Swedish University of Agricultural Sciences
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Influence of climatic drivers and long term N fertilization on the production and leaching of dissolved organic carbon (DOC) and dissolved organic nitrogen (DON) in a Norway spruce (Picea abies) forest stand

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Keywords: long-term nitrogen fertilization, dissolved organic carbon, dissolved organic nitrogen, spruce forest, climate

Abstract

The influence of long-term nitrogen (N) fertilization and climatic drivers on the production and leaching of dissolved organic carbon (DOC) and dissolved organic nitrogen (DON) was studied in a Norway spruce (*Picea abies* (L.) Karst.) forest stand. DOC and DON soil solution concentrations in the O horizon were roughly an order of magnitude larger than B horizon soil solution concentrations. Soil solution sampled in the O horizon did not seem to respond to N fertilization. In the B horizon, however, slightly elevated concentrations of DOC and DON were occasionally observed in the fertilized plots. There did not seem to be a substantial effect of N fertilization on soil solution concentrations of DOC and DON. A decisive influence of simple climatic drivers on the within-year dynamics of DOC and DON soil solution concentrations could not be determined in this work. The annual mean DOC concentrations were higher 2009 than 1995, which might reflect an influence of the tree stand development on DOC soil solution concentrations.

Keywords: long-term nitrogen fertilization, dissolved organic carbon, dissolved organic nitrogen, spruce forest, climate.

Table of contents

1 Introduction

1.1 Theoretical background

On a global scale, forest ecosystems constitute a major pool of organic carbon (Eswaran et al., 1993). Of this vast carbon pool as much as two thirds is found in the soil compartment (Dixon et al., 1984), and boreal forest soils constitute a large part (Schlesinger, 1984). Soil organic matter (SOM) is of a heterogenic nature with regard to its molecular characterization, reflecting both variation in the organic compounds from which it originates and processes governing its formation (Kalbitz, 2000; Sutton & Sposito, 2005). This carbon stock is subject to continuous degradation and re-polymerization by biologically mediated decomposition, as in enzymatic mediated organic matter degradation (Baldrian, 2008), and abiotic processes, as in precipitation phenomena (Romkens & Dolfing, 1998) or leaching of water-soluble compounds in recent litter (Qualls et al., 1991; Berg, 2003). The water-soluble fraction of soil organic matter is what is commonly referred to as dissolved organic matter (DOM), and is operationally defined as organic molecules passing through a filter of 0,2 µm or 0,45 µm mesh size. DOM plays an important part of the geochemical cycle of several major elements (Qualls et al., 1991) and acts as substrate for microbial growth and respiration (Schimel & Weintraub, 2003) and as a vector for metals, e.g. copper (Temminghoff et al., 1997). DOM also plays a role in soil formation, for instance podzolisation (Lundström et al., 2000). The carbon and nitrogen associated with this fraction is termed dissolved organic carbon (DOC) and dissolved organic nitrogen (DON), respectively.

On a landscape scale, DOC fluxes in catchments have been found to largely be governed by landscape factors such as wetland coverage (Kortelainen et al., 2006) or anthropogenic activity, such as forest clear cuts (Laudon et al., 2009). On a smaller scale, however, the factors governing DOC release from litter and more humified soil organic matter have been considered to stand mainly under biotic control (Zech et al., 1994; Kalbitz et al., 2000; Kalbitz et al., 2007). Although biotic processes are considered to be of dominating influence as to the formation of potential DOM (Kalbitz et al., 2000), the processes governing solubility of this organic matter released to soil solution have by the same authors been proposed to stand primarily under abiotic control, e.g. sorption/desorption and precipitation processes, thereby obscuring the effect of biotic control on concentrations of DOM in soil solution.

In a study conducted in Bavaria, Germany, Zech et al. (1994) showed that soil solution DOC increased by on average a factor two as the percolating water passed through the O horizon and entered the E horizon. The authors stressed the fact that DOC concentrations in soil solution were highest in conjunction with periods of rain following a dry period, events which have been proposed to coincide with short periods of increased mineralization, which corroborates the influence of the microbial community on the production of potential DOC. Cycles of wetting and re-wetting have in laboratory studies been shown to result in pulses of respiration, the so-called “Birch-effect” (Birch, 1958), and respiration rates coincide to some extent with the potential for leaching of DOC from decomposing litter in laboratory studies (Hansson et al., 2010).

In a study of spruce stands under Swedish conditions (Fröberg et al., 2004) the concentration of DOC in soil solution increased by just over a factor two as the water passed through the O horizon. The contribution from litter and the more humified Oe and Oa horizons to DOC leaching from the O horizon were collated, showing that recent litter, Oe and Oa horizons contribute 20%, 30% and 50% respectively to DOC leaving the O horizon. These observations points towards the importance of both fresh litter and older, more humified organic matter found in the O horizon as sources to DOC in soils, although other authors have argued that the more humified Oe and Oa horizons are of greater importance (Kalbitz et al., 2007).

Retention of DOC has been shown to primarily take place in underlying horizons, leaving low levels of DOC in the output water from the B horizon (Fröberg et al., 2006). Overall the B horizon serves as a sink to DOC and constitutes a large pool of organic carbon in the soil compartment with a long turnover time (Raich & Schlesinger, 1992; Fröberg et al., 2004). The chemical composition of DOM that precipitates in soil has been studied by Scheel et al (2008), and a preferential precipitation with aluminum of aromatic compounds was observed. The authors stress the consequent stabilization of the dissolved organic matter by these associations, and its implications for microbial availability of precipitated organic matter in lower soil horizons.

Anthropogenic activity, or consequences of such activity, can exert some influence on the turnover of SOM and thus the formation of DOC and DON. Contradictory results are found on the effect of input of inorganic nitrogen on DOC soil solution concentrations. Atmospheric deposition of N has been found to decrease decomposition rate in spruce stands (Magill & Aber, 1998), which could imply lower DOC concentrations. However, Berggren et al. (1997) found no apparent effect on DOC concentrations in soil solution sampled below the mor layer in studies with N fertilization. Further, laboratory incubations have shown no effect of N fertilization on the leaching of DOC from the mor layer (Sjöberg et al., 2003). DOC concentrations sampled below the mor layer does not represent the production of DOC, however, but rather the result of production, leaching, mineralization to CO_2 and sorption processes. N fertilization in Norway spruce stands in Sweden has increased the concentration of DON in soil solution both from the F and H layer compared to irrigation (Andersson & Berggren, 2005). Clear-cut harvesting of trees increases stream water DOC concentration on a watershed scale (Laudon et al., 2009) and thinning has been argued to increase microbial organic matter mineralization, as a result of increased incoming solar radiation and soil temperature (Zech et al., 1994), possibly leading to elevated concentrations of DOM in soil solution.

Fröberg et al. (2004) correlated DOC concentrations to the net primary production of the ecosystem as DOC flux was positively correlated with the aboveground standing biomass. As nitrogen typically is a limiting nutrient for tree growth in boreal forest stands and thus will influence net primary production, the authors argued that increases in nitrogen status of the soil will exert a positive influence on DOC leaching from the soil.

1.2 Modeling DOC and DON

Several dynamic models have attempted to describe DOC dynamics in soils, amongst others ECOSSE, CoupModel and DyDOC. The DyDOC model (Michalzik et al., 2003; also described in Tipping et al., 2005) addresses the dynamics of DOC in soils based on metabolism, sorption processes and mass flow in percolating water. Conceptually, DyDOC describes specific soil compartments (horizons 1, 2 and 3) in which respiration, sorption and mass flow are computed simultaneously. Soil water exists in micropores, which is held by tension against the gravitational force, or macropores, where water moves under the act of gravity. Exchange of DOC between micro- and macropores is modeled by an exchange constant, D_{exch}. Carbon input to the system takes place as litter input, root input (belowground) and throughfall. Organic carbon input is transformed into CO_2, substrate or DOC. Further, substrate can be transformed to Hum-1 (a humic component prone to microbial degradation) or CO_2, and Hum-1 can be further transformed to Hum-2 (a recalcitrant humic component) or CO_2. Metabolism of organic carbon in the soil is described by first order rate constants and Q10 relationships. Sorption processes in the micropore system are described by equilibrium partitioning constants, or K_d-values, and are dependent on ion speciation in soil solution and the nature of the solid phase. The DyDOC model has shown reasonable agreement with field experiments (Michalzik et al., 2003; Tipping et al., 2005).

1.3 Previous research at Stråsan research station

The Stråsan research station has been thoroughly described and discussed by Tamm (1991). The long-term application of fertilizers, especially nitrogen, has greatly increased tree growth (Engström & Ahl, 2004). Studies by Berggren et al. (1997) have been conducted at Stråsan research station 1995 - 1996, and nitrogen fertilization did not show an effect on DOC concentrations in leachates from the mor layer. These measurements are included in the data evaluation made in the present work, along with new measurements of DOC and DON in soil solution made during 2009. Fertilization treatments, both nitrogen, phosphorous and potassium fertilisation has shown to increase the amount of carbon (Hyvönen et al., 2008), and nitrogen (Andersson et al., 2001) in the soil profile.

1.4 Objectives with the study

In this work, the effect of different long-term nitrogen fertilization regimes on DOC and DON soil solution concentrations in a Norway spruce forest stand in central Sweden will be determined. Within year dynamics of DOC in leachates from the O horizon appear to be governed by temperature (Fröberg et al., 2004), but also precipitation dynamics (Zech et al., 1994), and the extent to which within-year dynamics of DOC and DON soil solution concentrations are governed by climatic drivers will explored statistically. Data for these investigations will be obtained from Stråsan research facility, a long term N fertilization field experiment located in the boreal landscape in central Sweden.

2 Material & methods

2.1 The Stråsan research station

The Stråsan research station (60º55’N, 16º01’E, 350 meters above sea level) is located in the boreal zone in the south east of Dalarna county, Sweden. The site is situated on a rather steep slope (≈20%). The soil type classifies as a haplic podzol and the parent material consists of stony glacial till (Berggren et al., 1997). The climate at the site can be described as a cool temperate climate with long winters and a short summer period. The annual mean temperature is 3.2 ºC and the annual mean precipitation sums up to 740 mm. The area currently receives low inputs of inorganic nitrogen (<5 kg N ha-1) through nitrogen deposition (www.ivl.se). Below, figure 1, is an overview of the layout of the fertilisation experiment.

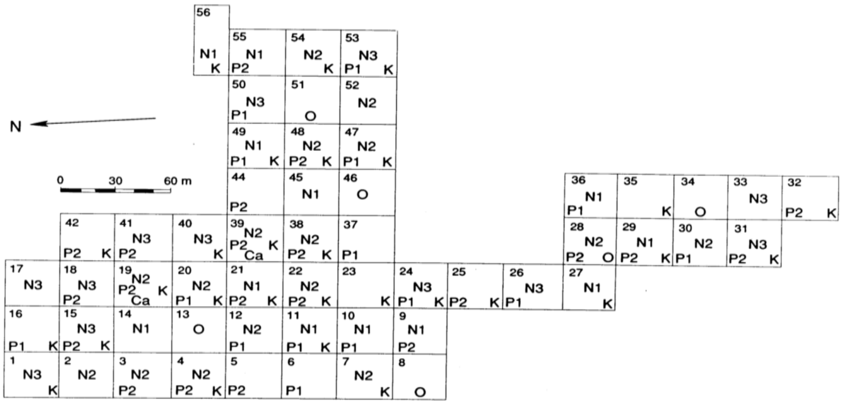

Figure 1. Overview of the Stråsan research facility. Plots are 30*30 meters and the total area ads up to 4.7 hectare. Modified from www.fiberskog.nu.

The tree stands of Norway spruce, *Picea abies* (L.) Karst., were planted in 1958 after clear-cutting the area (1954-1955), and a fertilization experiment was started in 1967. There are several fertilization treatments at the site, ranging from varying additions of inorganic nitrogen to the addition of other macro- and micronutrients (P, K, Mg, Ca, B, Zn, Cu, and Mn) alone, or in combination. For this study, only nitrogen fertilization regimes are considered. Nitrogen was applied as ammonium-nitrate (NH_4NO_3) at the start of the growing season. Each treatment consists of two replicates and the plot number can be seen to the top left of each square in figure 1. The fertilized plots considered in this study are 2, 13, 14, 33, 45, 46 and 52. Plots 13 and 46 are control plots, 14 and 45 the N1 treatment, 2 and 52 the N2 treatment and 33 is the N3 treatment respectively. Further description of the fertilization treatments are given in table 1.

Table 1. Nitrogen fertilization regimes employed at Stråsan research facility (kg N hectare^{-1})

Treatment year	**Duration (yrs)**	**Control**	**N1**	**N2**	**N3**
1967-1969	3	0	60	120	180
1970-1976	7	0	40	80	120
1977-1990	14	0	40	60	90
1991-1992	2	0	30	0	90
1993-2009	17	0	30	0	0
Sum (1967-2009):	43	0	1590	1760	2820

Although the N2 and N3 treatments were discontinued in 1990 and 1992 respectively, these treatments have received larger cumulative N-additions than the on-going N treatment, N1. The nitrogen fertilization treatments have resulted in increases of tree growth shown in table 2. Tree growth response to nitrogen fertilization has been substantial, highlighting the nitrogen limitation of net primary production of Swedish boreal forest ecosystems. For treatment N1 and N2, stem volume had increased by approximately 50% compared to control by spring 2010. Treatment N3 has resulted in less tree growth than N1 and N2 treatments, although the annual growth was high in the N3 treatment during early stages of stand development.

Table 2. Stand development (m^3 on bark ha^{-1}) of Norway spruce at Stråsan research site as influenced by N fertilization treatment. Data from Engström & Ahl (2004) unless otherwise shown

Year	Control	N1	N2	N3
1958	-	-	-	-
1972	4	22	17	23
1975	7	46	40	49
1978	13	76	71	80
1982	26	124	120	129
1986	46	172	175	169
1991	73	232	253	202
1996	108	281	300	234
2003[a]	233	340	350	274
2010[a]	286	425	442	295

[a] Data from inventories conducted at Stråsan research site by SLU, 2010.

If tree volumes removed by thinning (data not shown) is included, the difference between N fertilisation treatments and control is even larger.

Zero tension lysimeters (ZTL) were installed just below the O horizon, six in each plot, sampling water moving freely under the act of gravity. Further, Rhizon-type lysimeters (R) were installed in the bottom of the O horizon, six in each plot, which sampled mainly the meso- and micropore soil water using tension. These Rhizon-type lysimeters were only sampled during 1995. Tension lysimeters (TL) of Prenart-type were installed below the B horizon, four in each plot. The ZTLs were installed in September-October 1994, Rhizon lysimeters in June 1995, and Prenart cups in June 1994. The N3 plot was not sampled in 2009. Data on DOC, nitrate (NO3-), ammonium (NH4+) and total nitrogen (TN) concentration was sampled at two-week intervals during the growing season of 1995 and 2009. DON was calculated by subtracting nitrogen content in inorganic nitrogen species (nitrate-N, nitrite-N and ammonium-N) from the total nitrogen concentration. Other data on water chemistry were also analyzed during this period (absorbance at 254, 280 and 340 nm, cat-ions & anions and metals). During 1995, samples from all lysimeters were analyzed separately, but for samples taken during 2009 so-called “pooled samples” were analyzed. Pooled samples were obtained by pouring the samples gathered by each lysimeter into a large container from which a pooled sample was taken and analyzed. Pooling of samples thus renders volume weighted concentrations. If a lysimeter had a larger volume than 1 dm^3, only a 1 dm^3 sample from that specific lysimeter was added to the bulk sample.

Climate data have been collected from nearby observation stations and extrapolated from Åmotfors. Weather data for both 1995 and 2009 were calculated for Jädraås, approximately 26 km east, south-east of Stråsan research station. Potential evapotranspiration (PET) was calculated using the Penman-Monteith equation (Monteith, 1965) based on these weather data. Precipitation, temperature, wind speed, relative humidity, PET and solar radiation data have been compiled with daily resolution.

2.2 Statistical analysis

2.2.1 Effect of treatment

The effect of fertilization treatment on DOC and DON concentrations and leaching was studied. The effect of treatment was evaluated by ANOVA (Minitab Inc. 2007) and Tukey test at 95% confidence interval for pair wise comparisons.

By including data on DOC and DON soil solution concentrations from 2009, development with time of the ecosystem with regard to DOC and DON can be evaluated. Some increase of leaching of DON between 2009 and 1995 can be expected as a result of nitrogen fertilization of forest stands (Berggren et al., 1997). As nitrogen fertilization in treatments N2 and N3 were discontinued (1990 and 1992, respectively) this enables a study of the "memory effects" of nitrogen addition to Norway spruce forest stands. The capacity of these stands to approach the conditions of the control plots can be explored with regards to DOC and DON concentrations, as a qualitative measure of the ecosystems ability to recover from nitrogen amendment. The difference between years, 1995 and 2009, was tested by computing ANOVA on means of treatments. As the concentrations for 2009 are "pooled", the data for 1995 was recalculated as volume weighted concentration to make the comparison valid. However, this was not considered necessary for B horizon leachates due to the strong buffering of DOM concentrations in this soil horizon.

2.2.2 Effect of climate

Data was treated statistically to explore the extent to which simple climatic drivers govern within-year fluctuations of DOC and DON soil solution concentrations. The dataset was divided into subsets for each treatment in order to simplify the analysis. Possible governing climatic drivers on DOC and DON concentrations were analyzed by using stepwise multiple linear regression (JMP, 2008). Residuals were tested for normality by Shapiro-Wilks test. Autocorrelation was tested using the Durbin-Watson test and residuals were plotted against time to assess possible effects of time in the data. In previous work (Fröberg et al., 2004), the within year dynamics of DOC concentration in the O horizon were proposed to stand under the control of temperature. A time lag was observed, however, showing peaks in DOC concentration about two months later than peaks in temperature. Therefore, a function of temperature was included as an independent variable, representing the weekly temperature sum two months prior to the sampling occasion. The full set of predictor variables that were included in the statistical analysis are presented in table 3.

Table 3. Predictor variables included in statistical explorations of the control on within-year fluctuations of DOC and DON soil solution concentrations

Variable	Abbreviation	Resolution	Unit
Potential evapotranspiration	PET_d	daily	mm day^{-1}
Potential evapotranspiration	PET_w	weekly	mm $week^{-1}$
Precipitation	$Precipitation_d$	daily	mm day^{-1}
Precipitation	$Precipitation_w$	weekly	mm $week^{-1}$
Precipitation previous day	$Precipitation_p$	daily	mm day^{-1}
Temperature	$Temperature_d$	daily	ºC
Temperature sum	$Temperature_w$	weekly	ºC
Temperature previous day	$Temperature_p$	daily	ºC
Temperature sum 2 months prior	$Temperature_{w,2}$	weekly	ºC
Days without rain prior to sampling	Draught	daily	days

3 Results

3.1 Climate at Stråsan research station 1995 and 2009

The climate at the site can be described as a cool climate with long winters and a short summer period. The annual mean temperature is 3.2 ºC and the annual mean precipitation sums up to 740 mm. Precipitation and temperature regime, along with PET, is shown in figure 2. During 1995, precipitation was considerably lower than the annual average (566 mm), and mean temperature higher (4.7 ºC). During 2009, however, the cumulated precipitation was considerably higher (924 mm) than the long-term annual mean but the mean temperature was in line with the long term annual average (3.3 ºC).

The temperature sum during the study period (8th of June through 21th of November) in 1995 was 1546 °C and 1630 °C in 2009. In light of this fact, and from a closer examination of figure 2, it appears that there was a slightly warmer and considerably wetter climate at Stråsan research station during 2009 than in 1995 during the study period.

Potential evapotranspiration during 1995 is shown at the bottom of figure 2. Some general observations regarding the evaporative demand can be noted. Rather high rates of evapotranspiration occur during the period 1st of June until 1st of August, a period with low rainfall, and this period should thus represent a fairly dry summer. The daily potential evapotranspiration shows high correlation with daily mean temperature (r^2=0,61) but low correlation with daily precipitation (r^2=0,02) throughout the year.

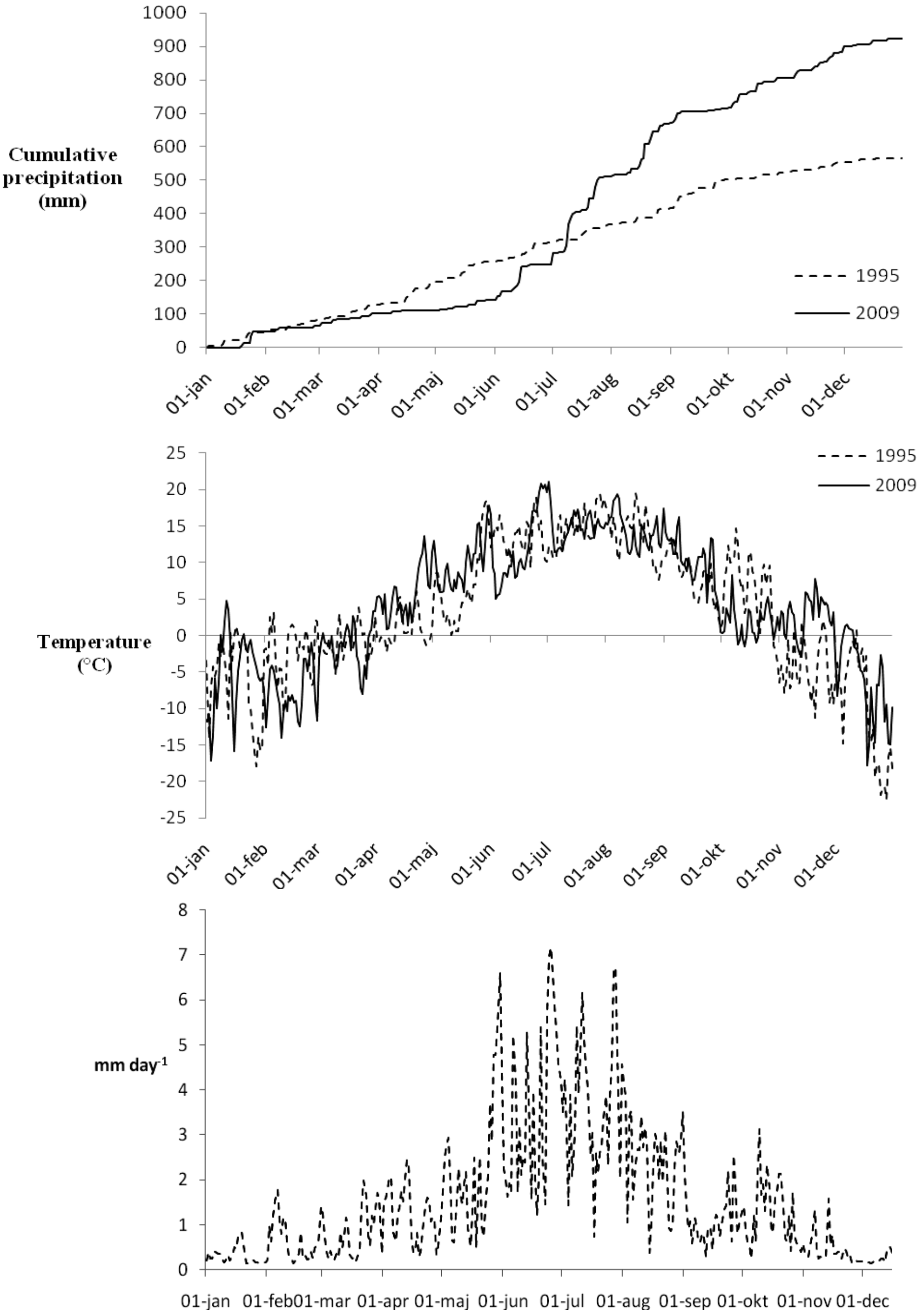

Figure 2. Climate at Stråsan research station n 1995 and 2009. Precipitation regime (top), daily mean temperature (middle) and daily PET (bottom). PET was only calculated for 1995.

3.2 Effect of fertilization treatment on soil characteristics

Increased soil N pools of fertilized plots have previously been reported (Andersson et al., 2004), and soil N pools have increased by up to a factor 3 by the year 1996. Also the soil content of carbon has increased by approximately a factor 2 by the year 1996 in the N1 treatment, as reported by Andersson et al. (2002) and tree growth has increased substantially (previously shown). As shown in table 4, increases in thickness of the litter, F and H horizons are observed as a result of the fertilization treatments, especially the H horizon.

Table 4. Depth of soil horizons per treatment at Stråsan research station in 1995. Values are means of six replicates ± standard deviation

Soil horizon	Control	N1	N2	N3
Litter	0,0 ±0,0	0,0 ±0,0	0,6 ±0,2	0,8 ±0,3
F	2,0 ±1,4	1,8 ±0,8	1,8 ±0,7	3,8 ±1,0
H	3,1 ±1,4	7,2 ±2,6	7,0 ±1,9	6,2 ±1,7
E	9,4 ±4,7	8,8 ±3,4	11,2 ±8,6	8,0 ±3,7
Bh	1,9 ±0,9	2,1 ±1,0	3,8 ±3,6	2,5 ±0,5
Bs1	11,0 ±3,8	10,1 ±2,4	10,8 ±3,2	10,2 ±3,2
Bs2	18,3 ±7,6	16,9 ±7,5	14,9 ±4,3	12,2 ±2,3

The increases in tree growth (table 2), F- and H-horizon depth (table 4) and soil organic matter build up of fertilized plots compared to control at Stråsan research site emphasizes the role of N as a limiting factor for primary production in Swedish boreal forests.

3.3 Seasonal fluctuations of DOC and DON in soil solution

3.3.1 DOC concentrations in soil solution below the O horizon

Dissolved organic carbon concentrations sampled below the O horizon during 1995 and 2009 are shown in figure 3 below. The DOC concentrations in soil solution sampled below the O horizon display large within-year variation. No apparent difference between treatments in 1995 can be observed. Results are in accordance with those reported by Andersson & Berggren (2002), where no apparent effect of N-fertilization on DOC soil solution concentrations was found.

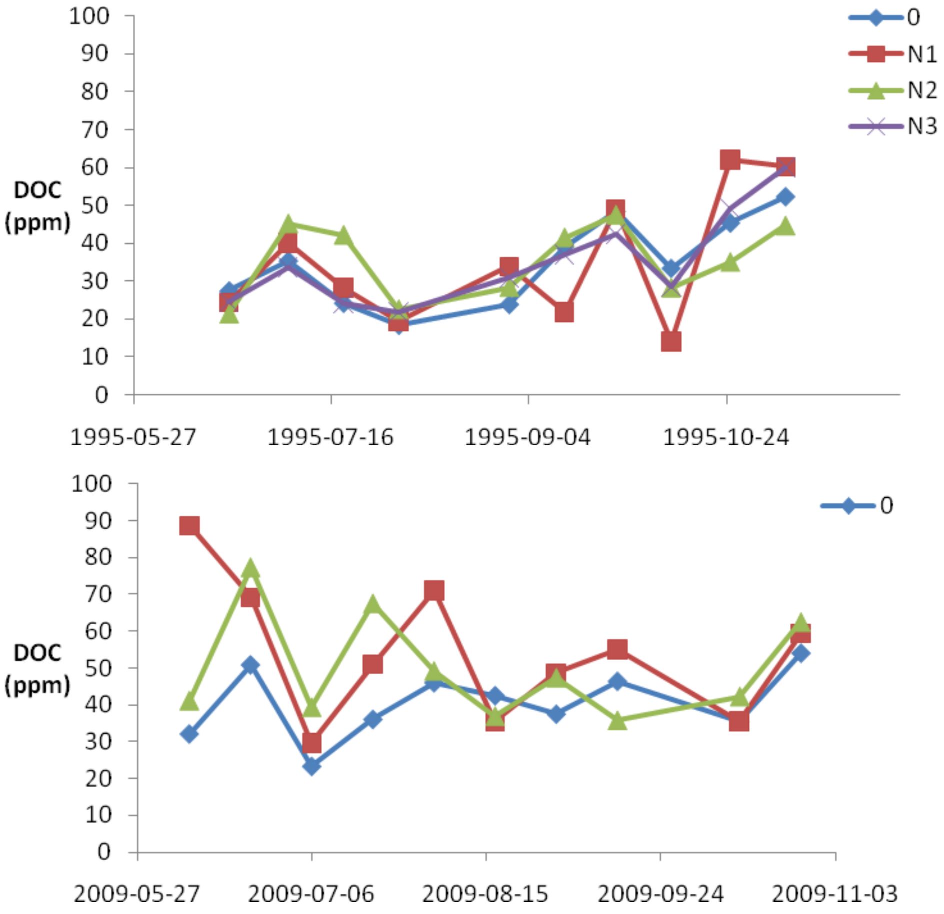

Figure 3. Seasonal fluctuation of DOC concentrations (means of six replicates in 1995, pooled concentrations in 2009) in soil solution sampled in the O-horizon from 1995-06-08 through 1995-11-12 (above) and 2009-06-08 through 2009-11-12 (below). Plot 33 (N3 treatment) was only sampled in 1995.

The DOC concentrations during 2009 were slightly elevated during early summer compared to the same period of 1995 and early summer of 2009 was considerably wetter than the same period 1995.

3.3.2 DOC concentrations in soil solution below the B horizon

DOC concentrations in tension lysimeters installed below the B horizon during 1995 and 2009 are shown below in figure 4. The magnitude and variation of DOC concentrations were considerably less in the B horizon, compared to the O horizon leachates.

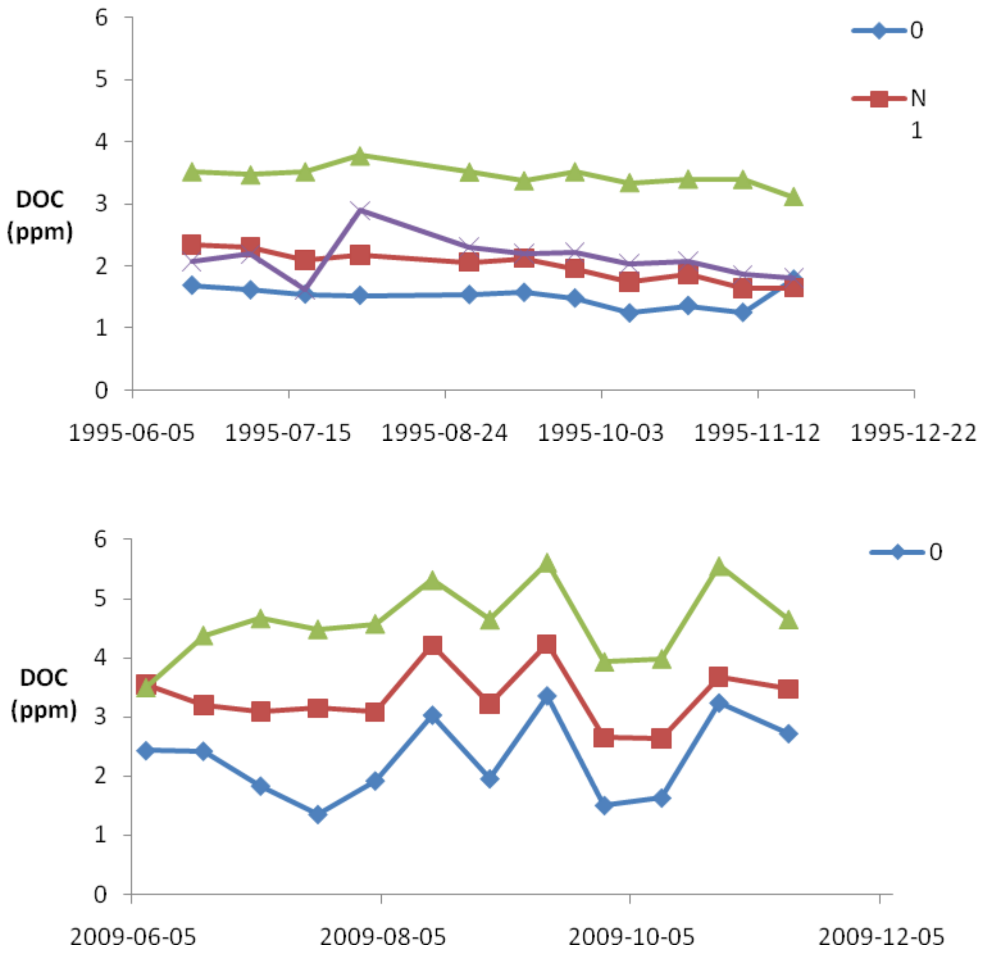

Figure 4. Seasonal fluctuation of DOC concentration (means of four replicates in 1995, pooled concentration in 2009) in soil solution sampled with tension lysimeters below the B horizon during 1995 (above) and 2009 (below). Plot 33 (N3 treatment) was only sampled in 1995.

From figure 4 above, N-fertilization appeared to exert a positive influence on the DOC concentration in soil solution sampled below the B horizon. The N1 and N2 treatments showed elevated concentrations compared to control at all sampling occasions. The concentrations were higher for all treatments during 2009, even in the control plots, compared to 1995. The N3 treatment showed concentrations comparable to those of the N2 treatment.

3.3.3 DON concentrations in soil solution below the O horizon

DON concentrations sampled in ZTL's below the O horizon during 1995 and 2009 are shown in figure 5 below. The N1 treatment showed extreme values during the early summer of 2009.

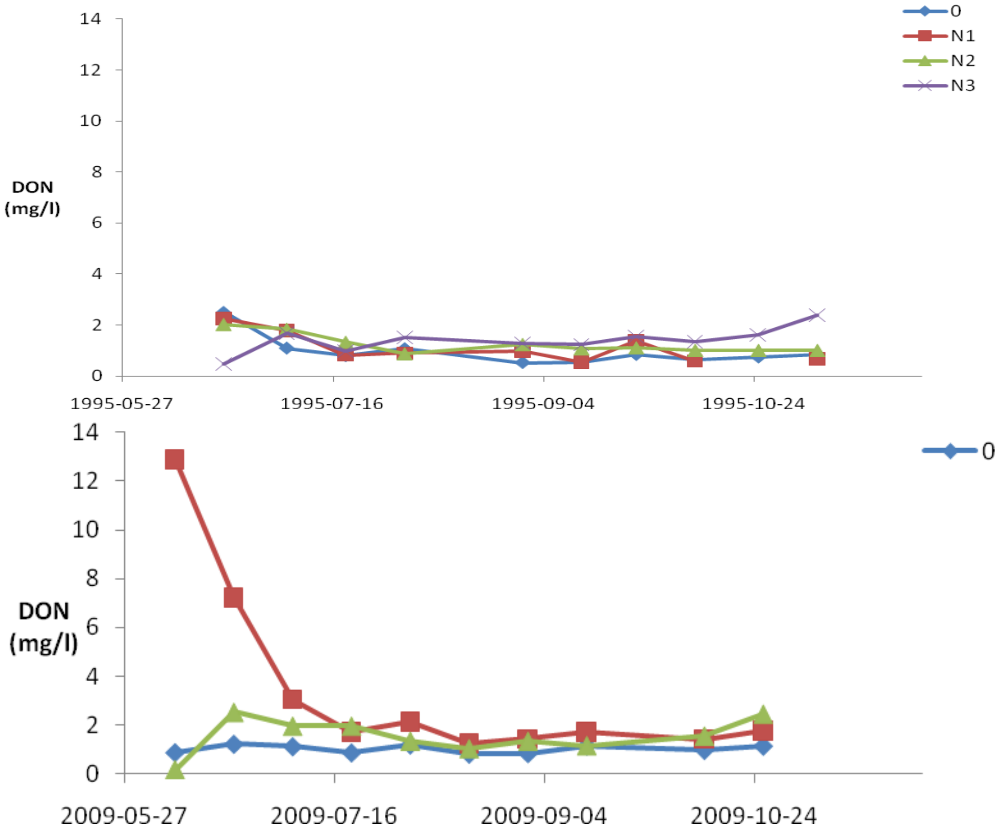

Figure 5. Seasonal fluctuation of DON concentration (means of six replicates in 1995, pooled concentrations in 2009) in soil solution sampled in the O-horizon from 1995-06-08 through 1995-11-12 (above) and 2009-06-08 through 2009-11-12 (below). Plot 33 (N3 treatment) was only sampled in 1995.

Treatments N3 appeared to show slightly elevated concentrations of DON during 1995. Further, during 2009 the concentrations in soil solution sampled from the O horizon showed very high DON concentrations in early summer, possibly as a result of the applied ammoniumnitrate during a period with large amounts of rainfall.

3.3.4 DON concentrations in soil solution below the B horizon

DON concentrations sampled below the B horizon during 1995 and 2009 are shown in figure 6 below. The concentrations are roughly one order of magnitude lower than in O horizon leachates.

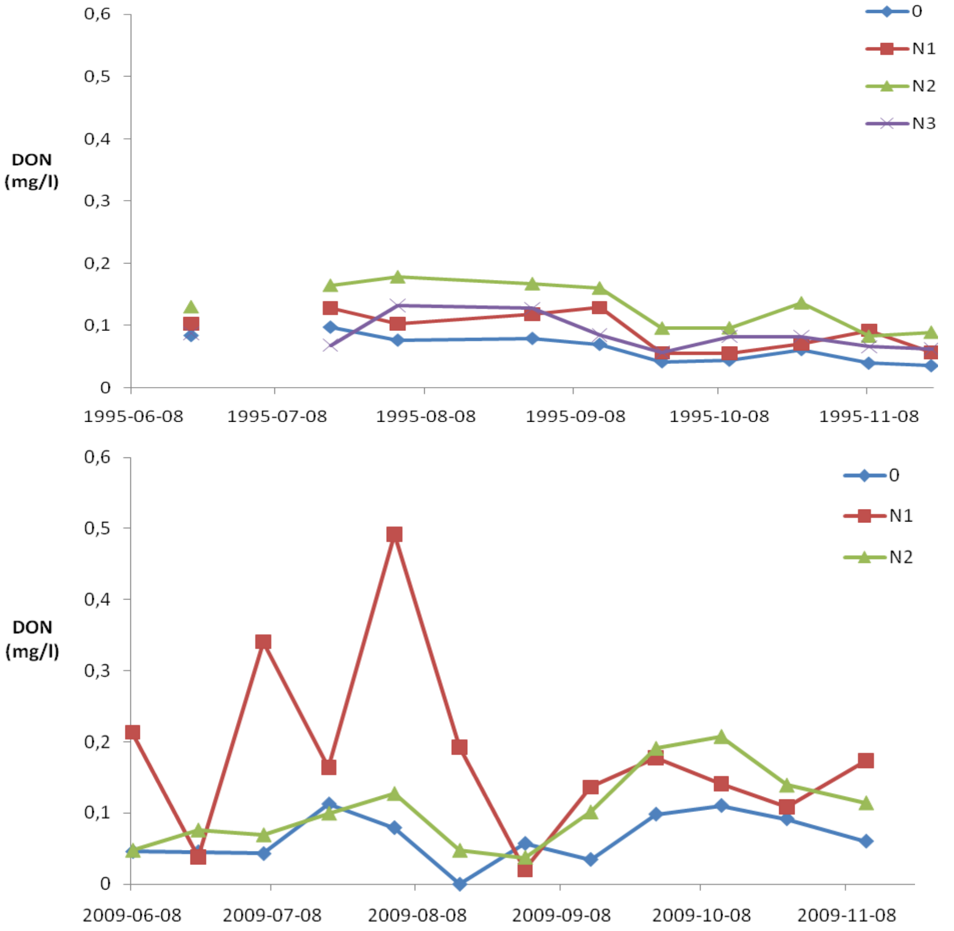

Figure 6. Seasonal fluctuation of DON concentration (means of four replicates in 1995, pooled concentration in 2009) in soil solution sampled below the B horizon from 1995 (above) and during 2009 (below). Plot 33 (N3 treatment) was only sampled in 1995.

If figures 5 and 6 are compared, N-fertilization (treatments N1, N2 and N3 during 1995) appeared to influence DON soil solution concentration in the B horizon foremost. Further, the high concentrations in the O horizon during early summer also manifest in the B horizon leachates, although with a time lag. This fact suggests that extreme events occurring in the O horizon soil solution chemistry affects soil solution concentrations later found in the B horizon. Further, it is clear, from figures 3 – 6 that the concentrations of DOC and DON in soil solution decrease dramatically when passing through the mineral soil. On average, soil solution concentrations found in lysimeters sampling below the B horizon were roughly one order of magnitude lower and much less variable than those found in the O horizon. This is most probably due to sorption in the mineral soil and illustrates an aspect of soil formation; i.e. the podzolisation process.

3.4 Statistical analysis of the effect of treatment and climatic drivers

3.4.1 Effect of N fertilization

Comparisons between treatments at individual sampling occasions are presented in appendix, table A1, A2 and A3. In general, differences between treatments are more pronounced in soil solution from the B horizon. Neither DOC, nor DON, concentrations sampled in the O horizon with ZTL's displays significant differences between N-fertilization treatments. Especially in O horizon leachates the comparison is influenced by the high variation in leachate concentrations. In soil solution sampled below the B horizon, treatments N1 and N2 showed significantly higher concentrations of both DOC and DON compared to control at several sampling occasions. Due to the risk of type-1 error, these results should be interpreted with some caution, however.

Considering that the N1 treatment is the only treatment still receiving N-fertilization it is interesting that the N2 treatment shows significant differences from control more frequently than the N1 treatment in the B horizon leachates. Furthermore, DOC concentrations in the B horizon tend to show a significant difference from control as frequently as DON concentrations; a fact that stands in contrast to earlier studies (Berggren et al., 1997; Andersson et al. 2002).

It seems that the annual mean concentrations of DOC and DON are considerably higher in 2009 than in 1995 (figures 3 – 6). A statistical analysis of variance on annual means of DOC having two levels of year (1995 and 2009) and three levels of treatment (control, N1 and N2) showed that the effect of year was significant for both O horizon leachates, p=0,027, and B horizon leachates, p=0,009 with regards to annual mean DOC soil solution concentration (table 5). There were no significant interaction effects between time and treatment for DOC soil solution concentrations in either soil horizon. This strengthens the observation that soil solution concentrations are, on average, higher in 2009 than in 1995.

Table 5. ANOVA for comparison of effect of treatment and year on DOC mean annual concentration. O horizon leachates, ZTL's (above), and B horizon leachates, TL's (below)

O horizon

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Treatment	2	99,75	99,75	49,88	0,61	0,579
Block	1	0,55	0,55	0,55	0,01	0,938
Year	1	775,95	775,95	775,95	9,51	0,027
Year*treat	2	132,01	132,01	66,00	0,81	0,496
Error	5	407,92	407,92	81,58		
Total	11	1416,17				

B horizon

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Treatment	2	9,7249	9,7249	4,8625	22,41	0,003
Year	1	3,8212	3,8212	3,8212	17,61	0,009
Year*treat	2	0,1912	0,1912	0,0956	0,44	0,666
Block	1	2,2620	2,2620	2,2620	10,42	0,023
Error	5	1,0850	1,0850	0,2170		
Total	11	17,0843				

Further, a statistical analysis of variance of annual mean DON soil solution concentrations, table 6, showed that the effect of year was significant for O horizon leachates, p=0,001, but not for leachates from the B horizon, p=0,382 with regards to DON. The interaction term year*treatment was significant for DON in the O horizon, as the effect of the N1 treatment was considerably higher than N2 during 2009.

Table 6. ANOVA for comparison of effect of treatment and year on DON mean annual concentration. O horizon leachates, ZTL's (above) and B horizon leachates, TL's (below)

O horizon

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Treatment	2	3,5670	3,5670	1,7835	19,47	0,004
Block	1	0,1328	0,1328	0,1328	1,45	0,282
Year	1	3,9585	3,9585	3,9585	43,21	0,001
Year*treat	2	2,8489	2,8489	1,4245	15,55	0,007
Error	5	0,4581	0,4581	0,0916		
Total	11	10,9653				

B horizon

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Treatment	2	0,012734	0,012734	0,006367	3,82	0,098
Year	1	0,001529	0,001529	0,001529	0,92	0,382
Year*treat	2	0,005672	0,005672	0,002836	1,70	0,279
Block	1	0,000133	0,000133	0,000133	0,08	0,789
Error	5	0,008331	0,008331	0,001666		
Total	11	0,028399				

To investigate the possible contribution of the wetter summer period of 2009 to the differences presented above, a statistical analysis of variance having two levels of time (wet period of 2009 and dry period of 2009) and three levels of treatment (control, N1 and N2) was performed. This analysis showed a lack of significance for the effect of time for DOC and DON in both O horizon and B horizon leachates, indicating that the rainy period of 2009 didn't give rise to the elevated mean annual concentrations of DOC or DON.

It would seem, from this comparison, that the difference between years cannot be attributed to the difference in precipitation regime between the two years. Currently no data on soil profile descriptions (soil C and N pools, amount of litter, depth of F and H horizons etc.) are available for 2009, but such data would pose a valuable addition when interpreting these findings.

3.4.2 Effect of climate

The data was analyzed statistically for governing factors of DOC and DON concentrations. For several subsets, climatic drivers showed no apparent control on DOC and DON concentrations (selection criteria $r^2_{adj.} > 0.6$) . Selected subsets, however, displayed some correlation between soil DOC and DON and climatic drivers, implying that these drivers are not merely incidental. Selected models are shown in appendix, table A5. The fitted models use different predictors and estimators between subsets which weaken the validity of the analysis and suggest that simple climatic drivers do not, solely, control DOC and DON soil solution with-in year dynamics.

Some general observations are worth noting. First, most selected models were constructed using O horizon soil solution leachates. This is to be expected as the B horizon represents a soil compartment which is, arguably, to a lesser degree affected by processes at the soil surface/atmosphere interface. Temperature and precipitation dynamics most certainly affect O horizon leachates to a greater extent than leachates from the mineral soil, as shown by for instance Fröberg et al. (2002). Furthermore, the lack of within-year variation of concentrations in the B horizon lends the data unfit for the analysis. It appears that concentrations in the mineral soil are to a greater extent regulated by sorption-desorption processes, “smoothing” the concentrations in the B horizon.

Temperature is included in all selected models, either as the daily mean temperature or the weekly temperature sum, indicating some effect of temperature on concentrations of both DOC and DON. Most estimators of daily temperature are positive, meaning that higher concentrations are observed during warmer days. Precipitation and potential evapotranspiration are more rarely included as predictors in the models, and the estimators for these predictors display a contradictory nature.

When comparing concentrations in the O horizon at individual sampling occasions, leachates from ZTL’s display significantly lower concentrations than leachates from Rhizon type lysimeters of both DOC and DON for some occasions, especially during the first three sampling occasions (appendix, table A4). This is in line with the conceptual framework of the DyDOC model, in which production of DOC takes place in the smaller pores and transport takes place in the larger pores. Water passing through the larger pores would occasionally moves too fast for the soil solution in smaller and larger pores to equilibrate, producing lower concentrations in ZTL’s than in Rhizon type lysimeters. The first three days of sampling in 1995 stand out in that they occur the day after rather high amounts of rainfall (3 – 8 mm), days with significant differences between ZTL’s and R lysimeters, which corroborates this hypothesis. Further, this shows, conceptually, the diluting effect of precipitation events on DOC soil solution concentrations and their differentiation between meso- and macropores.

4 Discussion

The effect of N fertilization on DOC and DON soil solution concentration appear to be of a complex nature. The effect of long-term N additions has been shown to include increases in tree growth, soil carbon- and nitrogen-pool build up (by 1995), increases in the depth of organic horizons but, to a fairly low extent, elevated DOC and DON leaching from the B horizon. The O horizon leachates do not display easily interpreted patterns of DOC or DON soil solution concentrations but rather a highly variable nature, both spatially and temporally (figures 3 – 6), implying that leaching of DOM from the O horizon is not influenced by the N fertilization.

Andersson & Berggren (2002) showed that N-fertilization led to increased concentrations of DON in the soil solution sampled from the O horizon, averaged over the whole year. Here the influence manifests in soil solution sampled from the B horizon and previous studies (Berggren et al. 1997) has shown that the long term N fertilization at Stråsan research site has lead to a system that was close to leaching of DON from the mineral soil by 1995. This pattern is further corroborated by the inclusion of data from 2009 (figures 5 and 6). The rather weak treatment effects in the B horizon could be regarded as an indirect effect of N fertilisation; probably increased acidity in fertilized plots, as reported by Nohrstedt (1992), or the increased tree stand volume in fertilized plots which could imply larger root litter production and/or root exudates. It is possible that a fraction of the turnover of old roots takes place below the lysimeters installed in the O horizon. The DOC and DON soil solution concentrations in the B horizon could thus be affected by the turnover of a larger pool of SOM and increased root exudates as a response to increased tree growth.

It appears, from the results in this study, that there is a difference in DOC and DON concentrations in soil solution between the years 1995 and 2009 which could not be attributed to climate. Rather, the effect of increasing concentrations with time could be interpreted with the development of plot characteristics in mind. The standing tree volume is considerably higher in the tree stands of 2009 compared to 1995, which would imply larger litter inputs and a larger abundance of roots. The carbon pools in the O horizon are probably larger in 2009 compared to 1995, although such data is at present not available. As reviewed by for instance Berg & Matzner (1997), N deposition might decrease the decomposition of humus. One would thus lend oneself to the suggestion that when an increased litter production due to N fertilisation occurs, the potential increase in DOC production is hampered by the addition of mineral N. On the other hand, when an increased production of litterfall takes place as a result of natural stand development with time, increased DOC concentrations are observed. This would further imply that the addition of mineral N might hamper the production of DOC, and poses an interesting topic in need of further research.

The lower concentrations of DOC and DON in B horizon leachates compared to O horizon leachates imply that considerable retention of DOC and DON takes place in underlying mineral soil horizons. This is most probably the effect of sorption of DOM. Overall the B horizon has been shown to serve as a sink to DOM and constitutes a large pool of organic carbon in the soil compartment with a long turnover time (Raich & Schlesinger, 1992; Fröberg et al., 2004), thus limiting the potential for leaching of DOC and DON from the forest stands.

The N fertilization treatments that ended in 1990 and 1992 (N2 and N3) have shown no conclusive pattern of the tendency of plots to approach the conditions found in the control. In part this is due to the lack of effect of treatment in the O horizon. However, the annual mean concentration of DON appear to be somewhat lower in the N2 plots during 2009 than 1995 in the B horizon leachates, perhaps pointing to the fact that some recovery is taking place (Appendix, figure A1). The annual mean concentration of DOC does not seem to diminish in the N2 plots in 2009 (appendix, figure A2), implying some discrepancy between DOC and DON soil solution concentrations.

The absence of correlation with climatic drivers implies that soil water fluxes, sorption/desorption and soil temperature dynamics, which have not been addressed in this work, are of great importance when considering soil DOM dynamics, as proposed by Kalbitz et al. (2000) and Fröberg et al. (2004). It could also point towards the lack of resolution in the weather data and possibly the inappropriateness of using such a “course” methodology for exploring variable processes such as DOC and DON soil solution dynamics. It would thus seem that the tracking of climatic drivers’ influence on DOC and DON soil solution concentrations would be better addressed by the means of dynamic modeling, such as the DyDOC model.

The O horizon leachates display large variation of DOC and DON soil solution concentrations within treatments. This might be due to the dynamics of waterfluxes through the horizon, imposed by the precipitation regime. As reviewed in Kalbitz et al. (2000) it is possible that preferential flowpaths in the O horizon would transport DOM to below-lying soil horizons in recurring pulses, occasionally rendering DOM concentrations of non-equilibrium and thus large variation in DOC and DON soil solution concentrations in gravity fed water passing through the O horizon. Further, the within-year variation of DOC and DON in the subsoil is much greater during 2009 than 1995. This could be the effect of the increased rainfall that takes place in early summer of 2009. During this period approximately 200 mm fall within four weeks time, a substantial amount of rainfall for such a short period. Although further inference of the importance of precipitation regime on “between-year effects” is difficult in the present work, some influence cannot be ruled out.

The DyDOC model computes DOC concentrations and fluxes in the forest soil compartments. Temperature governs several processes in the model; sorption-desorption phenomena, and biological transformations of DOC. Q10 relationships are expressed to exert some influence on the processing of organic matter in the model, suggesting positive correlation of DOC and temperature. A tendency towards higher concentrations during warmer periods was observed in this study; however, these findings seem to be of a somewhat circumstantial nature and might be the result of simple dilution effects. Further, the speciation of production and transport of DOC in micro- and macropores respectively, as in the DyDOC model, seems just, although the test for differences between pore water concentrations conducted in this work is not a strong test.

It appears, from this study, that the production and cycling of DOM in Norway spruce forest soils is a highly buffered system. DOC and DON concentrations sampled below the mor layer do not represent the production of DOC or DON, however, but rather the result of production, leaching, mineralization to CO_2 and sorption/desorption processes which makes deduction from soil water concentrations complex. As a pulse of rainfall enters the soil, DOM is leached out of the horizon and the concentration of DOC and DON in the larger pores decreases. But as soon as water transport through the profile halts, the DOC and DON which is desorbed and enters soil solution raises soil solution concentrations, buffering the effects of the rain pulse. With increasing temperature an increased microbial activity would possibly stimulate the formation of DOC and DON, but the sorption of these species would most certainly increase as soil solution concentration of DOC and/or DON rises, buffering the concentrations in the soil solution. These effects could easily obscure the effects of climatic drivers on the concentrations in soil solution, thereby not meaning that they are without importance, but rather that the processes governing production and transport are not easily observed without using a process-orientated, dynamic approach.

5 Conclusions and future perspectives

The soil solution concentrations of DOC and DON in Norway spruce stands do not seem to respond to long-term N fertilization in a uniform manner. O horizon soil solution concentrations do not display an effect of N fertilization on DOC or DON concentrations, possibly due to large spatial and temporal variation. The concentrations of DOC and DON are occasionally significantly higher in fertilized plots in the mineral soil, although this treatment effect most probably is of an indirect nature.

The DOC and DON concentrations in the mineral soil are less variable and of an order of magnitude lower than in the O horizon leachates. This is most likely an effect of sorption in the mineral soil which lessens the potential for leaching of DOC and DON from the mineral soil.

There does not seem to be a decisive effect of simple climatic drivers on the concentrations of DOC and DON in soil solution. In the O horizon, temperature seems to exert some influence on the concentration of DOC.

There appears to be an effect of development with time in long-term N fertilized tree stands. There is a difference in concentrations of DOC and DON between the years 1995 and 2009 which could not be attributed to the differences in weather between years. Therefore, it would seem that the development with time of plot characteristics (standing tree volume, annual litter fall, soil C and N, O horizon depth etc.) are important in regulating the annual mean soil solution concentration of DOC and DON. Data from 2009 on soil characteristics would pose an interesting addition to the evaluation of these findings.

To show more explicitly how the concentrations of DOC and DON are effected by N fertilization and climatic drivers at Stråsan research station, sorption/desorption reactions, water flows and soil temperatures would need to be considered. A dynamic approach with a model driven by climatic variables and considering sorption/desorption and water fluxes, could arguably provide a better framework within which these effects could be studied.

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8 Appendix

Table A1. Results from ANOVA and pair wise comparisons by Tukey test (95% confidence interval) of DOC and DON concentrations sampled below the O horizon (ZTL_lys_DOC and ZTL_lys_DON) and below the B horizon (TL_lys_DOC and TL_lys_DON) during 1995. Treatments with same letters do not display significant differences in concentration of DOC or DON

	Treatment	20th June	5th July	19th July	2nd Aug	30th Aug	13th Sept	26th Sept	10th Oct	25th Oct	8th Nov
ZTL_lys_DOC	0	a	a	a	a	a	a	a	a	a	a
	N1	a	a	ab	a	a	a	a	a	a	a
	N2	a	a	b	a	a	a	a	a	a	a
ZTL_lys_DON	0	a	a	a	a	a	a	a	a	a	a
	N1	a	a	a	a	a	a	b	a	a	a
	N2	a	a	a	a	a	a	ab	a	a	a
TL_lys_DOC	0	a	a	a	a	a	a	a	a	a	a
	N1	ab	ab	a	a	a	a	a	a	a	a
	N2	b	b	b	b	b	b	b	b	b	b
TL_lys_DON	0	a	-	a	a	a	a	a	a	a	a
	N1	a	-	ab	a	ab	a	ab	ab	a	a
	N2	a	-	b	b	b	a	b	b	b	a

Table A2. Results from ANOVA and pair wise comparisons by Tukey test (95% confidence interval) of DOC and DON concentrations sampled below the O horizon with tension lysimeters (R_lys_DOC and R_lys_DON) during 1995. Treatments with same letters do not display significant differences in concentration of DOC or DON

	Treatment	20th June	5th July	19th July	2nd Aug	30th Aug	13th Sept	26th Sept	10th Oct	25th Oct	8th Nov
R_lys_DOC	0	a	a	a	a	a	a	a	a	a	a
	N1	a	a	a	a	a	a	a	a	a	a
	N2	a	a	a	a	a	a	a	a	a	a
R_lys_DON	0	a	a	a	a	a	a	-	a	a	-
	N1	a	a	a	a	a	a	-	a	a	-
	N2	a	b	a	a	a	a	-	a	a	-

Table A3. Results from ANOVA and pair wise comparisons by Tukey test (95% confidence interval) of DOC and DON concentrations sampled below the O horizon (ZTL_lys_DOC and ZTL_lys_DON) and below the B horizon (TL_lys_DOC and TL_lys_DON) during 2009. Treatments with same letters do not display significant differences in concentration of DOC or DON

	Treatment	1	2	3	4	5	6	7	8	9	10	11
ZTL_lys_DOC	0	a	a	a	a	a	a	a	a	a	a	
	N1	a	a	a	a	a	a	a	a	a	a	
	N2	a	a	a	a	a	a	a	a	a	a	
ZTL_lys_DON	0	a	a	a	a	a	a	a	a	a	a	
	N1	a	b	b	a	a	a	a	a	a	a	
	N2	a	a	a	a	a	a	a	a	a	a	
TL_lys_DOC	0	a	a	a	a	a	a	a	a	a	a	a
	N1	b	a	a	ab	a	a	a	a	ab	a	a
	N2	b	a	a	b	a	a	a	a	b	a	a
TL_lys_DON	0	a	a	a	a	a	a	a	a	a	a	a
	N1	a	a	a	a	a	a	a	b	ab	a	a
	N2	a	a	a	a	a	a	a	b	b	b	a

Table A4. ANOVA results of concentrations of DOC and DON sampled from the O horizon with Zero tension lysimeters (ZTL_Lys) and Rhizon type lysimeters (R_Lys). Tests were for difference in concentration between lysimeters types. Pairwise comparisons were made by Tukey test at 95% confidence interval

Species and treatment	Lysimeter-type	1995-06-20	1995-07-05	1995-07-19	1995-08-02	1995-08-17	1995-09-14	1995-09-28	1995-10-10	1995-10-26
DOC_Control	ZTL_Lys	a	a	a	a	a	a	a	a	a
	R_Lys	b	a	b	b	a	a	a	a	a
DOC_N1	ZTL_Lys	a	a	a	a	a	a	a	a	a
	R_Lys	b	a	b	a	a	a	b	a	a
DOC_N2	ZTL_Lys	a	a	a	a	a	a	a	a	a
	R_Lys	b	a	b	b	a	a	a	a	a
DOC_N3	ZTL_Lys	a	a	a	a	a	a	a	a	a
	R_Lys	a	a	b	a	a	a	a	a	a
DON_Control	ZTL_Lys	a	a	a	a	a	a	a	a	a
	R_Lys	a	b	a	a	a	b	a	a	a
DON_N1	ZTL_Lys	a	a	a	a	a	a	a	a	a
	R_Lys	a	b	a	a	a	a	a	a	a
DON_N2	ZTL_Lys	a	a	a	a	a	a	a	a	a
	R_Lys	a	b	a	a	a	a	a	a	a
DON_N3	ZTL_Lys	a	a	a	a	a	a	a	a	a
	R_Lys	a	b	a	a	b	a	a	a	a

Table A5. Climatic controls on DOC and DON concentration in the soil profile. Selected models derived from stepwise multiple linear regression are on the form $Y = \beta_1 X_1 + \beta_2 X_2 .... \beta_n X_n + \beta_0$. *O horizon leachates with Zero tension lysimeters (ZTL_Lys) and tension lysimeters (R_Lys), B horizon leachates with tension lysimeters (TL_lys)*

Treatment	Y	$\beta_1 X_1$	$\beta_2 X_2$	$\beta_3 X_3$	$\beta_4 X_4$	$\beta_5 X_5$	β_0	R^2_{adj}
R_lys_1995_0	DON	0,059 Temperature$_d$	0,47 Precipitation$_d$	-0,025 PET$_w$	0,071draught	-	0,37	0,72
ZTL_lys_1995_N1	DON	0,14 Temperature$_d$	-0,026 Temperature$_w$	0,33 PET$_w$	-0,0030 Temperature$_{p,2}$	-	0,88	0,67
TL_lys_1995_0	DON	-0,0044 Temperature$_d$	0,00082 Temperature$_w$	0,0038 Precipitation$_p$	-	-	0,048	0,60
ZTL_lys_1995_0	DOC	*3,5* Temperature$_d$	*-0,72* Temperature$_w$	-	-	-	48	0,70
ZTL_lys_1995_N1	DOC	-0,64Temperature$_w$	-22 Precipitation$_d$	-4,7 PET$_d$	1,2968 PET$_w$	-4,4 draught	87	0,75