Responses of dissolved organic carbon to freeze-thaw cycles associated with the changes in microbial activity and soil structure

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Abstract. Arctic warming accelerates snowmelt, exposing soil surfaces with shallow or no snow cover to freeze-thaw cycles (FTCs) more frequently in early spring and the late autumn. FTCs influence Arctic soil C dynamics by increasing or decreasing the amount of dissolved organic carbon (DOC); however, mechanisms-based explanation of DOC changes considering other soil biogeochemical properties is limited in previous research. To understand the effects of FTCs on Arctic soil responses, we
10   designed microcosms with surface organic soils from Alaska and investigated several soil biogeochemical changes under seven-successive temperature fluctuations of freezing at -9.0±0.3℃ and thawing at 6.2±0.3℃ for 12 h each. Our study found that FTCs significantly changed the following soil variables: soil respiration, DOC and total dissolved nitrogen (TDN) contents, two DOC quality indices, micro-aggregate distribution, and small-sized mesopore volume. Multivariate statistical analyses supported that the FTCs improved soil structure and functions which led to facilitated DOC decomposition by soil microbes,
15   and changes in DOC quantity and quality by FTCs. This study showed that FTCs affected DOC characteristics without negatively impacting soil microbial respiration activity, as soil microbes had previously adapted to temperature fluctuations in the Arctic. Soil micro-aggregation enhanced by FTCs and the subsequent increase in soil respiration and small-sized pore volume could promote DOC decomposition, eventually decreasing the DOC content in the soil solution. This study provides a mechanism-based interpretation of how FTCs alter DOC characteristics in Arctic soil by incorporating its structural changes
20   and microbial responses, ultimately improving our understanding of Arctic soil C dynamics.

Keywords. Freeze-thaw cycles; dissolved organic carbon; soil microbial respiration; soil micro-aggregates; pore size distribution

1. Introduction

25   Arctic tundra soils store approximately 1,300±200 Pg of soil organic carbon (SOC) in permafrost (Čapek et al., 2015), which accounts for about 30% of global SOC pool (Xu et al., 2009). Recently, Arctic warming, four times faster than global warming (IPCC, 2019), have enhanced permafrost thaw, causing the previously stored SOC to be released into greenhouse gases (CO_2 and CH_4) and/or leaching dissolved organic carbon (DOC) (Estop-Aragonés et al., 2020). Particularly, DOC released from the active layer could be further decomposed by soil microorganisms into CO_2 and CH_4, which can lead to

30   positive feedback on permafrost thawing (Foster et al., 2016; Yi et al., 2015). Permafrost thaw also influences the Arctic
watershed by inflowing terrestrial-derived DOC into the surrounding lakes and seas (Al-Houri et al., 2009). The released DOC
in the active layer can horizontally migrate along the unfrozen vicinity between the frozen layers during the freezing phase;
additionally, it can infiltrate to the deeper active layer and upper permafrost during the thawing phase (Ban et al., 2016; Han
et al., 2018). Thus, the measurement for quantitative and qualitative DOC changes are necessary for understanding the
35   permafrost C dynamics responses to Arctic warming (Xu et al., 2009; Foster et al., 2016; Perez-Mon et al., 2020).

Moreover, increased temperature of Arctic regions accelerate snow melting (Henry, 2008; Førland et al., 2011; Kreyling et
al., 2008) and cause rainfall instead of snowfall (Henry, 2013; IPCC, 2014), leading to the absence of snow cover on the soil
surface (Callaghan et al., 1998; Heal et al., 1998). In Arctic regions, snow plays a key role in protecting the tundra soils against
dramatic temperature changes caused by harsh climate (Royer et al., 2021). Exposed soil surfaces lacking snow cover are
40   likely to undergo more frequent freeze-thaw cycles (FTCs) in the early spring and late autumn, because they are directly
influenced by the diurnal fluctuations of atmospheric temperature (Kreyling et al., 2008; Henry, 2013; Freppaz et al., 2007).
Climate models project that the air temperature of the Arctic may continue to rise owing to climate change, thereby enhancing
the occurrence of FTCs in permafrost soils within the near future (Henry, 2008; Groffman et al., 2011; Grogan et al., 2004).

Numerous studies have reported influences of FTCs on soil C availability, which is strongly related to CO_2 emissions and
45   microbial growth/activity in Arctic tundra soils (Sawicka et al., 2010; Schimel and Clein, 1996; Larsen et al., 2002; Männistö
et al., 2009; Lipson and Monson, 1998; Perez-Mon et al., 2020; Grogan et al., 2004; Foster et al., 2016; Schimel and Mikan,
2005; Sjursen et al., 2005; Yi et al., 2015). In surface soil, FTCs could increase the amount of DOC in soil solution, which was
interpreted as decreases in DOC utilization by soil microbes due to cell lysis under -7°C to -11°C of freezing temperature
(Sawicka et al., 2010; Schimel and Clein, 1996; Larsen et al., 2002). On the other hand, some studies have shown negligible
50   changes or decreases in the amount of DOC by FTCs, without negative responses of microbial biomass, community and
enzymatic activities (Männistö et al., 2009; Lipson and Monson, 1998; Perez-Mon et al., 2020). This was interpreted by that
soil microorganisms in the Arctic permafrost had already adapted to extreme temperature fluctuations for a long period of time;
thus, microbial DOC utilization for growth and activity could not be inhibited compared to non-permafrost regions, such as
forest, grassland, and cropland (Gao et al., 2018, 2021; Song et al., 2017). These controversial results suggest that further
55   research and evidence of DOC changes by FTCs are required for a better mechanism-based understanding of permafrost soil
C dynamics in the early spring and late autumn.

FTCs can indirectly affect the Arctic tundra DOC dynamics through soil structural changes such as fragmentation,
rearrangement, and aggregation of soil particles (Matzner and Borken, 2008; Liu et al., 2021; Zhang et al., 2016). Owing to
the phase transitions in soil water during FTCs, soil matrix cracks and the physical degradation of soil aggregates have been
60   reported in previous studies (Oztas and Fayetorbay, 2003; Wang et al., 2012; Murton et al., 2006; Hall and André, 2003). In
contrast, some researchers have found that FTCs enhance soil aggregate stability (Lehrsch, 1998) and small-sized aggregate
formation (50-250 and 500-1000 μm) (Li and Fan, 2014). Changes in soil aggregate distribution by FTCs likely affect soil
pore volume and spatial distribution (Lu et al., 2021; Al-Houri et al., 2009; Oztas and Fayetorbay, 2003; Viklander, 1998),

leading to alterations in soil water retention (Ma et al., 2019) and DOC release (Matzner and Borken, 2008; Song et al., 2017;
Gao et al., 2018; Feng et al., 2007). Additionally, these soil structural changes may affect microbe-mediated soil C
mineralization and utilization by improving the soil water and nutrient distribution (Athmann et al., 2013; Liang et al., 2019;
Sander and Gerke, 2007). However, the linkage of how structural changes by FTCs, such as the formation of aggregates and
pores with specific sizes, affect DOC changes has not been elucidated.

This study aimed to understand the effects of FTCs on Arctic tundra DOC dynamics by considering the changes in microbial
activity and soil physical structure. We designed two parallel sets of microcosms with surface organic soils from Alaska to
simulate temperature fluctuations during the early spring in the study area. One set of microcosms was established for
destructive sampling to investigate the temporal changes in soil respiration, soil enzyme activities, DOC characteristics, and
soil aggregate-size distribution. The other set of soil core incubation was prepared with re-packed soil to measure pore size
distribution (PSD) using soil water retention curves. We tested the following hypotheses: (1) FTCs alter DOC quantity and
quality without decreasing microbial activities of the soils previously adapted to temperature fluctuations of the Arctic, and (2)
change in soil micro-aggregation by FTCs enhance microbial activities and water-holding pores, eventually affecting DOC
characteristics.

2. Materials and methods

2.1. Soil preparation

The soil for microcosm incubation was sampled from the moist acidic tundra in Council (64.51°N, 163.39°W) on the Seward
Peninsular in Northwest Alaska. The average annual temperature and precipitation over the past 30 years (1981-2020) has
been -3.1°C and 258 mm, respectively (Alaska Climate Research Center). This site is a tussock tundra dominated by cotton
grass (*Eriophrum vaginatum*), blueberry (*Vaccinium uliginosum*), lichen, and moss (*Sphagnum* spp.). Soil sampling was
performed at three random locations chosen as replicates using a soil core sampler (SIPRI corer, John's Machine Shop,
Fairbanks, AK, USA), and the samples were stored at -20°C prior to microcosm incubation. The soil core samples were divided
into organic and mineral layers, of which the surface organic soils were collected and homogenized for basic soil analyses and
incubation experiments. The basic soil properties are summarized in Table 1.

2.2. Soil incubation with freeze-thaw cycles

Soil incubation was conducted with two parallel sets of microcosms: one for destructive sampling and the other for
monitoring soil PSD changes. For the destructive sampling set, 260 g of fresh soil in a 380 mL polypropylene bottle was used
to investigate the soil biogeochemical properties influenced by FTCs. The other incubation set used a re-constructed soil core
by placing 120 g of fresh soil in a 99 mL core cylinder (5 cm diameter × 5 cm height) to measure PSD alterations under FTCs
conditions. The FTCs were performed for seven days with the seven-successive temperature fluctuations of freezing at -9.0±0.3°C
and thawing at 6.2±0.3°C for 12 h each (Fig. S1). This temperature range is representative of early spring conditions at the

95 study site, where the average minimum and maximum temperatures from April to May are -8.5℃ and 7.1℃, respectively
(Alaska Climate Research Center). The control condition (CON) maintained an average spring temperature of -2℃ without
any fluctuations (Fig. S1). Three replicates were used for CON and FTC treatments of each microcosm set. All incubation sets
had soil bulk densities and volumetric water content adjusted to 0.72 g cm^{-3} and 0.50 cm^3 cm^{-3} (70% water-filled pore space),
respectively, similar to field-soil conditions (Table 1).

2.3. Soil analyses

All soil analyses, except for the PSD measurement, were conducted using the first incubation set for destructive sampling.
Soil respiration was measured by collecting gas samples daily. The incubation bottle was sealed with a cap for 60 min, and a
gas sample was collected from the headspace through a septum using 10 mL syringes (BD Luer-Lok tip, BD Company,
Franklin Lakes, NJ, USA). The CO_2 concentration was detected using gas chromatography (Agilent 7890A, Santa Clara, CA,
105 USA) with a hydrogen flame ionization detector. The CO_2 flux was calculated based on changes in headspace concentration
over the measured period using the following Eq. (1) (Troy et al., 2013):

$$\text{Flux} = \frac{dGas}{dt} \times \frac{V}{A} \times \frac{[P \times 100 \times MW]}{R} \times \frac{273}{T}, \tag{1}$$

where $dGas/dt$ is the change in the CO_2 concentration over time, V and A are the volume and area of the incubation bottle, P
is the atmospheric pressure, MW is the molecular weight of CO_2, R is a gas constant, and T is the absolute temperature.

110 Several soil biogeochemical properties were measured at the end of seven successive FTCs. Soil extracellular enzyme
activity was determined: two oxidases (peroxidase and phenol-oxidase) and four hydrolases (β-D-glucosidase, cellobiase, N-
acetyl-glucosaminidase, and aminopeptidase) involved in soil C and N cycling (Liao et al., 2022). These enzyme activities
were measured by fluorometric assays using L-3,4-dihydroxyphenylalanine (L-DOPA) solution for oxidases and
methylumbelliferyl (MUF)-linked substrates for hydrolases (Kwon et al., 2013). To quantify the available C and N in the soils,
115 we measured the DOC and TDN contents via water extraction. Twenty grams of fresh soil was extracted with 40 mL of distilled
water, filtered through a 0.45-µm filter, and measured using a Multi N/C 3100 analyzer (Analytik Jena, Jena, Thüringen,
Germany). The filtered samples were also used to estimate DOC qualitative indices. The specific ultraviolet absorbance at 254
nm (SUVA$_{254}$), which allowed for the estimation of DOC aromaticity, was calculated using UV absorbance at 254 nm (A$_{254}$)
divided by DOC concentration (mg C L^{-1}) and the path length (m) of the UV cuvette of the spectrometer (Eppendorf, Hamburg,
120 Germany) (Lim et al., 2021). The ratio of A$_{254}$ to A$_{365}$ (A$_{254}$/A$_{365}$) was used as a proxy that is negatively related to the molecular
weight of the DOC compounds (Berggren et al., 2018). For NH_4^+-N and NO_3^--N contents analysis, 5 g of fresh soil was
extracted using a 2 M KCl solution, and the filtrates were analyzed using an auto-analyzer (Quaatro, SEAL Analytical GmbH.,
Norderstedt, Schleswig-Holstein, Germany).

Soil aggregate fractionation was performed using density separation and a subsequent wet-sieving method at the end of
125 incubation (Kim et al., 2021; Yoo et al., 2017). Further, 20 g of air-dried and 2-mm sieved soil was mixed with 35 mL of
distilled water for 30 min. The soil-water mixture was allowed to stand overnight and was then centrifuged at 3,200 rpm for

10 min. The supernatant, which was considered to be a free-light fraction (<1.0 g cm^{-3}), was collected using pre-combusted glass microfiber filters (GF/A). The heavy fraction was wet-sieved using 1000, 250, and 53 μm sieves to separate water-stable aggregates into four size classes: mega-aggregates (1000-2000 μm), macro-aggregates (250-1000 μm), micro-aggregates (53-250 μm), and mineral-associated fractions (<53 μm). The wet-sieving procedure was performed by manually shaking each sieve 100 times over 2 min. All aggregate fractions remaining on the GF/A filters and sieves were transferred to an aluminum dish, dried in an oven at 60 °C for a week, and then weighed.

To estimate the PSD, soil water release curves were generated by the Hydrus-1D model equipped with van Genuchten soil-hydraulic equations, which can be applied to organic and mineral soil (Šimůnek et al., 2013). The modeling procedure requires the van Genuchten parameters, calculated using volumetric water content at field capacity and wilting point (Kameyama et al., 2012; Likos et al., 2014). The volumetric water content at field capacity was measured by the soil water content at a matric potential of -33 kPa using a sand box (Eijkelkamp Agrisearch Equipment, Santa Barbara, CA, USA) (Yoo et al., 2020), after saturating soil core samples. As the matric potential used in a sandbox was not enough to cover the whole soil water release curves, the volumetric water content at wilting point (-1,500 kPa) was calculated using the pedotransfer function from soil carbon content and bulk density (da Silva and Kay, 1997). Lastly, the PSD was estimated from the matric potential corresponding to each pore size using the Young-Laplace equation (Kim et al., 2021).

2.4. Statistical analyses

Analysis of variance (ANOVA) was performed using the GLM procedure (SAS 9.4, SAS Institute Inc., Cary, NC, USA) to compare the measurement data between the CON and FTC treatments. Least-square means were used to assess significant differences among treatments at $p<0.05$. Following the ANOVA, we performed a principal component analysis (PCA) using the "FactoMineR" package in RStudio 4.2.1 (Rstudio Inc., Boston, MA, USA) to verify whether soil variables with significant responses could discriminate the FTC soil from the CON soil. Pearson's correlation analysis was conducted using the CORR procedure (SAS 9.4) to examine the relationship between soil variables. Finally, structural equation modeling (SEM) was employed to illustrate the mechanisms by which soil biogeochemical variables influenced by FTCs contributed to changes in DOC characteristics. Based on the ANOVA and correlation analysis results, we started with a hypothetical model containing all plausible interaction pathways, from soil microbial and physical variables significantly affected by FTC to DOC content and qualitative indicators, which was further refined using SEM techniques (Kim et al., 2020). The SEM in this study was performed using the "lavaan" package (RStudio 4.2.1). For the model evaluation and selection, we adopted the chi-square value (χ^2), p-value from the chi-square test, root mean squared error (RMSE), goodness-of-fit index (GFI), and comparative fit index (CFI) to examine whether parameters and pathways estimated the model results with significance (Yang et al., 2022; Zhao et al., 2019; Kebonye et al., 2020). Larger χ^2 values are better, and a chi-square p-value <0.05 indicates a better fit. The RMSE values <0.05 and <0.10 indicate perfect and good fits of the model, respectively, and GFI and CFI values >0.90 are indicative of a good fit. RStudio was also utilized to examine the models' goodness of fit.

3. Results

3.1. Soil biogeochemical changes by freeze-thaw cycles

160   A higher level of soil CO_2 flux was observed in FTC than in CON during the incubation period (Fig. 1a). Accordingly, the cumulative CO_2 emission in the FTC soil was 3.6 g m^{-2} hr^{-1}, six-times higher than that in the CON soil (Fig. 1b). On the other hand, no significant differences ($p > 0.05$) in microbial extracellular enzyme activities were observed between the two treatments (Table 2).

165   FTCs changed the quantity and quality of DOC in the soil solution, as presented in Table 3. The DOC and TDN contents in the FTC soil were higher by 29% than those in the CON soil ($p > 0.05$). As a proxy for DOC quality, SUVA$_{254}$ was higher but A$_{254}$/A$_{365}$ was lower in the FTC soil than in the CON soil ($p < 0.05$). In contrast, no significant changes ($p > 0.05$) in inorganic N (NH_4^+-N and NO_3^--N) content were determined because of FTCs.

FTCs caused a significant difference in the water-stable aggregate distribution between the FTC and CON soils (Table 4).
170   In the FTC soil, the micro-aggregate formation increased by 20% compared to that in the CON soil, whereas the mass proportion of mineral-associated fractions decreased by 23% ($p < 0.05$). The soil water retention curves showed that the PSD differed significantly between the FTC and CON soils (Table 5). The volume of small-sized mesopores (0.2-10 μm) was significantly greater in the FTC soil than in the CON soil ($p < 0.05$).

3.2. Influencing variables deriving dissolved organic carbon changes by freeze-thaw cycles

175   PCA was conducted to discriminate the data between the two treatments using the following seven soil variables that had significant responses to FTCs: cumulative CO_2 emission, DOC and TDN contents, SUVA$_{254}$, A$_{254}$/A$_{365}$, micro-aggregates, and small-sized mesopores (Table 3, 4, and 5). The first two principal components (PCs) accounted for 93.4% of the data variability and could distinctly discriminate the FTC soil from the CON soil (Fig. 2). Pearson correlation analysis confirmed that DOC and TDN contents correlated highly with micro-aggregate proportion, small-sized mesopore volume, and cumulative CO_2
180   emission ($r > 0.800$, $p < 0.05$; Fig. 3). In contrast, SUVA$_{254}$ and A$_{254}$/A$_{365}$ showed no significant correlation with the soil variables ($p > 0.05$), except for a correlation between SUVA$_{254}$ and cumulative CO_2 emissions ($r = 0.839$, $p < 0.05$). Lastly, SEM was developed to understand how relationships between soil structural properties and microbial activity contribute to changes in DOC quantity and quality (Fig. 4). We found that the mass proportion of micro-aggregates did not directly affect DOC content, despite a significant correlation with each other (Fig. 4). The DOC content was directly affected by the volume of the small-
185   sized mesopores and the cumulative CO_2 emissions. The SUVA$_{254}$ was directly affected by cumulative CO_2 emissions and micro-aggregate formation without the effect of small-sized mesopores. Specifically, the main factor contributing to DOC quality was the cumulative CO_2 emissions.

4. Discussion

4.1. Effects of freeze-thaw cycles on dissolved organic carbon associated with microbial activities

190   The seven-successive FTCs increased CO_2 emission from the soil during incubation (Fig. 1) and also reduced DOC and TDN contents relative to those observed in the non-treated soil (Table 3). As expected, DOC content was significantly affected by FTCs; however, no decrease but an increase in soil respiration was observed. Our findings indicate that FTCs can enhance soil respiration by accelerating the decomposition of labile organic matter by soil microbes (Grogan et al., 2004; Han et al., 2018; Foster et al., 2016; Gao et al., 2021). The main reason for these results might be that soil microorganisms have already

195   adapted to the extreme cold climate, where the temperature frequently fluctuates in early spring and late autumn in the Arctic tundra (Perez-Mon et al., 2020; Koponen and Bååth, 2016; Walker et al., 2006; Song et al., 2017). In other words, soil microbes in the Arctic tundra can survive below temperatures of -7 to -11°C (Lipson et al., 2000; Männistö et al., 2009; Lipson and Monson, 1998), the general threshold for microbial cell lysis (Sawicka et al., 2010; Skogland et al., 1988; Soulides and Allison, 1961). These results suggest that responses of DOC to FTCs might be one of the factors affecting the Arctic tundra C cycle

200   with permafrost thaw. As the C-rich permafrost thaws, soil C availability in the Arctic tundra would increase dramatically, which can lead to a high risk of CO_2 release to the atmosphere (Estop-Aragonés et al., 2020).

Meanwhile, FTCs did not significantly change the activities of extracellular enzymes (Table 2), which are released by soil microbes to obtain C and N from recalcitrant soil organic matter such as cellulose, chitin, polypeptides, and lignin (Sinsabaugh, 2010; Liao et al., 2022). This may be because soil microbes preferentially utilize simple compounds that do not require

205   enzymes for degradation in the process of DOC decomposition enhanced by FTCs (Foster et al., 2016; Gao et al., 2021; Perez-Mon et al., 2020). Thus, the DOC quality indices, $SUVA_{254}$ and A_{254}/A_{365}, differed significantly between the FTC and CON soils (Table 3), indicating that complex substrates with high aromaticity and molecular weight remained in the dissolved organic matter after successive FTCs (Berggren et al., 2018; Yang et al., 2019).

We confirmed the first hypothesis that FTCs would change DOC quantity and quality without inhibiting the activities of

210   soil microbes previously adapted to temperature fluctuations in the Arctic. Furthermore, in Figs. 3 and 4, the Pearson correlation and SEM showed high and direct correlations of cumulative CO_2 emissions with DOC content and quality indices, especially $SUVA_{254}$. These results support a mechanism-based understanding that FTCs can enhance microbial respiration activity, eventually affecting DOC quantity and quality.

4.2. Effects of freeze-thaw cycles on dissolved organic carbon associated with soil structural properties

215   FTCs increased the mass proportion of macro-aggregates compared with those observed in the non-treated soil (Table 4). This might be related to the lower water content in the FTC soil (0.63±0.03 g water/g soil) than in the CON soil (0.71±0.01 g water/g soil), although the data showed marginally significant differences between two treatments ($p=0.069$). Soil water is a major factor determining the physical degradation of soil bodies or the formation of soil aggregates, which is influenced by repeated FTCs (Zhang et al., 2016). Several studies have shown that continuous volume changes between frozen and non-

220   frozen soil water due to FTCs can cause soil cracks and aggregate fragmentation (Ban et al., 2016; Groffman et al., 2011; Zhang et al., 2016). However, as indicated in our results, Grogan et al. (2004) reported that soil water could decrease by evapotranspiration during thawing periods of FTCs. This led to our speculation that the FTC soil with low soil water content had less impact by volume change between two phases of soil water, eventually mitigating the effect of soil physical fragmentation. Moreover, during the freezing periods of the FTCs, an unfrozen water film may form on the surface of the soil

225   particles, which can derive electrical charges and intensively include solutes excluded during icing (Sletten, 1988; Zhang et al., 2016). Those characteristics could allow the unfrozen-water film to function as a binding agent between soil particles, eventually enhancing soil micro-aggregation after seven-successive FTCs.

Soil micro-aggregates enhanced by FTCs affected DOC quantity and quality mainly through changes in the microbe-mediated mechanism rather than the physical mechanism (Fig. 4). Since soil structural dynamics derived from FTCs can be a

230   critical process for soil quality and function (Rabot et al., 2018), soil aggregate formation can improve soil structural stability and govern nutrient cycling and water retention, resulting in enhanced microbial activity (Bird et al., 2000; Yoo et al., 2017; Kim et al., 2021). Consequently, our findings suggest that the improvement of soil structure and function by FTCs contribute to DOC decomposition by soil microbes, thereby reducing the DOC content and increasing the DOC aromaticity in the FTC soil (Fig. 4).

235   On the other hand, FTCs can affect DOC quantity through physical mechanisms as well, such as soil aggregation and pore formation. We found that the significant difference in soil PSD affected by FTCs was in the volume of the small-sized mesopores (Table 5). These pores were strongly related to soil micro-aggregate formation (Figs. 3 and 5). Our findings indicate that the increase in micro-aggregate formation by FTCs probably created the corresponding-sized soil pores, small-sized mesopores, through the rearrangement and formation of soil pores (Peng et al., 2015; Dal Ferro et al., 2012; Zaffar and Lu,

240   2015). Furthermore, such pores are able to hold water surrounding the soil particles (Jim and Ng, 2018; Kim et al., 2021), possibly contributing to water film development on the soil particle surfaces. As mentioned previously, this water film can have electrical charges and condensed solutes during the freezing periods of the FTCs, serving as binding materials for soil micro-aggregation (Zhang et al., 2016). Thus, we speculate that the increase in mesopores by enhanced soil micro-aggregation may provide an opportunity for dissolved solute in the soil pore water to be adsorbed and occluded to the soils, thereby

245   decreasing the DOC content in the soil solution of the FTC treatment (Fig. 4).

We confirmed the second hypothesis that the change in soil micro-aggregation by FTCs would enhance microbial activity and water-holding pores, eventually affecting DOC characteristics. The FTCs led to an increase in soil micro-aggregate formation and consequent changes in soil microbial activity and pore distribution, eventually accelerating DOC decomposition and decreasing its content in the soil solution. Our findings could contribute to a mechanism-based understanding of the effect

250   of FTCs on DOC properties through changes in soil biogeochemical properties.

5. Conclusions

This study demonstrated Arctic tundra soil responses to FTCs, focusing on the changes in DOC characteristics associated with microbial activity and soil physical structure. We found that the following seven variables differed significantly by FTCs: soil respiration, DOC and TDN contents, two DOC quality indices, micro-aggregate distribution, and small-sized mesopore
255 volume. Multivariate statistical analyses, including PCA, Pearson correlation, and SEM, contributed to the mechanism-based interpretation of how FTCs altered DOC quantity and quality mediated by the changes in microbial activity and soil physical structure. As a result, FTCs altered the DOC quantity and quality without inhibiting soil microbial respiration activity because of soil microbes that have previously adapted to temperature fluctuations in the Arctic tundra. In addition, soil micro-aggregation enhanced by FTCs and the subsequent increase in soil respiration and small-sized pore distribution could promote
260 DOC decomposition, eventually decreasing the DOC content in the soil solution. In conclusion, we elucidated the effects of FTCs on DOC characteristics in Arctic tundra soils by incorporating soil structural changes and microbial responses. It needs to study further how the deeper active layer or ice-rich permafrost thaw under warming would affect the permafrost C dynamics with FTCs.

Data availability

265 All data can be provided by the corresponding authors upon request.

Author contributions

YJ Kim and JY Jung planned the campaign; YJ Kim and J Kim performed the measurement and analysed the data; YJ Kim wrote the manuscript draft; JY Jung and J Kim reviewed and edited the manuscript; JY Jung acquired the financial support for the project leading to this publication.

270 Competing interests

The authors declare that they have no conflict of interest.

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460 **Table 1: Physicochemical characteristics of the organic soil collected from the field site of Council, Alaska.**

Soil texture			Total C	Total N	DOC	TDN	SUVA$_{254}$	A$_{254}$/A$_{365}$	Bulk density	Volumetric water content
Sand	Silt	Clay								
(%, w/w)			(g kg^{-1} soil)		(mg kg^{-1} soil)		(L mg^{-1} m^{-1})		(g cm^{-3})	(cm^3 cm^{-3})
43.4	49.5	7.1	259.2	11.4	688.63	39.62	1.77	4.28	0.72	0.49
(2.5)	(2.9)	(0.7)	(21.8)	(1.2)	(50.36)	(2.59)	(0.14)	(0.11)	(0.07)	(<0.01)

Note: The numbers in parentheses are standard errors (n=3).

Table 2: Soil enzyme activities in the soils treated (FTC) and non-treated (CON) by freeze-thaw cycles.

	Peroxidase	Phenol-oxidase	B-glucosidase	Cellobiosidase	β-N-acetyl-glucosidase	Aminopeptidase
	(μmol g^{-1} soil min^{-1})		(nmol g^{-1} soil min^{-1})			
CON	12.02 (0.62)	1.19 (0.20)	0.074 (0.004)	0.132 (0.002)	0.073 (0.001)	0.764 (0.020)
FTC	15.20 (0.99)	1.10 (0.06)	0.004 (0.001)	0.133 (0.002)	0.071 (0.001)	0.737 (0.008)

Note: The numbers in parentheses are standard errors (n=3).

465

Table 3: Characteristics of dissolved organic matter and inorganic nitrogen contents in the soils treated (FTC) and non-treated (CON) by freeze-thaw cycles.

	DOC	TDN	SUVA$_{254}$	A$_{254}$/A$_{365}$	NO$_3^-$-N	NH$_4^+$-N
	(mg kg^{-1} soil)		(L mg^{-1} m^{-1})		(mg kg^{-1} soil)	
CON	659.91[b] (3.10)	39.01[b] (0.82)	1.81[a] (0.06)	4.02[b] (0.07)	0.03 (<0.01)	0.01 (<0.01)
FTC	467.04[a] (2.93)	25.44[a] (0.30)	2.93[b] (0.14)	3.72[a] (0.01)	0.03 (<0.01)	0.02 (0.01)

Note: The values with different letters denote significant differences between treatments at a p<0.05 level. The numbers in parentheses are standard errors (n=3).

470

Table 4: Aggregate size-density distribution in the soils treated (FTC) and non-treated (CON) by freeze-thaw cycles.

	Free-light fraction	Water-stable aggregates			
	(<1.0 g cm^{-3})	Mega-aggregate (1000-2000 μm)	Macro-aggregate (250-1000 μm)	Micro-aggregate (53-250 μm)	Mineral-associated fraction (<53 μm)
		(% by weight)			
CON	0.56 (0.05)	6.88 (0.83)	23.11 (0.90)	31.44[a] (0.96)	38.00[b] (2.08)
FTC	0.77 (0.08)	6.91 (0.76)	25.31 (1.44)	37.78[b] (1.81)	29.23[a] (2.26)

Note: The values with different letters denote significant differences between treatments at a p<0.05 level. The numbers in parentheses are standard errors (n=3).

475 **Table 5: Pore size distribution (PSD) in the soils treated (FTC) and non-treated (CON) by freeze-thaw cycles.**

	Total soil pore	Pore volume among the different-sized classes			
		Macropore	Mesopore		Micropore
		(>30 μm)	Large (10-30 μm)	Small (0.2-10 μm)	(<0.2 μm)
		(cm^3 g^{-1} soil)			
CON	1.066 (0.022)	0.294 (0.006)	0.118 (0.003)	0.498[a] (0.009)	0.155 (0.005)
FTC	1.112 (0.023)	0.298 (0.006)	0.118 (0.002)	0.532[b] (0.011)	0.163 (0.005)

Note: The values with different letters denote significant differences between treatments at a $p<0.05$ level. The numbers in parentheses are standard errors (n=3).
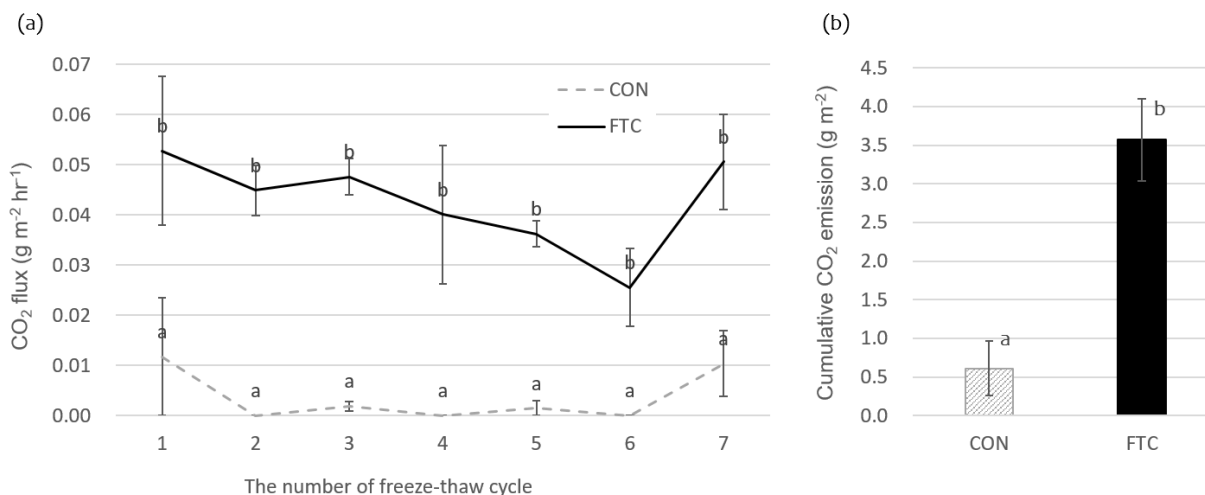
Figure 1: Temporal changes in CO_2 flux (a) and cumulative CO_2 emission (b) in the soils treated (FTC) and non-treated (CON) by freeze-thaw cycles. Different letters denote significant differences at a $p<0.05$ level (n=3).
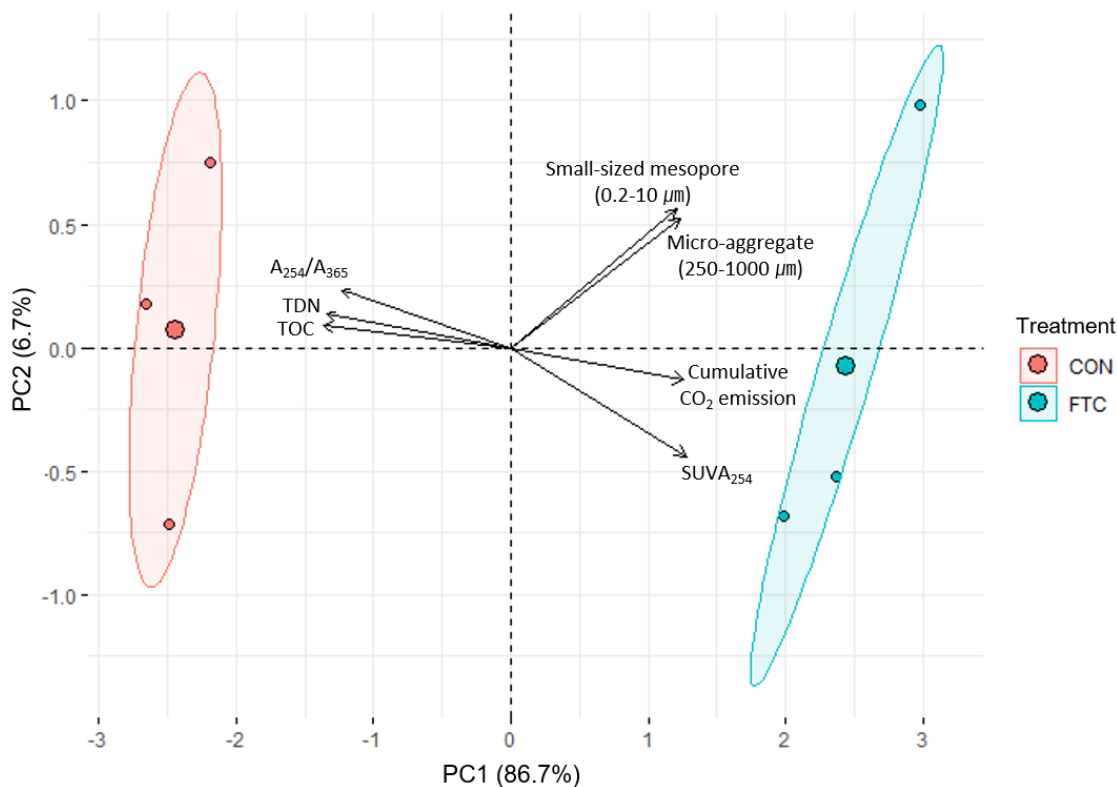
Figure 2: Principal component analysis (PCA) for the observed variables in the soils treated (FTC) and non-treated by freeze-thaw cycles (n=3).
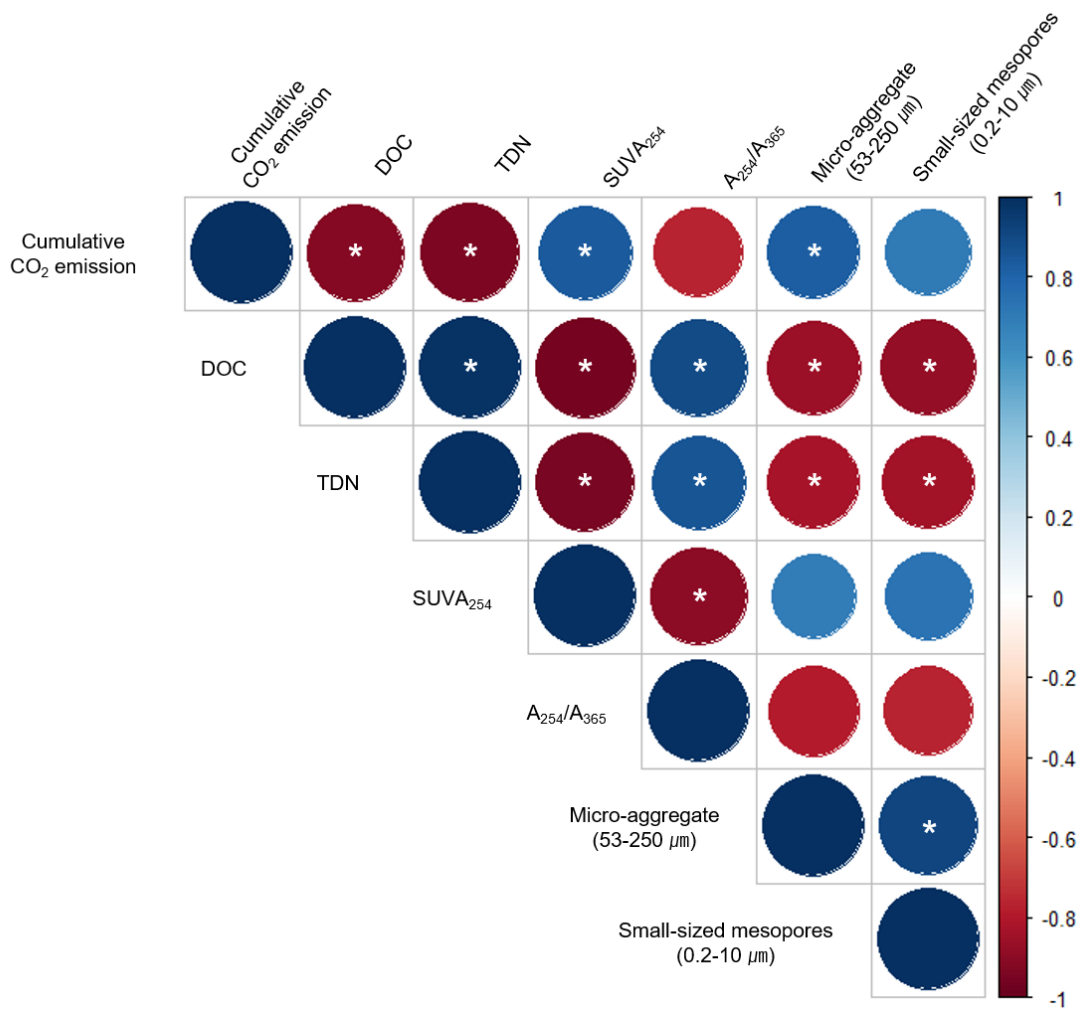
Figure 3: Correlation matrix between the observed variables in the soils treated (FTC) and non-treated (CON) by freeze-thaw cycles.
490   **The * denote significance at a p<0.05.**
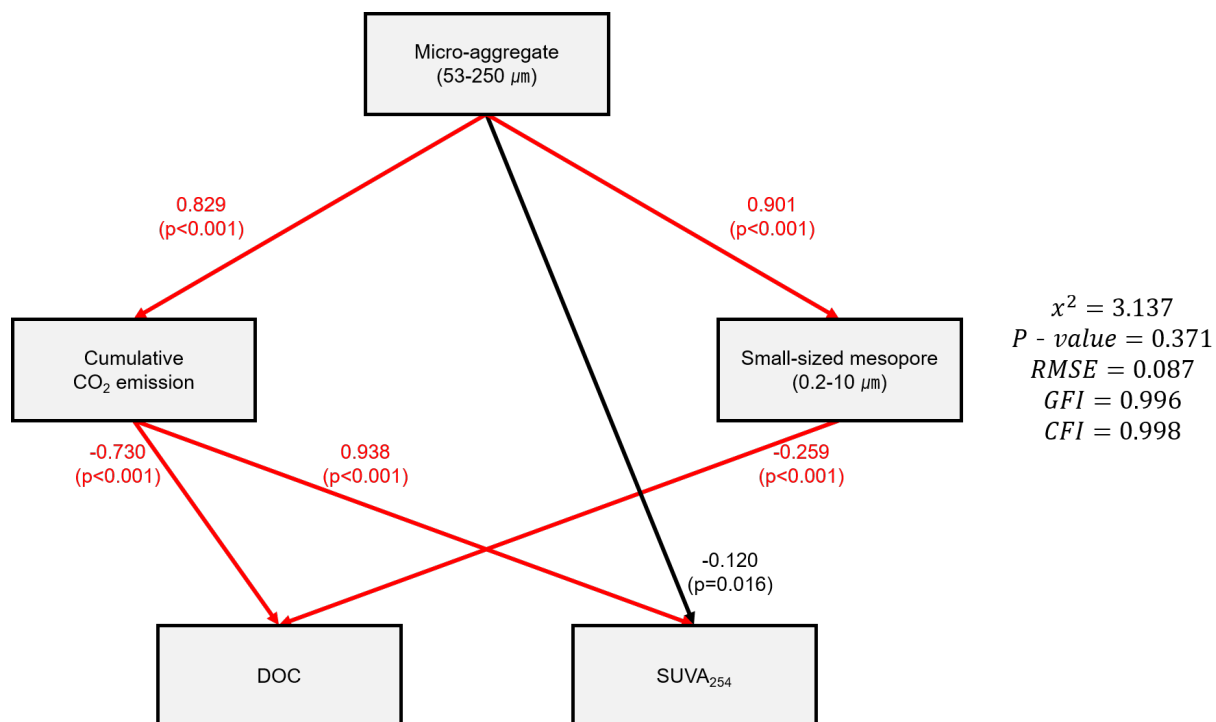
Figure 4: A conceptual diagram for the structural equation model analysis. The red and black arrows indicate the correlated pathway between at the significance levels of p<0.01 and p<0.05, respectively (n=3). Numbers denote standardized parameter values for the covariance relationship, with the sign indicating a positive and negative effect.