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The influence of soil frost on the quality of dissolved organic carbon in a boreal forest soil: combining field and laboratory experiments

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Abstract Riparian soils exert a major control on stream water dissolved organic carbon (DOC) in northern latitudes. As the winter climate in northern regions is predicted to be particularly affected by climate change, we tested the sensitivity of DOC formation to winter conditions in riparian soils using an 8 year field-scale soil frost manipulation experiment in northern Sweden. In conjunction with the field experiment, we also carried out a laboratory experiment based on three levels of four winter climatic factors: frost intensity, soil water content, frost duration and frequency of freeze–thaw cycles. We evaluated changes in lability of DOC in soil solution from lysimeter samples taken at different depths (10–80 cm) as well as from DOC extracted from soils in the laboratory, using carbon-specific ultraviolet absorbance at 254 nm ($sUVA_{254}$). In the field, significantly more labile DOC was observed during the spring and summer from upper horizons of frost-exposed soils, when compared to controls. In addition, the amount of labile DOC was positively correlated with frost duration at a soil depth of 10 cm. In the laboratory, frost intensity was the factor that had the greatest positive influence on DOC lability; it also reduced the C:N ratio which may indicate a microbial origin of the DOC. The laboratory experiment also demonstrated significant interactions between some of the applied climatic factors, such as frost intensity interacting with water content. In combination, field and laboratory experiments demonstrate that winter soil conditions have profound effects on DOC-concentration and quality during subsequent seasons.

Keywords Soil frost · $sUVA_{254}$ · Dissolved organic carbon · Water content · Riparian zone · CCF design

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Introduction

Dissolved organic carbon (DOC) is made up of a heterogeneous mixture of mainly humic substances with high molecular weight, but also includes low molecular weight constituents such as sugars, amino acids and organic acids. In soils, DOC originates from recently accumulated litter and humus, root exudates, and microbial biomass (Kalbitz et al. 2000). Terrestrially derived DOC plays a fundamental role in surface water chemistry, can influence biogeochemical cycling (Hruska et al. 2003; Shafer et al. 1997; Tipping 1993) and food web structure (Jansson et al. 2007) of aquatic ecosystems, and have consequences for catchment carbon balance (Cole et al. 2007;

Nilsson et al. 2008). The quality of DOC and the availability of nutrients are among the factors that control the growth efficiency of heterotrophic bacteria in streams and lakes (Berggren et al. 2007; del Giorgio and Cole 1998). The quality of DOC, in turn, depends on both the origin and state of oxidation (Kalbitz et al. 2000). For example, dissolved organic matter of microbial origin normally contains lower concentrations of aromatic compounds (McKnight et al. 1991) and has a lower C:N ratio compared to dissolved organic matter that originates from soils and higher plants (McKnight et al. 1997). In addition, climate can have important effects on DOC formation and export to surface waters (Erlandsson et al. 2008; Haei et al. 2010; Sarkkola et al. 2009). Recently, this fact has resulted in accelerated research efforts aimed at elucidating the mechanisms of soil DOC formation and its sensitivity to anticipated climate change (Davidson and Janssens 2006; Matzner and Borken 2008).

About 40% of the global soil carbon pool is stored at high latitudes (IPCC 2000), which include boreal regions characterized by distinct winters and seasonal snow cover. Snow cover accounts for a major part of the annual water budget in boreal landscapes, and also acts to reduce heat loss from the ground during the coldest months of the year (Stieglitz et al. 2003), thus exerting control over soil temperature and frost development (Mellander et al. 2007). Through these effects on key soil properties, snow cover influences biogeochemical processes during the winter, with consequences for DOC formation and export (Groffman et al. 2001; Liptzin et al. 2009; Williams et al. 1998).

Most of the boreal region is expected to be significantly affected by climate change, most notably during winter seasons (IPCC 2001). One important implication of this predicted climate perturbation is that the timing, extent and duration of snow cover are expected to change. Such changes have the potential to alter soil temperature, frost regime and the frequency of freeze–thaw cycles (FTCs) (Stieglitz et al. 2003). To better understand how surface waters (e.g. streams and lakes) will be influenced by a changing climate, it is important to identify the mechanisms regulating the size of the soil DOC pool and to determine how environmental variation influences the biogeochemical properties of DOC exported to adjacent aquatic habitats. At lower sub-zero temperatures (greater frost intensities), physical disruption of soil aggregates (Kalbitz et al. 2000) and the limited amount of liquid water (Öquist et al. 2009) can significantly affect the release and quality of DOC. Mortality of fine roots (Tierney et al. 2001) or lysis of freeze-damaged soil organisms (Soulides and Allison 1961), as well as an increased number of FTCs (Henry 2007) may also affect the soil DOC pool. To date, only a few field-scale studies have examined the effect of soil frost on DOC (Austnes et al. 2008; Fitzhugh et al. 2001; Groffman et al. 2001, 2010), and these have focused mainly on the release of DOC rather than its composition.

The riparian zone is a major source of terrestrial carbon entering adjacent streams (Bishop et al. 2004; McGlynn and McDonnell 2003; Seibert et al. 2009). In a previous field study, we demonstrated an increase in soil solution and stream water DOC concentrations ([DOC]) as a result of greater frost intensity and duration in a riparian zone in northern Sweden (Haei et al. 2010). To further improve our understanding of the mechanisms by which winter processes regulate the quality and quantity of DOC in boreal soils, which is subsequently transported to adjacent streams, we combined a long-term field experiment with a controlled laboratory experiment involving soil samples taken from the same site. The use of a long-term field experiment in combination with a laboratory experiment that examines the influence of key environmental controls under controlled conditions has not previously been reported in the literature. Our hypothesis was that the soil DOC pool and its lability are affected by winter climatic factors (i.e. soil frost intensity, soil water content, soil frost duration, and frequency of FTCs). In addition, this influence stems not only from the individual factors per se, but also from the interactions between them.

Materials and methods

Field manipulation of soil frost

The study site is the riparian zone of a small first order stream in the Krycklan catchment at Svartberget Experimental Forest (64°14′ N, 19°46′ E), 60 km northwest of Umeå, Sweden (Buffam et al. 2007). The experimental location has undergone extensive soil, hydrological (Laudon et al. 2004), and biogeochemical (Petrone et al. 2007) monitoring since 1996. These riparian soils have been showed to control stream water DOC concentrations (Köhler et al. 2009), organic carbon bioavailability (Ågren et al. 2008), dissolved inorganic carbon fluxes (Öquist et al. 2009), and stream metal loading (Klaminder et al. 2006). The experimental catchment is composed of 80 year-old Norway spruce (*Picea abies*), with an understory dominated primarily by blueberries (*Vaccinium myrtillus*). Ground cover is composed of moss-mats, mainly *Hylocomium splendens* and *Pleurozium schreberi* (Forsum et al. 2008). The annual mean air temperature (1980–2009) in the area is 1.7°C, with average air temperatures of −9.6 and 14.6°C in January and June, respectively. On average, the ground is covered by snow for 168 days per year (1980–2007) from mid November to early May. The average maximum snow depth is 76 cm (varying between 43 and 113 cm, 1980–2007). Average soil frost in a reference location is 16 cm (varying between 2.5 and 79 cm, 1992–2007). All of the climatic parameters are measured at a reference climate station, situated approximately 1 km from the study catchment.

The field manipulation of soil frost was initiated in the autumn of 2002, originally to explore the effects of variable temperature and frost regimes on soil DOC. The experimental design was reported in detail by Öquist and Laudon (2008) and Haei et al. (2010), but in short, three different soil frost treatments were applied in triplicate square plots of 9 m^2: shallow soil frost (SSF), deep soil frost (DSF) and ambient soil frost (ASF). In the SSF treatment, the soil surface was insulated using geotextile bags filled with Styrofoam pellets which limited soil frost development. The ASF (control) plots were kept under ambient conditions. In the DSF treatment, the plots were covered with roofs which prevented the accumulation of snow on the ground. At the end of winter, the snow that had accumulated on the roofs was placed back on the soil surface to maintain the water balance.

Soil solution was sampled at depths of 10, 25, 40, 60 and 80 cm by means of suction lysimeters at all plots, 8–15 times per year between 2003 and 2008. Soil temperature and water content (using time-domain reflectometry (TDR)) were continuously monitored at all depths. Dramatic reduction in water content acted as an indicator of soil freezing (Nyberg et al. 2001), and in combination with soil temperature was used to estimate the maximum soil frost depth. Maximum frost depth was calculated by linear interpolation between frozen- (temperature < 0°C and minimum water content) and unfrozen-soil layers (temperature > 0°C) (Haei et al. 2010; Öquist and Laudon 2008). Lysimeters were installed in the center of each sampling plot, 2.9 ± 0.5 m [mean ± standard deviation (SD)] away from the stream. One day prior to sampling, lysimeters were exposed to a 100-kPa vacuum and the initial portion of the sample was discarded prior to analysis. Soil solution samples were kept frozen until DOC analysis was conducted using a TOC-5000 Shimadzu analyzer. Ultraviolet (UV) absorbance in the wavelength range 190–510 nm was measured in 1-cm quartz cuvettes with a Hewlett Packard 8452A diode array spectrophotometer.

Laboratory multi-factor experiment

To better understand the winter factors affecting the composition and amount of soil water DOC after soil frost thaw, we investigated carbon-specific UV absorbance ($sUVA_{254}$; see below) and [DOC] in response to four individual factors (soil frost intensity, soil water content, soil frost duration and frequency of FTCs) and their interactions.

The experimental design was based on a central composite face-centered (CCF) model, using the Modde 9.0 statistical package (Umetrics, Umeå, Sweden). CCF design is often classified as a response surface modelling (RSM) design, corresponding to only three levels of each factor. In a CCF design, the experimental region is a cube in which the factor levels are maintained between the low and high defined levels, and the axial points are centered on the faces of the cube (Eriksson et al. 2008). The advantage of such a design is that fewer experiments are needed compared to a full factorial design. Our CCF design was centered on the four factors at three different levels, giving a total of 27 runs, with three replicated centre points (Table 1).

In order to mimic the natural conditions we collected the riparian soil samples just before the onset of the winter, in early December 2007. The soil samples were collected close to the site of the soil frost experiment and at the same distance from the stream. Samples were collected from the upper 30 cm and pooled. Roots and coarse debris were removed and the sample was sieved using a 3 mm mesh. Soil water holding capacity (WHC) was measured on four

Table 1 Factors and levels of climatic factors applied in the laboratory experiment, which was based on a CCF design

Factors	Frost intensity (°C)	Water content (WHC%)	Frost duration (months)	FTC (Cycles)
Levels	−12	30	2	1
	−6	60	4	4
	0	90	6	7

replicate samples, which were placed in plastic cylinders (height: 20 mm, diameter: 25 mm) with nylon mesh bottoms, soaked in water for 12 h, and drained for 1 h prior to drying at 105°C for 12 h (Ilstedt et al. 2000). The WHC was calculated as the mass ratio between the water content difference, as measured before and after drying, and the dry soil. Loss on ignition (LOI) was determined by ashing dried samples at 500°C for 4 h ($n = 3$). Soil samples were air dried at 12°C to adjust their water contents to 30, 60 and 90% WHC. After moisture adjustment, we placed samples in 250 ml sealed polypropylene jars and kept them at constant temperatures of −12 (±1), −6 (±1), or 0 (±1)°C using temperature-controlled freezers and an ice-bath, respectively. The temperature of the latter was maintained by regular addition of ice. Sealed jars were kept at these temperatures for 2, 4 or 6 months and exposed to different numbers of FTCs (1, 3 or 7 cycles) at equal time intervals for each frost duration period. Each cycle included thawing of the sample at 5°C for 48 h, and subsequent freezing at the initial temperature.

At the end of each freezing period (2, 4 and 6 months), we extracted the soil DOC with water, using an approach modified from Jones and Willett (2006): 25 g samples of soil were placed in clean high density polyethylene (HDPE) bottles with 125 ml MilliQ water. The HDPE bottles were shaken at 3.33 Hz for 1 h and centrifuged at 8000×g for 10 min at 20°C. Supernatants were collected and filtered through 0.45 µm mixed cellulose ester (MCE) filters prior to analyses. One subsample of the supernatant was immediately frozen before analysis of DOC and total dissolved nitrogen (TDN), using an IL-550 TOC-TN analyzer (Hach-Lange, Manchester, UK), as well as dissolved inorganic nitrogen (DIN; see below). On an additional unfrozen subsample, we measured UV–visible absorbance in the wavelength range of 190–510 nm.

DON was estimated by subtracting the DIN (NO_3^-–N and NO_2^-–N, NH_4^+–N) from TDN and the C:N ratio was calculated by dividing the DOC by the DON. C:N ratio can be used as an indicator for the quality of the organic matter and its origin (McKnight et al. 1991, 1997). The analyses of NO_3^-–N, NO_2^-–N and NH_4^+–N relied on the standard methods (Bendschneider and Robinson 1952; Chaney and Marbach 1962). NO_3^-–N and NO_2^-–N analyses were carried out using an Alpkem RFA 300 autoanalyzer (ALPKEM Corporation, Clackamas, USA).

Data analysis

For the soil solution samples from the field experiment, carbon specific ultraviolet absorbance ($sUVA_{254}$) was calculated by dividing the absorbance at the wavelength of 254 nm (m^{-1}) by [DOC] (mg C l^{-1}). $sUVA_{254}$ is positively correlated with the aromaticity of carbon (Vogt et al. 2004; Weishaar et al. 2003). Since the aromatic compounds are, generally, less bioavailable than aliphatic carbon (Perdue 1998), lower values of $sUVA_{254}$ are associated with more bioavailable DOC and a higher substrate quality (Berggren et al. 2009). We excluded the first year's data in order to minimize any artifacts resulting from the establishment of the experimental site and to give enough time for lysimeters to equilibrate. The data for the year 2006 were also excluded because of missing absorbance measurements. We assessed the soil frost treatment effect on the soil solution $sUVA_{254}$ using a non-parametric Mann–Whitney U-test in the SPSS 17.0 software (SPSS Inc., Chicago, IL, USA). We evaluated the analysis at the 5% significance level.

To evaluate the effects of the duration of soil frost as well as soil temperature on variation in $sUVA_{254}$ at a soil depth of 10 cm, we performed linear regression analyses using the SPSS 17.0 software (SPSS Inc., Chicago, IL, USA). These analyses were based on the average $sUVA_{254}$ at a depth of 10 cm in each plot (3 DSF, 3 SSF and 2 ASF), in response to the mean duration of soil frost as well as the soil temperature at the same depth, during the preceding winter. The duration of soil frost was defined as the number of days when the soil temperature was <0°C and the mean winter soil temperature was calculated for the period 1st November– 15th March, at a depth of 10 cm. Because of malfunctioning temperature probes, we excluded the data for one of the ASF treatment plots as well as the temperature data for the last winter in the other two ASF plots.

In the laboratory study, we identified significant winter-related factors and interactions influencing $sUVA_{254}$, the C:N ratio and [DOC] using multiple linear regression (MLR) models. The initial individual MLR models for $sUVA_{254}$, C:N and [DOC] were based on the four individual factors and all possible pairs of two way interactions (six pairs). Histograms were used to test whether the responses were normally distributed. As a result of these tests, we log-transformed $sUVA_{254}$ and [DOC], and neg-log-transformed C:N, to ensure normal distribution. On the basis of normal probability plots of residuals (outside ± 4 SD), one outlying experiment (out of the total 27 experiments) for the $sUVA_{254}$ model was excluded. We used coefficient plots at the 10% significance level to refine the initial model. We report the significant factors and interactions based on the 5% significance level. R^2_{adj} and Q^2 for each final model indicate to which extent the model can explain and predict, respectively, the variations in the studied variable.

We calculated the responses ($sUVA_{254}$, C:N ratio and [DOC]) and their 95% confidence intervals as affected by different interactions of factors using the function "Prediction" in the statistical package Modde 9.0, while keeping the other individual factors at their center levels.

Results

Field manipulation of soil frost

In the DSF treatment, snow removal resulted in deeper soil frost and lower temperatures compared to the ASF and SSF treatments. The average (±SD) maximum soil frost depths were 49 (6), 29 (3) and 4 (5) cm for the DSF, ASF and SSF treatments, respectively. Minimum temperatures (±SD) for these treatments were −5.2 (1.1), −2.2 (1.0) and −0.2 (0.4)°C at a depth of 10 cm. The mean value of $sUVA_{245}$ (March–October) in the top 40 cm was in the range 3.6–5.2 l mg^{-1} C m^{-1} and was generally lowest in the DSF plots and highest in the ASF plots (Table 2). At a depth of 10 cm, the $sUVA_{254}$ value

Table 2 Number of soil solution samples, $sUVA_{254}$ (4 year mean ± SD) in the spring and summer (March–October 2003, 2004, 2007 and 2008) and results of Mann–Whitney tests for the three soil frost treatments at five depths down the soil profile

Soil depth (cm)	Soil frost treatment	Number of soil solution samples	$sUVA_{254}$ (Mean ± SD) (L mg^{-1} C m^{-1})	*p*-value for the Mann–Whitney test		
				DSF & SSF	DSF & ASF	SSF & ASF
10	DSF	21	3.6 ± 1.1	0.21	0.01	0.15
	SSF	12	4.0 ± 0.5			
	ASF	25	4.5 ± 1.1			
25	DSF	6	3.8 ± 0.4	0.04	0.03	0.96
	SSF	36	4.9 ± 2.2			
	ASF	43	4.6 ± 1.3			
40	DSF	49	4.6 ± 0.9	0.37	0.48	0.59
	SSF	66	4.4 ± 0.9			
	ASF	63	4.8 ± 1.8			
60	DSF	54	4.3 ± 1.2	0.83	0.65	0.59
	SSF	58	4.5 ± 1.2			
	ASF	55	4.5 ± 1.2			
80	DSF	57	4.6 ± 1.2	0.20	0.44	0.34
	SSF	67	4.7 ± 1.7			
	ASF	33	5.2 ± 2.1			

for the DSF treatment was significantly lower compared to that for ASF. At a depth of 25 cm, the $sUVA_{254}$ value for the DSF treatment was significantly lower than for either SSF or ASF (Table 2). No statistical difference was found between the treatments in the deeper soil layers, although they displayed similar patterns to each other (Table 2).

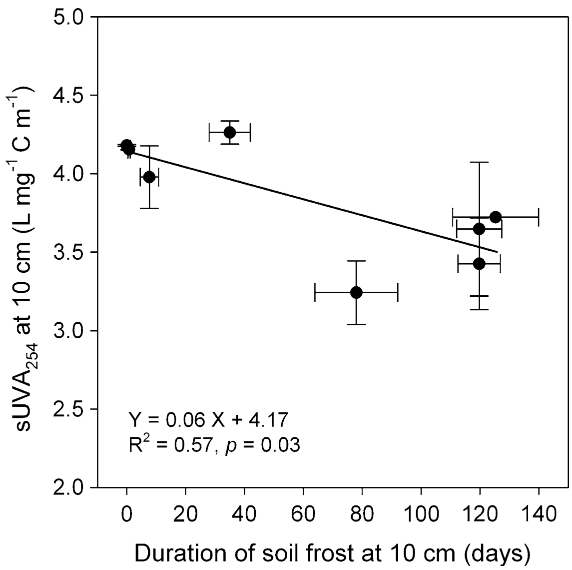


Fig. 1 Soil solution $sUVA_{254}$ at a depth of 10 cm during spring and summer in response to soil frost duration during the preceding winter. Each point indicates one plot (3 DSF, 3 SSF and 2 ASF). $sUVA_{254}$ (March–October) and the soil frost duration are averages for the years 2003, 2004, 2007 and 2008. Soil frost duration refers to the number of days with soil temperature < 0°C. The *bars* represent standard errors

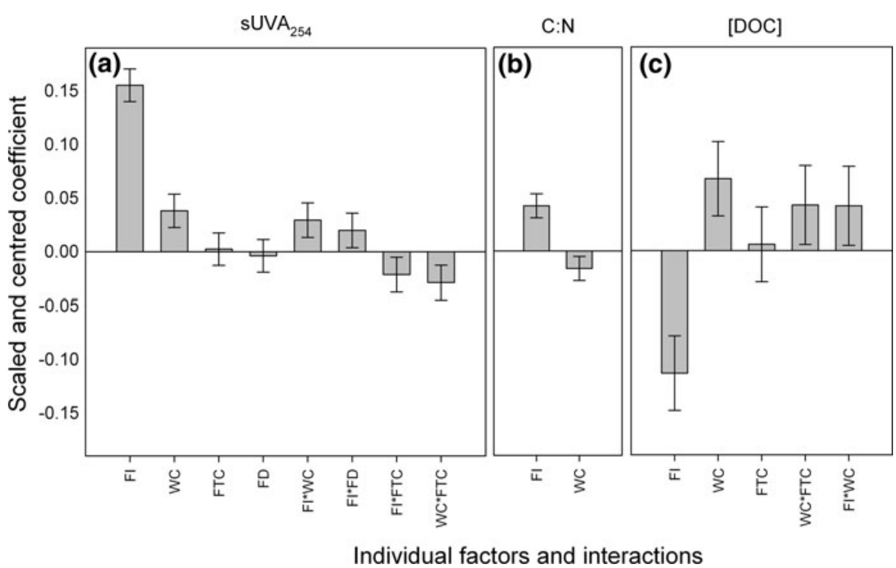
Longer periods of soil frost at a depth of 10 cm resulted in lower $sUVA_{254}$ values at the same depth during the following spring and summer (Fig. 1). About 60% of the variation in $sUVA_{254}$ could be explained by the duration of soil frost ($n = 8$, $R^2 = 0.57$, $p = 0.03$; Fig. 1). At a depth of 10 cm, $sUVA_{254}$ was positively correlated with the preceding winter's soil temperature ($R^2 = 0.41$). However, winter temperature did not produce a significant regression at the 5% level ($p = 0.09$).

Laboratory multi-factor experiment

The average (±SD) organic carbon content of the pooled soil sample was 18.3% (0.3). The final MLR models, based on the significant factors and interactions, revealed R^2_{adj} and Q^2 values of 0.87 and 0.64 for $sUVA_{254}$, 0.72 and 0.68 for C:N and 0.73 and 0.64 for [DOC].

The coefficient plot (Fig. 2a) for $sUVA_{254}$ showed that soil frost intensity (sub-zero temperatures) followed by soil water content were the two significant influential factors and that both were positively correlated with $sUVA_{254}$. There was a significant interaction between soil frost intensity and water content (Fig. 3a). Specifically, the effects of water content on $sUVA_{254}$ were more pronounced at lower frost intensities; higher water contents shifted $sUVA_{254}$ values upwards (towards higher aromaticity) (Fig. 3a). $sUVA_{254}$ was generally lower at the

Fig. 2 Scaled and centered coefficient plots for **a** $sUVA_{254}$, **b** C:N and **c** [DOC] for the final MLR model, showing the significant factors and interactions; *FI* frost intensity, *WC* water content, *FTC* freeze–thaw cycle and *FD* frost duration. The *bars* indicate 95% confidence intervals



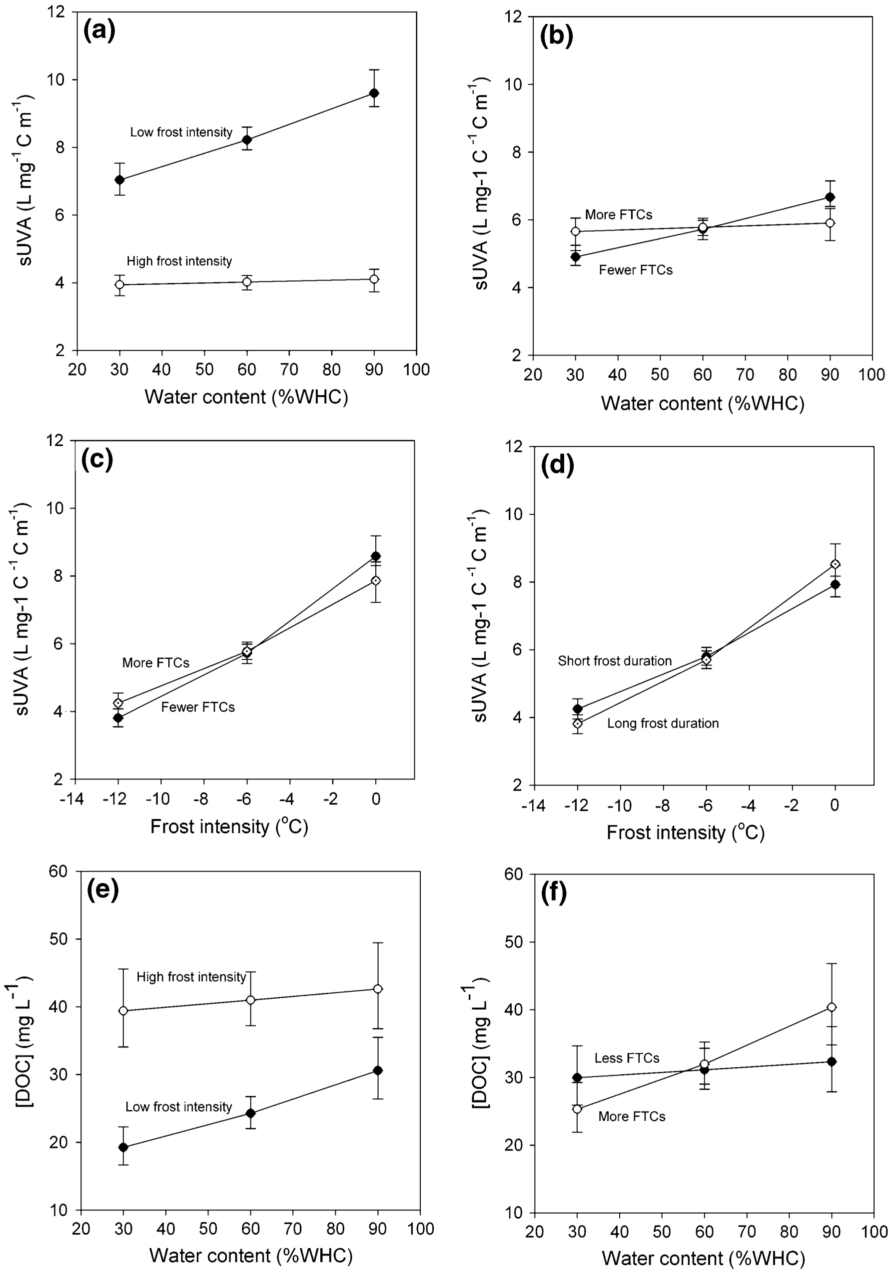


Fig. 3 Significant two way interactions for **a**–**d** $sUVA_{254}$ and **e**–**f** [DOC] in the laboratory experiment, designed on the basis of a CCF model and analyzed by MLR (95% significance level)

greater frost intensities compared to the control (0°C, Fig. 3a). The frequency of FTCs had no effect on $sUVA_{254}$, but there was a slight interaction with both soil water content and frost intensity (Fig. 3b, c). Permanent frost (fewer FTCs) increased $sUVA_{254}$ values (indicating lower DOC bioavailability) at higher water contents (Fig. 3b). Frost duration had no effect (Fig. 2a), but there was a slight interaction between soil frost duration and intensity (Fig. 3d). $sUVA_{254}$ showed a tendency towards higher values by increasing temperature from −12 to 0°C, at both short and long frost durations (Fig. 3d) .

Soil frost intensity and water content were the only significant factors influencing the C:N ratio: frost intensity was positively correlated and water content negatively correlated with C:N (Fig. 2b). None of the interactions in the model were significant with respect to the C:N ratio.

Frost intensity and water content were the only factors with significant effects on [DOC], and the interactions between water content and frost intensity as well as water content and FTC were significant (Fig. 2c). Frost intensity negatively affected [DOC], while the other three significant factors/interactions had a positive influence on [DOC] (Fig. 2c). There was a significant interaction between soil-frost intensity and water content (Fig. 3e). At lower frost intensities, [DOC] increased more with increasing water content, than at greater frost intensities (Fig. 3e). Ranges of [DOC] at the most intensive frost were generally higher than at the least intensive frost (Fig. 3e). The number of FTCs did not have a significant effect on [DOC] (Fig. 2c), but there was a slight interaction between FTC frequency and water content with respect to [DOC] (Fig. 3f). More frequent FTCs increased the [DOC] in the soils containing more water (Fig. 3f).

Discussion

Our field and laboratory experiments indicated that more intensive and longer lasting soil frost decreased $sUVA_{254}$ and hence increased the bioavailability of DOC in the soil solution during the following spring and summer. In the field experiment, the downward shift in $sUVA_{254}$ values in the upper 40 cm of the soil exposed to the DSF treatment, compared to ASF, could be explained by the higher soil frost intensity. DSF plots experienced deeper soil frost and longer periods of frost, as well as lower temperatures in the upper horizons. Plant root injuries caused by extreme temperatures (Weih and Karlsson 2002) and mortality of fine roots resulting from freezing (Tierney et al. 2001) may lead to the release of nutrients from below-ground organic matter. Since the below-ground organic matter associated with trees is mainly comprised of simple sugars and amino acids (Giesler et al. 2007; Scott-Denton et al. 2006), this may contribute to the increase in the bioavailable DOC (Berggren et al. 2009). Austnes et al. (2008) reported a tendency towards decreased $sUVA_{254}$, caused by cell lysis, in the soil water as a result of snow removal from a heathland system which normally experienced mild freezing conditions.

The design of our laboratory experiment, with soil collected in late autumn, soil temperatures down to −12°C, and a maximum “winter” of 6 months, aimed to simulate conditions as closely as possible to our field conditions. In addition, applying up to seven freezing cycles made it possible to detect the cumulative effect of multiple cycles, which may be important from a climate change perspective (Henry 2007). The results of our laboratory study supported the results of the field experiment; namely, that the bioavailability of DOC increased with increasing soil frost intensity. Duration of soil frost alone did not have a significant effect, but in combination with greater frost intensities (lower sub-zero soil temperature), it significantly affected the DOC quality. The lowest values of $sUVA_{254}$ in our laboratory experiment were within the ranges observed in soil solution samples from the frost manipulation experiment, but values increased to twice those recorded initially. We mainly observed these high values at high temperatures (Fig. 3a, c, d). Whether this was due to the homogenization of soils, removal of fine roots, or another experimental artifact is not known. However, the occurrence of the same trends in $sUVA_{254}$ values for both field and laboratory experiments strongly supports the idea that soil frost affects the quality of the DOC pool.

In the laboratory experiment, we observed a decrease in the C:N ratio at higher frost intensities. This result could indicate an increasing microbial origin of the dissolved organic matter, as this generally has lower C:N ratios than that originating from soils and higher plants (McKnight et al. 1997). At freezing temperatures, microorganisms can be metabolically active, but at lower rates than when temperatures are above freezing (e.g. Öquist et al. 2009; Panikov and Sizova 2006). Under such conditions, limitations associated with substrate availability and restricted O_2 diffusion caused by frost (Clein and Schimel 1995; Davidson and Janssens 2006; Öquist et al. 2007) may result in starvation and partial lysis of the soil microbial community (Austnes and Vestgarden 2008). As soils thaw, microbes may also be subjected to physiochemical shock associated with the abrupt shift in soil water potential; this process may also result in cell lysis (Jefferies et al. 2010), and subsequent production of low C:N organic matter.

Our laboratory experiment showed an increase in overall [DOC] as a result of more intensive soil frost. These results confirmed our observations in the field (Haei et al. 2010) and were consistent with results from other soil frost studies (Austnes and Vestgarden

2008; Henry 2007; Hentschel et al. 2008). We also observed that water content played an important role, as did the interaction between soil water content and frost intensity and the frequency of FTCs. Higher soil water content stimulated DOC production at higher frost intensities; in addition, the [DOC] increase resulting from more frequent FTCs was more pronounced when soils contained more water. In a study by Sparrman et al. (2004), however, they concluded that the water content prior to freezing did not influence liquid water present when soils were frozen. Instead, higher initial water content prior to freezing resulted in more ice which could, conceivably, increase physical damage to elements such as soil organic matter and microbial populations. In addition, our results highlighted the fact that different aspects of interactions between variables, such as the interaction between frost intensity and soil water content and the interaction between soil water content and frequency of FTCs, are highly important; a topic that has received insufficient attention previously.

The simultaneous study of multiple factors in our laboratory experiment, and the significant interactions between these variables, demonstrates the complexity of conditions affecting the amount and composition of DOC in soils. The few existing studies in the literature that have discussed the effects of winter climate conditions on DOC have mainly focused on individual factors or, at most, on the combination of two factors (e.g. Hentschel et al. 2008). It was therefore difficult to compare our results with these previous works. We found frost intensity (sub-zero soil temperature) to be by far the most influential factor increasing the bioavailability of DOC. In contrast, Hentschel et al. (2008) found no significant change in the quality of DOC, evaluated by specific UV absorbance at 280 nm and humification indices based on fluorometry, using a more intensive frost treatment (down to −13°C). They even found a tendency towards more humified substances, a change that was confirmed by the higher levels of lignin-derived phenols in the same experiment and reported by Schmitt et al. (2008). We found a lower C:N ratio and increased [DOC] as a result of more intensive frost, while Hentschel et al. (2008) found no significant impact of lower frost temperatures on the C:N ratio, but an increase in [DOC] after 2 week frost treatments at −8°C and −13°C in the laboratory. Austnes and Vestgarden (2008) reported reduced aromaticity (lower $sUVA_{254}$) and a lower DOC:DON ratio, as well as increased [DOC] as a result of permanent frost at −5°C for a period of about 2 months. However, such conditions were not directly comparable to our laboratory conditions as the duration of permanent frost in their studies was less than or only as long as the shortest frost period considered in our study. In addition we exposed our samples to more extreme frost intensities (down to −12°C) than Austnes and Vestgarden (2008).

There have been studies on soil freeze–thaw events in both the field and laboratory (Henry 2007; Matzner and Borken 2008), but most attention has been paid to the response of nutrients, carbon dioxide, and microbial activity and biomass. There have been a few studies on the response of forest soil DOC to FTCs (e.g. Austnes and Vestgarden 2008; Grogan et al. 2004; Hentschel et al. 2008), but since different methodologies have been used, it is difficult to draw general conclusions (Henry 2007). In our laboratory experiment, the frequency of FTCs interacted with both soil water content and frost intensity (Fig. 3b, c); multiple FTCs did not have a profound impact on DOC quality, but they did increase the [DOC] in soils with a higher water content (Fig. 3f). Ivarson and Sowden (1970) found that freezing increased the total amount of extracted free sugars and amino acids, but found no significant difference between the effects of single and multiple FTCs. Austnes and Vestgarden (2008) reported no significant decrease in $sUVA_{254}$ and DOC:DON, but a significant increase in [DOC], as a result of slow soil freeze–thaw cycling (a 123 h period at −5°C during 2 weeks). Grogan et al. (2004) observed a significant increase in [DOC] and decrease in microbial biomass carbon after multiple FTCs as compared to a single deep freeze treatment (min soil temp ~−5°C), in subarctic heath tundra mesocosms. Hentschel et al. (2008) did not report any change in the amount and spectroscopic properties of DOC in the O horizon (39% (v/v) water content) as a result of multiple FTCs in the laboratory. In contrast, we found that water content was an influential factor that exhibited a significant interaction with the frequency of FTCs. It has been suggested that repeated FTCs damage microorganisms and adversely affect their ability to decompose organic matter (Schimel and Clein 1996), which may contribute to the increased [DOC]. At higher water contents this effect becomes even more pronounced.

Conclusions

Our results suggest that the DOC in the riparian soil system is sensitive to variation in winter conditions related to soil frost. With a changing climate, the development of the snow pack and its effects on winter soil temperatures will probably be altered, which may affect the quality and quantity of DOC. However, the overall effect of such changes is not easy to foresee. Any climate related change to the soil organic matter pool in the riparian zone may affect the water quality in adjacent streams. Such impacts are likely to be pronounced in the northern boreal region, which is considered particularly susceptible to projected future climate change.

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