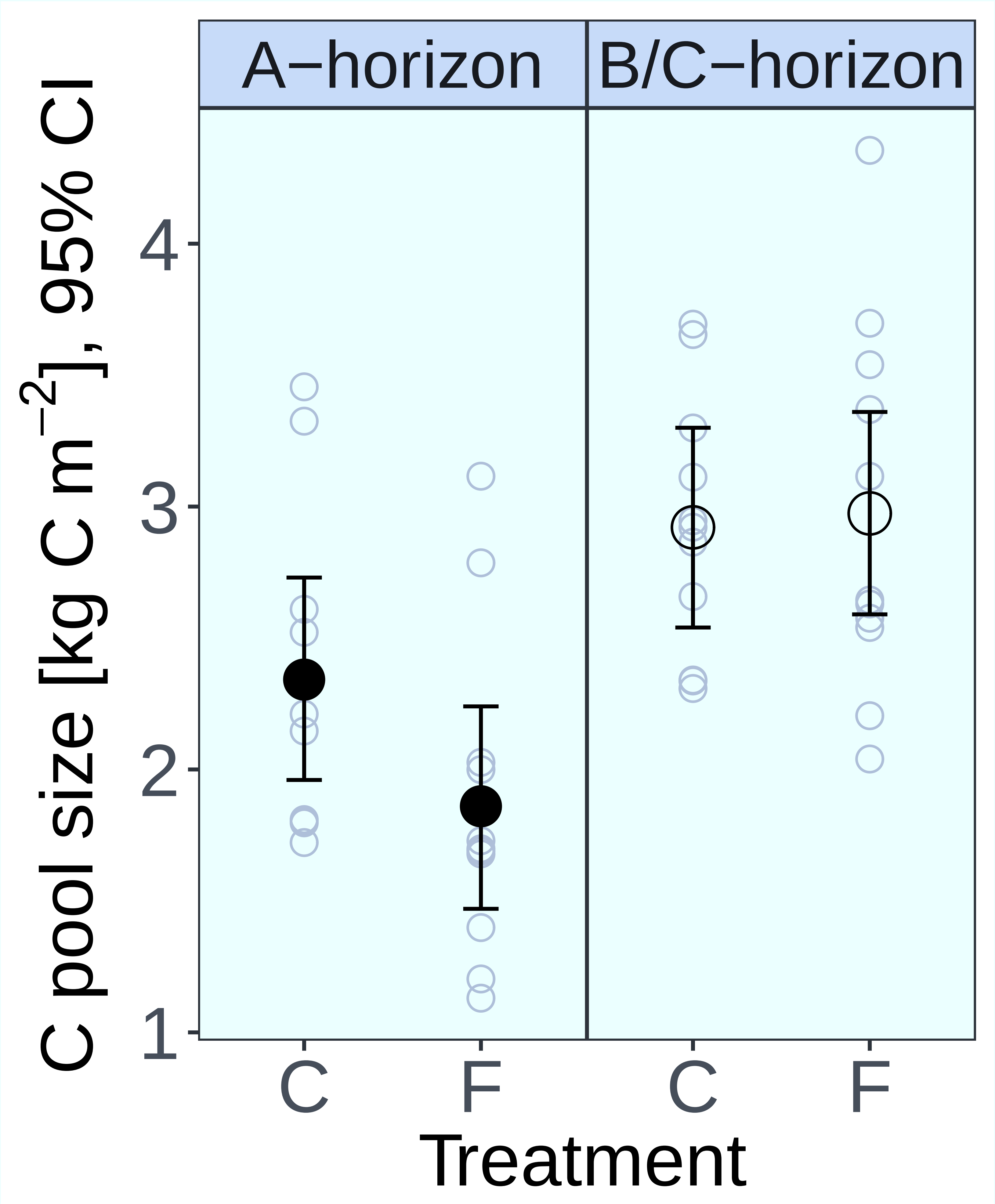
Table 1

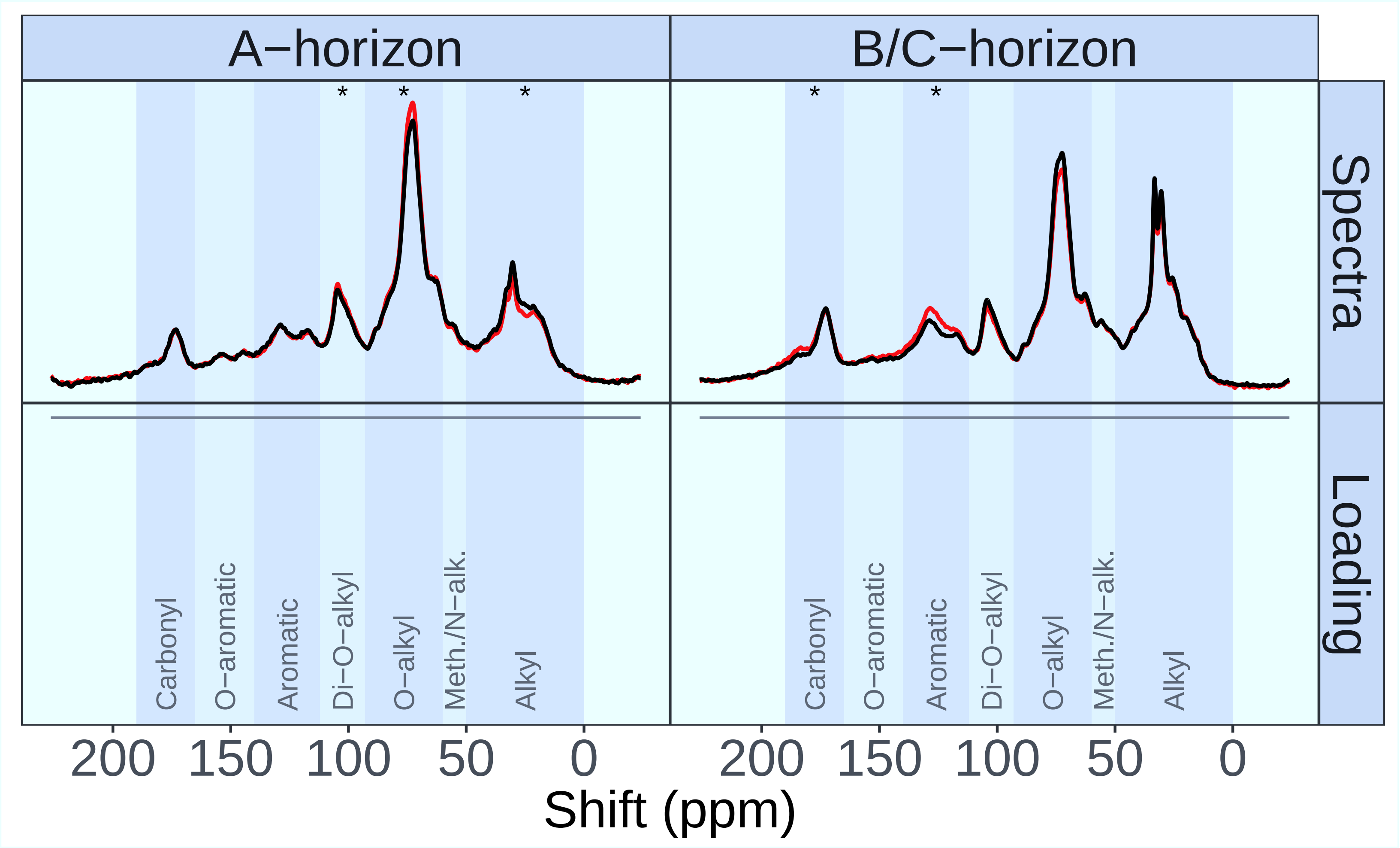
Response	*Horizon*	*Effect size*	*Lower 95% CI*	*Upper 95% CI*	*p-value*
%C	***A***	**-2.0563**	**-3.6623**	**-0.4503**	**0.0205**
%C	*B/C*	0.9525	-0.06051	1.96552	0.1113
%C	*B/C (HF-treated)*	1.8646	-0.4052	4.1344	0.0729
kg C m^{-2}	***A***	**-0.48285**	**-0.89685**	**-0.06886**	**0.03152**
kg C m^{-2}	*B/C*	0.05238	-0.42044	0.52520	0.8201

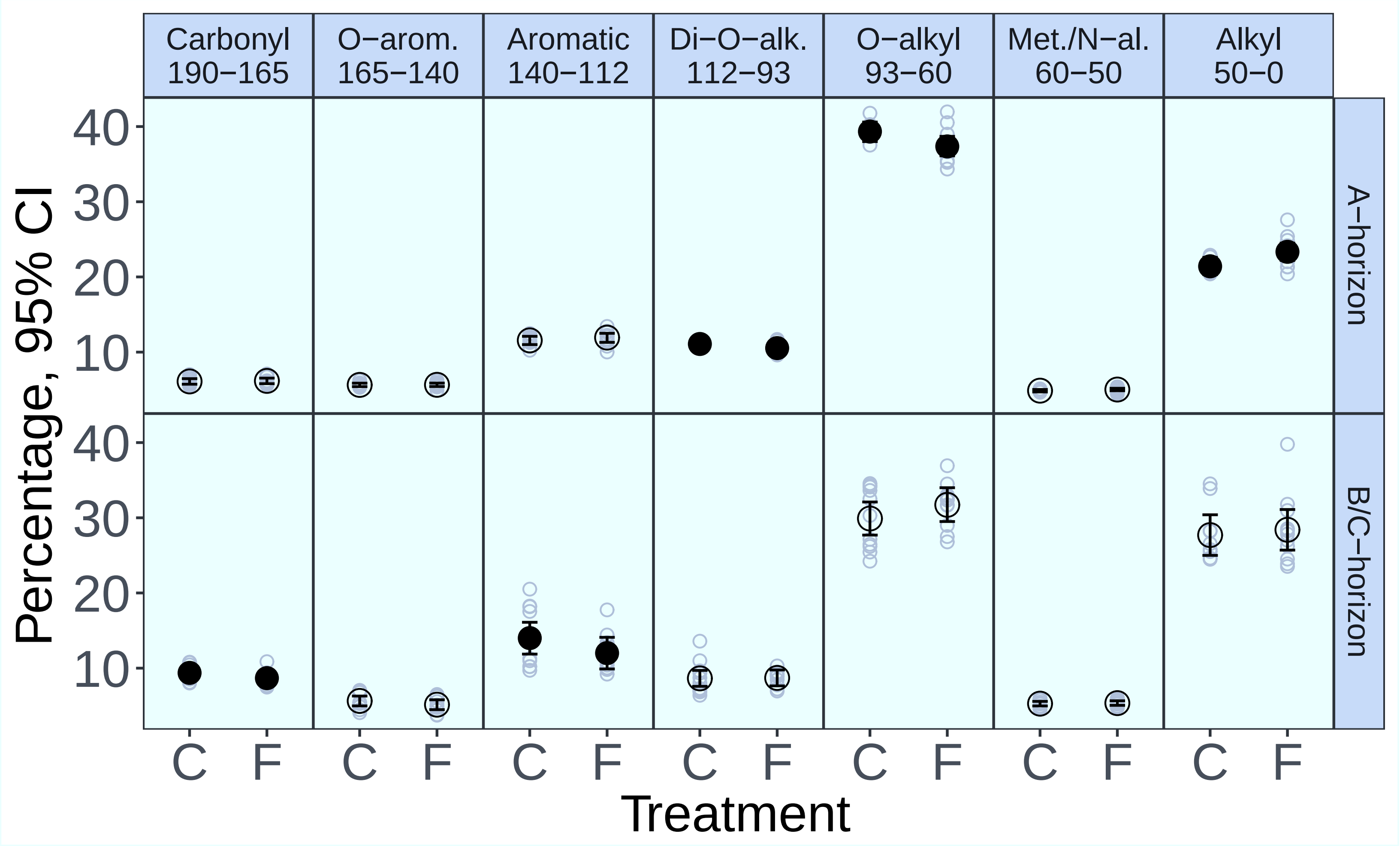
Table 2

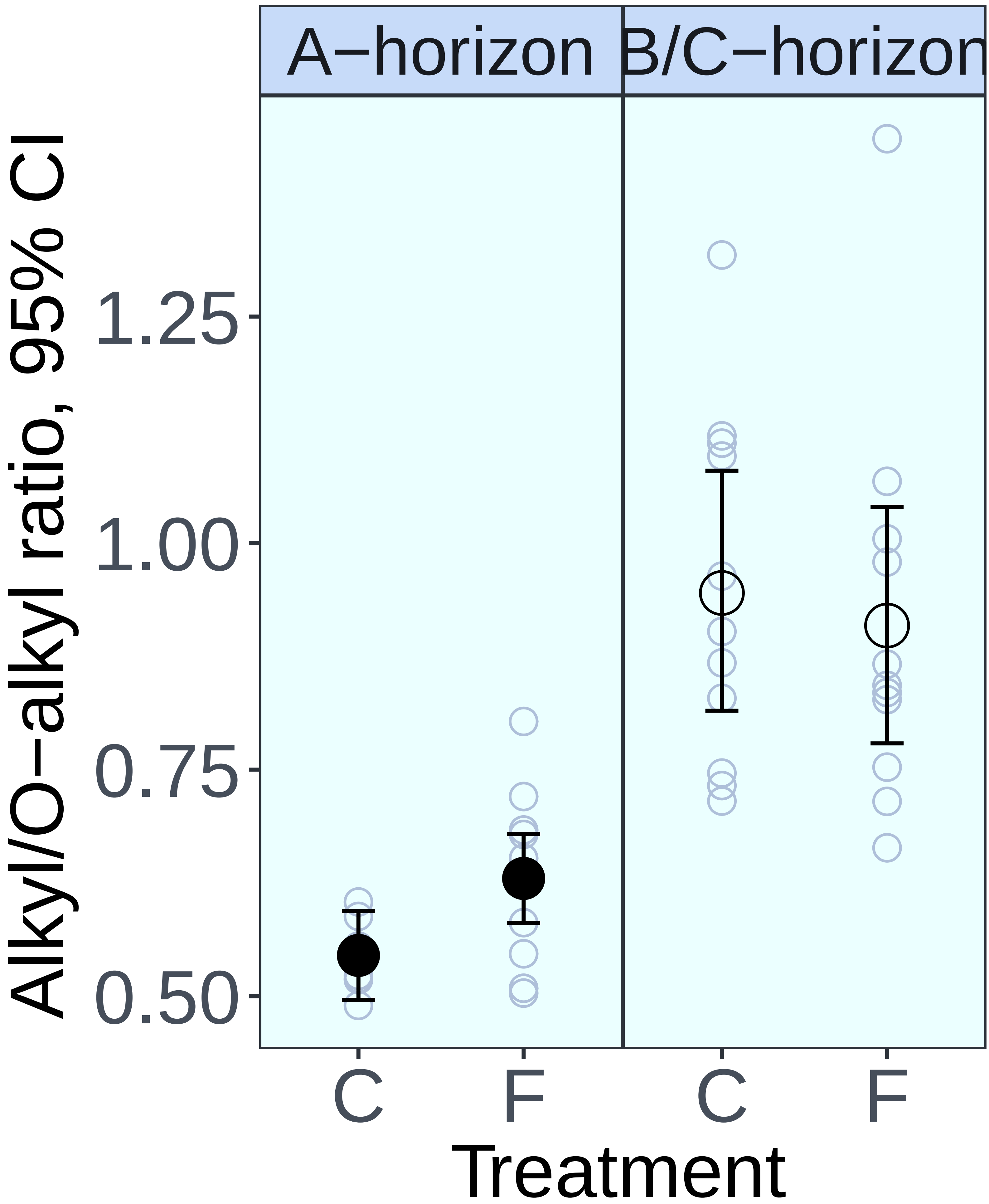
Horizon	*Integral*	*Region*	*Effect size*	*Lower 95 %CI*	*Upper 95% CI*	*p-value*
A	*Carbonyl*	*190-165*	0.000683	-0.0009593	0.0023253	0.3987
A	*O-aromatic*	*165-140*	0.0001435	-0.00281	0.003097	0.92
A	*Aromatic*	*140-112*	0.003705	-0.001253	0.008663	0.1437
A	***Di-O-alkyl***	***112-93***	**-0.005542**	**-0.009067**	**-0.002018**	**0.007272**
A	***O-alkyl***	***93-60***	**-0.019813**	**-0.032911**	**-0.006714**	**0.009047**
A	*Methoxy/ N-alkyl*	*60-50*	0.001589	-0.0006453	0.0038233	0.1522
A	***Alkyl***	***50-0***	**0.019235**	**0.009103**	**0.029367**	**0.002273**
A	***alkyl/ O-alkyl***	***ratio***	**0.085**	**0.03685**	**0.13316**	**0.003633**
B/C	***Carbonyl***	***190-165***	**-0.006956**	**-0.012728**	**-0.001184**	**0.02721**
B/C	*O-aromatic*	*165-140*	-0.0050147	-0.0104986	0.0004692	0.08004
B/C	***Aromatic***	***140-112***	**-0.019838**	**-0.037232**	**-0.002445**	**0.03473**
B/C	*Di-O-alkyl*	*112-93*	0.0006397	-0.0134508	0.0147301	0.9257
B/C	*O-alkyl*	*93-60*	0.018268	-0.009001	0.045538	0.1859
B/C	*Methoxy/ N-alkyl*	*60-50*	0.0007683	-0.0028917	0.0044283	0.6674
B/C	*Alkyl*	*50-0*	0.006997	-0.011176	0.025171	0.4351
B/C	*alkyl/ O-alkyl*	*ratio*	-0.03617	-0.17731	0.10497	0.6006


A−horizon
B/C−horizon
Percentage C, 95% CI
25
20
15
10
5
C
F
C
F
Treatment


A−horizon
B/C−horizon
C pool size [kg C m^{-2}], 95% CI
4
3
2
1
C
F
C
F
Treatment


A−horizon
B/C−horizon
Spectra
Loading
*
*
*
*
*
Carbonyl
O−aromatic
Aromatic
Di−O−alkyl
O−alkyl
Meth./N−alk.
Alkyl
200
150
100
50
0
Shift (ppm)


Carbonyl
190−165
O−arom.
165−140
Aromatic
140−112
Di−O−alk.
112−93
O−alkyl
93−60
Met./N−al.
60−50
Alkyl
50−0
A−horizon
B/C−horizon
Percentage, 95% CI
10
20
30
40
C
F
Treatment


A−horizon
B/C−horizon
Alkyl/O−alkyl ratio, 95% CI
1.25
1.00
0.75
0.50
C
F
C
F
Treatment

Supporting Information for

Soil organic carbon depletion and degradation after long term non-growing season warming in High Arctic Svalbard

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Figure S1. Snow depth

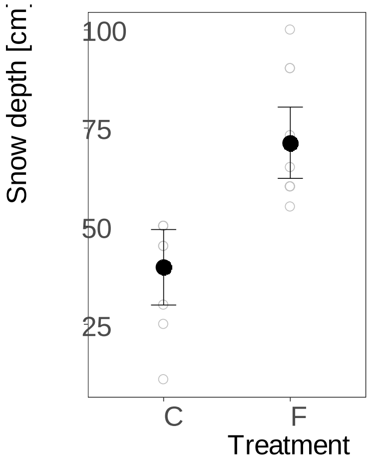

Figure S1: Snow depth in control (C) and snow fence (F) areas from each plot (raw data, grey open circles) and modelled with 95% confidence intervals (black circles). Measurements were undertaken between 4 and 12 May 2017, i.e. almost two years after the soil sampling for this study. However, we observed that snow depths in the study site are uniform across seasons and are confident that these measurements are representative for all years preceding soil sampling. Data were obtained by digging snow pits and analysed with linear mixed effects models analogous to other analyses in the main article (see there).

Figure S2. Active layer thickness/ maximum thaw depth

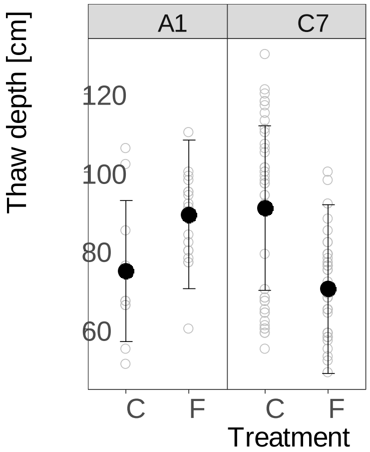

Figure S2: Active layer thickness/ maximum thaw depth from two of eleven plots (coded A1 and C7) in control (C) and snow fence (F) areas from each plot (raw data, grey open circles) and modelled with 95% confidence intervals (black circles). Measurements were undertaken between 4 and 07 September 2011, i.e. towards the end of the growing season four years prior to soil sampling for this study, in these two plots only. Data were obtained by probing with a 1cm diameter steel rod. Statistical analyses were performed with linear mixed effects models analogous to other analyses in the main article (see there) with snow fence treatment and plot (here only A1 and C7) and their interaction as significant fixed effects.

Figure S3. Soil temperature

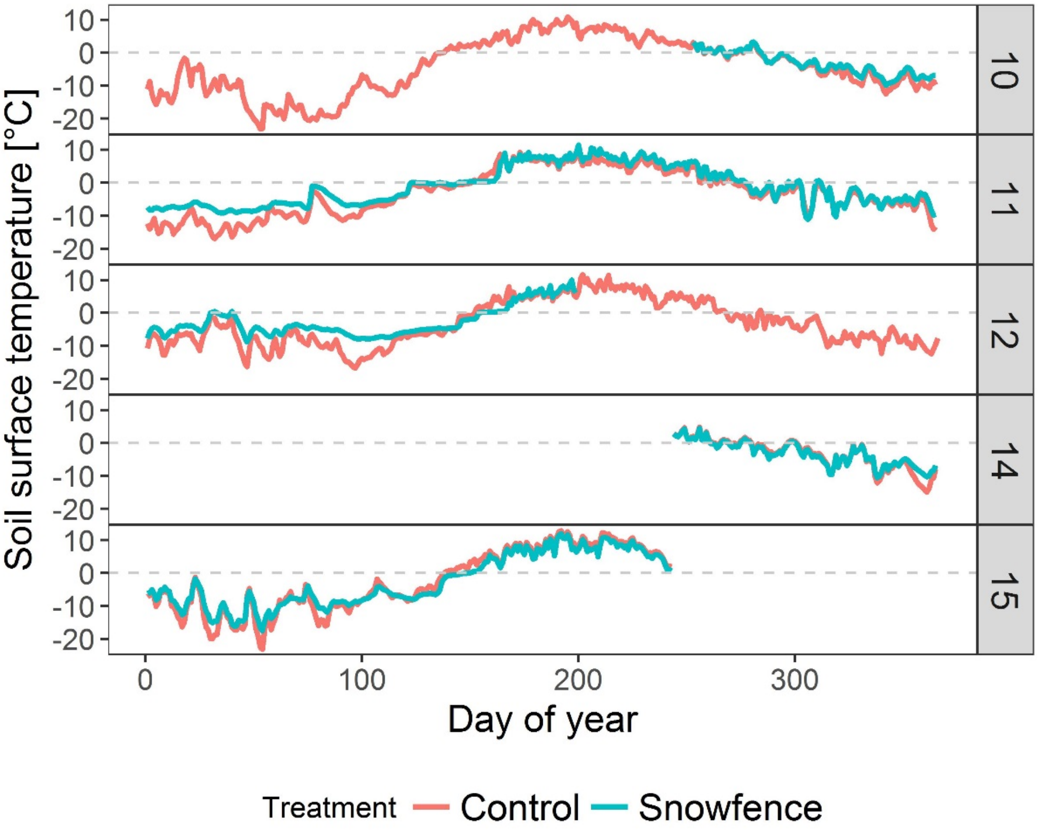

Figure S3: Soil surface temperature averaged over hourly measurements and 2 plots in 2010-12 and 10 plots in 2014-15 by Tiny Tag thermistors and data loggers at around 2 cm soil depth during years 2010 to 2015. Missing data in the figure are caused by logger failure, later deployment or because the data were not downloaded at the time of writing. The average effect of the snow fence treatment during the non-growing season (i.e. when thermistors measured below 0 degC) was 1.86 degC (95% CI: 1.83 – 1.9 degC; $p < 0.00001$; linear mixed effects model based on hourly raw data analogous to other models in the main article).

Figure S4. Soil moisture

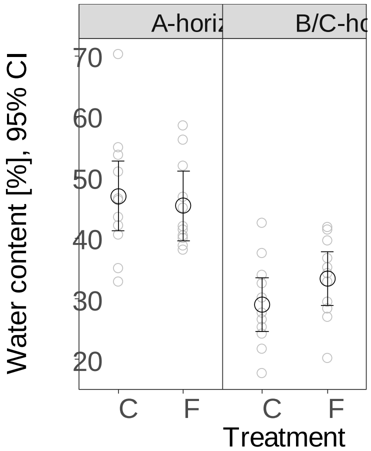

Figure S4: Soil moisture from all soil samples in control (C) and snow fence (F) areas from each plot (raw data, grey open circles) and modelled with 95% confidence intervals (black circles). Data were obtained by comparing sample weights before and after drying (see main article text). Statistical analyses were performed with linear mixed effects models analogous to other analyses in the main article (see there) and snow fence treatment effects were found to be non-significant (p = 0.5399 and 0.05592 for A- and B/C-Horizon, respectively). However, other studies from the same field site found that soil moisture in the upper 6cm was slightly higher shortly after snow melt, i.e. at the beginning of the growing season, while these changes disappeared quickly as the growing season progressed (Semenchuk, Christiansen, Grogan, Elberling, & Cooper, 2016).

References for supporting information

Semenchuk, P. R., Christiansen, C. T., Grogan, P., Elberling, B., & Cooper, E. J. (2016). Long-term experimentally deepened snow decreases growing-season respiration in a low- and high-arctic tundra ecosystem. *Journal of Geophysical Research: Biogeosciences*, *121*(5), 1236–1248. https://doi.org/10.1002/2015JG003251

Semenchuk, P. R., Elberling, B., & Cooper, E. J. (2013). Snow cover and extreme winter warming events control flower abundance of some, but not all species in high arctic svalbard. *Ecology and Evolution*, *3*(8), 2586–2599. https://doi.org/10.1002/ece3.648