

Turnover and fluxes of carbon and nitrogen in a
spruce forest under natural and extreme
meteorological conditions

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Summary

Climate models predict an increase in the intensity and frequency of extreme meteorological climate events like extended summer droughts, heavy rainfall or intensive frost periods with largely unknown effects on microbial activity and physico-chemical soil properties and their impact on availability of soil organic matter. The influence of drying/rewetting (A/W) and freezing/thawing (G/A) events on solution chemistry and leaching losses of soils is barely known.

This thesis aimed to study the effects of A/W and G/A events on soil solution chemistry and solute fluxes, in particular, of dissolved organic carbon (DOC) and inorganic nitrogen (NH_4^+ , NO_3^-) in a podzol soil under a Norway spruce forest. A field experiment was designed to study the effects of (i) summer drought by exclusion of natural throughfall and subsequent rewetting and of (ii) soil frost by removal of natural snow cover. In complementary laboratory experiments with undisturbed soil columns, (i) drying/rewetting cycles were simulated with different rewetting intensities and (ii) freezing/thawing cycles were induced using different freezing temperatures. In the second part of this work, total C and N stocks as well as radio-carbon signatures of soil organic carbon (SOC) from different soil horizons and density fractions were investigated. Based on $\Delta^{14}\text{C}$ signatures of bulk SOC and SOC fractions, turnover times (TT), input and accumulation rates of SOC were calculated using a non-steady or steady state model. Specific UV absorbance and emission fluorescence (lab experiment) and radio-carbon signatures (field experiment) of DOC were determined to identify sources and dynamics of DOC. The N net mineralisation was defined by the *in situ* coring method.

TT of 3-10 years in the Oi and Oe horizon highlighted the potential of rapid changes in the mineralisation of SOC in case of climate changes. Most of the SOC of the Oa horizon was accumulated in the free particulate organic matter fraction (FPOM, 59-84 %) with slow TT of 90- 160 years. Accumulation rates of $3.8\text{-}7.8 \text{ g C m}^{-2} \text{ a}^{-1}$ during the past decade indicated the organic layer as a net CO_2 sink. Mineral associated organic matter (MAOM) was the most abundant fraction in the B horizon with over 40% of the total SOC and TT of 390- 2170 years. Recalcitrance of soil organic matter (SOM) and/ or stabilization processes slowed down the turnover times (130- 880 years) of the FPOM fraction in the mineral horizons.

A/W increased the DOC concentrations in the organic layer and upper mineral soil. More DOC was released from the organic layer to the mineral soil. However, the effects on total

DOC leaching were smaller due to reduced water fluxes. Specific UV absorbance and emission fluorescence detected a switch in the release of easily decomposable DOC to hardly decomposable DOC during the wetting phase. Prolonged summer drought and incomplete rewetting due to hydrophobicity of SOM in the organic layer and upper mineral horizon reduced net N mineralisation as well as concentrations and fluxes of the NH_4^+ and NO_3^- . The net nitrification rate in the organic layer was more negatively influenced than net ammonification, indicating that nitrifiers are more sensitive to drought stress than ammonifiers.

The effect of soil frost strongly depended on soil freezing temperature. Only soil frost at temperature below -8°C led to short periods of additional DOC production in the organic layer. Spectroscopic properties and $\Delta^{14}\text{C}$ signatures of DOC implied a disruption of soil aggregates and desorption of older DOC from the mineral associated organic matter fraction of the Oa horizons by G/A events. Severe soil frost below -8°C inhibited the activity of nitrifiers and ammonifiers with decreased NH_4^+ and NO_3^- concentrations and fluxes in the mesocosm experiment. A delayed (by 4 months) increase in NO_3^- concentration in the upper soil horizon by moderate soil frost (-5°C) was attributed to reduced immobilisation by heterotrophic microorganisms.

Summarised, drying and the effect of hydrophobicity led to long-term, severe soil frost to short-term reduction in N mineralisation and N leaching. The effect of increased NO_3^- concentrations as delayed response to G/A needs further research in case of potentially changes in the N balance. Drying as well as freezing induced changes in the soil structure and properties and led to increased DOC concentrations. Moderate soil temperature had much less effects on C and N in this temperate forest soil.

The results of this thesis demonstrated the potential of extreme meteorological events on the quality and availability of dissolved C and N. Both, A/W and G/A cycles decreased C and N mineralisation, increased the sink strength of the soil by the accumulation of SOC and N, considering constant C and N litter input. However, optimal temperature and moisture conditions in other seasons could compensate the sink strength of soils. This work underpins the need for holistic and long-term investigations to understand and model the impact of extreme meteorological conditions on the dynamics of dissolved C and N.

Zusammenfassung

Aktuelle Klimaprognosen gehen von einer zunehmenden Häufigkeit extremer Wetterbedingungen aus. Solche Ereignisse, wie etwa Sommerdürren, Starkregen und das Auftauen und Gefrieren von Böden beeinflussen die mikrobielle Aktivität und die physicochemischen Eigenschaften des Bodens und dies führt zu Veränderungen in der Verfügbarkeit bodenorganischer Substanz (SOM). Die Auswirkungen von Austrocknungs-/Wiederbefeuchtungs- (A/W) und Gefrier-/Auftauereignissen (G/A) auf die Zusammensetzung der Bodenlösung und den Austrag bzw. die Verlagerung von Mineralstoffen aus der organischen Auflage in den Mineralboden wurde bisher kaum untersucht.

Ein Schwerpunkt dieser Arbeit lag in der Untersuchung von Kohlenstoff (DOC) und Stickstoff (NH_4^+ , NO_3^-) in der gelösten Phase sowie deren Stoffflüsse bei A/W- und G/A in einem Podzolboden eines Fichtenwaldes. Im Feldversuch wurde (i) Sommertrockenheit durch Ausschluss des Niederschlages und (ii) der Bodenfrost durch Entfernen der natürlichen Schneedecke experimentell herbeigeführt. Zusätzlich wurden im Labor an ungestörten Bodensäulen A/W und G/A Ereignisse gezielt nachgestellt. Dabei wurde (i) wiederholtes A/W mit unterschiedlichen Intensitäten, sowie (ii) wiederholtes G/A bei unterschiedlichen Temperaturen betrachtet. Der zweite Teil dieser Arbeit bestand in der Bewertung der C und N Vorräte, sowie in der Bestimmung der $\Delta^{14}\text{C}$ Signatur der einzelnen Bodenhorizonte und deren Dichtefractionen. Es wurden Umsatzzeiten (TT), Eintrag und Akkumulationsraten des organischen Kohlenstoffs mit Hilfe der $\Delta^{14}\text{C}$ Signatur unter Verwendung von non- steady und steady state Modellen ermittelt. Veränderungen des DOC wurden mit Hilfe der UV-Absorptions- und Fluoreszenzspektren im Laborversuch und Radiokarbonbestimmung (DO^{14}C - Altersdatierung) im Freiland erfasst. Die Nitrifikations- bzw. Ammonifikationsrate wurde mittels *in situ* Coring-Methode bestimmt.

Der organische Kohlenstoff (SOC) der Oi and Oe Horizonte zeigte mit TT von 3- 10 Jahren ein Potential für erhöhte Mineralisation bei klimatischen Veränderungen. Der Oa Horizont war gekennzeichnet durch einen hohen Anteil an ungebundenen partikulären organischen Materials (FPOM, 59-84 %) und langsamerer TT (90- 160 Jahre). Die berechnete Akkumulationsrate von 3.8- 7.8 g C m⁻² a⁻¹ in den letzten 10 Jahren weist die organische Auflage als Kohlenstoffsenke aus. Das mineralisch assoziierte organische

Material (MAOM) überwog im B Horizont mit über 40 % des gesamten SOC und zeigte TT von 390- 2170 Jahren. Verschlechterte Abbaubedingungen und/ oder Stabilisierungsprozesse im Mineralboden führten zu langsameren TT (130- 880 a) der FPOM Fraktion ($1-3 \text{ kg C m}^{-2}$) im Vergleich zur organischen Auflage.

A/W-Ereignisse erhöhten die DOC Konzentrationen in der organischen Auflage und den oberen Mineralbodenhorizonten. Während der Austrocknung kam es nur zu geringen Wasserflüssen, dennoch konnte eine Verlagerung von DOC aus der organischen Auflage in den Mineralboden festgestellt werden (Freiland: K $5-8 \text{ g/m}^2\text{a}^{-1}$, AW $5-17 \text{ g/m}^2\text{a}^{-1}$). Die Analyse der UV-Absorptions- und Fluoreszenzspektren ergab eine Änderung der DOC Qualität von leicht verfügbarem Substrat direkt nach Wiederberegnung hin zu aromatischen und komplexeren Verbindungen am Ende der Beregnung. Andauernde Trockenheit und eine unvollständige Wiederbefeuchtung nach Austrocknung (aufgrund von Hydrophobizität von SOM der organischen Auflage) verursachte eine geringere N- Mineralisation mit abnehmenden NH_4^+ und NO_3^- Konzentrationen und Flüsse in den oberen Bodenhorizonten. Die Netto- Nitrifikation der organischen Auflage verminderte sich stärker als die Netto- Ammonifikation und verdeutlichte damit die höhere Empfindlichkeit der Nitrifikanten gegenüber Trockenstress.

Der G/A Effekt ist sehr stark von der Bodenfrosttemperatur abhängig. Bodenfrost unterhalb einer Temperatur von -8°C führte zu einem kurzzeitigen Anstieg der DOC Konzentrationen in der organischen Auflage. Spektroskopische und DO^{14}C - Analysen ließen dabei auf das Aufbrechen von Bodenaggregaten, die Freilegung und Desorption von älterem DOC aus der MAOM Fraktion des Oa Horizontes unter Bodenfrostbedingungen schließen. Bodenfrost unterhalb -8°C hemmte die Aktivität der Ammonifikanten und Nitrifikanten und führte zu verringerten NH_4^+ und NO_3^- Konzentrationen und Flüsse im Laborversuch. Erhöhte NO_3^- Konzentrationen, die in den oberen 20cm Bodenschichten vier Monate nach Bodenfrost (-5°C) auftraten, sind wahrscheinlich auf eine geringere NO_3^- Immobilisierung durch heterotrophe Mikroorganismen zurückzuführen.

Zusammenfassend kann man festhalten, dass Austrocknung einschließlich Hydrophobizität längerfristig und intensiver Bodenfrost kurzfristig die N- Mineralisation, N- Verfügbarkeit und den N Austrag in N-gesättigten Böden reduzieren. Die drei Monate nach dem G/A Ereignis beobachtete erhöhte NO_3^- Konzentration, bedarf weiterer Unter-

suchungen. Austrocknung und Frost führten zu Änderungen in der Bodenstruktur und der Bodeneigenschaften mit einer erhöhten Verfügbarkeit an DOC. Moderate Bodentemperaturen haben dabei nur einen sehr geringen Einfluss auf C und N in diesem Waldökosystem.

Die Ergebnisse dieser Arbeit unterstreichen den potentiellen Einfluss extremer Wetterereignisse auf die qualitative Zusammensetzung und Bioverfügbarkeit von gelösten C und N. Eine reduzierte C- und N- Mineralisation als Reaktion des Ökosystems auf A/W und G/A würde zu einer erhöhten Senkenfunktion des Bodens durch Akkumulation an SOC und N führen, sofern die C- und N- Einträge konstant bleiben. Dagegen können optimalere Temperatur- und Bodenwasserbedingungen in anderen Jahreszeiten den Abbau an C und N forcieren und damit die Senkenfunktion ausgleichen. Diese Arbeit verdeutlicht den Bedarf an ganzheitlichen und langfristigen Forschungsansätzen um die Einflüsse von extremen Wetterbedingungen auf die C- und N- Dynamik in Böden verstehen und modellieren zu können.

Chapter 1

Synopsis:

Turnover and fluxes of carbon and nitrogen in a spruce forest under natural and extreme meteorological condition

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1.1. Introduction

Background

Climate models predict that present greenhouse gas emission will cause further warming and other changes in the global climate system (IPCC 2007). Global air temperature is likely to rise between 1.8°C and 4.0°C during this century. With this increase, an increasing frequency of extended summer droughts and periods of intense precipitation are expected. Frequency and intensity of soil frost are expected to change in the future due to widespread reduction of snow covers that prevent soil frost (Hosaka et al., 2005; IPCC 2007).

Drying/rewetting and freezing/thawing is anticipated to affect C and N mineralisation and nutrient fluxes in soils. Large quantities of carbon and nitrogen can be sequestered, released to the atmosphere and leached by soil and biota. There is a growing interest in the future role of soils as sink or source for C and N in view of climate change (reviewed by Matzner and Borcken 2007, Borcken and Matzner 2008). Small changes in the soil organic carbon (SOC) and nitrogen (N) pool are expected to affect the global C and N budget and atmospheric concentration. The need for estimation of SOC and N stock and understanding of net terrestrial C and N sequestration has become essential to understand the cumulative effects of these climate changes.

Soil carbon

Forest soils are of special interest for C storage in the global carbon cycle, as they provide a significant sink for atmospheric CO₂. The organic horizon plays an important role in the dynamic of carbon fluxes. It is considered to be a source for plants and microorganism as well as a reservoir for carbon. In terrestrial ecosystems, 1500 PgC (1Pg= 10¹⁵g) are stored in soils and 500 PgC in plants. This is slightly more than twice the amount of carbon (C) present in the atmosphere as CO₂ (730 PgC) (Schlesinger 1990).

The amounts of soil organic carbon (SOC) and their turnover times vary with depth, litter quality and climate (Schimel et al., 1994). In the forest floor, soil C sequestration range from less than a year to a few decades (Trumbore et al., 1996; Quideau et al., 2001). Stabilisation mechanisms like chemical recalcitrance, formation of aggregates and interaction with soil minerals lead to carbon pools with different turnover times (van Lützwow et al., 2006). The various functions in the soil and the global C cycle (ac-

tive, passive pool, sink/source for trace gases) need an exact differentiation of SOM in functional pools (van Lützow et al., 2007). Density fractionation allows the separation in free (FPOM), occluded particulate organic matter (OPOM) and mineral associated organic matter (MAOM) pools. The use of radiocarbon analysis helps to describe the dynamics of accumulation and turnover of SOC pools (Trumbore 2000; Gaudinski et al., 2000).

Dissolved organic carbon (DOC) is important for the transport of carbon into the mineral soil (Michalzik et al. 2003). DOC is a leaching product from plants (canopy and roots), litter and humus and it is generated by microbial activity. The measured DOC concentration is the net effect of production, mineralisation and sorption-desorption processes. In temperate forest soils, the flux of DOC from the organic layer to the mineral soil is substantial (115- 398 kg ha⁻¹ a⁻¹), whereas the C fluxes leaving the mineral soil are smaller (5- 66 kg ha⁻¹ a⁻¹) (Kalbitz et al., 2000; Michalzik et al., 2001). Laboratory experiments have indicated that most mineral soils have a high capacity to adsorb and stabilize DOC (Qualls and Haines 1992a; Kaiser et al., 1996; 2005). DOC collected in the mineral soil is chemically much more similar to soil organic matter than to fresh litter leachates reconfirm the suggestion of significant sorption and exchange processes (Karlton et al., 2005; Fröberg et al., 2006; Kalbitz et al., 2007, Sandermann and Armundson 2009). Also the decomposition of DOC has been shown to be slow and partial (Qualls and Haines 1992b) which limit the transport of DOC to deeper soil horizons (Nelson et al., 1994). The fact of high sorption and stabilization of C in the mineral soil has questioned the importance of DOC fluxes in the belowground C cycle.

$\Delta^{14}\text{C}$ as a tracer for soil organic dynamics

Radiocarbon formed in the atmosphere is rapidly converted to CO₂ and enters the vegetation via photosynthesis. Aboveground thermonuclear weapon tests from 1945- 1963 resulted in the enrichment of ¹⁴C in the atmosphere (Fig. 1.1a). Interaction between soil, vegetation and atmospheric CO₂ led to distinct $\Delta^{14}\text{C}$ values in different soil horizons (Fig. 1.1a). $\Delta^{14}\text{C}$ measurements are a useful tool for studying cycling of C in terrestrial ecosystems. Old and stable soil organic carbon (SOC) consists of a low $\Delta^{14}\text{C}$ signature compared to young and recent SOC. The creation of new substrate via impact of extreme meteorological conditions results therefore in measurable changes in DO^{14}C .

The calculation of the mean turnover time (TT) of SOC from its radiocarbon signature has been carried out by steady-state or non-steady-state models (Gaudinski et al., 2000; Trumbore 2000). In some instances, two different TT were found to yield identical values of ^{14}C (Fig. 1.1b). Turnover time, the average time of the carbon entering to the soil, is increased with increasing stability and density of the soil, which increased with soil depth.

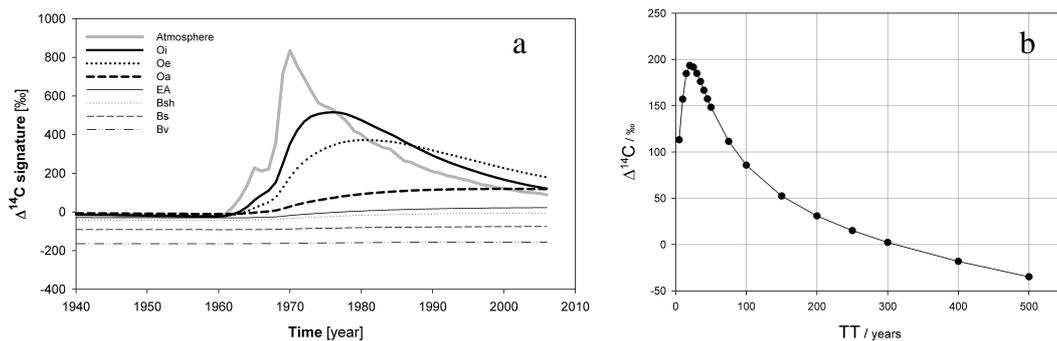


Figure 1.1 (a) The time record of ^{14}C signature of the atmosphere and soil organic carbon in organic layer and mineral horizon from 1940- 2010 as estimated from a non-steady and steady model (Chapter 2). (b) Values of ^{14}C and estimated turnover times (TT) (steady state model) of C for samples collected in 2006 (Chapter 2).

Soil nitrogen

The average global pool of N in the upper 1m depth ranges between 133- 140 Pg N (Batjes, 1996). The amount of N is depending on processes, which add or remove N from the ecosystem, e.g. N deposition, N fertilization, biological fixation, denitrification and N leaching.

The production of ammonium (NH_4^+) and nitrate (NO_3^-) in the organic layer by microbial processes of gross ammonification and gross nitrification forms accessible minerals for plants. In temperate forest soils, nitrification is regulated by intensive competition for NH_4^+ between plants, heterotrophic microbes and nitrifiers (Aber et al., 1989). However, net nitrifications, which include gross nitrification and NO_3^- consuming processes is important for the regulation of N losses from the ecosystems along hydrological and gaseous flow paths. The balance between gross nitrification, inorganic N uptake by plants and inorganic Immobilisation by microbes can differ by changed atmospheric N deposition, anthropogenic management and climate change, thus leading to enhanced nitrate concentration and N loss from ecosystem (Borken and Matzner

2004, Callesen 2007). Nitrogen losses have been found to reduce nutrient stocks, but also affect groundwater and streamwater quality by NO_3^- leaching and atmospheric chemistry by N_2O losses.

The average fluxes from the organic layer to the mineral soil range between 1- 13 kg NH_4^+ $\text{ha}^{-1} \text{a}^{-1}$ and 2- 68 kg NO_3^- $\text{ha}^{-1} \text{a}^{-1}$, whereas the NH_4^+ and NO_3^- losses in the mineral soil are lower with 0.1- 5 kg $\text{ha}^{-1} \text{a}^{-1}$ and 0.1- 40 kg $\text{ha}^{-1} \text{a}^{-1}$ (Gundersen et al., 1998; Fitzhugh et al., 2001; Solinger et al., 2001; Callesen et al., 2007).

Impact of extreme meteorological conditions on DOC, NO_3^- , NH_4^+ via soil solution

Temperature and water are the main drivers for many biological and chemical processes and thus ecosystem functioning. Drying/rewetting and freezing/thawing of soil are important events, which affect C and N mineralisation and C and N trace gas emission from soils (Matzner and Borken 2008, Borken and Matzner 2009). While studies on effects of changes of dry periods and rewetting intensities as well as freeze intensities have been mainly focused on CO_2 - und N_2O -emissions, the impact of extreme weather on soil solution chemistry are still uncertain.

Effects of drying/rewetting: In general, drought limits the water and substrate availability, which directly restricts the activity of soil microorganisms, and therefore reduces the amount of C and N cycled in the ecosystem (Kieft et al., 1987; Goebel et al., 2005; Ford et al., 2007). Borken et al. (2006) postulated that temperate forest soil works as a transient C sink during extended drought, which is reduced by rewetting events. Rewetting of dry soil results in sudden increases of C and N Mineralisation due to several reasons (summarised in Borken and Matzner, 2008): (i) accumulation of organic material by reduced Mineralisation (van Gestel et al, 1991), death of microorganisms (van Gestel et al., 1993) and fine roots (Gaul et al., 2008a) and increased release of organic material via (ii) desorption from the soil matrix and (iii) disruption of soil aggregates (Lundquist et al., 1999). In laboratory experiments, rewetting effects were detectable inbetween 2-6 days (Franzluebbers et al., 2000; Fierer and Schimel 2002), whereas in the field remoistening of topsoils and resulting effects were observed over weeks or months depending on the hydrophobicity, intensity and duration of precipitation (Borken et al., 1999).

Laboratory and field experiments have shown increased DOC concentrations (Lammersdorf et al., 1998; Lundquist et al., 1999a; Fierer and Schimel 2002) and a change in the quantity and chemical properties of DOC (Christ and David 1996b; Kalbitz et al.,

2000; Schmitt et al., 2009) after soils were exposed to drying/ rewetting. The effects of drought on inorganic N in temperate forest soil are less clear. A short ammonium peak followed by elevated nitrate concentration was found after rewetting (Lamersdorf et al., 1998). N fluxes were either increased (Xu et al., 1998; De Visser and van Bremen 1995) or unaffected (O'Neill et al., 2003) with decreasing soil moisture.

Effects of freezing/thawing: C and N Mineralisation are temperature sensitive processes, which decrease at lower temperature, however, root and microbial processes continue in cold and frozen soil (Edwards and Cresser 1992, Clein and Schimel 1995). Following soil freezing, the rates of Mineralisation and decomposition of SOM are increased as a result of inputs of labile OM via (i) physical disruption of aggregates (van Bochove et al., 2000; Freppaz et al., 2008) (ii) increased fine root and microbial mortality (Christensen and Christensen 1991; Tierney et al., 2001; Gaul et al., 2009b) and (iii) decreased uptake by roots (Fitzhugh et al., 2001). Soil freezing therefore results in increased C and N concentrations in soil solution.

Additional C losses via CO₂ do not or just marginally occur after freezing/thawing events (Larsen et al., 2002; Grogan et al., 2004; Groffmann et al., 2006; Goldberg et al., 2008; Muhr et al., 2009b). In two laboratory studies a positive effect of soil frost on DOC production was observed (Wang and Bettany 1993, Fitzhugh et al 2001). An increase of DOC concentrations in soil solution in the field and at deeper soil after freezing/thawing has not been reported yet. Decreasing microbial C and elevated fine root mortality is leading to additional input of carbon into the soil (Tierney et al., 2001; Grogan et al., 2004; Gaul et al., 2009b).

Observations in N Mineralisation after soil frost are contradictory. Some studies reported higher NO₃⁻ concentrations in soil solution during the following year after soil frost (Boutin and Robitaille 1995; Mitchell et 1996; Fitzhugh et al., 2001; Callesen et al., 2007). Other studies found less affected NO₃⁻ concentrations (Fitzhugh et al., 2001; Neilson et al., 2001; Müller et al., 2002; Freppaz et al., 2007; Kaste et al., 2008). It is possible that the kind of tree species, soil condition and N soil status influence the respond to soil frost (Fitzhugh et al., 2001; Matzner and Borken 2008).

1.2. Objectives

The interdisciplinary Research Group 562 ‘Dynamics of soil processes under extreme meteorological boundary conditions’ of the German Research Foundation (DFG) comprising soil scientists, hydrologists, plant ecologist and microbiologists was established in 2005 to illuminate consequences of drying/rewetting (A/W) and freezing/thawing (G/A) on element turnover and fluxes, structure of the soil microbial community and biogeochemical processes in a Norway spruce forest soil. This thesis focused on C and N in case of stock, element transport in soil solution and related Mineralisation processes.

The objectives of this thesis were follows:

- (1) *to quantify the stock and heterogeneity of SOC and N and to determine the turnover rates of SOC in a forest soil.*

Nine randomly distributed soil pits were dug for area-based determination of rock volume, bulk density, SOC and N contents. In density fractions (free-, occluded particulate, mineral associated organic matter) of the Oa, EA, Bsh, Bs and Bv horizons, the carbon and nitrogen contents were determined. Radiocarbon (^{14}C) measurements in SOC were used to assess the turnover times, the C input rate and C accumulation in organic layer and mineral soil horizons.

- (2) *to study the temporal and spatial pattern of the DOC concentrations and DO^{14}C signature in throughfall and soil solution in order to improve our understanding of DOC sources and dynamics in a Norway spruce stand.*

During a two-years study, seasonal changes in DOC concentrations and $\Delta^{14}\text{C}$ signatures of DOC in throughfall and soil solution beneath the organic layer and at 90 cm soil depth were determined. A comparison of $\Delta^{14}\text{C}$ signatures of DOC and SOC allowed identifying the potential source of DOC.

- (3) *to quantify the effects of drying/rewetting on concentrations and fluxes of DOC, NH_4^+ and NO_3^- as well as net- N Mineralisation at different wetting intensities.*

To study drying/rewetting effects, a temporary roof construction was installed on three experimental plots to prevent infiltration of throughfall, followed by controlled rewetting in the field. Additionally, a drying/rewetting experiment with undisturbed soil columns was performed in the laboratory with a control and three wetting intensities of 8, 20 and 50 mm (n=4). The hypothesis stated that drying/rewetting decrease solute fluxes of NH_4^+ and NO_3^- as well as N net Mineralisation and increase DOC fluxes. It was expected that increasing wetting intensity will result in decreased N and DOC fluxes because of hydrophobic surfaces in the topsoil after desiccation.

- (4) *to quantify the effect of freezing/thawing on concentrations and fluxes of DOC, NH_4^+ and NO_3^- as well as the dynamics of N net Mineralisation rates in organic and mineral soil horizons*

To investigate the effects of freezing/thawing, soil frost was induced by removal of snow cover under field conditions from Dezember 2005 to February 2006. In a complementary approach the effect of repeated freezing/thawing experiments with undisturbed soil columns in the laboratory at temperature of -3°C , -8°C and -13°C was studied. It anticipated that freezing and thawing would increase solute fluxes of DOC, NH_4^+ and NO_3^- as well as N net Mineralisation and that low freezing temperature increase this effect.

1.3. Experimental set up

Site description

The Coulissenhieb II site was a mature Norway spruce forest (*Picea abies*, L.) in the Fichtelgebirge, Germany (50°8'N, 11°52'E; 770m a.s.l.), completely covered by ground vegetation, mainly *Deschampsia flexuosa* (L.) Trin. and *Callamagrostis villosa* (Chaix.) J.F.Gmel. The soil was classified as Haplic Podzol according to the FAO soil classification (IUSS, 2006) with a well stratified mor-like organic layer of 7-10 cm thickness comprising Oi, Oe and Oa horizons (Fig. 1.2). The mean annual precipitation was about 1160 mm and the mean annual air temperature is 5.3 °C.

Soil properties are detailed in Chapter 2. The SOC and N content of the soil decreased with increasing depth from 35.8 % C in the Oi horizon to 2.4 % in the Bv horizon, and from 1.7 % N to 0.2 % N. The soil stored 3.8 kg C m⁻² and 0.09 kg N m⁻² in the organic layer and 11.4 kg C m⁻² and 0.6 kg N m⁻² in the mineral soil.

Design of the laboratory experiments

Two laboratory experiments with undisturbed soil columns were conducted to study the effects of sequential drying/rewetting and freezing/thawing with focus on trace gases, the transport in soil solution and changes in organic matter quality. This thesis concentrated on soil solution chemistry and fluxes of DOC, NH₄⁺ and NO₃⁻. The soil columns were taken in April 2005 using polyacrylic cylinders with a diameter of 17.1 cm. Two types of soil columns, one including Oi, Oe and Oa horizons, and the other including organic horizons plus mineral soil (Oi, Oe, Oa, EA, Bsh and Bs horizons), were used.

In the first experiment, three cycles were performed for the drying-rewetting experiment (Fig. 1.3). The two types of columns were divided into four treatments, i.e. a con-

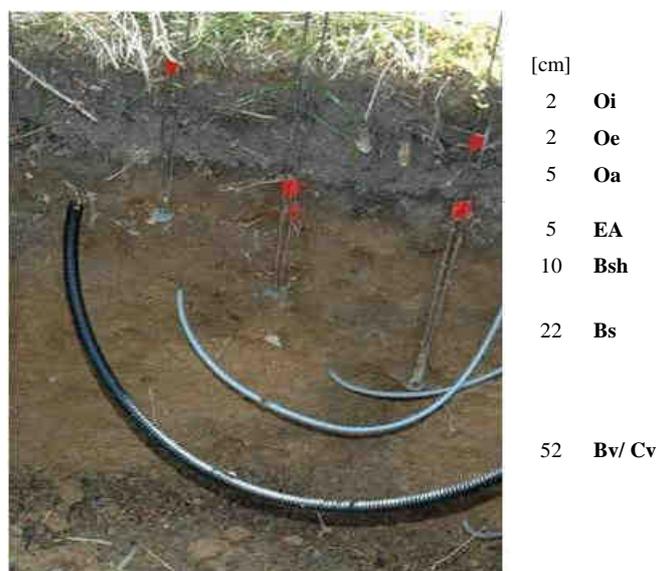


Figure 1.2 Soil profile of Coulissenhieb II: Haplic Podzol with mor-like organic layer

control and three wetting intensities of 8, 20 and 50 mm, each with four replications. The soil columns, except the control columns were intensively desiccated at temperatures between 20 and 25°C by ventilation with dry air from top and bottom over 42 days (drought period). The controls were wetted with 4 mm day⁻¹ for 25 days, the 8 mm treatment with 8 mm day⁻¹ for 12.5 days, the 20 mm treatment with 20 mm day⁻¹ for 5 days and the 50 mm treatment with 50 mm day⁻¹ for 2 days during the wetting intensive phase. Subsequently, irrigation continued with a rate of 4 mm day⁻¹ for 2 weeks (wetting phase) in all treatments. After further 33 days the columns were repeatedly desiccated for 42 days.

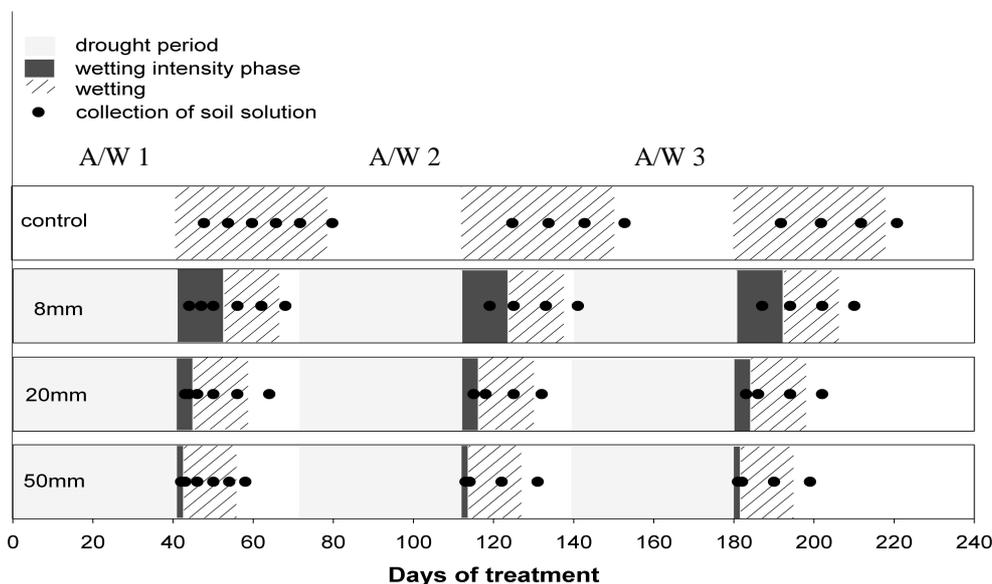


Figure 1.3 Drying-rewetting (A/W) experiment: Each cycle consisted of drying period over 42 days at 20–25°C, wetting intensive phase of 8mm (12.5d), 20mm (5d) and 50 mm (2d) and a wetting of 4mm for 20days. The control was not desiccated but wetted with 4 mm d⁻¹ for 45 days.

In the second experiment, three freezing/thawing (G/A) cycles were executed. An overview of this experiment is shown in Fig. 1.4; the soil cores were allocated randomly to four different temperature regimes: 5°C, -3°C, -8°C and -13°C (n=4). The frost treatments were frozen for 14 days in the freezer at different temperature regimes. After seven days of thawing at +5°C, all columns were irrigated with 4 mm d⁻¹ for 20 days and percolates were sampled every 4–5 days. After further 20 days, the frost treatments were frozen again.

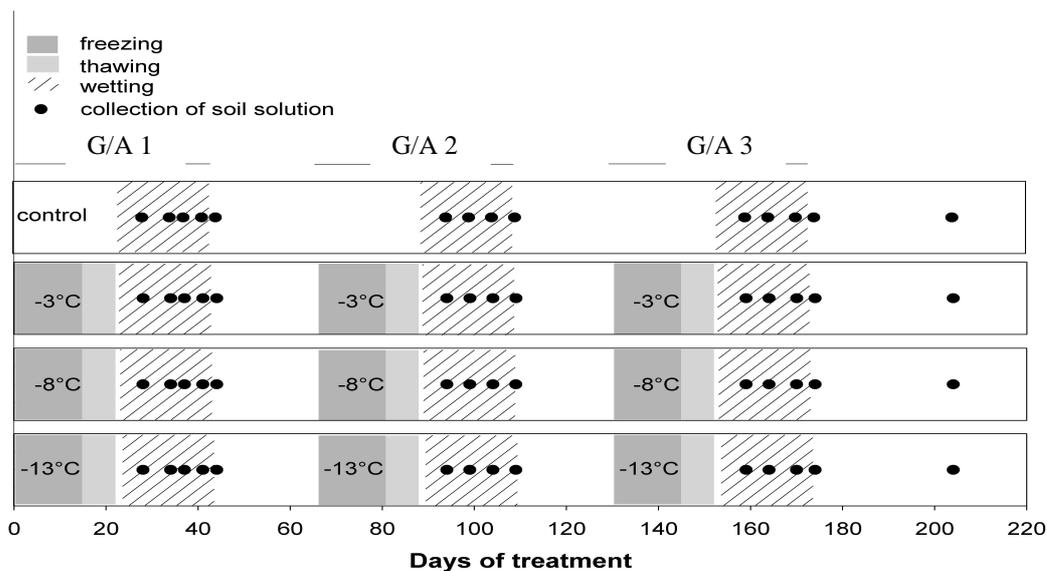


Figure 1.4 Freezing/thawing (G/A) experiment: Each cycle consisted of freezing for two weeks at -3 , -8 or -13°C . After a thawing phase of 7 days all columns were wetted with 4mm artificial throughfall solution per day for 20 days.

The irrigation solution was similar to the long-term average throughfall in the Norway spruce stand at the Fichtelgebirge (Matzner et al., 2004). We applied artificial throughfall with the following components (mg l^{-1}): Na^{+} 0.69, K^{+} 2.8, Ca^{2+} 1.4, Mg^{2+} 0.2, NH_4^{-} -N 1.4, NO_3^{-} -N 1.4, SO_4^{-} -S 2.3, PO_4^{-} -P 0.06, Cl^{-} 1.4 and a pH of 4.4.

Design of the field experiments

The experiment was carried out in a mature Norway spruce forest (*Picea abies*, L.) of an age of 140 years at the Coulissenhieb II research site, located in the Fichtelgebirge in southern Germany. Three control plots, three freezing/thawing plots (G/A) and three drying/rewetting plots (A/W) were established in the summer of 2005 (Fig. 1.5). Leachates from the forest floor were collected below the Oa horizon by three plate lysimeters and three ceramic suction cups each at 20 cm soil depth and below the rooting zone at 90 cm soil depth were installed per plot. Soil solutions were collected bi-weekly, filtered with $0.4 \mu\text{m}$ cellulose-acetate filters and stored at $+2^{\circ}\text{C}$ until chemical analysis. Additionally, N net mineralisation was determined by the in situ coring method.

At each G/A plot, plastic nets (mesh width 1cm) were used to cover the organic layer to avoid disturbance of the soil surface during snow removal. Snow was manually removed to induce soil frost, between the end of December and the beginning of February 2006. In total, the removed snow was equal to 147 mm of throughfall.

At each A/W plots, 400m² woody roof constructions were installed about 3m above the organic layer. The roofs were covered from 24.06.2006 to 09.08.2006 (47 days) with transparent polyvinylchloride (PVC) panels to dry-down the soil. A 0.4m deep plastic sheet was installed around the A/W plots to reduce lateral movement of soil water and water uptake by roots from outside the A/W plots. Subsequent to the throughfall exclusion (67mm), the soil was rewetted for 2 days with springwater in 150m distance with the following composition (mg l⁻¹): Na⁺ 3.07, K⁺ 1.58, Ca²⁺ 5.35, Mg²⁺ 1.3, NH₄⁻ N 0.02, NO₃⁻-N 2.43, SO₄⁻-S 5.5, PO₄⁻ P <0.05, Cl⁻ 2.85 and a pH of 5.0. After rewetting, the roofs were removed.

The results of the drying/rewetting-experiment were not included in a publication but are part of the synopsis.

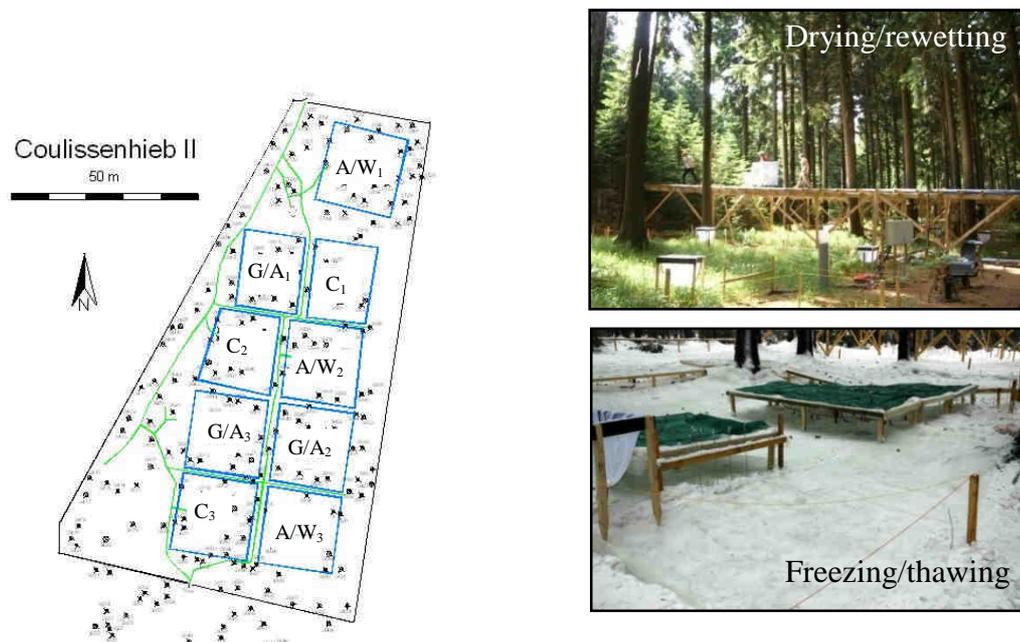


Figure 1.5 Design of the field experiments at the Coulissenhieb II site: three control (C), three freezing/thawing (G/A) and three drying/rewetting plots (A/W), each of an area of 20m x 20m.

1.4. Synopsis

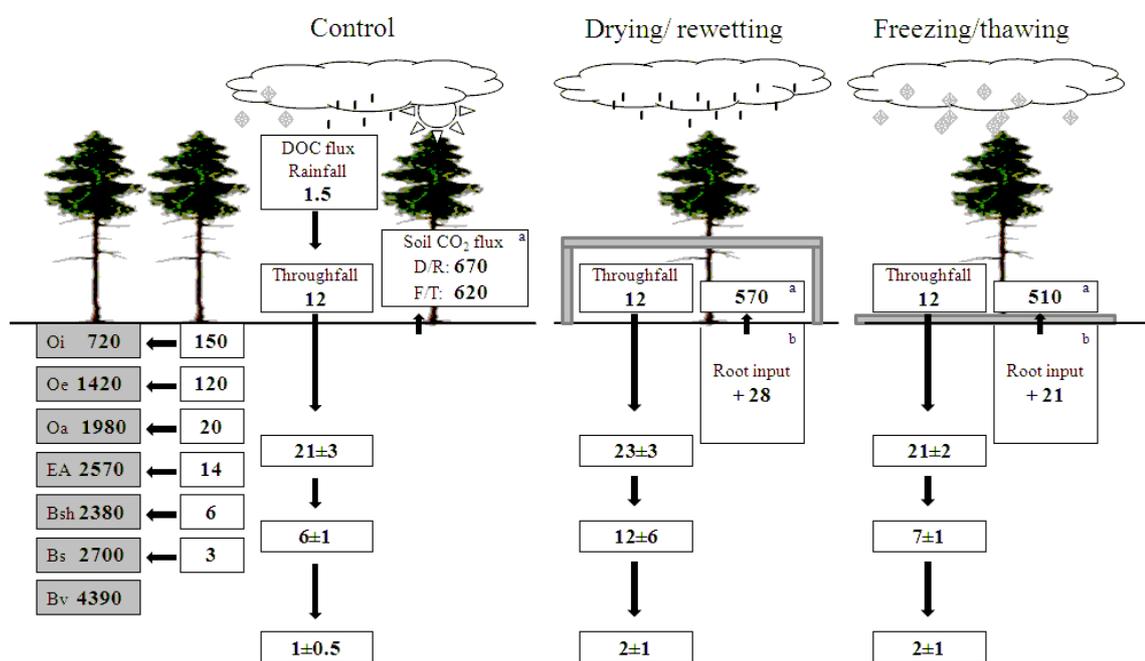
Soil organic carbon and total nitrogen under non-manipulated conditions (Chapter 2 and Chapter 3)

The Coulissenhieb II study site was characterised by well stratified mor-like organic horizon. It consisted of heterogen organic carbon (2.8- 4.9 kg C m⁻²) and nitrogen (0.05- 0.16 kg N m⁻²) stock in the organic layer (Fig. 1.6), attributing to thickness levels between 7 cm and 10 cm. The low C/N ratio of 18 in the Oa horizon as well as high atmospheric N deposition rates and leaching losses (Matzner et al., 2004) indicated N saturation of this forest site. Based on a non-steady state model, soil carbon pools (leaf and root litter) in the Oi and Oe horizons has fast turnover times (TT) with 3-10 years and small accumulation rates of <0.1 and 0.3 g C m⁻² a⁻¹ (Table 2.3). Based on this result the carbon pools in the Oi and Oe horizons achieved steady-state since 40- 60 years. In contrast, the Oa horizon acted as a small sink for soil organic carbon (SOC). Most of the Oa horizon was in form of free particulate organic matter (FPOM) and not associated with minerals. The rate of turnover was slow enough (90- 160 years) that the annual C input (20 g C m⁻² a⁻¹, Fig. 1.6) led to annual accumulation rates of 4-8 g C m⁻² a⁻¹ during the past decade.

The mineral horizon provided valuable C and N reservoir with 8.6- 16.2 kg C m⁻² and 0.5- 0.9 kg N m⁻² down to 62 cm soil depth. The TT of SOC (Table 2.2) decreased with increasing depth and bulk density. The large heterogeneity was attributable to the varying thickness (43- 60 cm), rock fraction (12- 29 % by volume) and the amount of fine earth (312- 512 kg m⁻²). The TTs of the FPOM fraction were slow (70- 1200 years) and it showed that recalcitrance of SOM, aggregate formation and interaction with soil minerals were important processes in this forest soil (Poirier, 2005; Kögel-Knabner et al., 2008).

However, decomposition of soil organic matter (SOM) is temperature sensitive processes and implies a high susceptibility to changes in meteorological condition (Trumbore et al., 1996). About 25% of the SOC pool and 10% of the N pool was held in the top 10cm, the layer which was most prone to changes in temperature and soil moisture. At that time, the organic layer was a small sink for organic carbon, but the turnover of non-stabilized SOM decreased with increasing temperature and might turn the soil from a small sink to a transient C source. Provided that the FPOM fraction of 0.7- 1.1 kg C m⁻²

(9- 16 %) and 0.02- 0.04 kg N m⁻² (5- 8 %) in the B horizons is accessible for microbial attack, the soil has a large potential for C and N losses. With increasing depth the TT increase, the C input rates decreased and the response to changes in meteorological condition decreased too. The need for better understanding of C and N cycling in the mineral soil became essential to understand cumulative effects in view of climate change. $\Delta^{14}\text{C}$ revealed no answer of the question “Acts the mineral horizon as a net C sink or source?”.



^a Muhr and Borken (2009), Muhr et al. (2009)

^b Gaul et al. (2008a, b)

Figure 1.6 Carbon budget (g m⁻²) and annual carbon input rates (g m⁻² a⁻¹) (see Chapter 2), DOC and CO₂ fluxes as affected by drying/rewetting and freezing/thawing at the study site Coullissenhieb II. Annual carbon input was calculated with a non-steady state model (organic layer) and steady state model (mineral soil horizons). DOC flux was calculated from May 2006 to April 2007, because the water fluxes are similar at all treatments from 1. May 2006 on. CO₂ fluxes, which include heterotrophic and autotrophic respiration, were calculated for 2006 by Muhr and Borken (2009) and Muhr et al. (2009). CO₂ measurements were simultaneously measured on control vs. drying/rewetting and control vs. freezing/thawing plot at different days and intensity. These differences in the design explain the different CO₂ fluxes for 2006. The effect of C input to the soil via root death following drying/rewetting and freezing/thawing were determined by Gaul et al. (2008a, b).

Dissolved organic carbon (DOC) and their natural variation (Chapter 3)

The DOC release from the organic horizon is depending on atmospheric deposition of organic substances (Avery et al., 2006), biological degradation of plant residues (Guggenberger and Zech 1994) and leaching of substances of fresh litter (Qualls and Haines 1991). These processes are controlled by temperature, soil moisture, pH and leaching rate (Christ and David 1996b, Michalzik and Matzner 1999).

The throughfall DOC followed no seasonal trend (Fig. 3.2). The observed independence of DOC concentrations from air temperature and amount of throughfall contradicted other findings on regulating factors (Lindroos et al., 2008). Nevertheless, different DOC concentrations but identical DOC fluxes in 2006 and 2007 pointed out that the amount of precipitation was an important factor regulating the DOC fluxes in throughfall (Starr and Ukonmaanaho 2007). DO^{14}C signature from -52 ‰ to 41 ‰ indicated that throughfall DOC was influenced by deposition of fossil C originating from combustion of fossil fuel (Fig. 3.4). A mass balance approach revealed that fossil DOC contributed 8- 10 % to total throughfall DOC in the 1st quarter of 2006 and 2007. The positive values in the summer month resulted mainly from the release of recently synthesized DOC in case of higher biological activity. DOC from throughfall was available C for microorganisms and decompose rapidly in the organic layer, therefore did not contribute to the DOC leaching from the organic layer (Michalzik et al., 2001; 2003). Otherwise, the older carbon would have acted as an important long- term carbon sink because its turnover is probably slower.

Seasonal variations in DOC concentrations of organic layer percolates have been found in several studies (e.g. Michalzik and Matzner 1999; Solinger et al., 2001; Kaiser et al., 2002; Fröberg et al., 2006). At the Coulissenhieb II site, a pronounced seasonality with low concentrations after snowmelt and highest concentrations in summer was found (Fig. 1.7). Increased substrate availability for DOC production via fresh litter input (Qualls and Haines 1991) had no effect of the DOC concentration in the Oa horizon. DOC concentrations followed slightly the pattern of soil temperature. Similar maxima DOC concentrations in both years at different soil moisture condition and changed seasonal pattern indicated that physico-chemical processes (sorption-desorption) dominated the actual concentrations additionally to the strong temperature dependence (Fröberg et al., 2006). Furthermore, the DO^{14}C measurements at different

times suggested that leached DOC comes out from different carbon pools (Fig. 3.3). Disruption of soil aggregates due to intensive drying, incomplete remoistering due to hydrophobicity of organic matter and preferential flow (Bogner et al., 2008) led to desorption of DOC (Lundquist et al., 1999a) from the MAOM fraction (from -26 ‰ to 38 ‰). It contributed to the leaching of older DOC and was the responsible mechanism for the occurrence of the negative DO^{14}C beneath the organic layer in 2006. It was assumed, that the Oa horizon itself was the main source for the leached. Another potential source effecting the DO^{14}C

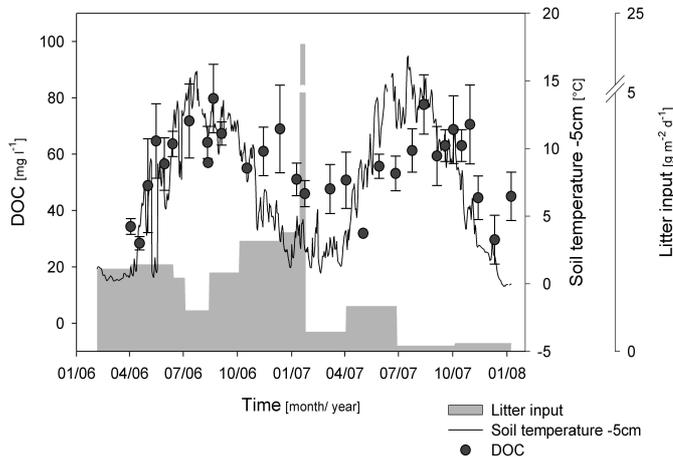


Figure 1.7 DOC concentrations in soil leachates from the forest floor and daily litter (needles, cones and branches) input.

signature of organic layer might be the input of fossil DOC, but the fate of infiltrating fossil DOC in the soil remains unknown. Under constant moisture conditions, decomposition of organic matter from fresh litter or roots enriched the DO^{14}C signature towards more positive values, which explained the modern DO^{14}C leached from the Oa horizon in 2007.

Annual DOC fluxes, CO_2 fluxes and C input rates are summarised in Fig.1.6. The DOC fluxes in throughfall represented a large C input with an annual flux of $12 \text{ g C m}^{-2} \text{ a}^{-1}$ (2006) to the organic layer. Additional C of $9 \text{ g C m}^{-2} \text{ a}^{-1}$ was mobilized through the organic layer, whereas the upper 10cm mineral soil retained $15 \text{ g m}^{-2} \text{ a}^{-1}$ of leached DOC. Assuming steady state, i.e. C loss (heterotrophic respiration, DOC leaching) was equal to C input (root litter, DOC), DOC input contributed 8 % ($=15 \text{ g m}^{-2} \text{ a}^{-1}$) in 0-10 cm and 14 % ($=21 \text{ g m}^{-2} \text{ a}^{-1}$) in 0-90 cm mineral soil to the annual C input. This estimate was lower compared to a field study of Sandermann and Amundson (2009), where DOC movement and retention were responsible for 20 % of the total mineral soil C stock. This drought induced isotopic shift in 90 cm soil depth (like beneath the Oa horizon) indicated that different SOM fractions of the Bv horizon and overlaying hori-

desorption of DOC (Lundquist et al., 1999a) from the MAOM fraction (from -26 ‰ to 38 ‰). It contributed to the leaching of older DOC and was the responsible mechanism for the occurrence of the negative DO^{14}C beneath the organic layer in 2006. It was assumed, that the Oa horizon itself was the main source for the leached. Another potential source effecting the DO^{14}C

zons acted as potential DOC source that depends on flow paths of soil water and the amount of infiltrating water. Nevertheless, small concentrations of DOC in 20 cm and 90 cm soil depth implied that the mineral soil is an effective DOC sink. Al and Fe oxides/hydroxides and clay minerals retain large amount of DOC in Podzols (Kalbitz et al., 2000). Thus, annual net C losses were dominated by respiratory losses as CO₂.

DOC, NH₄⁺ and NO₃⁻ as affected by drying/rewetting (Chapter 4)

Soil drying in laboratory and field experiment led to a strong reduction in the soil water content and causes in changes in the soil structure and induces hydrophobicity of soil surfaces (Muhr and Borken, 2008). Rewetting or regeneration of matric potential took several weeks back to the level of the control. An explanation could be water repellency and preferential flow patterns in the organic layer (Bogner et al., 2008). As long as matric potential does not recovered microbial activity remain slower. However, it was found that A/W effectively increased DOC concentrations and strongly reduced the NH₄⁺ and NO₃⁻ concentrations of the organic layer independently from wetting intensities (Figs. 4.2, 4.3, 4.4). The effects on enhanced DOC fluxes were smaller due to reduced water fluxes during the time period considered, although the experiment showed a small amount of additional DOC input from the organic layer to the mineral soil (e.g. field experiment: K 5-8 g m²a⁻¹, AW 5-17 g m²a⁻¹).

Drying and rewetting resulted in an increase of DOC concentrations during the laboratory and field experiment. Additionally decreased CO₂ emission (Muhr et al., 2008; 2009) suggested a decrease in C Mineralisation. It is well known that during drying microorganisms become inactive or die (e.g. Mikha et al., 2005; Xiang et al., 2008). The shift of more plant to microbial sugar as less plant sugar was transformed to microbial sugars (Schmitt et al., 2010) corroborate the belief of weaker adaptation of the microbial population to drought. It seemed that drought stress not only affected the organic horizon but also the mineral horizon down to 20cm soil depth. In the laboratory experiment, A/W led to increased DOC concentrations by more than 60- 90 % and the field experiment induced increased DOC concentrations soil depth by factor 2- 3 in the three-months after rewetting (10/06- 1/07) in 20 cm. Decreased soil respiration (Fig. 1.6; Muhr et al., 2008; Froitzheim personal communication) as well less production of microbial sugar (Schmitt et al., 2010) in the mineral soil indicated decreasing microbial activity during drying/rewetting. Physiological or nutritional stress, i.e. decrease plant

sugar was most pronounced in the EA horizon (Schmitt et al., 2010). Decreased C Mineralisation and thus less C Immobilisation explained the increase of DOC concentrations in 20cm soil depth (Fig. 1.9). Nevertheless, it is assumed that the adsorption of DOM to mineral surfaces is far more important than the decomposition of DOM in mineral soils (Kalbitz et al., 2000). The aspect of increasing DOC concentrations and fluxes in the upper soil horizons implied that the stabilization of DOC by adsorption and precipitation with dissolved aluminium (Scheel et al., 2007) and might affect the storage of mineral soil (Fig. 1.6).

Table 1.1 Soil N stocks (n=9), annual NO_3^- and NH_4^+ fluxes (n=3) in throughfall and different depths down to the Bv horizon in the control, drying/rewetting and freezing/thawing plots from May 2006 to April 2007.

Horizon	N Stock g m^{-2}	NO_3^- -N $\text{g m}^{-2} \text{ a}^{-1}$			NH_4^+ -N $\text{g m}^{-2} \text{ a}^{-1}$		
		Control	Drying/ rewetting	Freezing/ thawing	Control	Drying/ rewetting	Freezing/ thawing
Throughfall		1.0	1.0	1.0	1.0	0.9	1.0
Oi	27 (3)						
Oe	62 (33)						
Oa	158 (120)	2.4 (0.3)	2.7 (0.7)	2.9 (0.3)	0.1 (0.1)	0.1 (0.1)	0.1 (0.1)
EA	134 (81)						
Bsh	106 (49)	3.0 (1.1)	2.3 (0.4)	3.6 (1.7)	0	0.1 (0.2)	0
Bs	131 (60)						
Bv	302 (123)						
90cm		2.5 (0.2)	1.9 (0.6)	2.0 (0.6)	0	0	0

The production of NH_4^+ and NO_3^- by microbial processes was strongly reduced due to drying/rewetting. The laboratory and field experiments indicated that drying/rewetting decrease the *in situ* N net Mineralisation and the NO_3^- concentrations (Figs. 1.8, 1.9, 4.2, 4.3). Increasing NH_4^+ to NO_3^- ratios and diminished N_2O and NO production (Muhr et al., 2008a; Goldberg and Gebauer 2009a) indicated a reduction of nitrification rate during drought. Incomplete remoistering have contributed to long- lasting reduced microbial activity due to water stress and low substrate after rewetting. Increased fine root

biomass and necromass after drying/rewetting (Gaul et al., 2008a) possible decreased NO_3^- availability in the field due to an increased root N uptake.

Decreased NH_4^+ availability was also a factor which influenced the net nitrification (Muhr et al., 2010). NH_4^+ concentrations in the field were always extremely low ($<2\text{mg l}^{-1}$) due to microbial immobilisation,

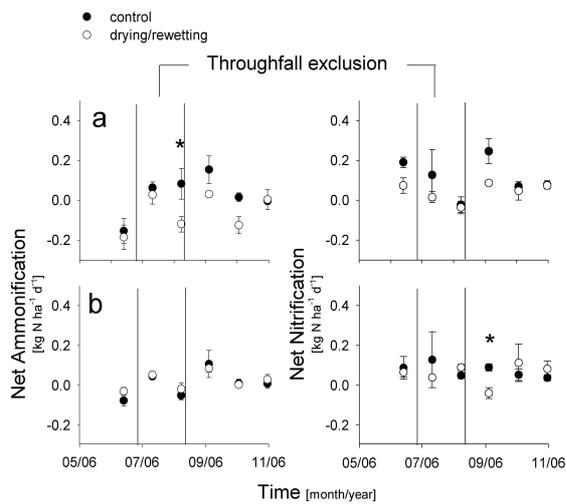


Figure 1.8 Impact of drying/rewetting of net ammonification and net nitrification in organic (a) and mineral (b) horizon. Error bars indicate standard errors ($n=3$).

plant uptake and abiotic sinks as consumptive processes (Hart et al., 1994).

In the laboratory experiment, reduced ammonification and microbial immobilisation of NH_4^+ were probably the explanations for the reduced NH_4^+ concentrations. The increase in NH_4^+ to NO_3^- ratios in the mineral soil signified a reduced nitrification rate and implied also a high sensitivity of nitrifiers in the mineral soil to drought stress (Tietema et al., 1992; Smolander et al., 2005).

The effects on C and N leaching in the form of DOC, NO_3^- and NH_4^+ were low and showed only small variations based on large differences among the soil pits. Due to the increased fine root mortality (Gaul et al., 2008a) and decreased CO_2 emission (Muhr et al., 2009a) during drying/rewetting, organic C accumulated and enhanced the C storage in this spruce forest (Fig. 1.6). Prolonged summer drought likely increased C sequestration in the subsoil via small additional transport of DOC to greater soil depth. However, the fate of subsoil DOC on larger time scale remains unknown. In case of high temperature dependence, higher maximal temperature might lead to an increased C mineralisation in the upper soil horizons. A/W showed a tendency to decrease NO_3^- fluxes (Table 1.1). In agreement with another manipulation study in a N saturated forest (Xu et al., 1998) and the results from the field and laboratory experiment (Figs. 1.8, 4.4), reduced N Mineralisation in the uppermost mineral soil conserved soil organic nitrogen and reduced N losses via leaching as a result of prolonged summer drought, considering constant C and N litter input.

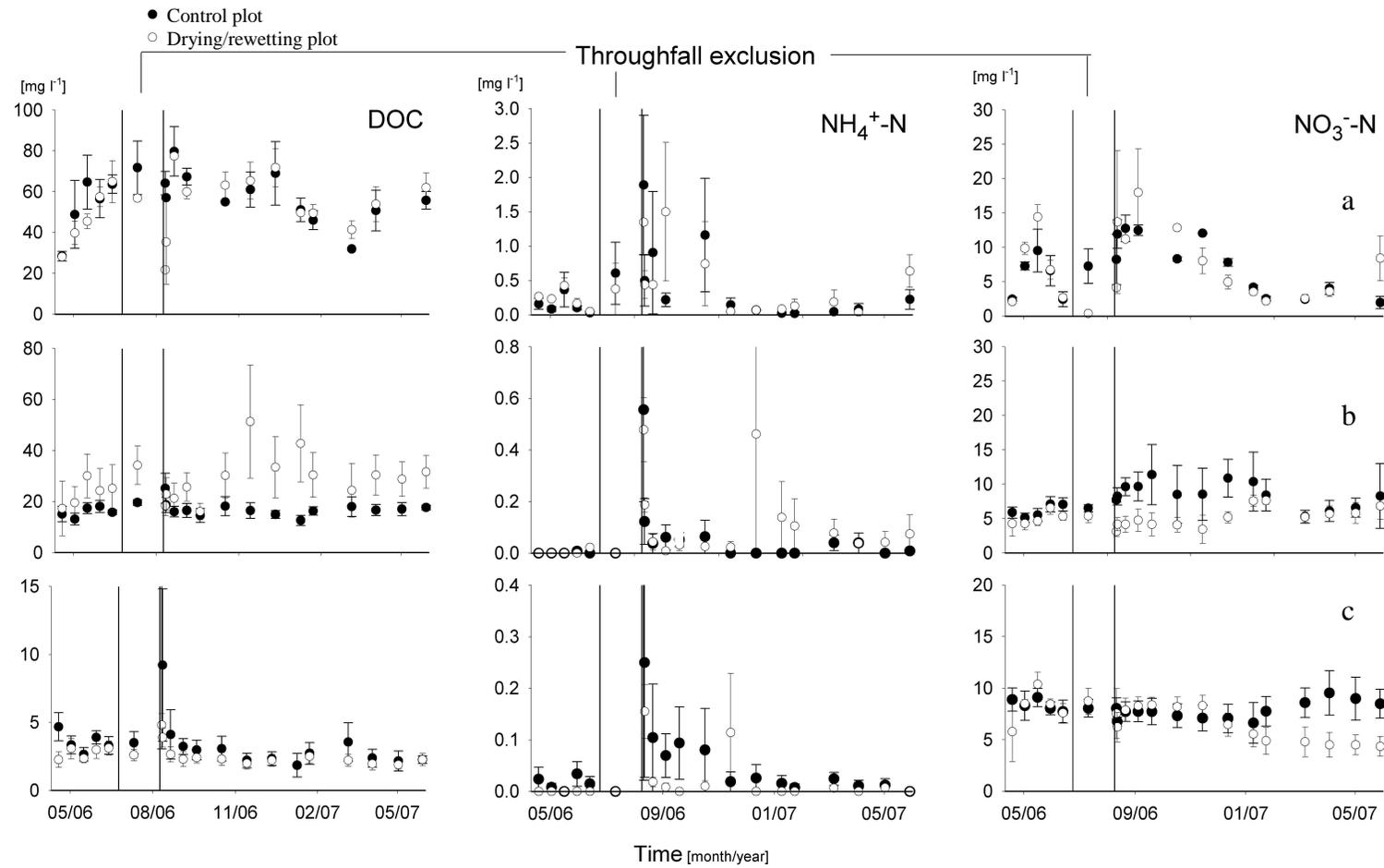


Figure 1.9 Concentrations of DOC, NH_4^+ and NO_3^- of (a) organic layer, (b) 20cm soil depth and (c) 90cm soil depth. Error bars represent standard errors (n=3)

DOC, NH_4^+ and NO_3^- as affected by freezing/thawing (Chapter 5 and Chapter 6)

In the laboratory experiment, DOC concentrations and fluxes substantially increased in the organic layer of soil columns, which were frozen at -8°C and -13°C . Nevertheless, the enhanced release of DOC was a strong single effect and the relevance for DOC fluxes remained low. The -3°C treatment, which was close to the condition of the field experiment with lowest soil temperature of -5°C , was less affected. These results confirmed the findings from the snow removal experiment of Fitzhugh et al. (2001), where a response of DOC concentrations to soil freezing at -5°C was also not apparent. The higher DOC concentrations at freezing temperature lower than -8°C were attributed to stronger physical disruption of SOM. These implications were often discussed throughout the literature (Soulides and Allison 1961; Christensen and Christensen 1991; Edwards and Cresser 1992; van Bochove et al., 2000). The release of less humified material at the beginning of thawing (Fig. 5.1) as well slightly enhanced lignin solubility (Schmitt et al., 2008) supported the assumption that during freezing a proportion of DOM became available through physical disruption of aggregated soil. The exposure of new soil surfaces enhanced both the release of DOC by percolating water and the decomposition of exposed organic matter. I suggested that a major source of extra DOC during thawing was due to lysis of microbial biomass induced by soil frost. DO^{14}C signature as a tracer for the origin of DOC would increase by lysis of microbial biomass as it comprises relatively young carbon. Unchanged spectroscopic properties (Fig. 5.1) and DO^{14}C signatures (Fig. 6.5) did not confirm enhanced mortality of microorganisms during soil frost. These findings were in accordance with results of high microbial resistance to freezing/thawing from Lipson and Monson (1998) and Grogan et al., (2004) for tundra and alpine soils, but contradicted the result of significant mortality of microbes at temperatures below -5°C by Edwards and Cresser (1992).

In contrast to DOC, the NH_4^+ and NO_3^- losses were always lower after soil frost and the effects were stronger with lower frost temperature. The reason for decreased NH_4^+ and NO_3^- losses might be reduced activity of microorganisms, which were integrated in the N cycle. Decreasing concentrations of microbial sugars with increasing frost intensity indicated reduced microbial activity (Schmitt et al., 2008). The missing increase in N_2O emission compared to CO_2 during thawing in the same laboratory experiment (Goldberg et al., 2008) indicated that the microbial population involved in the N cycle

was much stronger disturbed by soil frost as compared to the C cycle. Additionally, reduced $\text{NO}_3^-/\text{NH}_4^+$ ratios implied a higher sensitivity of net nitrification than net ammonification to severe freezing temperatures. However, our results from the laboratory experiment covered the initial response in-between 30 days after frost. In the field experiment, the NH_4^+ concentrations were relatively low compared to NO_3^- and no immediate freezing/thawing effect on the release of NH_4^+ and NO_3^- was detectable after thawing. The reason for the relative small effects was the constant microbial activity due to moderate freezing temperatures. Soil areas, which remain unfrozen, still possessed liquid water films with greater concentrations of substrates allowing the microbial population to survive and to maintain their activity (Edwards and Cresser 1992, Wang and Bettany 1993). The missing response for N Mineralisation in the field (Fig. 6.3), coupled with the moderate freezing event ($< -5^\circ\text{C}$) induced by the snow manipulation experiment, was consistent with the result of the laboratory experiment. The latter demonstrated that N Mineralisation decreased much more when the soil was subjected to severe soil freezing lower than -8°C . As mentioned before, these results were confirmed by Larson et al. (2002) and Schimel (2004), but contradicted the more often observed increased N Mineralisation after soil frost (Matzner and Borcken 2008). The delayed increase in NO_3^- concentration in the organic layer and 20cm soil depth (Fig. 6.4) confirmed results from Fitzhugh et al. (2001) and Callesen et al. (2007). The higher NO_3^- concentrations were caused by changes in the microbial community, namely fungi were more negatively affected than bacteria due to soil frost (Schmitt et al., 2008). ^{14}C measurements revealed significant reduction in heterotrophic respiration (Muhr et al., 2009a), which implied a reduced immobilisation of NO_3^- by growing heterotrophic microorganism. Increased nitrification or reduced denitrification as a cause of increasing NO_3^- concentrations were excluded, because soil emission of NO and N_2O were similar during this time (Goldberg et al., 2010). The assumption of enhanced mortality of microorganisms was not confirmed of the basis of unchanged DO^{14}C signature (Fig. 6.5). Reduced N uptake by fine roots or increased fine root turnover (Tierney et al., 2001) were unlikely because of similar fine root biomass and even higher root production on the freezing/thawing plot (Gaul et al., 2008b).

The freezing/thawing in laboratory and field experiment showed the need of a defined soil temperature to increase the fluxes of DOC after thawing of frozen soil. An

increased leaching of DOC after thawing occurred only at temperature lower than -8° . At higher soil temperature the DOC fluxes were unaffected. Assuming the simulation of long-term extreme soil frost due to reduction in snow cover (Hosaka et al., 2005; IPCC 2007) holds true, increasing leaching of DOC as well as a pronounced CO_2 pulse would be possible in the field (Chapter 5; Goldberg et al., 2008). Soil frost led to an increased C sequestration in this forest soil. Nevertheless, the long-term effects of reduced C emission in the summer following the freezing/thawing were more important to the annual C balance in this forest soil than the time period of freezing/thawing (Fig. 1.6; Muhr et al., 2009b). The laboratory experiment suggested, if soil frost was more intensive and frequent, the availability and loss of NH_4^+ and NO_3^- decreased after freezing/thawing. The delayed effect of increasing NO_3^- concentration in the field experiment had only low effects on the annual leaching rate because of low water fluxes during that time. However, this delayed effect could be much more pronounced at severe freezing temperature (Callesen et al., 2007; Kaste et al., 2008). The rate of short and long-term effects of severe freezing on annual leaching losses requires further research.

1.5. Conclusion

Both summer drought and severe soil frost increased the availability of DOC in the upper soil horizons. Whereas the fluxes of DOC were unaffected by soil frost, drying/rewetting induced a greater rate of DOC leaching at 20cm and 90cm soil depth. The ^{14}C signature of DOC was a useful indicator of seasonal and interannual variations in terms of the C source. Physical disruption of soil aggregates, and thus, exposure of previously inaccessible substrate, was likely the dominant mechanism explaining the increased availability of relatively old C in drier years. Considering the radiocarbon signature, there was no evidence for increased mobilization of young DOC from dead microbial biomass or increased fine root mortality. The increase ratio of plant to microbial sugars indicated decreased microbial DOC production and enhanced desorption. A/W and G/A events resulted in a decreased availability of NH_4^+ and NO_3^- . The lack of response to freezing/thawing in the field experiment confirmed earlier findings of a more sensitive N net Mineralisation, which decreased at severe soil frost than moderate soil frost to -5°C . The long-term effect of soil frost, increasing NO_3^- concentrations in 20cm soil depth could be much more pronounced at severe soil frost causing additional leaching losses of NO_3^- .

In conclusion, the organic layer of the spruce forest soil worked as a small net sink with C accumulation mainly in the Oa horizon. The results of laboratory and field experiment indicated a decreased C and N Mineralisation in form of enhanced DOC and decreased NH_4^+ and NO_3^- availability in the organic layer and upper mineral horizon due to drying/ rewetting as well as freezing/thawing.

It is expected, that if the climate simulation of enhanced drought periods and extreme soil frost holds true (IPCC 2007), the C and N sequestration and sink strength of this Podzol soil will increase as long as litter input does not decrease. Moreover, the potential shift in the availability of DOC and inorganic N due to drying/rewetting and freezing/thawing could affect the microbial activity as well as productivity and diversity of forest ecosystems. Experimental manipulations of climatic parameters provide useful process information on hourly to annual time scales, but the response of forest ecosystems on decadal time scales is highly uncertain. In this context, the recovery of organisms and processes following severe summer droughts and soil frost is poorly understood. Moreover, future changes in the vegetation, plant productivity, forest management or other driving factors could overcome increasing frequency of extreme meteorological events.

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Chapter 2

Stock, turnover time and accumulation of organic matter
in bulk and density fractions of a Podzol soil

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Summary

Temperate forest soils store large amounts of organic matter and are considered as net sinks for atmospheric carbon dioxide. Information about the sink strength and the turnover time of soil organic carbon (SOC) is required to assess the potential response of soils to climate change. Here we report on stocks, turnover times (TT) and accumulation of SOC in bulk soil and density fractions from genetic horizons of a Podzol in the Fichtelgebirge, Germany. Stocks of SOC, total nitrogen and exchangeable cations determined in nine quantitative soil pits strongly varied with stone content and thickness of horizons in both the organic layer and the mineral soil. On the basis of radiocarbon signatures, mean turnover times of 4, 9 and 133 years, respectively, were calculated for Oi, Oe and Oa horizons from three soil pits, using a non-steady-state model. The Oa horizons accumulated $4\text{--}8\text{ g C m}^{-2}\text{ a}^{-1}$ whereas the Oi and Oe horizons were close to steady-state during the past decade. Free particulate organic matter (FPOM) was the most abundant fraction in the Oa and EA horizons with TT of 70–480 years. In the B horizons, mineral associated organic matter (MAOM) dominated with over 40% of total SOC and had TT of 390–2170 years. In contrast to other horizons, MAOM in the Bsh and Bs horizon had generally faster TT than occluded particulate organic matter (OPOM), possibly because of sorption of dissolved organic carbon by iron and aluminium oxides/hydroxides. Our results suggest that organic horizons with relatively short turnover times could be particularly vulnerable to changes in climate or other disturbances.

2.1. Introduction

The accumulation of soil organic matter (SOM) is a characteristic feature of temperate and boreal forest ecosystems. Common among the soil types of the temperate and boreal zone are Podzols covering 485 million ha throughout the world (IUSS Working Group WRB, 2006). These soils developed under heather or coniferous forests and store large amounts of SOM as a result of slow decomposition processes and accumulation of SOM in organic and spodic horizons. Referring to 1 m soil depth, Batjes (2002) estimated that Podzols had the second-largest soil organic carbon (SOC) stocks in Europe after Histosols. There is reason for concern that climate change will turn these soils from a net sink into a net source for atmospheric carbon dioxide (CO₂).

Soils contain different organic matter fractions with varying stability, turnover time (TT) and temperature sensitivity (Trumbore, 2000; Kögel-Knabner et al., 2008). Specific SOM fractions are more vulnerable to climate change and other disturbances than others, but the characterization of SOM fractions depends on the methodological approach. Physical fractionation techniques are less destructive than chemical fractionation procedures and relate more directly to structure and function of SOM (Christensen, 2001). Density fractionation allows the separation of free particulate (FPOM), occluded (OPOM) and mineral-associated organic matter (MAOM). The FPOM fraction mainly contains recognizable plant material and fungal hyphae and responds quickly to changes in carbon (C) inputs and environmental conditions (and represents an active pool), whereas the OPOM and MAOM vary strongly with differences in soil structure and mineralogy (Baisden et al., 2002; von Lützow et al., 2006; Crow et al., 2007). Compared with FPOM, slower turnover of OPOM is attributed to chemical recalcitrance, humification and physical stabilization by occlusion (Poirier et al., 2005; Kögel-Knabner et al., 2008). MAOM is the dominating fraction in mineral soil horizons and has very slow turnover rates because of stabilization by interaction with mineral surfaces, and iron/aluminium (Fe/Al) oxides and hydroxides (Torn et al., 1997; Kögel-Knabner et al., 2008). However, a critical issue of the density fractionation is the removal of soluble and less degraded substrate during density fractionation (Crow et al., 2007). This soluble fraction was generally discarded and therefore not characterized and considered in SOC models.

The mean TT of SOC in bulk soil or fractions can be calculated from its radiocarbon signature ($\Delta^{14}\text{C}$), SOC stock or C input, and by using a steady-state or non-steady-state model (Gaudinski et al., 2000; Trumbore, 2000). The TT of SOC generally increases with increasing stability and density of the fraction. Estimates of TTs based on bulk mineral soil, however, may lead to misleading interpretations (Davidson et al., 2000). The radiocarbon signature of bulk mineral soil is governed by MAOM, but labile SOC fractions of bulk soil with short TTs could rapidly respond to global warming.

While gaseous and solute C losses from soils have been intensively investigated, little is known about the C input and net accumulation of SOC in specific soil horizons. In undisturbed coniferous forests, accumulation of SOC takes place mainly in the organic layer whereas the mineral soil seems close to steady-state and thus changes are barely

detectable on a decadal time scale (Ågren et al., 2008; Trumbore, 2000). When tree biomass increases or remains at current levels, SOC stocks will also increase, but the accumulation rate is small in mature forests (Ågren et al., 2008). A chronosequence study by Schlesinger (1990) suggests an average accumulation rate of 0.7–12.0 g C m⁻² a⁻¹ for boreal and temperate soils developed during the past 10 000 years. The C accumulation rate is relatively large in the initial period of soil genesis, but slows down with increasing age.

The balance between litter input and losses by microbial respiration and leaching controls the build-up of organic horizons. Above-ground litterfall in coniferous forests ranges between 735 and 8575 kg ha⁻¹ a⁻¹ along a climatic gradient from north Scandinavia to Spain (Berg and Meentemeyer, 2001). Estimates for root litter input are less reliable because of methodological difficulties. Wutzler and Mund (2007) modelled root litter production for spruce and estimated fine root production of 890–1830 kg ha⁻¹ a⁻¹.

Area-based estimates of stocks and accumulation or loss rates of SOC and nitrogen (N) in bulk soil and density fractions require representative sampling procedures in the field. The spatial variation in rock content, bulk density and thickness of soil horizons cause large uncertainties in soil surveys. Estimates of stocks of elements in soils are based on fine earth (<2 mm), but large rock fragments make it difficult to assess the amount of fine earth in genetic horizons on larger scales (Corti et al., 2002).

Only a few studies provide an area-based analysis of SOC and nutrient stocks or of TTs of SOC in bulk or density fractions of genetic horizons. The objectives of our study were: (i) to quantify the stocks and heterogeneity of SOC and nutrients in a mountain Podzol, (ii) to determine SOC and N stocks in different density fractions, (iii) to calculate the TT of bulk soil and SOM fractions from their $\Delta^{14}\text{C}$ signatures by a steady-state or non-steady-state model, and (iv) to assess the input and accumulation of C in the organic horizons.

2.2. Methods

Site description

The study was performed in a mature Norway spruce forest (*Picea abies* L.) at an elevation of 770 m above sea level in the Fichtelgebirge, SE Germany (50°08'N, 11°52'E).

The mean annual air temperature is 5.3°C with warm summers and cold continental winters. Mean annual precipitation is approximately 1160 mm (Foken, 2003).

The natural vegetation consists of beech (*Fagus sylvatica* L.) and silver fir (*Abies alba* Mill.) (Gerstberger et al., 2002). According to the forest administration, the area was almost completely cleared between the 16th and 18th centuries in order to supply the mining industry with construction wood and charcoal. The region was afforested with spruce trees during the mid-19th century. Tree rings indicate a tree age of 140 years in 2008 for our study area. The patchy ground vegetation is dominated by *Deschampsia flexuosa* (L.) and *Calamagrostis villosa* (Chaix).

The Fichtelgebirge comprises large granite formations surrounded by metamorphic rock series of gneiss, mica schists and phyllites. Erosion and solifluction formed the typical rock and boulder fields. Podzolic soils developed from deeply weathered granite and are overlain with a relatively thick humus layer (Gerstberger et al., 2002). Base saturations between 52 % in the EA horizon and 40 % in the Bsh horizon indicate former application of lime to counteract soil acidification (Hentschel et al., 2007). Carbonates, however, were not chemically detectable in any soil horizons.

Sampling

Within an area of 1 ha, nine soil pits of 0.7 m × 0.7 m were dug for area-based determination of rock volume, bulk density (BD) and element contents down to the Cv horizon. The pits were randomly distributed, and limited to between-tree and between-boulder areas. The organic (Oi, Oe, Oa) and mineral (EA, Bsh, Bs, Bv) horizons were consecutively removed and separately weighed. The volume of stones was estimated by measuring the girth of the stones at different positions and their total weight. A grid of 0.7 m × 0.7 m with 10 cm × 10 cm increments was fixed over the surface of the soil pit to measure the height of each removed soil horizon.

For each horizon, large stones, coarse roots and soil material were separately weighed in the field. Subsamples of each horizon were sieved (<2 mm) and then dried at 105°C over 48 hours to determine the gravimetric water content and the stone fraction. The bulk density of fine earth (<2 mm) was calculated by dividing its mass by the averaged volume of horizon minus the volume of rocks and coarse roots. The rock weight was converted to rock volume using a density of 2.45 g cm⁻³ for parent granite at the site.

Analyses

Soil pH was determined in a 0.01 M CaCl₂ solution (soil:solution ratio 1:2.5). Exchangeable cations (Na⁺, K⁺, Ca²⁺, Mg²⁺, Al³⁺, Fe³⁺) of the Oa and mineral soil horizons were extracted with 1 M NH₄Cl solution and then analysed by ICP-OES (Varian Vista-Pro, Mulgrave, Victoria, Australia). The amount of exchangeable H⁺ was calculated from the difference between the pH-value of NH₄Cl solution and the extracts. For C and N analysis a CNS analyser (Heraeus Elementar Vario EL, Hanau, Germany) was used.

Radiocarbon signatures of above-ground litter, bulk soil (including organic and mineral soil horizons) and density fractions were determined by accelerator mass spectrometry (AMS). Subsamples of 1 mg C were combusted in 6 mm sealed quartz tubes with 60 mg CuO oxidizer and 1 cm silver wire for 2 hours at 900°C. The resulting CO₂ was purified from water and non-condensable compounds. Afterwards, CO₂ was reduced to graphite using the zinc reduction method where TiH₂ and Zn with Fe act as catalysts at 550°C for 7.5 hours (Xu et al., 2007). All preparations took place at the Department of Soil Ecology at the University of Bayreuth. The graphite targets were analysed by the Keck-CCAMS facility of the University of California, Irvine, with a precision of 2–3 ‰. Radiocarbon data are expressed as Δ¹⁴C (‰ deviation is from the ¹⁴C/¹²C ratio of oxalic acid standard in 1950). The samples were corrected to a δ¹³C value of –25 ‰ to account for any mass-dependent fractionation effects (Stuiver and Polach, 1977).

Density fractionation of soil

Soil samples of the Oa and all mineral horizons from three randomly chosen soil pits were fractionated by density separation. Dry soil samples were dispersed in sodium polytungstate solution (SPT, Sometu, Berlin, Germany) using a similar procedure to that described in detail by John et al. (2005). Dry soil samples (<2 mm, 60°C) were fractionated at densities of 1.6 g cm⁻³ and 2.0 g cm⁻³. For the Oa and EA horizons, 10 g soil and 40 ml of SPT with a density of 1.6 g cm⁻³ were gently shaken. After sedimentation, the solution was centrifuged at 5085 g for 1 hour (Varifuge 3.2RS). The supernatant was filtered through a 0.45 μm pre-washed cellulose acetate filter (Schleicher and Schuell, Dassel, Germany) and the FPOM fraction < 1.6 g cm⁻³ was washed with 200 ml deionized water. Then the pellet was dispersed with 2.0 g cm⁻³ SPT and 10 glass beads and was shaken for 16 hours and then centrifuged at 5085 g for 1 hour. The supernatant with particles < 2.0 g cm⁻³ (OPOM) was filtered and washed (200 ml water) through

0.45 μm cellulose acetate filters. The pellet thus contained the mineral-associated organic matter fraction $> 2.0 \text{ g cm}^{-3}$ (MAOM). To remove the salt, the pellet was washed three times with deionized water. For the Bsh, Bs and Bv horizons, 20 g soil and 80 ml of SPT were used. The FPOM, OPOM and MAOM fractions and the used SPT solution were freeze-dried and then finely ground with a ball mill for analyses of SOC and N contents.

Turnover time (TT) of organic carbon

The TT SOC in each fraction was calculated from its radiocarbon signature. Following Gaudinski et al. (2000), we used a non-steady state model for the Oi, Oe and Oa horizons. We assumed that the buildup of the organic layer primarily started after reforestation in 1867, but we cannot ignore the possibility that older organic matter is included in the present Oa horizon (see below). The $\Delta^{14}\text{C}$ signature of fresh spruce litter from the year 2006 ($86 \pm 1\text{‰}$) was close to the $\Delta^{14}\text{CO}_2$ signature in the atmosphere (88.5‰) in the year 2000 (Levin et al., 2008), indicating an average age of six years. Hence, this shift in the $\Delta^{14}\text{C}$ was included in the calculation of TT.

The C input (I) in $\text{kg C m}^{-2} \text{ a}^{-1}$ added by litter production in each year t (since 1867, time of reforestation) is represented in a sigmoid equation (e.g. Böttcher and Springob, 2001; Ågren et al., 2008):

$$I_t = \frac{I_{2006}}{1 + e^{-\frac{t-1900a}{15}}} \quad (2.1)$$

The term '1900a' in Equation 2.1 is the year of maximal increase of litter production and the value '15' describes the slope of the graph.

The SOC stock and the $\Delta^{14}\text{C}$ of each organic horizon in 2006 were then calculated using Equations 2.2 and 2.3 (modified after Gaudinski et al., 2000). We assumed zero initial SOC in 1867 in the Oi and Oe horizons. For the Oa horizon the model revealed an existing SOC stock between 0.5 and 0.9 kg C m^{-2} in 1867 differing within the three soil pits.

$$C_{2006} = C_{1867} \cdot e^{-k \cdot (2006-t)} + \sum_{t=1867}^{t=2006} I \cdot e^{-k \cdot (2006-t)} \quad (2.2)$$

where C is the SOC stock in a specific year (kg C m^{-2}), I is the annual input rate ($\text{kg C m}^{-2} \text{ a}^{-1}$), k is the decay constant of organic matter and reciprocally proportional to TT , and t is the calendar year.

$$F_{m(2006)} = \frac{\sum_{t=1867}^{t=2006} (F_{atm(t-6)} \cdot C_t)}{\sum_{t=1867}^{t=2006} C_t} \quad (2.3)$$

where F_m is the $^{14}\text{C}/^{12}\text{C}$ ratio in the fraction pool per year normalized to oxalic acid standard and F_{atm} is the $^{14}\text{C}/^{12}\text{C}$ ratio of the atmosphere normalized to an oxalic acid standard ($\Delta^{14}\text{CO}_2$ signature of litter input in year t is equivalent to F_{atm} six years before). For the existing C pool of the Oa horizon in the year 1867 we assumed a $F_{m(1867)}$ of 0.996 which is equivalent to the average $\Delta^{14}\text{C}$ signature of tree rings between 1852 and 1867.

From Equations 2.1 and 2.2 and the measured C stock in each organic horizon, we estimated the turnover time ($1/k$) and C input rate (I) of the organic layer. Modern $\Delta^{14}\text{C}$ has two possible TT s for each $\Delta^{14}\text{C}$ value (Trumbore, 2000). The $\Delta^{14}\text{C}$ of the Oa horizons (107–132‰) corresponds to TT s of either 4–7 or 100–160 years. Given the type and thickness of the organic layer we expect longer TT s for the Oa horizons to be more realistic.

For the mineral soil horizons, we used a time-dependent, steady state model as presented in Gaudinski et al. (2000):

$$F_m(t) \cdot C(t) = I \cdot F_{atm}(t) + (1 - k - \lambda) \cdot F_m(t-1) \cdot C(t-1), \quad (2.4)$$

where λ is the radioactive decay constant for ^{14}C , and equal to 1/8267 year.

If the pool of SOC is at steady-state, then $I = kC(t)$ and $C(t) = C(t-1)$ reduce Equation 2.4 to:

$$F_m(t) = k \cdot F_{atm}(t) + (1 - k - \lambda) \cdot F_m(t-1), \quad (2.5)$$

For the values of F_{atm} , we used radiocarbon data from dendrochronologically dated wood samples (1844 to 1958) published by Stuiver et al. (1998) and atmospheric $\Delta^{14}\text{CO}_2$ contents after 1959 (Levin et al., 2008). A steady-state model was also used for the MAOM fraction of the Oa horizon because we assumed that the MAOM fraction in the Oa horizon is partly a result of biotic or abiotic mixing processes with mineral soil.

Statistics

The soil characterization was made with nine replicates and the data are presented as the mean and standard deviation (Table 2.1). STATISTICA 6.0 was used to display the heterogeneity of SOC and N stocks between the nine soil pits (Fig. 2.1). The density fractionation and determination of $\Delta^{14}\text{C}$ were made on three of the nine pits (Table 2.2, Fig. 2.2).

2.3. Results

Variation of physical and chemical properties in soil pits

The 7– 10 cm thick organic layer consisted of litter (Oi), fermented (Oe) and humified (Oa) horizons, whereas the 43– 60 cm thick mineral soil comprised EA, Bsh, Bs and Bv horizons. The mean bulk density increased from 0.07 g cm^{-3} in the Oi horizon to 1.17 g cm^{-3} in the Bv horizon (Table 2.1). Mean volumetric rock contents increased from 7 % in the Oa horizon to 25 % in the Bv horizon with large variations among individual soil pits.

The SOC and N contents of the soil decreased with increasing depth from 45.8 % C in the Oi horizon to 1.4 % C in the Bv horizon and from 1.7 % N to 0.2 % N, respectively (Table 2.1). The C/N ratio ranged between 19 and 27 in the organic layer whereas C/N ratios (21– 22) were almost constant in the EA, Bsh and Bs horizons. SOC stocks increased from 2.8– 4.9 kg C m^{-2} in the organic layer (sum of Oi, Oe and Oa horizons) to 2.2– 7.9 kg C m^{-2} in the Bv horizon (Fig. 2.1a). Nitrogen stocks increased from 0.05– 0.16 kg N m^{-2} in the organic layer to 0.17–0.51 kg N m^{-2} in the Bv horizon (Fig. 2.1b).

Table 2.1 Mean thickness, bulk density of fine earth (BD), volumetric rock fraction (RF), amount of fine earth, pH (CaCl₂), organic C and total N contents, C/N ratio, and stocks of exchangeable cations in genetic horizons of a Norway spruce soil at the Fichtelgebirge. Numbers in parentheses are standard deviations of the means (n = 9)

Horizon	Thickness /cm	BD /g cm ⁻³	RF /Vol.%	Fine earth /kg m ⁻²	pH	C /%	N /%	C/N	Stock /g m ⁻²						
									H ⁺	Na ⁺	K ⁺	Mg ²⁺	Ca ²⁺	Al ³⁺	Fe ³⁺
Oi	2.1 (0.3)	0.07 (0.01)				45.8 (2.7)	1.7 (0.2)	27 (2.3)							
Oe	2.2 (0.7)	0.15 (0.05)				42.1 (18.9)	1.8 (0.6)	22 (2.0)							
Oa	4.9 (1.5)	0.25 (0.09)	7 (4)	11.3 (2.9)	3.3 (0.2)	21.2 (6.9)	1.1 (0.3)	19 (1.7)	0.5 (0.7)	0.9 (1.1)	1.9 (1.1)	1.3 (0.7)	41.2 (44.8)	6.4 (4.3)	1.1 (1.0)
EA	5.2 (2.3)	0.60 (0.05)	11 (7)	28.8 (13.1)	3.4 (0.2)	8.3 (2.6)	0.4 (0.2)	21 (3.4)	0.4 (0.3)	1.0 (0.8)	2.1 (1.3)	1.4 (1.1)	50.4 (53.1)	15.4 (9.1)	1.4 (0.7)
Bsh	5.3 (1.8)	0.75 (0.04)	15 (5)	40.1 (17.1)	3.6 (0.2)	6.0 (1.2)	0.3 (0.1)	22 (2.2)	0.4 (0.3)	1.4 (0.8)	2.0 (0.6)	1.3 (1.1)	48.1 (37.24)	32.7 (19.3)	4.6 (3.1)
Bs	11.4 (4.6)	0.79 (0.06)	18 (7)	78.5 (32.6)	3.8 (0.1)	3.6 (0.8)	0.2 (0.0)	21 (1.2)	0.2 (0.1)	1.6 (0.8)	2.8 (1.0)	1.0 (0.6)	42.8 (39.0)	59.4 (27.2)	3.0 (2.4)
Bv	30.5 (9.1)	1.17 (0.09)	25 (9)	292.0 (102.3)	4.2 (0.1)	1.4 (0.5)	0.2 (0.3)	8 (1.2)	0.2 (0.1)	18.1 (17.7)	10.8 (4.6)	0.8 (0.5)	21.7 (14.5)	114.9 (56.3)	0.7 (0.5)
Sum	59.8 (5.7)								1.6 (1.1)	24.9 (17.2)	19.6 (5.0)	5.2 (1.9)	198.5 (158.3)	226.0 (71.0)	10.2 (4.5)

The total amounts of SOC and N stored in the nine soil pits were 13.1– 20.3 kg C m⁻² and 0.7– 1.1 kg N m⁻², where the organic layer contributed 19– 35% to total SOC and 5– 14% to total N.

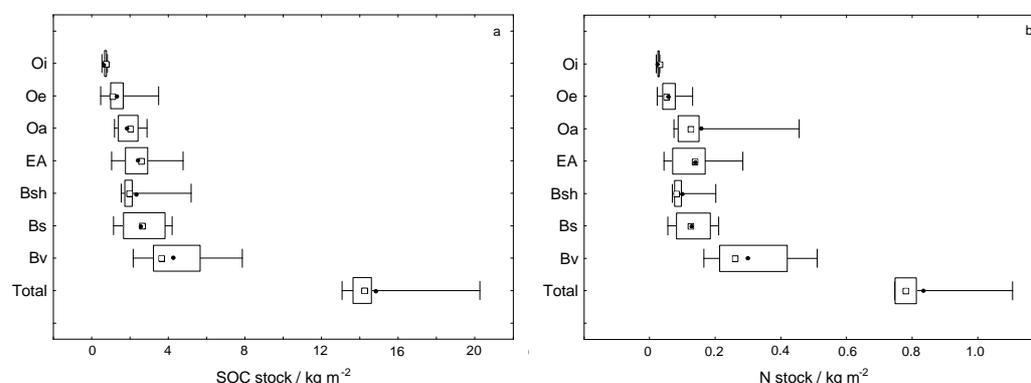


Figure 2.1 Box plots representing soil heterogeneity of (a) SOC and (b) N stocks in different genetic horizons of nine soil pits. The open square within the box marks the median, the black circle the mean and the boundaries of the box indicate the 25th and 75th percentile. Whiskers indicate the minimum and maximum SOC and N stocks.

C and N in density fractions

The mean recovery of total soil mass after density fractionation varied between 92 % in the Oa and 98 % in the Bv horizon (data not shown). These losses coincided with the mean recovery of SOC between 90 and 96 % (Fig. 2.2a). Some SOC was lost in the particulate fraction during density fractionation but 2– 6% of SOC was dissolved in the SPT solution. The mean recovery of N was smaller and ranged between 84 and 99 % (Fig. 2.2b).

Consistently throughout the three soil pits, SOC contents ranged between 17 and 38 % C in the FPOM and OPOM fractions, whereas the MAOM fraction contained no more than 2.7 % C (data not shown). In the Oa and EA horizons, the major part of SOC was associated with FPOM (Oa = 1.6 kg C m⁻² and EA = 1.1 kg C m⁻²), whereas MAOM dominated the Bsh, Bs and Bv horizons with 0.8–2.2 kg C m⁻² (Fig. 2.2). The SOC stock of the OPOM fraction was the largest in the EA horizon (0.8 kg C m⁻²) followed by Bv (0.6 kg C m⁻²), Bsh (0.6 kg C m⁻²), Oa (0.4 kg C m⁻²) and Bs horizons (0.3 kg C m⁻²). Overall, the soil stored 2.7– 5.2 kg C m⁻², 2.3–3.3 kg C m⁻² and 4.0–4.9 kg C m⁻² in the FPOM, OPOM and MAOM fractions, respectively.

The N stock in the FPOM fraction decreased with depth from 0.09 kg N m⁻² in the Oa horizon to 0.01 kg N m⁻² in the Bv horizon (Fig. 2.2b). In the B horizons, the

MAOM fraction yielded the largest N stocks, contributing 74 to 83 % to total N in the respective horizon. The amount of N stored in the OPOM fraction varied between 0.01 and 0.04 kg N m⁻² with 9– 16% of the total N in the soil.

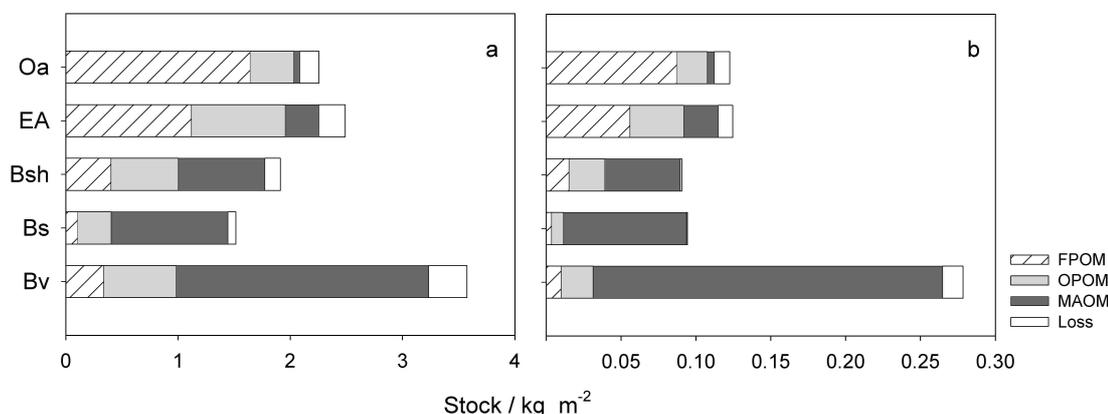


Figure 2.2 Stocks of SOC (a) and total N (b) in the three density fractions (FPOM, OPOM, MAOM) in genetic soil horizons from three soil pits. 'Loss' indicates the amount of organic matter lost during density fractionation.

$\Delta^{14}\text{C}$ signatures and turnover times of soil organic C

Mean radiocarbon signatures indicate modern C in litterfall ($87 \pm 1\%$), in the Oi ($114 \pm 8\%$) and Oe ($161 \pm 16\%$) horizons, but a mixture of modern and pre-bomb C in the Oa horizon ($119 \pm 12\%$) (Fig. 2.3). The EA horizon had a large range in $\Delta^{14}\text{C}$ signature from -45 to 92% , indicating that considerable amounts of modern C were incorporated in some EA horizons. Within the B horizons, the $\Delta^{14}\text{C}$ signature continuously decreased from -14% to -145% . The vertical decrease in the $\Delta^{14}\text{C}$ signature below the Oi horizon coincides with the decrease in the SOC content.

In Pits 1 and 2, the TT of SOC in bulk soil increased consistently with soil depth from the organic horizons to the Bv horizon (Table 2.2). By contrast, Pit 3 had a less negative $\Delta^{14}\text{C}$ signature in Bsh (-28.7%) compared with the EA horizon (-45.0%), resulting in a difference of 110 years in TT. The shorter TT of bulk SOC indicates that the Bsh horizon accumulated more 'young' C than the Ea horizon.

Similar to the bulk soil, $\Delta^{14}\text{C}$ signatures of density fractions generally decreased with soil depth in the order FPOM > OPOM > MAOM (Table 2.2). One exception is the FPOM fraction of the Bv horizon that had consistently younger SOC (6.8 to -66.8%)

Table 2.2 Radiocarbon signature of bulk soil and density fractions of three pits expressed in ‰. The turnover times expressed in years (given in parentheses) were calculated based on the radiocarbon signature with a steady-state or non-steady-state model (Gaudinski, et al. 2000). Two turnover times were calculated for bulk soil and density fractions with modern radiocarbon signature.

Horizon	Pit 1				Pit 2				Pit 3			
	Bulk	FPOM	OPOM	MAOM	Bulk	FPOM	OPOM	MAOM	Bulk	FPOM	OPOM	MAOM
O _i	105.1 (3)*				121.0 (5)*				115.2 (5)*			
O _e	157.8 (9/90)*				179.0 (10/50)*				147.6 (8/120)*			
O _a	132.0 (7/100)*	150.9 (9/90)*	70.4 (120)*	38.0 (180)	117.8 (6/140)*	108.0 (4/160)*	28.4 (240)*	-25.9 (400)	107.1 (4/160)*	124.5 (6/140)*	48.8 (170)*	28.1 (210)
EA	91.6 (100)	119.8 (6/70)	43.7 (170)	12.1 (260)	22.5 (230)	34.7 (190)	-2.5 (320)	-13.4 (370)	-45 (570)	-31.9 (480)	-64.3 (710)	-48.0 (590)
Bsh	-7.9 (350)	25.3 (220)	-41.0 (540)	-28.4 (460)	-4.9 (330)	13.8 (250)	-27.6 (450)	-16.9 (390)	-28.7 (460)	-26.9 (450)	1.2 (300)	-20.4 (410)
Bs	-56.9 (650)	-95.2 (970)	-138.9 (1390)	-48.1 (590)	-75.3 (800)	-31.0 (480)	-149.3 (1500)	-63.9 (710)	-57.3 (660)	-120.0 (1200)	-124.4 (1250)	-43.8 (560)
Bv	-156.2 (1580)	-66.8 (730)	-182.9 (1880)	-207.2 (2170)	-155.6 (1570)	6.8 (280)	-130.7 (1310)	-180.2 (1850)	-124.4 (1250)	-30.2 (470)	-106.7 (1080)	-141.5 (1420)

with faster TT than the respective FPOM fraction of the overlaying Bs horizon (-31 to -120 ‰). There was either an input of 'young' SOC in the FPOM fraction of the Bv horizon or reversely an input of 'old' SOC or stabilization mechanisms in the FPOM fraction of the Bs horizon. Another exception is the reverse order of $\Delta^{14}\text{C}$ signatures and TT in the OPOM and MAOM fractions of the Bsh (except in Pit 3) and the Bs horizon. In these horizons, MAOM had faster TT (390- 710 years) compared with the OPOM fraction (450- 1500 years). The shift in the TT of the MAOM fraction from the Bs horizon to the Bv horizon was relatively large in all three soil pits.

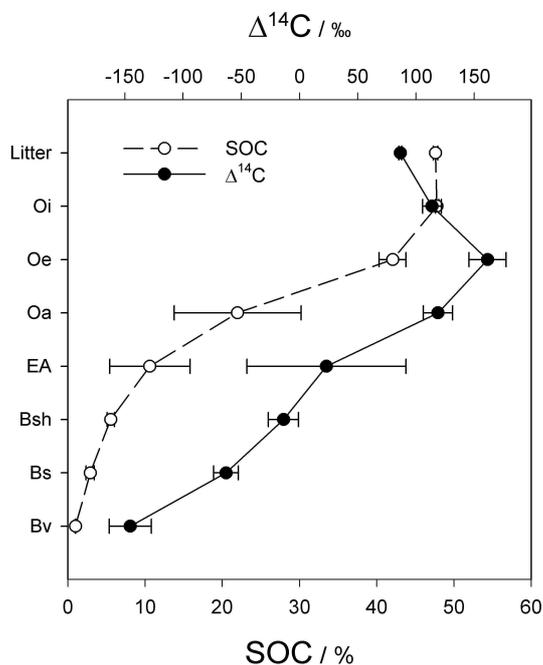


Figure 2.3 Vertical pattern of $\Delta^{14}\text{C}$ (‰) and total SOC (%) in litter, organic and mineral soil horizons. Error bars represent the standard deviation of the mean ($n = 3$)

2006. C input rates in the Oe ($80 \text{ g C m}^{-2} \text{ a}^{-1}$) and Oa (20 and $21 \text{ g C m}^{-2} \text{ a}^{-1}$) horizons were similar in Pits 1 and 2. For the Oe horizon of Pit 3, we calculated (with a TT of 8 a) a much larger C input of $200 \text{ g C m}^{-2} \text{ a}^{-1}$, which reflects the large C stock of 1.7 kg C m^{-2} . Here, the C input in the Oe horizon is greater than in the Oi horizon ($150 \text{ g C m}^{-2} \text{ a}^{-1}$). Again, Pit 3 differs with respect to the C input of $9 \text{ g C m}^{-2} \text{ a}^{-1}$ in the Oa horizon.

The modelled accumulation of SOC indicates that the Oi and Oe horizons are approaching a steady-state with C accumulation smaller than $0.3 \text{ g C m}^{-2} \text{ a}^{-1}$ in the last

The isotopic balance approach revealed a wide spectrum for the lost fraction, ranging from modern SOC with mean $\Delta^{14}\text{C}$ of 477 ‰ to relatively old SOC (-201 ‰) (data not shown). There was no systematic pattern with soil depth or for specific

C input and storage in organic horizon

The annual C input to organic horizons and the FPOM fractions of the Oa horizon were calculated from the non-steady-state model (Equation 2.2) and using the respective SOC stock and TT (Table 2.3). Assuming a sigmoid increase of litter input (Equation 2.1), the estimated C input in the Oi horizon by above-ground litter varied between 130 and $180 \text{ g C m}^{-2} \text{ a}^{-1}$ in

Table 2.3 SOC stock, turnover time and annual C input calculated by a non-steady-state model of the organic layer of three soil pits. SOC accumulation was calculated by difference of modelled SOC stock within last 10 years (see Fig. 4b). For the O_a horizon, the model revealed an existing carbon pool of 0.5-0.9 kg C m⁻² in 1867. Turnover time and C input given in parenthesis result from either ¹⁴CO₂ of the atmosphere fixed before or after the ¹⁴C-bomb peak in 1965 by the forest ecosystem (see Fig. 2.4c).

Horizon	SOC stock			Turnover time			C input			SOC accumulation		
	/kg C m ⁻²			/a			/g C m ⁻² a ⁻¹			/g C m ⁻² a ⁻¹		
	Pit 1	Pit 2	Pit 3	Pit 1	Pit 2	Pit 3	Pit 1	Pit 2	Pit 3	Pit 1	Pit 2	Pit 3
O _i	0.68	0.77	0.78	3	5	5	180	130	150	<0.1	<0.1	<0.1
O _e	0.78	1.09	1.72	9/(90)	10/(50)	8/(120)	80/(10)	80/(30)	200/(30)	0.2	0.3	0.3
O _a	1.48	1.89	0.90	(7)/100	(5)/140	(4)/160	(210)/20	(270)/21	(220)/9	5.5	7.5	3.5
O _a (FPOM)	0.87	1.60	0.61	(9)/90	(4)/160	(6)/140	(100)/14	(380)/16	(97)/7	4.7	6.2	3.0

10 years (e.g. Pit 2, Fig. 2.4). The Oa horizon accumulated small amounts of SOC between 3.5 and 7.5 g C m⁻² a⁻¹ (Table 2.3), where most of that SOC was accumulated in the FPOM fraction (3.0- 6.2 g C m⁻² a⁻¹).

2.4. Discussion

Heterogeneity of soil chemical properties

The Podzol at our study site contained 13.1- 20.3 SOC kg m⁻² and 0.7- 1.1 N kg m⁻² down to a mean mineral soil depth of 60 cm (Fig. 2.1). These values are smaller than the mean SOC (29.6 kg m⁻²) and N stock (1.96 kg N m⁻²) in the top 1 m of European Podzol soils (Batjes, 2002). Even small SOC and N contents in the subsoil may considerably contribute to total stocks. Several authors (e.g. Canary et al., 2000; Harrison et al., 2003) have emphasized that soils should be sampled to a maximum depth for accurate estimates of total SOC and N stocks. However, big rocks made it impossible to increase the depth of the soil pits, and thus total SOC and N stocks are relatively small at our study site.

Large amounts of SOC (19- 35% of the total) and N (5- 14% of the total) highlight the importance of the organic layer as SOC and N reservoirs in Podzols. Under coniferous forests, Podzols may accumulate large amounts of SOM in the organic layer within decades, making them vulnerable to climate change and other disturbances. An accurate, area-based estimate to detect changes of SOC and N stocks is hampered by the enormous spatial heterogeneity. In our study, varying thickness (7- 10 cm) was the main reason for the large heterogeneity in C and N stock of the organic layer, whereas bulk density, SOC and N contents were less variable.

The mineral soil down to 60 cm stores more SOC and N at our site than the organic layer. Because of slow TTs (see below), changes in the SOC stocks are generally small unless strong disturbances of the soil structure accelerate the decay of SOM. Changes in the N stock, however, could possibly be much faster in forests with large atmospheric N deposition. In view of climatic or other environmental changes, SOC and N stocks of mineral horizons are required for better understanding of C and N cycling in soils. At our site, the Bv horizon stored approximately twice as much SOC and N than the EA, Bsh and Bs horizons. The large heterogeneity of SOC and N stocks in the whole miner-

al soil is attributed to differences in the thickness (43- 60 cm), rock fraction (12- 29 % by volume) and the amount of fine earth (312- 512 kg m⁻²) among the soil pits.

SOC and N in density fractions

The portion of the FPOM fraction decreased and the portion of the MAOM fraction to total SOC stock increased with soil depth, whereas the OPOM fraction was almost evenly distributed throughout the soil pits (Fig. 2.2). Large FPOM fractions in the Oa and EA horizons point to low rates of degradability of spruce litter and reduced microbial activity compared with other litter types and less acid soils (John et al., 2005). Provided that the FPOM fraction of 0.7- 1.1 kg C m⁻² (9- 16%) and 0.02- 0.04 kg N m⁻² (5- 8%) in the B horizons is accessible for microbial attack, the soil has a large potential for C and N losses. However, the slow TT of FPOM suggests that it contains not only fresh and undecomposed material, but also charred plant debris (black carbon) as identified by Marschner et al. (2008). 'Recalcitrant' compounds such as lignin, lipids and their derivatives could constitute a considerable proportion of the FPOM fraction, although their TTs are much faster than previously thought (Marschner et al., 2008). Another mechanism, the stabilization of DOC by precipitation with dissolved aluminium (Scheel et al., 2007), could possibly contribute to the slow TT of the FPOM fraction from the mineral soil. Our results contradict the concept that the FPOM fraction corresponds to the active SOC pool with TT <10 years.

The N content and C/N ratio of SOM fractions may play an important role in their stability. Decreasing C/N ratios in the order OPOM > FPOM > MAOM (not shown) are in accordance with the observation by Golchin et al. (1994). The large N content of MAOM is primarily related to the advanced degradation of organic C compounds. However, an N fertilization experiment suggests that 'newly' added N is partly retained in slow SOM pools, enhancing thereby its stability against microbial decay (Hagedorn et al., 2003). If this stabilization mechanism is relevant, then the size and TT of SOM fractions should have been altered under the large N deposition rate at our study site during recent decades.

The Oa horizon is generally not considered for density fractionation although this horizon often contains a mineral fraction. Here, MAOM contributes 33 % to total mass in the Oa horizon, but the SOC stock of 2.3 % was relatively small. Despite the small SOC stock in the OPOM and MAOM fractions, their $\Delta^{14}\text{C}$ signatures influence the TT of the

Oa horizon (Table 2.2). In the case of Pit 2, however, all three density fractions had smaller $\Delta^{14}\text{C}$ signatures than the bulk Oa horizon. A non-characterized fraction of SOC and N was lost by the density fractionation either as particulate or as dissolved forms in SPT solution. The mass balance approach implied that the missing fraction varied from modern SOC with a mean $\Delta^{14}\text{C}$ signature of 477 ‰ to relatively old SOC (-201 ‰), representing a SOC stock of approximately 0.9- 1.3 kg C m⁻² down to 60 cm depth. Crow et al. (2007) found extractable young organic matter in deciduous forest soil and assumed that less degraded substrates were removed during density fractionation. Hence, the lost fraction seems to play an important role in the turnover of SOM in forest soils.

Turnover time (TT), input and accumulation of SOC in organic horizons

The estimated annual C input in the Oi horizon by above-ground litterfall varied between 130 and 180 g C m⁻² a⁻¹ in 2006. A mean annual litter input of 107 g C m⁻² a⁻¹ (assuming a C content of 50%) was measured in an adjacent Norway spruce stand of the same age (Berg, 2004). This input rate, however, does not include branches, twigs, cones and ground vegetation, which may contribute considerably to total above-ground litter input.

The C input in the Oe and Oa horizons comprises the transfer of partly decomposed and humified above-ground litter from the Oi or Oe horizon, respectively, and the input of root litter. In case of the Oe and Oa horizons, root litter input probably increased with the build-up of these horizons. The present input of 200 g C m⁻² a⁻¹ in the Oe horizon of Pit 3 can be explained by the contribution from root litter, cones or twigs to the buildup of this relatively large SOC stock. In fact, the total mass of live coarse roots and cones was 2.5 to 4.5 times greater in Pit 3 than in Pit 1 and 2.

In the first decades of afforestation, SOC stocks increased rapidly in the Oi and Oe horizons and reached nearly steady-state after 80- 100 a (Fig. 2.4). In the past decade, the rate of SOC accumulation was smaller than 0.1 g C m⁻² a⁻¹ for the Oi horizon and approximately 0.3 g C m⁻² a⁻¹ for the Oe horizon. The fast TT of SOC (3- 10 years) highlights the potential of these horizons to respond rapidly to an increase in temperature. It has been demonstrated that along an elevation gradient, the TT of SOC in the topsoil is controlled by temperature (Trumbore et al., 1996).

The radiocarbon signature indicates that the Oa horizon was partly formed from SOC before afforestation. In our model, the SOC stock did not increase during the first 30 years after afforestation, which we attribute to the delay in the production and transfer of humified organic matter in the Oe horizon. In the present Oa horizon, the turnover rate (100- 160 years) is slow enough to allow the annual C input to result in a SOC accumulation of 3.5- 7.5 g C m⁻² a⁻¹. Almost all of this C input is accumulating in the FPOM fraction whereas the OPOM and MAOM fractions yield SOC close to a steady-state condition.

Overall, SOC accumulation rate of the organic layer (3.8- 7.8 g C m⁻² a⁻¹) is in good agreement with estimates of 2- 7 g C m⁻² a⁻¹ for a mixed deciduous forest (Gaudinski et al., 2000). Slightly larger accumulation rates of 12- 13 g C m⁻² a⁻¹ were estimated for coniferous soils in Sweden (Ågren et al., 2008). One reason for small accumulation rates at our study site could be the application of lime to the soil surface, which is often reported to improve soil conditions and thus to increase the mineralization of SOM (e.g. Persson et al., 1989; Fuentes et al., 2006).

Turnover time (TT) of SOC in mineral soil fractions

The TT of SOC in bulk samples increased with soil depth and exhibited little variation among the three soil pits. As with Pits 1 and 2, many studies have found increasing TT with increasing soil depth and density (e.g. Bol et al., 1999; Gaudinski et al., 2000; Trumbore, 2000; Certini et al., 2004; Eusterhues et al., 2007), pointing to increasing stabilization of SOC by minerals at greater depth.

The FPOM, OPOM and MAOM fractions indicate the existence of C pools with different turnover times that are associated with the degree of degradation and humification (Baisden et al., 2002; John et al., 2005). For the EA and Bv horizons, TT followed a typical pattern in the order FPOM < OPOM < MAOM. In the Bsh and Bs horizons, however, the MAOM fraction had faster TT than the OPOM fraction. We attribute this finding to the sorption of DOC with a less negative $\Delta^{14}\text{C}$ signature by Fe and Al oxides/hydroxides in the MAOM fraction. Sorption of DOC in spodic horizons is a typical process in Podzols and largely contributes to the SOC stock of the Bsh and Bs horizons in the long run. The intrinsic TT of the MAOM fraction in these spodic horizons is probably much faster, as predicted by our model approach. Hentschel et al. (2009) reported radiocarbon signatures of -65 to +38‰ for DOC in the soil solution below the Oa

horizon at our study site. The radiocarbon signatures of DOC decreased to between -25 and -265‰ at 90 cm mineral soil depth and these are within the range of the FPOM fraction. The time span between the formation of DOC from 'old' particulate SOM and sorption by the soil matrix in the Bsh and Bs horizons could be relatively short.

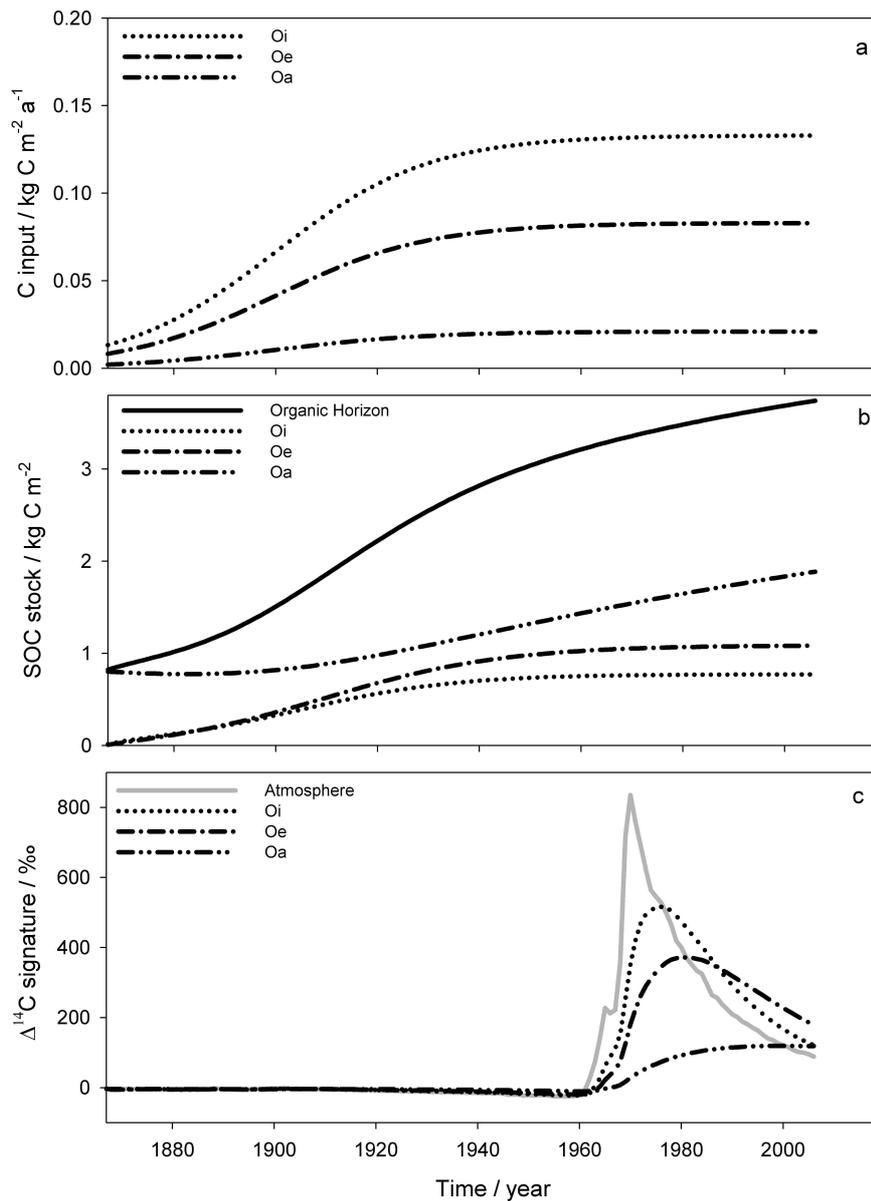


Figure 2.4 Carbon inputs from litter (a), SOC stocks (b), and ¹⁴C signature of the atmosphere and of SOC (Pit 2 only) (c) in organic horizons from 1867 to 2006 as estimated from a non-steady-state model.

A substantial shift in the $\Delta^{14}\text{C}$ signature and TT of the MAOM fraction was observed from the Bs to the Bv horizon in all three soil pits. Again, this shift supports the sorption of DOC in the MAOM fraction of the Bsh and Bs horizons. Turnover times of 1080-2170 years in the OPOM and MAOM fractions of the Bv horizon may be explained by small input of particulate and dissolved organic matter and strong physical and chemical stabilization.

2.5. Conclusion

The present soil was a small sink for atmospheric CO_2 in the order of 4- 8 $\text{g C m}^{-2} \text{a}^{-1}$ during the past 10 years and might be a net CO_2 sink of similar order in the near future at similar boundary conditions. Most of the SOC accumulated in the FPOM fraction of the Oa horizon whereas other organic and mineral soil horizons were in, or close to, steady-state. Sorption of DOC in the Bsh and Bs horizons seems to affect the radiocarbon signature of the MAOM fraction, but turnover times of 390- 710 years mask the true contribution to accumulation of SOC in these spodic horizons.

The C and N stocks of the organic layer are vulnerable to changes in climate conditions or other disturbances. The turnover time of non-stabilized SOM will probably decrease with increasing temperature and turn the soil from a small sink to a transient source.

The density fractionation method revealed some uncertainties. Firstly, a portion of SOM was either lost as particulate or dissolved SOM in the order of up to 10 % (SOC) and 16 % (N). This lost fraction is generally not characterized, but might participate in the C and N cycles of soils. Secondly, the FPOM fraction, often associated with the active pool of SOC, had slow turnover times on the decadal-centennial time scale in all soil horizons. It seems that recalcitrance of SOM or stabilization processes cause a slow turnover of the FPOM fraction in this forest soil.

The spatial variation of SOC and total N stocks in genetic horizons and density fractions was relatively large in this rocky forest soil, highlighting the importance of representative sampling approaches. The radiocarbon signatures of bulk soil and density fractions exhibited a small variation among the three pits, except the EA horizon where differences in bioturbation have apparently affected the spatial heterogeneity. Even in hete-

ogeneous soils, measurements of the $\Delta^{14}\text{C}$ signature provide a powerful tool for the assessment of C accumulation in forest soils.

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Chapter 3

Dynamics of dissolved organic ^{14}C in throughfall and soil solution of a Norway spruce forest

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Summary

Dissolved organic carbon (DOC) is an important component of the C cycle in forest ecosystems, but dynamics and origin of DOC in throughfall and soil solution are yet poorly understood. In a 2-year study, we analyzed the radiocarbon signature of DOC in throughfall and soil solution beneath the Oa horizon and at 90 cm depth in a Norway spruce forest on a Podzol soil. A two-pool mixing model revealed that throughfall DOC comprised mainly biogenic C, i.e. recently fixed C, from canopy leaching and possibly other sources. The contribution of fossil DOC from atmospheric deposition to throughfall DOC was on average 6% with maxima of 8–11% during the dormant season. In soil solution from the Oa horizon, DO^{14}C signature was highly dynamic (range from -8% to +103%), but not correlated with DOC concentration. Radiocarbon signatures suggest that DOC beneath the Oa horizon originated mainly from occluded and mineral associated organic matter fractions of the Oa horizon rather than from the Oi or Oe horizon. Relatively old C was released in the rewetting phase following a drought period in the late summer of 2006. In contrast, the DO^{14}C signature indicated the release of younger C throughout the humid year 2007. In soil solutions from 90 cm depth, DO^{14}C signatures were also highly dynamic (-127‰ to +3‰) despite constantly low DOC concentrations. Similar to the Oa horizon, the lowest DO^{14}C signature at 90 cm depth was found after the rewetting phase in the late summer of 2006. Because of the variation in the DO^{14}C signatures at this depth, we conclude that DOC was not equilibrated with the surrounding soil, but also originated from overlaying soil horizons. The dynamics of DO^{14}C in throughfall and soil solution suggest that the sources of DOC are highly variable in time. Extended drought periods likely have a strong influence on release and translocation of DOC from relatively old and possibly stabilized soil organic matter fractions. Temporal variations as well as the input of fossil DOC needs to be considered when calibrating DOC models based on DO^{14}C signatures.

3.1. Introduction

Dissolved organic carbon (DOC) plays an important role in the carbon cycle of terrestrial ecosystems and for the transfer of organic C from terrestrial to aquatic systems (Neff and Asner 2001; Cole et al., 2007). The production and leaching of DOC in terrestrial ecosystems affects the concentration, composition and age of DOC in aquatic sys-

tems (Raymond and Bauer 2001; Sickman et al., 2010; Tipping et al., 2010). The origin and quality of DOC as well as the dynamics of DOC concentrations in terrestrial ecosystems have therefore an impact on DOC concentrations in rivers and lakes (Evans et al., 2007; Roulet and Moore 2006).

As other land use systems, forests are relevant for the quantity and quality of surface water and groundwater. Pronounced seasonal and inter-annual variations of DOC concentrations and fluxes have been reported for different compartments of forest ecosystems (Buckingham et al., 2008; Michalzik and Matzner 1999; Solinger et al., 2001). Leaching of DOC from forest canopies represent a C flux to the soil that is mainly used as C and energy source by microorganisms (Michalzik et al., 2001). Besides precipitation, seasonal pattern of temperature could influence concentration and composition of throughfall DOC since biological, temperature-dependent processes (e.g., budding, pest infection, pollination) affect the production of DOC in the canopy. Fluxes of DOC in organic layers are often larger than throughfall input due to net production of DOC in organic horizons. In contrast, only small concentrations and fluxes of DOC are observed in deeper mineral soil horizons (Michalzik et al., 2001; Hentschel et al., 2007).

The origin, composition and function of DOC in different compartments of forest ecosystems are still a matter of debate. In throughfall, DOC may result from the leaching of organic substances from plant tissues as well as from atmospheric deposition of organic substances. The latter may include marine and terrestrial organic C emissions as well as emissions from the combustion of fossil fuels and biomass (Avery et al., 2006). However, systematic analyses on the origin of DOC in throughfall are not available to our knowledge.

Concentrations and fluxes of DOC in forest soils are the result of multiple factors and processes. Water fluxes are considered as a main driver of DOC fluxes among soil horizons and of DOC output by leaching. DOC concentration, which determines DOC flux resulting from water transport, is partly controlled by sorption/desorption processes (Guggenberger and Kaiser 2003). Further factors controlling DOC concentrations and fluxes are leaching from plant litter, production, immobilization and mineralization of

DOC by microorganisms (Kalbitz et al., 2000; Neff and Asner 2001). Recent studies suggest that a large fraction of DOC is sorbed and stabilized by mineral surfaces rather than mineralized by soil organisms (Kaiser and Guggenberger 2000; Sanderman and

Amundson 2009). The residence time of sorbed DOC, however, is rather short, i.e. years-decades, unless it is sorbed by juvenile mineral surfaces (Guggenberger and Kaiser 2003).

In soil solution from B and C horizons, the concentration of DOC is relatively low and constant throughout the year (e.g., Borken et al., 1999; Fröberg et al., 2006). An unsolved question is to what extent DOC in these horizons originates from vertical translocation or desorption and production in the respective soil depth. Isotopic signature of DOC can be helpful to answer this question. In a field study with ^{13}C labelled spruce litter, the analysis of DO^{13}C revealed only a minor proportion of litter DOC in percolates below the Oe and Oa horizon of a Podzol in a Norway spruce forest (Fröberg et al., 2007a). Hence, most of the DOC was produced in the respective organic horizons themselves. In the same and another Norway spruce stand on Podzol, the DO^{14}C signature of soil solution below the B horizon was similar to the ^{14}C signature of bulk soil from the B horizon (Fröberg et al., 2006). Despite similar ^{14}C signatures, DOC is not necessarily desorbed from the B horizon alone. Both, DOC and soil organic matter (SOM) comprise a mixture of different constituents of varying ^{14}C signatures. Different results were reported for a Podzol in a forested watershed (Trumbore et al., 1992), and for Mediterranean forest and grassland soils (Sanderman and Amundson 2009). In both studies, DOC from different mineral soil horizons exhibited much younger ^{14}C signatures than the respective bulk soil. In addition to vertical translocation, the difference in the ^{14}C signature of DOC and bulk soil could be caused by equilibration between DOC and specific SOM fractions.

The few studies on DO^{14}C in forest ecosystems have not considered dynamics of ^{14}C signature, but used the ^{14}C signature from single sampling events or even from water extracts. Seasonal and interannual changes of DO^{14}C signatures, however, are of relevance for tracing the origin of DOC and for predicting DOC dynamics in soils by simulation models (Michalzik et al., 2003). The goal of this study was to use the temporal pattern of DO^{14}C signatures, concentrations and fluxes of DOC in throughfall and soil solutions in order to improve our understanding of DOC sources and dynamics in a Norway spruce site.

3.2. Methods

Site description

The Coulissenhieb II site is a mature Norway spruce forest (*Picea abies* L.) in the German Fichtelgebirge, adjacent to the Coulissenhieb I site which has been subject of long-term biogeochemical studies (Matzner 2004). The mean annual precipitation is about 1,160 mm and the mean annual air temperature is 5.3°C. The soil has a sandy to loamy texture and is classified as Haplic Podzol according to the FAO soil classification (IUSS 2006). The well stratified, morlike organic layer has a thickness of 7–10 cm, comprising Oi, Oe and Oa horizons (Table 3.1). The organic layer is almost completely covered by ground vegetation, mainly *Deschampsia flexuosa* (L.) and *Callamagrostis villosa* (Chaix). Soil properties are described by Schulze et al., (2009). In short, organic C content of the soil decreases with increasing depth from 46.6% C in the Oi horizon to 1.3% C in the Bv horizon. The soil stores 3.8 kg C m⁻² in the organic layer and 11.4 kg C m⁻² in the mineral soil down to 62 cm depth (Table 3.1).

Table 3.1 Thickness of soil horizons, bulk density (BD), organic C content, organic C stock and radiocarbon signature ($\Delta^{14}\text{C}$) of a Podzol at the Fichtelgebirge after Schulze et al. (2009). Error bars represent the standard deviation of the mean (n=9, $\Delta^{14}\text{C}$ n=3).

Horizon	Thickness [cm]	BD [g cm ⁻³]	C [%]	C stock [kg C m ⁻²]	$\Delta^{14}\text{C}$ [‰]
Oi	2.1 (0.1)	0.07 (0.00)	45.8 (0.9)	0.7 (0.1)	114 (8)
Oe	2.2 (0.2)	0.15 (0.02)	42.1 (6.3)	1.4 (0.9)	162 (16)
Oa	4.9 (0.5)	0.25 (0.03)	21.2 (2.3)	2.0 (0.6)	119 (13)
EA	5.2 (0.8)	0.60 (0.02)	8.3 (0.9)	2.6 (1.3)	23 (68)
Bsh	5.3 (0.6)	0.75 (0.01)	6.0 (0.4)	2.4 (1.2)	-14 (13)
Bs	11.4 (1.5)	0.79 (0.02)	3.6 (0.3)	2.7 (1.2)	-63 (11)
Bv	30.5 (3.0)	1.17 (0.03)	1.4 (0.2)	4.4 (1.9)	-145 (18)

About one-third of trees were removed from the study site in 2007 after a partial damage of trees by a storm event in January 2007. Except for throughfall samplers, the soil and installations (see below) were not damaged by the hurricane. The three damaged throughfall samplers were replaced and installed at similar locations about 1 week after the hurricane. Aboveground litter input was very large (562 g m^{-2}) due to this disturbance. Afterwards, litter fall was reduced by one-third from April to December 2007 (329 g m^{-2}) compared to the respective period in 2006.

Sampling

Three plots each of 400 m^2 were established and equipped on an area of about 1 ha at the Coulissenhieb I site in the summer of 2005. Throughfall and soil solutions were sampled between January 2006 and January 2008. Throughfall was continuously collected in 1 m height with three conical funnels (upper diameter of 20.2 cm) per plot. Each funnel was connected with a 5 l sampling flask and equipped with fine polyethylene fibre to exclude litter input into throughfall solution. Funnels and sampling flasks were replaced every second week. Throughfall solution of each 2-week sampling interval was used for chemical analyses. Prior to chemical analyses, throughfall solution from all nine samplers was merged to one mixed sample per sampling period (i.e., 25 and 23 mixed samples in 2006 and 2007, respectively). The amount of throughfall of all sampling flasks was used for calculation of 4-weekly and annual fluxes of throughfall volume and DOC.

Solution from the organic layer was collected below the Oa horizon using three plate lysimeters per plot. The plate lysimeter was made of a plastic bowl with a 50 μm pore-size polyethylene membrane on top. Each plate lysimeter had a surface area of 176 cm^2 that was connected to a vacuum pump. A suction of -10 kPa was applied for 1 min every 5 min throughout the whole experimental period. Solution of three plate lysimeters was continuously collected in one 2 l plastic flask per plot that was stored in an underground container. Water volume of each flask was measured biweekly and was used for calculation of water fluxes. We cannot exclude that water fluxes were underestimated at few occasions when the 2 l flasks were filled before the 2-week sampling interval. Total solution of the first 2-week sampling interval was stored in a climate chamber at 5°C and then mixed with total solution of the second 2-week sampling interval for chemical analyses. Apart from this scheme, solutions were biweekly analyzed

during the snowmelt in April 2006 and after the drought period in 2006. Four-weekly DOC fluxes were calculated from water fluxes and respective DOC concentrations.

Three ceramic suction cups per plot were installed below the rooting zone at 90 cm soil depth. These suction cups were operated at a continuous suction of -25 kPa. The sampling procedure and sampling intervals for soil solution from suction cups and plate lysimeters were identical. Samples from three suction cups per plot were merged to one mixed sample using the same 2 l flasks. In March 2008, groundwater from a well at 10 m depth and spring water were sampled at three occasions. The well was located about 50 m east from the study site and the spring about 100 m west from the study site. All water samples including throughfall and soil solution were filtered with prewashed 0.45 μm cellulose-acetate filters and stored at 2°C until chemical analysis.

Chemical analyses

DOC was determined by high temperature combustion and subsequent determination of CO_2 (Elementar, high-TOC). Prior to ^{14}C analyses, volume-weighted subsamples of soil solution were merged to 2–3 months samples per plot ($n = 3$) whereas volume-weighted subsamples of throughfall were merged to one representative sample of the study site per quarter. The ^{14}C signature of groundwater was determined at three dates. Radiocarbon signature of DOC was determined by accelerator mass spectrometry (AMS). Subsamples (1 mg C) of freeze-dried DOC were oxidized in 6 mm sealed quartz tubes with 60 mg CuO and 1 cm silver wire for 2 h at 900°C. The resulting CO_2 was purified from water and noncondensable compounds. Afterwards, CO_2 was reduced to graphite using the zinc reduction method (Xu et al., 2007). All preparations took place at the Department of Soil Ecology at the University of Bayreuth. The graphite targets were analyzed by the Keck-CCAMS facility of University of California, Irvine with a precision of 2–3‰. Radiocarbon data are expressed as $\Delta^{14}\text{C}$ (‰ deviation from the $^{14}\text{C}/^{12}\text{C}$ ratio of oxalic acid standard in 1950). The samples have been corrected for a $\delta^{13}\text{C}$ value of -25‰ to account for any mass dependent fractionation effects (Stuiver and Polach 1977). Tests of thermonuclear weapons between the 1950s and the early 1960s have almost doubled the ^{14}C content (835‰) of the atmosphere (Levin et al., 1985). The atmospheric ^{14}C level has steadily decreased after a moratorium on atmospheric testing mainly due to combustion of fossil fuels. Bomb ^{14}C is still present in the atmosphere as indicated by a $\Delta^{14}\text{C}$ signature of 47‰ in 2007 (Levin et al., 2008). The atmospheric pre-

bomb $\Delta^{14}\text{C}$ level since the industrialization in 1750 as measured by tree-ring analyses varied between 4.1 and -22.8‰ (Stuiver et al., 1998).

Biogenic and fossil DOC in throughfall

Throughfall may contain DOC from leaching of plant tissues in the canopy (hereafter ‘biogenic DOC’) and DOC from deposition of soot and other organic particles (hereafter ‘fossil DOC’). We assume a mean residence time of 3 years for C in plant tissues after photosynthetic fixation before it is released as biogenic DOC in throughfall. This assumption is based on half live of Norway spruce needles of about 3 years at our study site (Schulze et al., 2009). Consequently, biogenic DOC had ^{14}C signatures of 70‰ in 2006 and of 64‰ in 2007 equivalent to the average ^{14}C signature of CO_2 in the atmosphere at the Jungfrauoch, Switzerland, in 2003 and 2004 (Levin et al., 2008). Provided that biogenic and fossil DOC have different ^{14}C signatures a two-pool mixing model can be used to separate their portion to total DOC.

$$\Delta^{14}\text{C}_{\text{sample}} = x \cdot \Delta^{14}\text{C}_{\text{fossil}} + (1 - x) \cdot \Delta^{14}\text{C}_{\text{biogenic}} \quad (3.1)$$

where $\Delta^{14}\text{C}_{\text{sample}}$ is the measured DO^{14}C signature of throughfall, x the portion of fossil DOC in throughfall, $\Delta^{14}\text{C}_{\text{fossil}}$ is the DO^{14}C of fossil fuel carbon depleted in ^{14}C (-1,000‰). We cannot exclude that the portion of biogenic DOC is overestimated at deposition of soluble non-fossil organic particles with ^{14}C signatures $>-1,000\text{‰}$.

Temperature and matric potential

Air temperature was hourly recorded at 2 m above ground. At each plot, soil temperature was automatically recorded in 30 min intervals below the Oa horizon and the Bv horizon using one sensor per horizon. Volumetric water contents 6 cm below the Oa horizon were measured every 30 min with a time domain reflectometer (TDR probe) and converted into matric potentials using a calibration function for this specific horizon (Zuber 2007). Soil matric potential in 90 cm mineral soil depth was simultaneously recorded using self-constructed and calibrated tensiometers.

Statistical analysis

Statistical analyses were performed using STATISTICA 6.0. Linear regressions were made between DOC concentrations of throughfall and soil solution and throughfall, soil moisture and air temperature. Relationships were further tested by the Spearman correlation.

3.3 Results

Throughfall and air temperature

DOC concentration in throughfall was $18.0 \pm 2.2 \text{ mg l}^{-1}$ in 2006 with a maximum of 60 mg l^{-1} in July (Fig. 1). In 2007, average DOC concentration of throughfall was smaller by $11.5 \pm 1.8 \text{ mg l}^{-1}$. As the year 2006 had much less precipitation (868 mm throughfall) than 2007 (1,152 mm throughfall) (Fig. 2a), annual DOC fluxes were almost identical in 2006 ($129.5 \text{ kg C ha}^{-1} \text{ a}^{-1}$) and 2007 ($132.5 \text{ kg C ha}^{-1} \text{ a}^{-1}$) (Fig. 3.2b).

Mean annual air temperature was similar in 2006 (7.1°C) and in 2007 (7.5°C), however, the seasonal pattern of air temperature was different between the years (Fig. 3.2a). Mean winter air temperature was considerably lower in 2005/2006 (-3.8°C) than in 2006/2007 (1.2°C) whereas the summer of 2006 was warmer (14.1°C) compared to 2007 (12.6°C). DOC concentration in throughfall did not correlate with the amount of throughfall or air temperature (not shown).

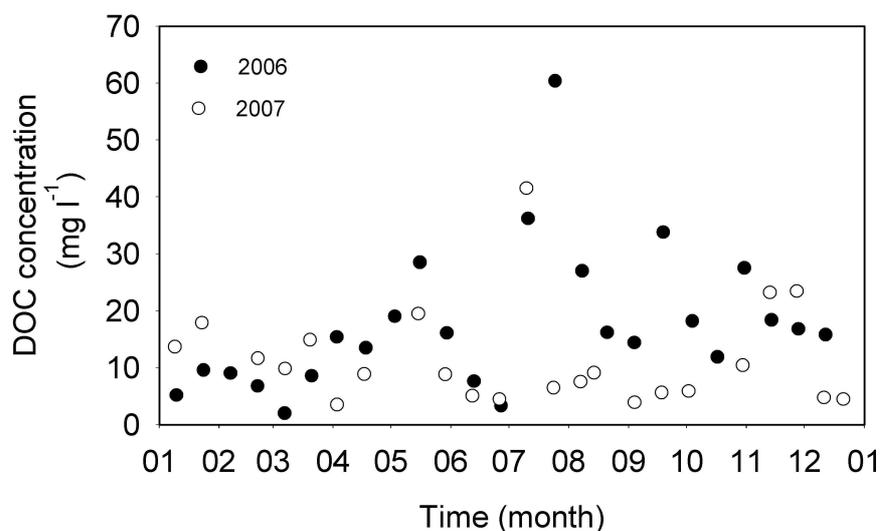


Figure 3.1 Concentrations of DOC in throughfall from biweekly sampling intervals during 2006 and 2007.

DO^{14}C signatures of throughfall varied between -52 and 41% throughout the study period (Fig. 3.2c), indicating a strong variation in the origin of DOC. Negative $\Delta^{14}\text{C}$ signatures occurred only during the dormant season from October to March and for the period from October to December 2006, representing the dominance of pre-bomb C in throughfall DOC. In contrast, throughfall DOC contained bomb C during growing seasons. Based on Eq. 3.1, the portion of fossil DOC in throughfall varied between 2 and

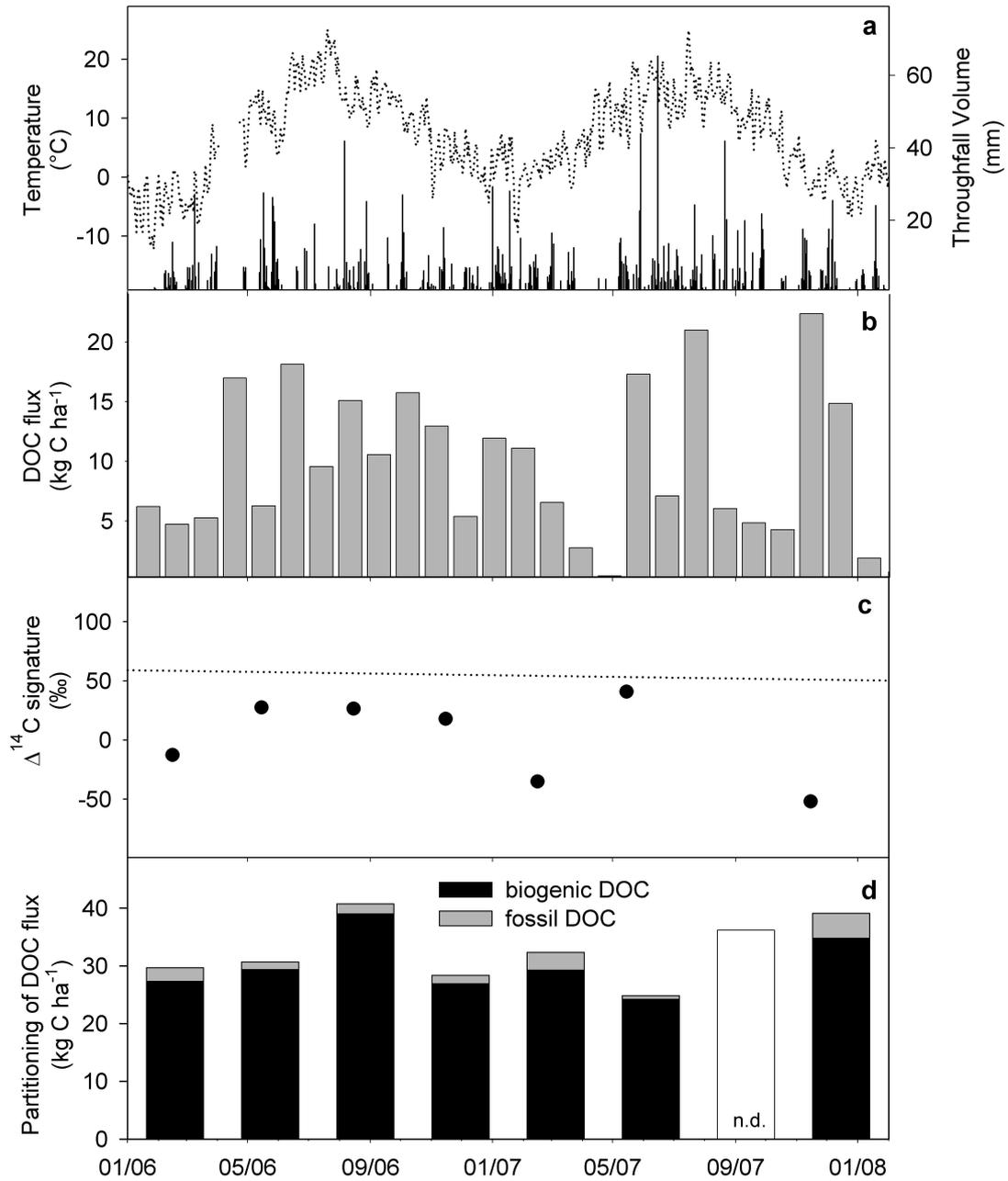


Figure 3.2 (a) Daily means of air temperature and throughfall volume, (b) four-weekly DOC fluxes, (c) mean quarterly radiocarbon signature, and (d) quarterly proportion of biogenic and fossil DOC in throughfall during 2006 and 2007. The dotted line indicates the ¹⁴C signature of atmospheric CO₂ at the Jungfraujoch (Levin et al., 2008). n.d. = not determined.

11% (Fig. 3.2d). Largest fluxes of fossil DOC were consequently found for the quarters from October to December 2007 (4.3 kg C ha^{-1}), from January to March in 2006 (2.3 kg C ha^{-1}) and 2007 (3.0 kg C ha^{-1}). On a 2-year average, throughfall DOC comprised about $124 \text{ kg biogenic C ha}^{-1} \text{ a}^{-1}$ and $7 \text{ kg fossil C ha}^{-1} \text{ a}^{-1}$. The concentration of DOC had no clear influence on its $\Delta^{14}\text{C}$ signature (Figs. 3.1, 3.2c).

Oa horizon

Soil temperature exhibited a strong seasonal pattern with lowest values during soil frost in the cold winter of 2005/2006. Mean annual soil temperatures were 6.5°C in 2006 and 7.4°C in 2007 (Fig. 3.3a). The matric potential in the O horizon varied between -22 and -310 kPa throughout the study period, except during a dry period between June and October 2006 where it decreased to a minimum of $-2,572 \text{ kPa}$ in early August (Fig. 3.3a).

Mean concentrations of DOC below the organic layer ($28.4\text{--}79.8 \text{ mg l}^{-1}$) were above those of throughfall concentration (Figs. 3.2b, 3.3b). In the cold winter of 2006, DOC concentration decreased from $50\text{--}57 \text{ mg l}^{-1}$ to a minimum of 28 mg l^{-1} in April after melting of about $40\text{--}50 \text{ cm}$ snow cover. In the dry summer of 2006, mean DOC concentration increased and peaked at 80 mg l^{-1} in August 2006, but then decreased during autumn and winter to a minimum in March 2007 (32 mg l^{-1}). A similar seasonality was visible in 2007 although matric potential exhibited no seasonal trend (Fig. 3.2a). A weak correlation was found between DOC concentration and soil temperature ($y = 1.8x + 44$, $r^2 = 0.43$, $p=0.001$) (not shown).

Annual DOC flux was greater in 2006 (455 kg ha^{-1}) than in 2007 (322 kg ha^{-1}) despite smaller throughfall volume in 2006 (Figs. 3.3c, 3.2a). The DOC flux peaked in April 2006 after the snowmelt when the soil was water-saturated. Small DOC fluxes occurred during the drought periods in July 2006 and April/May 2007 and during the frost period in December 2007/January 2008.

With one exception, positive DO^{14}C signatures indicate bomb C in the range from 15 to 103‰ (Fig. 3.3d). The only mean negative DO^{14}C signature (-8‰) occurred in the late summer/early autumn of 2006 after rewetting of dry soil. Prior to this minimum, the second lowest DO^{14}C signature (15‰) was found in the dry and warm period from July to September 2006. In the wet year 2007, the DO^{14}C signatures were consistently higher compared to 2006 and partly above the ^{14}C signature of atmospheric CO_2 , indicating the

release younger DOC in 2007 than in 2008. Again, DO^{14}C signature was not correlated with DOC concentration.

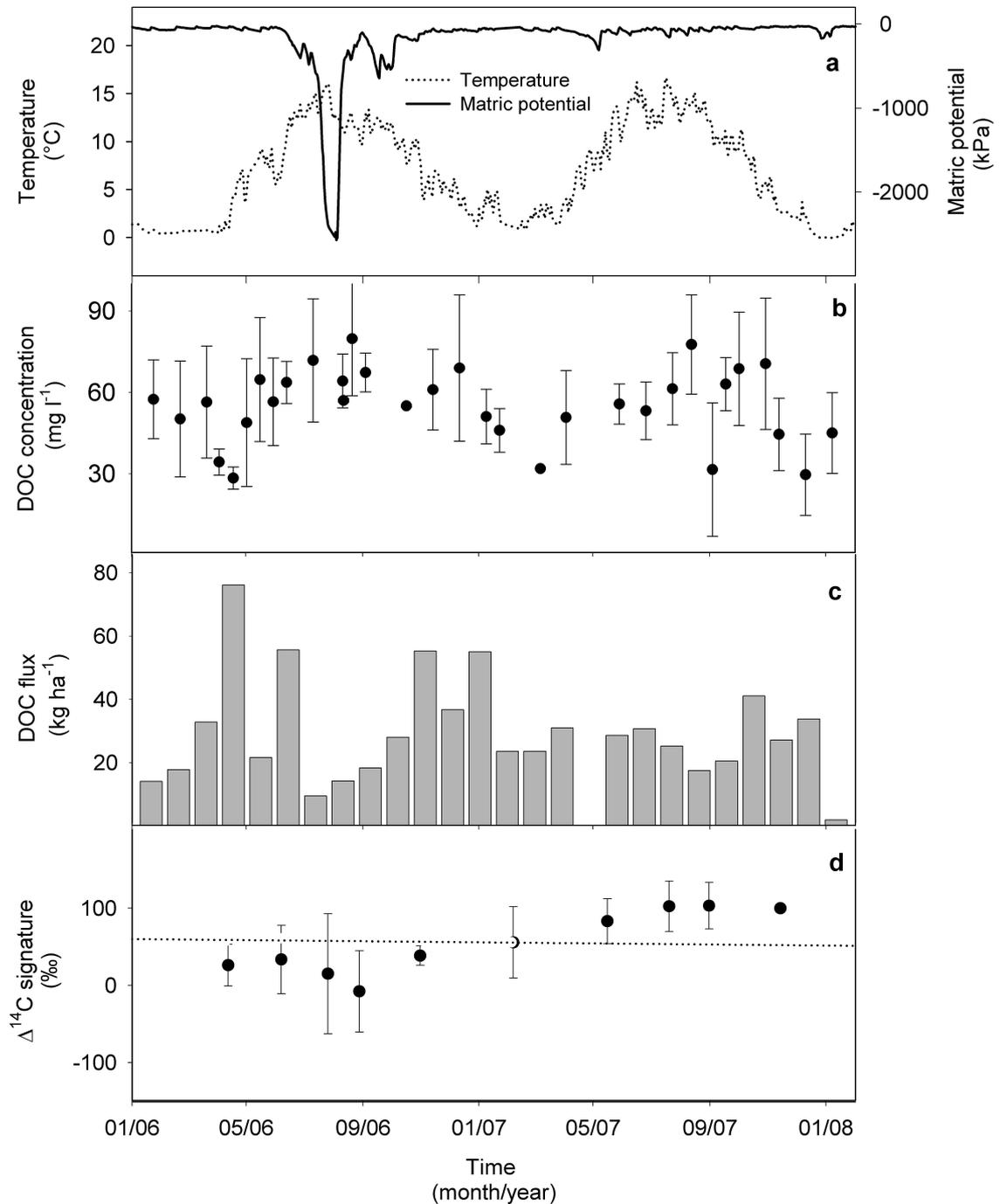


Figure 3.3 (a) Daily means of soil temperature and matric potential, (b) four-weekly mean DOC concentrations, (c) four-weekly means DOC fluxes, and (d) mean bimonthly radiocarbon signatures of DOC in the soil solution beneath the Oa horizon during 2006 and 2007. The dotted line indicates the ^{14}C signature of atmospheric CO_2 at the Jungfraujoch (Levin et al., 2008). Error bars represent the standard error of the mean ($n = 3$).

Water samples from 90 cm soil depth, well and spring

Because of the snow cover in the cold winter of 2005/2006 soil temperature reached its minimum of 1.5°C in early April 2006 shortly after snowmelt (Fig. 3.4a). In the mild winter of 2006/2007, minimum soil temperature of 2.8°C occurred in February of 2007. Mean annual soil temperatures were 6.4°C in 2006 and 7.4°C in 2007. Soil matric potential in 90 cm depth ranged between 6 and -20 kPa and was not significantly reduced during the dry summer of 2006.

Mean DOC concentration (3.0 ± 0.1 mg l^{-1}) was generally small and showed little variation among the three plots (Fig. 3.4b). Notable DOC concentrations were measured in August 2006 after rewetting of dry soil. This singular peak coincided with the maximum DOC concentration in the solution from the organic layer. The enhanced spatial variation is attributed to the large DOC concentration (20.2 – 41.6 mg l^{-1}) of one plot. No correlations were found between DOC concentration in 90 cm soil with soil temperature, matric potential and amount of throughfall (not shown).

DOC in 90 cm depth consisted of much older C with ^{14}C signatures ranging between -127 and 12‰ with no clear seasonal trend (Fig. 3.4c). Positive ^{14}C signatures were analyzed at two periods from May and July 2006 and from September to December 2007, pointing to the presence of bomb C below the rooting zone. Consistent with solution from the organic layer, lowest DO^{14}C signatures were detected after the dry and warm summer of 2006. However, the lowest value occurred later in the last quarter of 2006. DO^{14}C signature from one plot (-277‰) was mainly responsible for this minimum value and the pronounced spatial variability in 2006. The other two plots had DOC signatures between -53‰ and -21‰ from July to December 2006. Both, spatial and seasonal variability of DO^{14}C signatures were much smaller in 2007. DO^{14}C signatures were positively correlated with ln-transformed DOC concentrations ($y = 86.4 \ln(x) - 136$, $r_2 = 0.29$, $p = 0.009$) in 90 cm soil depth (not shown), indicating that the portion of ‘younger C’ increased with DOC concentration.

Groundwater at 10 m depth had a mean DOC concentration of 0.79 ± 0.03 mg l^{-1} and a mean ^{14}C signature of $-133 \pm 34\text{‰}$ (data not shown). The difference between the mean DO^{14}C signature of groundwater and soil solution at 90 cm (-41‰ from 2006 to 2007, Fig. 3.4c) indicate either input of older DOC from other sources than soil solution or

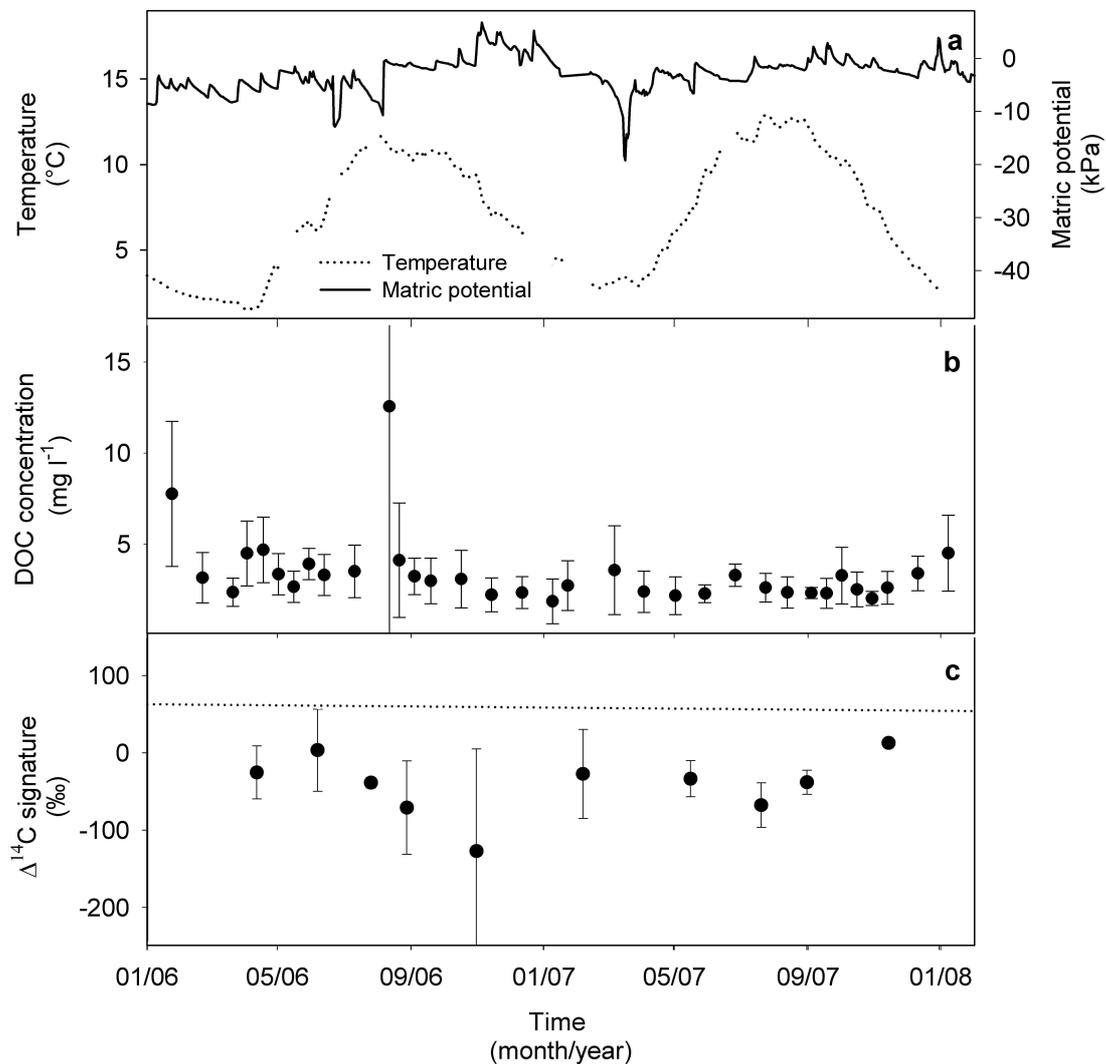


Figure 3.4 (a) Daily means of soil temperature and matric potential, (b) four-weekly means of DOC concentrations, and (c) bimonthly means of radiocarbon signatures of DOC in the solution from the mineral soil at 90 cm depth during 2006 and 2007. The dotted line indicates the ^{14}C signature of atmospheric CO_2 at the Jungfrauoch (Levin et al., 2008). Error bars represent the standard error of the mean ($n = 3$).

preferential decay/sorption of younger soil solution DOC along the pathway below 90 cm. The relevance of DOC decay/sorption during transportation is supported by the difference in DOC concentration between groundwater ($0.79 \pm 0.03 \text{ mg l}^{-1}$) and soil solution ($3.0 \pm 0.1 \text{ mg l}^{-1}$).

Spring water had a similar DOC concentration of $0.85 \pm 0.09 \text{ mg l}^{-1}$ to groundwater (data not shown). The mean ^{14}C signature of $-226 \pm 52\text{‰}$ indicates that DOC resides for some thousands years in the soil-groundwater system before it is released by the spring.

3.4. Discussion

DOC in throughfall

Our results suggest that the seasonal pattern of DOC concentration in throughfall is not controlled by air temperature or the amount of throughfall. Both, low and high DOC concentrations may occur after periods of light or heavy rainfall. The difference in mean annual DOC concentration between 2006 and 2007 can be attributed to the removal of one-third of all trees after the hurricane in January 2007. Canopy coverage is a main factor of concentrations and fluxes of DOC in throughfall. Clarke et al., (2007) found a decrease of DOC concentrations and fluxes with decreasing litterfall as a proxy for canopy coverage along a chronosequence of Norway spruce stands. Despite consistently lower DOC concentrations in 2007, elevated throughfall water volume in 2007 resulted in annual DOC fluxes being similar between the years. The annual DOC flux of $133 \text{ kg C ha}^{-1} \text{ a}^{-1}$ at our study site is on the upper range of throughfall fluxes ($40\text{--}160 \text{ kg C ha}^{-1} \text{ a}^{-1}$) reported for various temperate forests (Michalzik et al., 2001). The DOC flux at our site was relatively large as conifers seem to release more DOC than deciduous trees under same climatic conditions (Borken et al., 2004).

The radiocarbon signatures of DOC in the 1st quarter of 2006 (-13‰) and 2007 (-35‰) as well as in the 4th quarter of 2007 (-52‰) display relatively old carbon that was not alone released by the canopy. We assume that deposition of DOC from combustion of fossil fuels altered the radiocarbon signature of DOC in winter throughfall. According to the mass balance approach, between 5 and 11% or 1.4 and 4.3 kg C ha^{-1} of throughfall DOC originated from combustion of fossil fuels during the dormant seasons in 2006 and 2007. The contribution of fossil fuel was smaller in the 2nd to 3rd quarters, although relevant for annual DOC fluxes. Our estimates are likely biased because deposition of C with a different ^{14}C signature as from the combustion of firewood might represent an additional and unaccounted source of DOC in throughfall. Firewood is increasingly used in Germany as an alternative energy source during recent years. Consequently, the amount of biogenic DOC from the canopy was overestimated by the two pool mixing model. The contribution of ‘firewood DOC’ could be estimated by a three-pool mixing model using the isotopic signatures of ‘canopy DOC’ and of soot particles released by combustion of firewood.

In samples of single rain events, fossil DOC contributed between 4 and 24‰ to total DOC based on a ^{14}C signature of contemporary living material for biogenic DOC (Avery et al., 2006). According to Avery et al. (2006) the proximity of anthropogenic combustion sources and the wind direction influence the content of fossil fuel DOC in rainwater. The relative portion of fossil DOC is smaller in our quarterly throughfall samples because of the input of biogenic DOC from the canopy that was not reduced after the hurricane in 2007. Total input of fossil DOC, however, is likely greater in throughfall than in rainwater due to dry and wet deposition of organic particles and solutes in the canopy. The change of the DO^{14}C signature in throughfall is possibly not only affected by the input of fossil DOC via rainwater, but also by the leaching of biogenic DOC with different ^{14}C signature. A seasonal shift in the ^{14}C signature could result from predominantly leaching of recently synthesised C during growing season and enhanced leaching of older DOC during dormant season. However, varying biogenic DOC sources and DO^{14}C signatures would have had limited influence on the partitioning of DOC fluxes as fossil DOC is depleted in ^{14}C . For example, the portion of fossil DOC would be less than 27% as long as biogenic DOC has ^{14}C signatures $<300\%$. Based on the ^{14}C signature of atmospheric CO_2 , biogenic DO^{14}C signatures of 300–1,000‰ could only occur if DOC is released from plant tissues that were synthesized between 1962 and 1980 (Levin et al., 1985).

The fate of infiltrating fossil DOC in the soil remains unknown. Despite its age it is not necessarily recalcitrant or accumulates in the soil. However, even small inputs of fossil DOC could potentially affect the ^{14}C signature of DOC in the organic layer or mineral soil.

DOC below the Oa horizon

In agreement with other studies (e.g., Michalzik and Matzner 1999; Solinger et al., 2001; Dawson et al., 2002) DOC concentration below the organic layer exhibited a seasonal pattern with maximum in summer/autumn and minimum in early spring. DOC concentration followed slightly the seasonal pattern of soil temperature, suggesting that temperature has a small effect on the production of DOC. Even soil moisture seems to play a minor role in the control of DOC concentration. The seasonal pattern and level of DOC concentration were similar in both years although water availability in the organic layer was different during the growing season of 2006 and 2007. Release of DOC from

Table 3.1 Thickness of soil horizons, bulk density (BD), organic C content, organic C stock and radiocarbon signatures ($\Delta^{14}\text{C}$) of bulk soil and density fractions (FPOM = free particulate organic matter, OPOM = occluded organic matter, MAOM = mineral associated organic matter) of a Podzol at the Fichtelgebirge after Schulze et al. (2009). Error bars represent the standard deviation of the mean (n=9, n=3 for radiocarbon analyses, n.d. = not determined).

Horizon	Thickness	BD	C	C stock	$\Delta^{14}\text{C}_{\text{Bulk}}$	$\Delta^{14}\text{C}_{\text{FPOM}}$	$\Delta^{14}\text{C}_{\text{OPOM}}$	$\Delta^{14}\text{C}_{\text{MAOM}}$
	[cm]	[g cm ⁻³]	[%]	[kg C m ⁻²]	[‰]			
Oi	2.1 ± 0.1	0.07 ± 0.00	45.8 ± 0.9	0.7 ± 0.1	114 ± 8	n.d.	n.d.	n.d.
Oe	2.2 ± 0.2	0.15 ± 0.02	42.1 ± 6.3	1.4 ± 0.9	162 ± 16	n.d.	n.d.	n.d.
Oa	4.9 ± 0.5	0.25 ± 0.03	21.2 ± 2.3	2.0 ± 0.6	119 ± 13	128 ± 22	49 ± 21	13 ± 34
EA	5.2 ± 0.8	0.60 ± 0.02	8.3 ± 0.9	2.6 ± 1.3	23 ± 68	41 ± 76	-8 ± 54	-16 ± 30
Bsh	5.3 ± 0.6	0.75 ± 0.01	6.0 ± 0.4	2.4 ± 1.2	-14 ± 13	4 ± 27	-22 ± 22	-22 ± 6
Bs	11.4 ± 1.5	0.79 ± 0.02	3.6 ± 0.3	2.7 ± 1.2	-63 ± 11	-82 ± 46	-138 ± 13	-50 ± 11
Bv	30.5 ± 3.0	1.17 ± 0.03	1.4 ± 0.2	4.4 ± 1.9	-145 ± 18	-30 ± 37	-140 ± 39	-176 ± 33

particulate soil organic matter by physicochemical processes likely controls the concentration of DOC at different water contents (Fröberg et al., 2006). The amount of water extractable DOC from the organic layer of our study site increased with increasing amount of water (Schulze, unpublished data). Hence, water flux is an important driver of DOC fluxes from the organic layer into the mineral soil. We assume that the hydrological conditions after the snowmelt in 2006 display a specific situation. The DOC flux was much greater in 2006 than in 2007 although throughfall volume was opposite in these years.

The seasonal and interannual variation of DO^{14}C signature demonstrates that DOC originated from different carbon pools. In the late summer of 2006, the DO^{14}C signature decreased to $-8 \pm 30\%$ after some rainfall events which is below the ^{14}C signature of bulk organic C in the Oa horizon (Table 3.1). A potential source with a considerable effect on the DO^{14}C signature of the organic layer is fossil DOC from throughfall. According to a mass balance approach as given in Eq. 3.1, fossil DOC input of 1% (is equivalent to $3 \text{ kg C ha}^{-1} \text{ a}^{-1}$) would reduce the ^{14}C signature of total DOC by -10%. However, the fate of fossil DOC in the soil is unknown and the DO^{14}C signatures of throughfall and the Oa horizon were seasonally decoupled.

Another source of DOC is old C associated with mineral soil. Because of varying thickness of the Oa and Ea horizon it was impossible to separate completely these horizons at the installation of the lysimeter plates. We assume that DOC originated partly from the EA horizon although the amount of the EA horizon should have been small compared to the amount of the organic layer. Density fractionation of the Oa horizon revealed only a negative ^{14}C signature for mineral associated OM in one of three soil pits whereas lighter density fractions as well as mineral associated OM of the two other pits had positive ^{14}C signatures (Schulze et al., 2009). The portion of C in the mineral associated fraction of the Oa horizon was small (3%) compared to C in free particulate (72%) and occluded OM (25%). Nevertheless, it seems that this small fraction or very old occluded organic matter contributed to DOC during the re-wetting period in the summer of 2006. Disruption of soil aggregates due to drying and wetting and add-on desorption of DOC (Lundquist et al., 1999) from the mineral associated organic matter might be the responsible mechanisms for the occurrence of the negative DO^{14}C signature. Isotopic fractionation by microbial decomposition of ‘young’ organic matter or

other processes would have enriched the ^{14}C signature towards more positive values. Carbon that was photosynthetically fixed during the past 50 years has more positive ^{14}C values as presently fixed C because of the decline of $^{14}\text{CO}_2$ in the atmosphere since terminating atmospheric nuclear bomb tests. Radiocarbon signature of respired CO_2 also provides in some cases evidence of enhanced availability and release of 'old carbon' during the natural summer drought in 2006 at our study site (Muhr and Borcken 2009) and following artificial re-wetting of a dry forest soil under laboratory conditions (Borcken et al., 2006).

One would expect leaching of DOC from the whole organic layer into the mineral soil after some intensive rain events. Soil moisture of the O horizon, however, was not recovered during autumn 2006 despite of 345 mm rain between August and November. Remoistening of the organic layer was incomplete at this site, most likely due to hydrophobicity of organic matter and preferential flow (Bogner, personal communication). These transient changes of physicochemical soil properties have possibly contributed to the leaching of 'old DOC' in 2006. Soil drought could trigger the mobilization/desorption of old, i.e. protected organic matter because of the new spatial orientation of organic molecules under hydrophobic conditions.

Constant soil moisture promotes microbial activity and C mineralization of younger C pools (Christ and David 1996) and perhaps the release of young DOC from live roots. The improved conditions for microbial activity and root turnover in 2007 may explain the rather consistent DO^{14}C signatures (83–103%) which were closer to the ^{14}C signature of the bulk Oa horizon. Fröberg et al. (2003) also found bomb C in DOC from organic horizons of a Norway spruce stand, where the ^{14}C signatures of DOC were smaller than those of the respective bulk soil. Fresh litter as well as Oi and Oe horizons contribute a minor portion to DOC leached from the Oa horizon (Fröberg et al., 2005; 2007b). Thus, the Oa horizon itself is the main source of DOC beneath the Oa horizon. Specifically, ^{14}C signatures of free particulate and occluded organic matter correspond to the DO^{14}C signatures in 2007.

DOC in 90 cm soil depth

The DOC concentrations in 90 cm soil depth were relatively small and constant throughout the entire study period. In Podzols, DOC is strongly adsorbed by Al and Fe oxides/hydroxides and clay minerals which results in low DOC outputs (Kalbitz et al.,

2000). An exceptional increase of DOC concentration was observed after a dry period and some rain events in August 2006. This increase could be an indication of rapid movement of water and DOC from upper soil horizons through the soil profile.

Like the Oa horizon, the DO^{14}C signature in 90 cm depth decreased during the growing season of 2006, indicating the exposure of 'old' DOC. However, in contrast to the Oa horizon, this drought induced isotopic shift cannot be attributed to a specific soil horizon. Besides the drought/rewetting period in 2006, DO^{14}C signatures of -68 to 13% suggest that different OM fractions of the Bv and overlaying horizons could have acted as potential source for DOC. Given the dynamics of DO^{14}C signatures the source strength of specific soil horizons and OM fractions varied seasonally and inter-annually. The intensity of summer drought is possibly a factor of inter-annual variations as flow paths of soil water change with soil moisture. Preferential flow along roots and rock surfaces has been identified as a relevant and rapid transport of soil water at our site (Bogner et al., 2008). According to Bundt et al. (2001) roots generate organic compounds and thereby change the composition of DOC along preferential flow paths. Provided that these compounds have ^{14}C signatures similar to fresh litter, roots might influence the DO^{14}C signature in the Bv horizon. Microbial decomposition of dead roots is possibly another source of 'young' DOC in the subsoil.

The small concentration of groundwater DOC indicates that the soil below 90 cm depth is an effective net DOC sink. If the ^{14}C signature (-133%) of groundwater DOC is consistent throughout the year and representative for this forest stand then there is a substantial change of DOC composition during the passage from the subsoil to the well. Microbial decay and sorption/desorption of DOC may occur down to the well since the bedrock is partly weathered up to 30 m depth in this area.

3.5 Conclusion

Combustion of fossil fuels contributed between 5 and 7% to annual throughfall DOC, but the fate of fossil DOC in the soil remains unknown. The temporal variation of the DO^{14}C signature in soil solution beneath the Oa horizon and at 90 cm depth points to different sources within a year and between years. DOC beneath the organic layer originated mainly from the Oa horizon whereas DOC from throughfall, Oi and Oe horizon was quickly decomposed and/or sorbed by the Oa horizon. DOC at 90 cm depth likely

represented a mixture of DOC from the surrounding soil and overlaying soil horizons including the Oa horizon. The seasonal and inter-annual variation of the DO^{14}C signature may be attributed to water movement and downward transport of DOC along preferential flow paths. The shift in the DO^{14}C signature beneath the Oa horizon and at 90 cm depth after the dry summer of 2006 provides evidence for desorption of very old C following re-wetting of dry soil. Alternatively, strong preferential sorption or decay of young DOC could also affect the DO^{14}C signature in the deep mineral soil following summer droughts. In conclusion, repeated measurements over longer time periods are required when DO^{14}C is used to calibrate soil C models. Sorption and desorption of DOC with varying DO^{14}C signatures could alter the ^{14}C signature of particulate organic matter, and thus, challenge our understanding of organic C turnover in forest soils.

3.6. Acknowledgements

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Chapter 4

Leaching losses of inorganic N and DOC following
repeated drying and wetting of a spruce forest soil

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Summary

Forest soils are frequently subjected to dry-wet cycles, but little is known about the effects of repeated drying and wetting and wetting intensity on fluxes of NH_4^+ , NO_3^- and DOC. Here, undisturbed soil columns consisting of organic horizons (O columns) and organic horizons plus mineral soil (O + M columns) from a mature Norway spruce stand at the Fichtelgebirge; Germany, were repeatedly desiccated and subsequently wetted by applying different amounts of water (8, 20 and 50 mm day⁻¹) during the initial wetting phase. The constantly moist controls were not desiccated and received 4 mm day⁻¹ during the entire wetting periods. Cumulative inorganic N fluxes of the control were 12.4 g N m⁻² (O columns) and 11.4 g N m⁻² (O + M columns) over 225 days. Repeated drying and wetting reduced cumulative NH_4^+ and NO_3^- fluxes of the O columns by 47- 60 and 76- 85%, respectively. Increasing NH_4^+ (0.6- 1.1 g N m⁻²) and decreasing NO_3^- fluxes (7.6- 9.6 g N m⁻²) indicate a reduction in net nitrification in the O + M columns. The negative effect of dry-wet cycles was attributed to reduced net N mineralisation during both the desiccation and wetting periods. The soils subjected to dry-wet cycles were considerably drier at the final wetting period, suggesting that hydrophobicity of soil organic matter may persist for weeks or even months. Based on results from this study and from the literature we hypothesise that N mineralisation is mostly constrained by hydrophobicity in spruce forests during the growing season. Wetting intensity did mostly not alter N and DOC concentrations and fluxes. Mean DOC concentrations increased by the treatment from 45 mg l⁻¹ to 61- 77 mg l⁻¹ in the O columns and from 12 mg l⁻¹ to 21- 25 mg l⁻¹ in the O + M columns. Spectroscopic properties of DOC from the O columns markedly differed within each wetting period, pointing to enhanced release of rather easily decomposable substrates in the initial wetting phases and the release of more hardly decomposable substrates in the final wetting phases. Our results suggest a small additional DOC input from organic horizons to the mineral soil owing to drying and wetting.

4.1. Introduction

Climate models have projected changes in the global water cycle as a result of rising surface temperature on the earth (IPCC 2001). It is expected that an increasing frequency of extreme weather periods attend the long-term climatic changes during this cen-

ture. Extended summer droughts and periods of intense precipitation may have dramatic implications not only for the vegetation but also for biogeochemical processes and nutrient fluxes in soils. In the summer of 2003, an exceptional heat wave and rain deficit had a strong impact on plant productivity in many European regions. Ciais et al. (2005) stressed that such extreme drought events could reverse forest ecosystems from carbon sinks to net carbon sources because of stronger reductions in gross primary productivity than in ecosystem respiration. Under less extreme conditions, many plants have a limited ability to sustain their metabolism during drought periods through water uptake from groundwater or moist soil horizons while most soil microorganisms are directly exposed to water stress in the top soil. A reduced nutrient availability and mineralisation of soil organic matter due to water stress may potentially limit plant growth since photosynthetic carbon assimilation of plants is less constrained by drought (Körner 2006).

In addition to the effect on vegetation, drought may trigger changes in microbial activity, physical and chemical soil properties, which can be still effective in terms of mineralisation of soil organic matter and leaching losses during the wetting period. The death or inactivity of microorganisms due to water stress is one reason for low or inhibited N mineralisation in soils during drought (Kieft et al., 1987). Repeated drying may severely reduce the size, activity or composition of microbial community, and thus, the N mineralisation and nitrate losses in sandy soils with low nitrifier population (Franzuebbers et al., 1994). Another reason for low N mineralisation is the decreasing accessibility of organic matter for microorganisms at low water content through hydrophobicity and encapsulation of soil organic matter and adsorption of hydrophobic substances on mineral surfaces (Lützow et al., 2006). These physical effects may persist, particularly in organic soil horizons, for weeks or even months following wetting of dry soil. Water repellent soils resist water infiltration into the soil matrix and can lead to the development of irregular wetting and preferential flow paths or runoff (Dekker and Ritsema 2000). In this context, little attention has been paid to wetting intensity, which is here defined as the amount of added water per day. Both, heavy rainstorms and moderate rainfalls occur after long drought periods and could affect the hydrophobicity of soils as well as the transport of dissolved compounds in soil water.

Wetting of dry soil generally enhances the N mineralisation, but the extent varies with soil properties, soil treatment, intensity and frequency of drying and wetting. Sev-

eral mechanisms have been discussed in order to explain the mineralisation pulse following wetting. Increasing N mineralisation has been attributed to the exposure of easily decomposable compounds from the death of microbial organisms and fine roots, aggregate disruption and desorption of surfaces (Bottner 1985; Deneff et al., 2001; Kieft et al., 1987; Van Gestel et al., 1993). Some microorganisms might be adapted to water stress by production of intercellular solute substrate which can be catabolised and produce a pulse of C and N at increasing soil moisture (Kieft et al., 1987).

This wetting pulse, however, might have been overrated in some studies, considering periods of reduced N mineralisation rates during desiccation. On the contrary, the wetting pulse cannot always compensate for the reduction in N mineralisation during dry conditions as reported for the F layer from a Douglas fir stand (Pulleman and Tietema 1999). Comparisons of continuously moist soils with soils subjected to repeated dry/wet cycles indicate elevated N mineralisation rates in moist soils (Franzuebbers et al., 1994; Fierer and Schimel 2002). In forest stands, simulated summer droughts and subsequent wetting did even not release a NO_3^- pulse, suggesting that nitrification was not severely stimulated by wetting (Tietema et al., 1997). Hence, properties and treatment of soil as well as the experimental design including the conditions during drying and wetting and the definition of a control may largely affect the evaluation of such effects on N mineralisation.

Fluxes of dissolved organic carbon (DOC) are much smaller than the release of CO_2 from soils, but DOC or dissolved organic matter (DOM) may play an important role in many biochemical soil processes and sequestration of organic carbon. Although soil drought is a frequent phenomenon only few studies are available on the effects of drying and wetting on DOC fluxes and properties. Laboratory incubations suggest an increasing DOC production with increasing soil moisture (Christ and David 1996). However, despite lower water fluxes annual DOC flux increased or remained almost constant in the top soil of a Norway spruce forest following wetting of dry soil (Borken et al., 1999). The origin and quality of the additional released DOC was unclear in the study. Lundquist et al. (1999) suggested that increasing DOC concentrations in two agricultural soils subjected to drying and wetting may be partly ascribed to the release of organic substrates from roots. Hence, the release of additional organic substrates as described above might not only affect the fluxes of DOC but also the quality of DOC.

A growing number of laboratory studies on drying and wetting of soils have been published during past years, but many studies used disturbed soil samples, no controls and varying wetting intensities. Little is known about the effect of wetting intensity on soil processes although heavy precipitation irregularly occurs in nature and could affect soil processes and translocation of solute compounds within the soil. The aim of this study was to assess the effect of repeated desiccation and different wetting intensities on NH_4^+ , NO_3^- , total N and DOC fluxes and properties of undisturbed soil columns from a mature Norway spruce forest. In addition to a permanently moist control with 4 mm day^{-1} , three different wetting intensities of 8, 20 and 50 mm day^{-1} were established in order to simulate moderate to extreme precipitation events following soil drought. Two types of soil columns, consisting of organic horizons (O columns) and organic plus mineral soil horizons (O + M columns), were investigated to evaluate the effect of dry–wet cycles on organic layer and mineral soil.

We hypothesised that (1) repeated drying and wetting reduce the NH_4^+ , NO_3^- and total N fluxes, enhance the DOC fluxes and alter the quality of DOC, and that (2) the NH_4^+ , NO_3^- , total N and DOC fluxes decrease with increasing wetting intensity. We expect lower inorganic N and DOC fluxes with increasing wetting intensity at same total amount of added water because of hydrophobic surfaces after desiccation.

4.2. Methods

Undisturbed soil columns were sampled in a 135-year-old Norway spruce forest (*Picea abies*) at the Fichtelgebirge (870 m a.s.l.) in Germany. Mean annual precipitation is about 1.160 mm and mean annual air temperature is 5.3°C (Foken 2003). The forest floor is almost completely covered by ground vegetation, mainly *Deschampsia flexuosa* (L.) Trin. and *Calamagrostis villosa* (Chaix.) J. F. Gmel. The soil has a sandy loam texture and is classified as a Haplic Podsol according to the FAO soil classification (IUSS 2006) with a moder of 6–10 cm thickness consisting of Oi, Oe and Oa horizons. Nine soil pits each of 0.5 m² were quantitatively sampled by horizon for determination of soil properties at this study site (Table 4.1). The C and N contents of the soil decrease with increasing depth from 18 to 0.4 % at -55 cm and from 1.0% to <0.05 % at -55 cm, respectively. The C/N ratio of 18 in the Oa horizon as well as high atmospheric N deposition rates of 27.9 kg N ha⁻¹ a⁻¹ and leaching losses of 15.5 kg N ha⁻¹ a⁻¹ in 2004 (Calle-

sen et al., 2007) indicate N saturation of this forest site. The pH (H₂O) follows a rather small vertical gradient from 4.0 in the Oa horizon to 4.5 in the Bv/Cv horizon. Large amounts of exchangeable Ca and Mg concentrations down to the Bs horizon point to relatively favourable growth conditions for the vegetation. The base saturation decreases from 52 % in the Oa horizon to 12–16 % in the subsoil as a result of lime application. The stand, however, was not directly limed, but received unknown doses of dolomite (CaMgCO₃) in 1994 and 1999 when a helicopter frequently flew over this stand (and lost thereby part of the loading) in order to lime adjacent forest stands. Carbonate was analytically not detectable in samples of the top soil from nine soil pits.

The soil columns were taken in April 2005 using polyacrylic cylinders with a diameter of 17.1 cm and heights of 15 or 30 cm. The cylinders were carefully driven by hand into the soil by gradually removing of the surrounding forest floor and mineral soil to avoid physical disturbances of the soil structure. Two variants, Oi, Oe and Oa horizons (hereafter O columns) and organic plus mineral soil horizons including the Oi, Oe, Oa, Ea, Bsh and Bs horizons (hereafter O+M columns) were used in this experiment. The thickness of the O horizons ranged between 8 and 12 cm and the thickness of the O plus mineral soil horizons ranged between 17 and 23 cm. The soil columns were stored at 5°C at field moisture over 1–2 weeks before the first drying/wetting cycle.

Both, the O and O+M columns were divided into four treatments, i.e. a control and three wetting intensities of 8, 20 and 50 mm, each with four replications (Table 4.2). Three drying/wetting cycles were performed on the treated columns, whereas the controls were not desiccated and remained without irrigation in a cooling chamber at 15°C during the drying intervals. All other soil columns were intensively desiccated at temperatures between 20 and 25°C by ventilation with dry air from top and bottom over 42 days. After each drying phase all O and O+M columns were placed on polyethylene plates or ceramic plates, respectively, and were afterwards stored in the cooling chamber at 15°C some hours before wetting. The controls were wetted with 4 mm day⁻¹ for 25 days, the 8 mm treatment with 8 mm day⁻¹ for 12.5 days, the 20 mm treatment with 20 mm day⁻¹ for 5 days and the 50 mm treatment with 50 mm day⁻¹ for 2 days during the intensive wetting phase. Subsequently, irrigation continued with a rate of 4 mm day⁻¹ for 2 weeks (post irrigation) in all treatments. The soil columns of the 8, 20 and

Table 4.1: Mean chemical properties of soil profiles in the Norway spruce stand at the Fichtelgebirge (n=9).

Horizon	Depth (cm)	pH		C — (%) —	N	C/N	Ca ²⁺	Mg ²⁺	Na ⁺	K ⁺	Al ³⁺	H ⁺	CEC _{eff}	BS (%)
		cH ₂ O	CaCl ₂											
O _a	5	4.0	3.3	18.2	1.0	17.7	94.4	7.6	1.4	3.4	74.1	16.7	206	52
Ah	-5	4.3	3.4	7.4	0.4	19.2	60.9	3.6	1.0	1.9	66.9	15.5	152	44
Bsh	-12	4.3	3.4	5.5	0.3	19.7	70.9	2.3	1.1	1.3	97.6	8.3	190	40
Bs	-18	4.6	3.7	3.4	0.2	20.5	30.1	1.1	0.9	1.0	87.8	3.0	126	26
Bv	-55	4.6	4.1	1.3	0.1	12.3	3.7	0.2	1.1	1.0	41.3	0.6	48	12
Bv/C	< -55	4.5	4.0	0.4	<0.05	8.3	2.7	0.2	3.0	1.1	35.3	0.5	43	16

50 mm treatments remained for 33 days in the cooling chamber and were then repeatedly desiccated for 42 days. All soil columns received 156 mm during each drying/wetting cycle adding in total 468 mm over 225 days.

Table 4.2 Experimental design of each drying/wetting cycle (n=3) for the control, 8mm, 20mm and 50mm treatments with different rewetting intensities.

Parameter	control	8mm	20mm	50mm
Number of drying days	0	42	42	42
Intensive wetting (mm days ⁻¹)	4	8	20	50
Number of intensive wetting days	25	12.5	5	2
Post irrigation (mm days ⁻¹)	4	4	4	4
Number of post irrigation days	14	14	14	14
Added water per cycle (mm)	156	156	156	156
Number of wetting days per cycle	39	26.5	19	16

The irrigation solution was similar to the long-term average of throughfall in the Norway spruce stand at the Fichtelgebirge (Matzner et al., 2004). We applied artificial throughfall with the following components (mg l⁻¹): Na⁺ 0.69, K⁺ 2.8, Ca²⁺ 1.4, Mg²⁺ 0.2, NH₄⁺-N 1.4, NO₃⁻-N 1.4, SO₄²⁻-S 2.3, PO₄³⁻-P 0.06, Cl⁻ 1.4 and a pH of 4.4. The water was applied in 2 mm portions to the soil surface using a fine nozzle during the intensive wetting and post irrigation phase. A negative pressure of -450 mbar was applied for 5 s every hour to the polyethylene plates to collect soil solution from the O columns. A constant negative pressure of -400 mbar was applied to the ceramic plates to collect soil solution from the O+M columns. In the first dry-wet cycle, two percolates were analysed after 50 and 100 mm wetting and four during the post-irrigation phase. In the second and third cycle, two percolates were analysed after 50 and 100 mm wetting and two during the post-irrigation phase. The percolates were completely collected and comprise therefore the mean concentration of each sampling interval, which were defined by the irrigation rate.

The percolates were stored at 2°C and measured for pH and electric conductivity. After filtering within 5 days using cellulose–acetate-filters <0,45 µm (Schleicher and Schuell) at a negative pressure of -450 mbar, the percolates were analysed for dissolved organic carbon (DOC), total nitrogen (tN), ammonium (NH₄⁺-N) and nitrate (NO₃⁻-N). DOC and tN were determined by high temperature analyser (Elementar, high-TOC),

NH_4^+ by flow injection analyser (MLE, FIA-LAB) and NO_3^- by an ion chromatograph (DIONEX, DX500 Chromatography system). Concentration of dissolved organic nitrogen (DON) was calculated as the difference between total N and inorganic N. The fluxes were calculated by multiplying the concentration of DOC, NH_4^+ -N, NO_3^- -N and tN in the percolates with the measured water flux of each columns.

The aromaticity and complexity of DOC from the O columns was estimated by specific UV absorbance at 280 nm (UVIKON 930, BIOTEK Instruments; (Chin et al., 1994)). Further, emission fluorescence (SFM25, BIOTEK Instruments) was measured to calculate the humification indices of DOC (Zsolnay et al., 1999).

All soil columns were finally separated into organic, A and B horizons for determination of gravimetric water content, water holding capacity (WHC), organic C and total N content. Subsamples of 50–100 g were oven dried at 60°C (organic horizons) and 105°C (mineral horizons) over 48 hours to estimate final gravimetric water contents. Weighing of the soil columns during the experiment allowed to estimate the temporal course of total water contents during the drying and wetting phases. Samples of the organic, A and B horizons were homogenised, sieved (2 mm), dried (40°C) and ground to analyse organic C and total N contents using a CNS elemental analyser (Vario EL, elemental). The average C and N amounts were 5.5 kg C m⁻² and 0.3 kg N m⁻² in the O columns and 10.6 kg C m⁻² and 0.5 kg N m⁻² in the O+M columns. C and N amounts were not significantly different among the treatments.

Data were analysed using STATISTICA 6.0. Differences in NH_4^+ , NO_3^- , tN and DOC concentrations between the treatments (control, 8, 20 and 50 mm) were tested using the non-parametric Kruskal-Wallis test because of the skewed distribution of the data. When the Kruskal-Wallis Test was significant at $\alpha=0.05$, the Mann-Whitney U Test was used as a post hoc test to compare gradually the differences between two treatments. According to the Bonferroni-Hochberg method, a procedure for adjustment of multiple independence significance tests, the significance level of the Mann-Whitney U Test was compared and corrected with α/k , $\alpha/k-1$ to $\alpha/k-5$, where k is the number of pairs (k=6) for each parameter. The lowest P value of the Mann-Whitney U Test was adjusted by the lowest adjusted significance level (α/k) and the second lowest P value was compared with the second lowest significance level ($\alpha/k-1$). The Tukey HSD test

was performed to detect differences in cumulative DOC, tN, NH_4^+ and NO_3^- -fluxes (normal distribution) among the treatments, for each cycle and the sum of all cycles.

4.3. Results

Gravimetric water contents

The gravimetric water contents of the controls were relatively constant in the O columns (Fig. 4.1a) with 1.7 g g^{-1} and in the O + M columns (Fig. 4.1b) with 0.8 g g^{-1} throughout the experiment as exemplarily shown for the third dry–wet cycle. Based on day 0, the drought treatment reduced the gravimetric water content in the O columns from about 1.1 to 0.1 g g^{-1} and in the O + M columns from about 0.7 to 0.2 g g^{-1} , indicating that the mineral soil was moderately dry whereas the O horizons were extremely dry. Wetting increased the gravimetric water content of all dry soils, however, the moisture level of the control columns was not reached in the treatments despite the large amount of added water (156 mm). On average, the final gravimetric water contents of the 8, 20 and 50 mm treatments were about 50 and 30% lower than the controls, respectively. In other words, the WHC was severely reduced by the drought treatments by 17- 33 % in the O columns and by 30- 40 % in the O + M columns (Table 4.3).

Concentrations of NH_4^+ -N and NO_3^- - N in soil solution

NH_4^+ concentrations continuously increased from 2- 4 mg N l^{-1} to 7- 12 mg N l^{-1} in all treatments of the O columns during the first cycle (Fig. 4.2). A significant, on average higher NH_4^+ concentration of 15 mg N l^{-1} ($p < 0.05$) was observed in the control during the second and third cycle whereas the 8, 20 and 50 mm treatments showed relatively low concentrations of 6- 8 mg N l^{-1} .

The O + M columns showed an opposite pattern than O columns throughout the 3 cycles (Fig. 4.3). The mean NH_4^+ concentration of 0.1 mg N l^{-1} was significantly lower ($p < 0.05$) in the control compared to the 8, 20 and 50 mm treatments (1.5 - 2.0 mg N l^{-1}) during the first cycle. In the second and third cycle, NH_4^+ concentrations gradually increased in the 8, 20 and 50 mm treatments, but wetting intensity had only a significant effect in the second cycle. Similarly, mean NH_4^+ concentrations of the control increased in the second cycle (0.3 mg N l^{-1}) and third cycle (1.6 mg N l^{-1}).

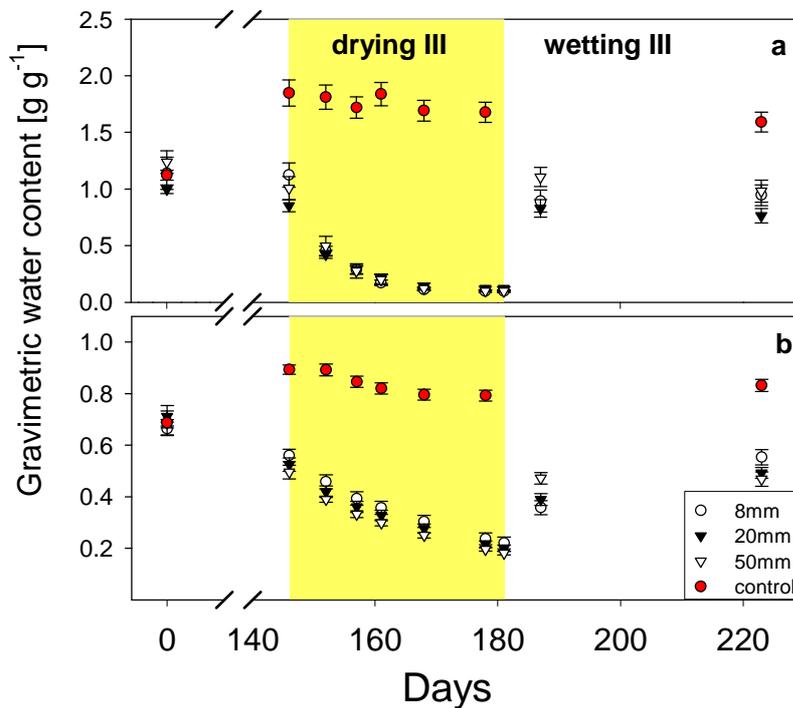


Figure 4.1 Temporal course of the mean gravimetric water contents during the third drying and wetting cycle of a O and b O+M columns. Error bars indicate the standard error of the mean ($n=4$).

In the control of the O columns, mean NO_3^- concentrations markedly increased during the first cycle and remained on a high level (21–30 mg N l^{-1}) during the second and third cycle (Fig. 4.2). Drying affected the NO_3^- concentrations in the 8, 20 and 50 mm treatments of both the O and O+M columns (Figs. 4.2 and 4.3). Mean NO_3^- concentrations of the O columns moderately increased from about 3 mg N l^{-1} (cycle 1) to 4.5–6.0 mg N l^{-1} (cycle 2) and 4.1–7.0 mg N l^{-1} (cycle 3). NO_3^- concentrations of the treatments increased with each drying/wetting cycle in the O+M columns and were generally higher than in the O columns. Wetting intensity had little effect on NO_3^- concentrations; only the 8 mm treatment of the O+M columns (Fig. 4.3) had significantly higher concentrations than the 20 mm (cycle 2 and 3) and 50 mm treatment (cycle 3).

Table 4.3 Mean net fluxes of DOC, DON, NH_4^+ , NO_3^- , total N, cumulative water fluxes and mean water holding capacity (WHC) following three drying/wetting cycles of the O and O+M columns. Potential net fluxes of the mineral soil (M) were calculated from the differences between O and O+M columns. Negative fluxes indicate a net sink of the mineral soil. Within different rewetting intensities, fluxes sharing the same letters are not significantly different at $\alpha=0.05$.

Parameter	O columns				O+M columns				(M)			
	control	8mm	20mm	50mm	control	8mm	20mm	50mm	control	8mm	20mm	50mm
$^1\text{CO}_2$ (g C m ⁻²)	101	94	98	95	151	117	110	112	50	23	12	17
DOC (g C m ⁻²)	18.6 ^a	23.9 ^a	20.7 ^a	25.9 ^a	4.9 ^a	7.6 ^a	6.3 ^a	6.7 ^a	-13.7 ^a	-16.3 ^a	-14.4 ^a	-19.2 ^a
DON (g N m ⁻²)	0.4 ^a	0.3 ^a	0.3 ^a	0.4 ^a	0.1 ^a	0.2 ^a	0.1 ^a	0.2 ^a	-0.3 ^a	-0.1 ^a	-0.2 ^a	-0.3 ^a
Ammonium (g N m ⁻²)	4.9 ^a	2.6 ^b	2.0 ^b	2.0 ^b	0.2 ^a	1.3 ^b	1.0 ^b	0.8 ^b	-4.7 ^a	-1.3 ^b	-1.0 ^b	-1.2 ^b
Nitrate (g N m ⁻²)	7.5 ^a	1.8 ^b	1.1 ^b	1.2 ^b	11.2 ^a	3.6 ^b	1.8 ^c	2.4 ^c	3.7 ^a	1.8 ^b	0.7 ^b	1.2 ^b
Total N (g N m ⁻²)	12.7 ^a	4.8 ^b	3.7 ^b	3.8 ^b	12.0 ^a	5.2 ^b	3.0 ^c	3.4 ^c	-0.7 ^a	0.4 ^a	-0.6 ^a	-0.4 ^a
Total water flux (mm)	411	365	353	365	415	298	304	304				

¹⁾ Cumulative CO₂ fluxes during the three wetting cycles were provided by J. Muhr (personal communication)

Fluxes of NH_4^+ , NO_3^- and total N

Except for NH_4^+ fluxes in the O + M columns, the highest net fluxes of NH_4^+ , NO_3^- and total N were observed in both the O and O + M columns of the control and increased with each cycle (Fig. 4.4). The O + M columns of the 8, 20 and 50 mm treatments had significantly higher NH_4^+ fluxes ($p < 0.05$) in the first and second cycle than the control. The difference was not significant in the third cycle because the NH_4^+ flux strongly increased in the control. Overall, the net NH_4^+ fluxes of the 8, 20 and 50 mm treatments were reduced by 47–60% in the O columns, but were 4 to 6 times higher in the O + M columns respective to the control (Table 4.3).

Under control conditions, the O and O + M columns released large amounts of NO_3^- while the net fluxes were significantly reduced by 76–85% in the O columns and by 68–84% in the O + M columns following drying and wetting. Moreover, the NO_3^- fluxes in the 8, 20 and 50 mm treatments of the O columns were smaller ($1.1\text{--}1.8 \text{ g N m}^{-2}$) than the NH_4^+ fluxes ($2.0\text{--}2.6 \text{ g N m}^{-2}$). Total N fluxes were dominated by NH_4^+ and NO_3^- fluxes whereas dissolved organic nitrogen (DON) was of less importance ($0.2\text{--}0.4 \text{ g N m}^{-2}$) and not affected by the treatments.

Concentrations and fluxes of DOC in soil solution

In the first cycle, DOC concentration of the O columns significantly increased ($p < 0.05$) in the 8 and 50 mm treatment whereas the 20 mm treatment was not significantly different to the control (Fig. 4.2). Mean DOC concentrations of the 8 and 50 mm treatment were 48 and 60% higher than the control (63 mg l^{-1}). A general decline was observed in the second cycle with mean DOC concentration of $63\text{--}66 \text{ mg l}^{-1}$ in the 8, 20 and 50 mm treatments and 44 mg l^{-1} in the control, but the differences were insignificant. In the third cycle, mean DOC concentrations further decreased in all treatments, except the 50 mm treatment, which had a similar concentration as in the second cycle. All treatments were significantly different in the order: 50 mm (71 mg l^{-1}) > 20 mm (49 mg l^{-1}) > 8 mm (43 mg l^{-1}) > control (27 mg l^{-1}).

Drying and wetting had a similar effect on DOC concentrations in the O + M columns as observed for the O columns (Fig. 4.3). The 8, 20 and 50 mm treatments had 60 to 90% higher DOC concentrations ($p < 0.05$) than the control during the first cycle, but wetting intensity had no effect on DOC concentrations. In contrast to the O columns, mean DOC concentrations of all treatments gradually increased during the second and

third cycle. However, the increase was stronger in the 8, 20 and 50 mm treatments than in the control.

Neither drying and wetting nor the wetting intensity had an effect on DOC fluxes in the O and O + M columns throughout the three cycles, except for the 50 mm treatment of the O columns which had 52 to 124 % higher DOC fluxes than the other treatments in the third cycle (Fig. 4.4). Despite higher DOC output rates in the 8, 20 and 50 mm treatments and smaller water fluxes than in the control, the fluxes were not statistically different because of large variation of DOC fluxes within the treatments (Table 4.3).

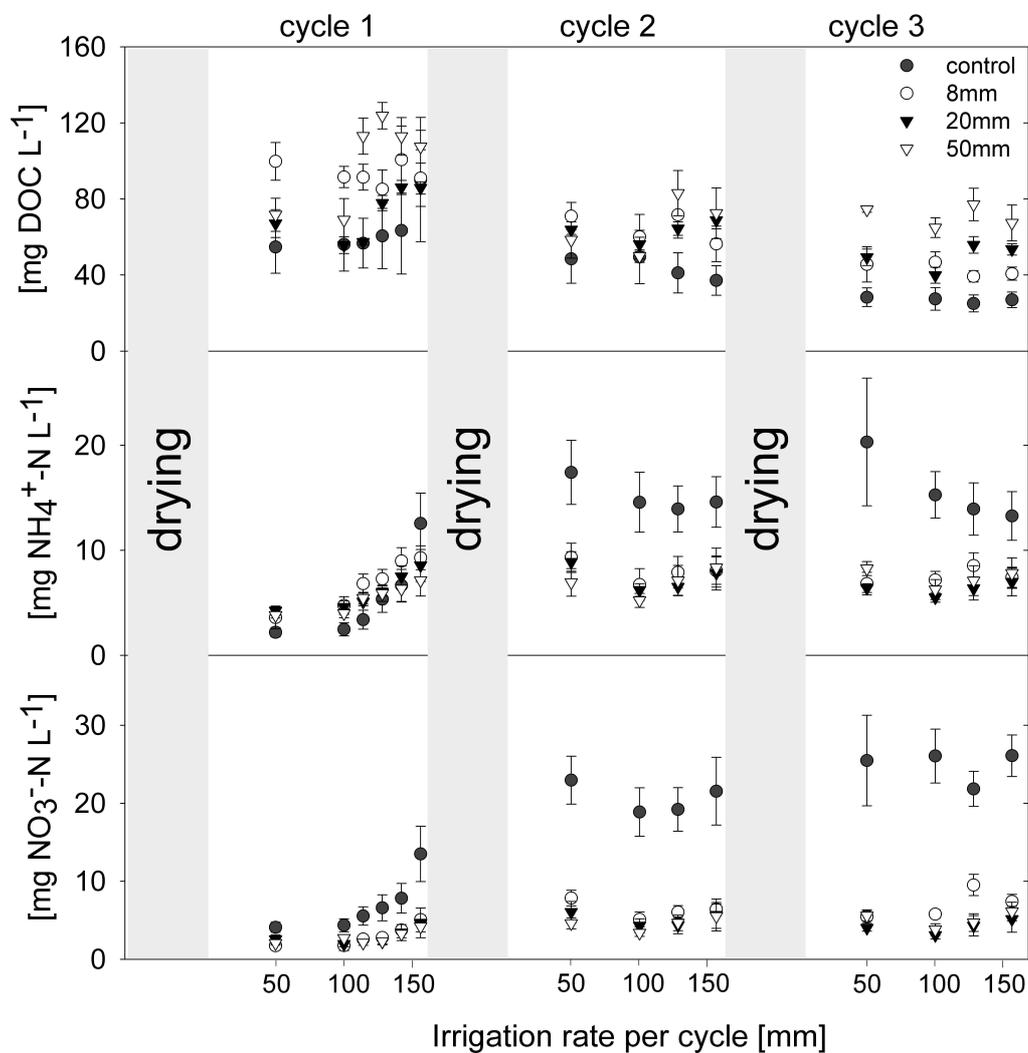


Figure 4.2 Mean concentrations of dissolved organic carbon (DOC), NH_4^+ and NO_3^- in the soil solution of the O columns during three drying/wetting cycles. Error bars indicate the standard error of the mean ($n=4$).

Spectroscopic properties of DOC

Specific UV absorbance of DOC from the O columns significantly decreased from 0.42 (control) to about 0.31 in the 8, 20 and 50 mm treatments after initial wetting in the first cycle (Fig. 4.5). To the end of the first post irrigation a strong increase in specific UV absorbance indicates substantial changes in the aromaticity and complexity of DOC. Similar patterns were observed in the second and third cycle, but the initial differences to the control were smaller as compared with the first cycle. Wetting intensity affected the initial specific UV absorbance of the 8 mm (second and third cycle) and 20 mm treatments (third cycle) while the 50 mm treatment remained constant throughout three cycles.

The initial and final humification index coincided with the specific UV absorbance in the first and second cycle. The largest differences in the humification index between the control and the other treatments were found in the third cycle, indicating an increasing mobilisation of humified substrates by repeated drying and wetting. Overall, spectroscopic analysis revealed an enhanced release of easily available substrates immediately after wetting and an increasing release of less decomposable compounds during the wetting periods.

4.4. Discussion

Concentrations and fluxes of inorganic N

Net N mineralisation was strongly reduced by repeated drying and wetting in the O and O+M columns. We have probably underestimated the net effect on net N mineralisation because the columns were dried 5- 10°C above the incubation temperature of the controls (15°C). The reductions in net N mineralisation occurred in the O horizons while the total N fluxes (Table 4.3) suggest that the mineral soil per se was not affected by the treatments. It is likely that the drying/wetting effect was weaker in the mineral soil than in the O horizons because of incomplete and inhomogeneous desiccation of the mineral soil. A stronger desiccation of the mineral soil could have reduced nitrification whereas ammonification is almost completely located in the organic layer.

In agreement with our study, periodic drying and wetting reduced cumulative N mineralisation of plant material (Franzluebbers et al., 1994) and an agricultural soil (Mikha et al., 2005). A flush or even an unchanged release of NH_4^+ or NO_3^- relative to the

control was not observed during the wetting periods as is described in other studies (e.g., Cabrera 1993; Miller et al., 2005; Seneviratne and Wild 1985; Van Gestel et al., 1993). The reduction in N mineralisation cannot be explained by gaseous N losses via nitrification and denitrification because NO_x and N_2O fluxes also declined as a result of drying and wetting in our experiment (J. Muhr et al., 2008). We attribute the differences in N concentrations and fluxes between the controls and treatments not only to drying and wetting effects but also to the optimised moisture conditions in the controls. A lower water content could have reduced the N mineralisation rate in the controls, and thus, the net effect of drying and wetting.

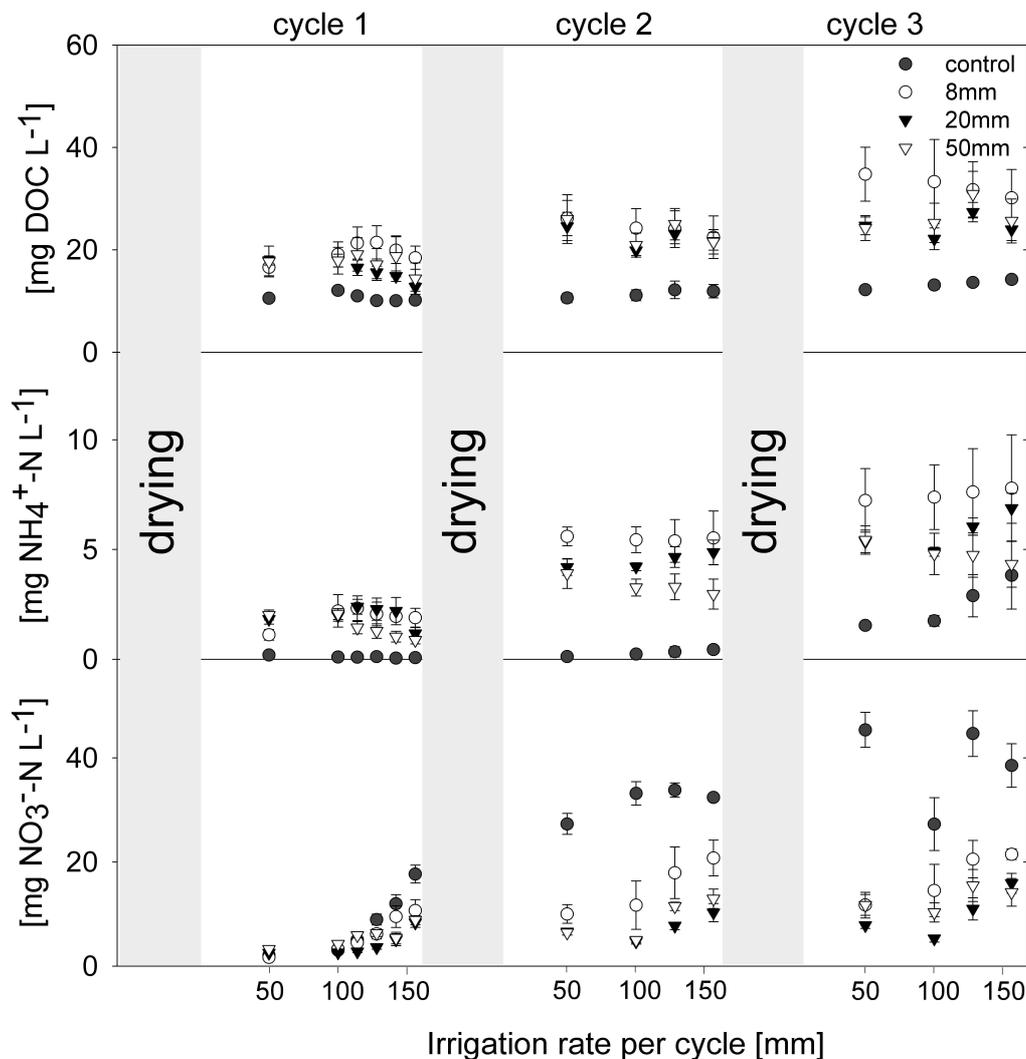


Figure 4.3 Mean concentrations of DOC, NH_4^+ and NO_3^- in the soil solution of the O+M columns during three drying/wetting cycles. Error bars indicate the standard error of the mean (n=4).

We discuss first the results of the O columns and afterwards the O + M columns because (1) organic and mineral soil horizons responded differently to drying and wetting and (2) leaching losses of the O horizons affected concentrations and fluxes of the mineral soil. The continuous increase in NH_4^+ concentrations could be the result of increasing ammonification, decreasing nitrification or immobilisation during the first wetting period in all treatments of the O columns. A small ammonifier population at the beginning of the experiment is likely since the soil columns were taken in early spring. It is well known that ammonification is largely controlled by temperature during growing seasons (Koerselmann et al., 1993).

The rather small differences in NH_4^+ concentrations between the control versus the 8, 20 and 50 mm treatments suggest a small drought effect during the first cycle. It is interesting that the NH_4^+ concentrations remained relatively unchanged during the second and third cycle whereas the control reached a higher concentration level. These elevated NH_4^+ concentrations may have been a result of both continuing growth of the ammonifier population and accumulation of NH_4^+ during the second desiccation period meanwhile the control was constantly moist and not irrigated. Under moist conditions, gross N mineralisation, i.e. ammonification, is positively correlated with C mineralisation in forest soils (Hart et al., 1994). Cumulative CO_2 fluxes of the O columns comprising the three wetting cycles were statistically not different between the treatments and the control (Table 4.3). Provided that gross N mineralisation and C mineralisation also coincided during the wetting periods in the 8, 20 and 50 mm treatments, the decrease in NH_4^+ fluxes could be attributed to increased microbial NH_4^+ immobilisation in the O horizons rather than to increasing gross nitrification. In contrast, decreasing NO_3^- concentrations and fluxes suggest a decrease in gross nitrification, considering that microbial NO_3^- immobilisation was not observed in coniferous forest floor material (Tietema 1992).

We assume that reduced water availability during the wetting periods diminished the net N mineralisation in the O horizons. The mean gravimetric water content of the control (165 %) was not nearly reached at the end of the wetting periods due to hydrophobicity of soil organic matter. Tietema et al. (1992) reported a linear increase in net N mineralisation of coniferous litter with increasing gravimetric water content up to 140 %. If this relationship is transferable to our soils NH_4^+ concentration should have in-

creased in the 8, 20 and 50 mm treatments after overcoming the hydrophobicity. However, our results indicate that hydrophobicity of organic horizons may persist for weeks or even months after wetting.

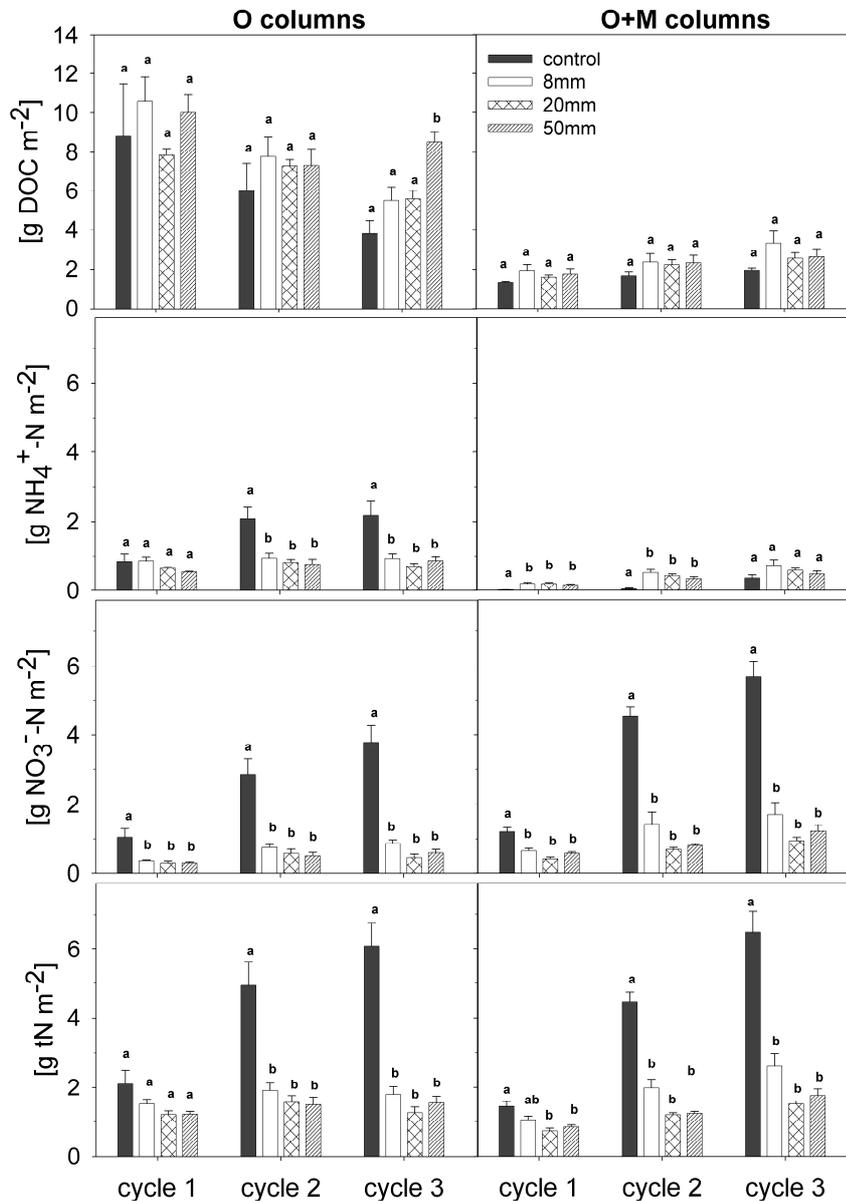


Figure 4.4 Leaching losses of DOC, NH_4^+ , NO_3^- and total N of the O and O+M columns during three drying/wetting cycles. Within each cycle, fluxes sharing the same letter are not significantly different at $\alpha=0.05$.

NO_3^- concentrations in the O columns mainly followed the pattern in NH_4^+ concentrations throughout the three cycles although the differences in NO_3^- concentrations were much larger between the control and the other treatments. Considering the reduced NH_4^+ production, nitrification was apparently also disturbed by desiccation of the O

horizon and reduced water availability because the ratios of NO_3^- to NH_4^+ were much smaller in the 8, 20 and 50 mm treatments (0.6- 0.9) during the second and third cycle as compared to the control (1.3 and 2.0). Nitrification seems to be even more sensitive to drought stress than ammonification (Smolander et al., 2005; Tietema et al., 1992). An explanation might be the slow growth rate of autotrophic nitrifiers while heterotrophic organisms including heterotrophic nitrifiers may respond much faster to wetting.

In all treatments, high NH_4^+ concentrations and fluxes point to incomplete nitrification in the O horizons and to considerable NH_4^+ inputs into the mineral soil. Although large amounts of NH_4^+ entered the mineral soil in the control, NH_4^+ concentrations were extremely low in the percolates of the O + M columns during the first and second cycle. Hence, NH_4^+ was almost completely removed by microbial immobilisation and nitrification. The increase in NH_4^+ concentration in soil solution during the third cycle point to a reduced nitrification rate or to delayed growth of ammonifiers. Ammonification plays generally a minor role in mineral soils due to small amounts of easily decomposable organic matter; however, clipped roots may have slightly increased substrate availability in the soils.

Both elevated NH_4^+ and reduced NO_3^- concentrations indicate disturbance of the nitrifier population in the mineral soil although part of the population survived repeated desiccation. Constant ratios of NO_3^- to NH_4^+ concentrations suggest no increase in the disturbance effect during the three cycles. Differences in NH_4^+ concentrations among the wetting intensities are likely a result of dilution because the concentrations decreased with increasing wetting intensity.

A precondition for elevated N mineralisation following drying and wetting is the mobilisation of available organic N (Appel 1998). The availability of organic N was obviously not enhanced in the O and O + M columns provided that at least a part of these organic N compounds are water-soluble. Fluxes of dissolved organic N (DON), calculated from the difference between total and inorganic N, were not significantly altered by the treatments (Table 4.3).

Concentrations, fluxes and properties of DOC

Cumulative DOC fluxes of both the O and O + M columns did not significantly increase in the treatments (Fig. 4.4, Table 4.3), but because of reduced water fluxes, DOC concentrations were mostly significant higher as compared to the control. A similar result

was reported by Boriken et al. (1999) who found no increase in annual DOC flux at 10 cm mineral soil depth due to a strong reduction in annual water flux owing to drying and wetting of a Norway spruce stand. One year later, however, annual DOC flux significantly increased by 29 % at only slightly reduced water flux. Lundquist et al. (1999) proposed several mechanisms for the increase in DOC of an agricultural soil subjected to frequent drying and wetting. Firstly, degradation of DOC declines because of reduced microbial populations. Secondly, DOC production increases as a result of enhanced turnover of microbial biomass and condensation of microbial products. Thirdly, enhanced release of previously encapsulated organic matter due to disruption of soil structure. The latter mechanism would not explain the increase in DOC concentration in the O horizons and contradicts the preferential sorption of DOC in many mineral soils. Increased disruption of the structure of particular organic matter and of microbial biomass could explain the increase in DOC mobilisation in our experiment.

In the first and second cycle, reduced specific UV absorbance and humification index of DOC in the O columns give a hint that a larger fraction of initially released DOC originated from dead microbial biomass (Zsolnay et al., 1999). It is likely that microorganisms mainly utilised this dead biomass during the initial phase of wetting and that only a small portion was released as DOC. This assumption is supported by slightly increased CO₂ fluxes following wetting of dry soils (Muhr et al., 2008). The preferential consumption of dead biomass and other light components during initial wetting would explain the latter increase in specific UV absorbance and humification index of DOC. In the third cycle, however, increased specific UV absorbance and humification index indicate a general reduction in dead microbial biomass and other easily decomposable compounds. Generally decreasing DOC concentrations and fluxes suggest that this pool was not fully renewed during repeated drying phases and it seems that stronger humified compounds dominated the composition of DOC.

The significant increase in DOC fluxes in the 50 mm treatment of the O columns during the third cycle remains unclear because DOC fluxes of all other treatments including the control gradually declined from cycle to cycle. We can only speculate that a larger portion of DOC was consumed and not leached in the control, 8 and 20 mm treatments. The number of irrigation days was higher in these treatments, suggesting

improved moisture conditions for microbial consumption of DOC between the initial wetting period and the post-irrigation period.

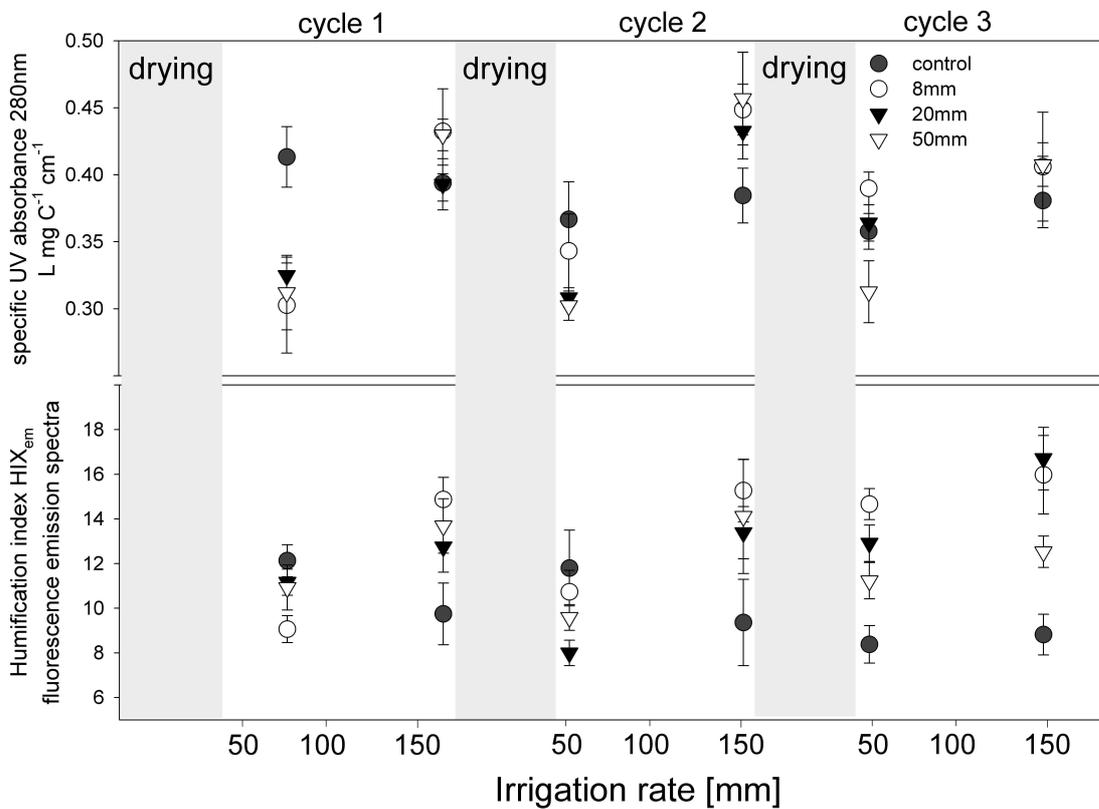


Figure 5.5 Mean specific absorbance at 280nm and humification indices of DOC in soil solution of differently treated O columns after 50mm and 148mm irrigation in three drying/wetting cycles. Error bars indicate the standard error of the mean (n=4).

4.5. Conclusion

Dry-wet cycles reduce net N mineralisation not only due to water stress during the drying phase, but also as a result of incomplete remoistening during the wetting phase. Hydrophobicity of organic layers may reduce N mineralisation and N availability in many temperate forests during growing seasons, which could affect the nutrition of trees. On the other hand, dry-wet cycles could considerably reduce leaching losses of N in N saturated forests. It is still unclear whether the activity or additionally the population of ammonifiers and nitrifiers was severely affected by drought stress since the water content of the controls was never reached in the soils subjected to drying and wetting. We attribute the increase in DOC concentrations not only to microbial turnover but also to physical-chemical processes following drying and wetting. The switch in the release of

relatively easily decomposable DOC during the initial wetting phase to more hardly decomposable DOC during the final wetting phase suggests a small pool of light C substrates that becomes available in the spruce forests soils following drying. In the long run, even small increases in the mobilisation of DOC from O horizons might affect the C storage of mineral soils in spruce forests, considering the sorption capacity for DOC in mineral soil horizons.

4.6. Acknowledgements

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Chapter 5

Repeated freeze-thaw events affect leaching losses of
nitrogen and dissolved organic matter in a forest soil

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Summary

Freezing and thawing may substantially influence the rates of C and N cycling in soils and soil frost was proposed to induce NO_3^- losses with seepage from forest ecosystems. Here we test the hypothesis that freezing/thawing triggers N and dissolved organic matter release from a forest soil after thawing and that low freezing temperatures enhance the effect. Undisturbed soil columns were taken from a soil under a Norway spruce site either comprising only O horizons or O horizons + mineral soil horizons. The columns were subjected to 3 cycles of freezing/thawing at temperatures of -3°C , -8°C , -13°C . The control columns were kept at constant $+5^\circ\text{C}$. Following the frost events, the columns were irrigated for 20 d at a rate of 4 mm d^{-1} . Percolates were analyzed for total N, mineral N and dissolved organic carbon (DOC). The total amount of mineral N extracted from the O horizons in the control amounted to 8.6 g N m^{-2} during the experimental period of 170 d. Frost reduced the amount of mineral N leached from the soil columns with -8°C and -13°C being most effective. In these treatments only 3.1 and 4.0 g N m^{-2} were extracted from the O horizons. Net nitrification was more negatively affected than net ammonification. Severe soil frost increased the release of DOC from the O horizons, but the effect was only observed in the first freeze-thaw cycle. We found no evidence for lysis of microorganisms after soil frost. Our experiment did not confirm the hypothesis that soil frost increases N mineralization after thawing. The total amount of additionally released DOC was rather low in relation to the expected annual fluxes.

5.1. Introduction

Freezing and thawing of soils is a common phenomenon at higher latitudes and in mountainous regions, the frequency and intensity being dependent on regional climate and the thickness of an insulating snow cover. Freezing and thawing may substantially influence the rates of C and N cycling in soils. Increased soil emissions of CO_2 and N_2O after thawing of frozen soil are often reported (van Bochove et al., 2000; Koponen et al., 2004; Ludwig et al., 2006). Reports on frost effects on N mineralization and N solute fluxes are contradicting. DeLuca et al. (1992) found a doubling of N net mineralization in thawed arable soils, similar to the findings of Herrmann and Witter (2002). In case of forest soils, Neilsen et al. (2001) observed an increase of N mineralization in Oa layers while N mineralization was unaffected in the Oe and A horizons. Increased N minerali-

zation as an effect of soil frost was also reported for an arctic soil by Grogan et al. (2004) and for different alpine soils by Freppaz et al. (2007). In contrast, a number of studies in forest or arctic soils reported N mineralization to be unaffected or even lower after soil frost (Groffman et al., 2001; Larsen et al., 2002; Schimel et al., 2004).

Freezing and thawing of soils might also induce the leaching of NO_3^- from the ecosystem. Mitchell et al. (1996) and Watmough et al. (2004) found soil frost to be a driver of high NO_3^- concentrations in small streams. In a German Norway spruce site, NO_3^- fluxes with seepage increased after an exceptional soil frost in the following year (Callesen et al., 2007). An increase of soil solution N following soil frost was also substantial in other studies (Boutin and Robitaille, 1995; Fitzhugh et al., 2001).

The freezing temperature should have an effect on the C and N turnover after thawing since the physical disruption of aggregates should increase, while the amount of remaining unfrozen water decreases (Edwards and Cresser, 1992). Furthermore the microbial necromass might also increase with decreasing freezing temperatures (Herrmann and Witter, 2002) providing easily available substrate after thawing.

The availability of substrate for microorganisms might limit the effects of freezing and thawing, if the latter occurs at higher frequency. The observed effects of frost on C and N mineralization were often highest in the first cycles and decreased in later ones as shown for arable soils by Herrmann and Witter (2002). Similar findings were reported from tundra (Schimel and Clein, 1996) and forest soils (Kurganova and Tipe, 2003; Goldberg et al., in preparation).

Fluxes of dissolved organic carbon (DOC) with soil solutions are generally much smaller than the release of CO_2 from soils, but DOC plays an important role in many soil processes and might be involved in the burst of N_2O emissions observed after thawing (Sehy et al., 2004) as well as in the sequestration of soil organic carbon (Kalbitz and Kaiser, submitted). While a large body of literature on DOC has evolved in the past years, effects of soil frost on DOC concentrations and fluxes have not been reported yet.

The objectives of this study were (1) to determine the effects of repeated freeze-thaw events on NH_4^+ , NO_3^- , dissolved organic N (DON), and DOC concentrations and fluxes in a forest soil and (2) to compare the effects of different frost temperatures. We hypothesized that freezing/thawing (a) triggers N mineralization and DOC release after thawing and (b) that low freezing temperatures enhance the effect.

5.2. Methods

Undisturbed soil columns were taken in a 135 y-old Norway spruce (*Picea abies*) forest at the Fichtelgebirge (770 m asl.) in Germany. Mean annual precipitation is approx. 1160 mm and mean annual air temperature is 5.3°C (Foken, 2003). The soil has a sandy to loamy texture and is classified as Haplic Podzol according to the FAO soil classification (IUSS and WRB, 2006) with a well stratified mor-like forest floor of 6-10 cm thickness.

Table 5.1 Mean chemical properties of nine soil profiles in the Norway spruce stand at the Fichtelgebirge.

	Depth (cm)	pH		C — (g kg ⁻¹) —	N	C/N	CEC _{eff} (mmol _c kg ⁻¹)	BS %
		H ₂ O	CaCl ₂					
Oa	5	4.0	3.3	182	10.3	17.7	197	54
EA	-5	4.3	3.4	74.1	3.85	19.2	145	47
Bsh	-12	4.3	3.4	54.9	2.79	19.7	185	41
Bs	-18	4.6	3.7	34.0	1.66	20.5	124	27
Bv	-55	4.6	4.1	12.7	1.03	12.3	48	12
Bv/C		4.5	4.0	4.03	0.48	8.3	43	16

CEC_{eff} = effective cation- exchange capacity ; BS = base saturation

For determination of soil properties, nine soil pits at the site were sampled by horizon. The C and N contents of the soil decrease with increasing depth from 18% in the Oa horizon to 0.4% in the Bv horizon and from 1.0% N to <0.05% N, respectively (Table 5.1). The pH (H₂O) ranges from 4.0 in the Oa horizon to 4.5 in the Bv horizon. The base saturation decreases from 54% in the Oa horizon to 12-16% in the subsoil indicating past lime applications to the surface during forest management.

The soil columns were taken in April 2005 using polyacrylic cylinders with a diameter of 17.1 cm. The cylinders were carefully driven by hand into the soil by gradually removing the surrounding forest floor and mineral soil to avoid physical disturbances of the soil structure. Two types of soil columns, one including Oi, Oe and Oa horizons (hereafter called O columns) and the other including organic horizons plus mineral soil (Oi, Oe, Oa, EA, Bsh and Bs horizons, hereafter called O+M columns) were used in this

experiment. In total 32 columns were taken and stored at 5°C for six weeks up to the beginning of the experiment. The soil water content during the experiments was close to field capacity, which is 39 vol % for the O horizon and approx. 50 vol % for the mineral soil.

The soil columns were subjected to four treatments, each with four replicates: frost at -3°C, -8°C and -13°C and a control treatment kept permanently at +5°C. The soil columns were frozen for 14 d in the freezer. After freezing, all columns were placed on polyethylene plates or ceramic plates, respectively. After seven d of thawing at +5°C, all columns were irrigated with 4 mm d⁻¹ for 20 d. With this irrigation rate the water filled pore space at field capacity was percolated approx. 2 times (O columns) and once (O+M columns) after each cycle. Percolates were sampled every 4-5 d. In total, three freeze-thaw cycles were performed. After irrigation and prior to the next freezing, the columns were kept for 20 d at +5°C without further irrigation and without collecting percolates. The irrigation solution was similar to the long-term average throughfall in the Norway spruce stand at the Fichtelgebirge (Matzner et al., 2004). A negative pressure of -450 mbar was applied for 5 s every hour to the polyethylene plates to collect soil solution from the O columns. A constant negative pressure of -400 mbar was applied to the ceramic plates to collect soil solution from the O+M columns.

After filtration within 5 d using cellulose-acetate filters <0.45µm (Schleicher and Schuell), the percolates were analysed for DOC, total nitrogen (tN), ammonium (NH₄⁺-N) and nitrate (NO₃⁻-N). Dissolved organic C and N were determined by high temperature combustion and subsequent determination of CO₂ and NO_x (Elementar, high-TOC), NH₄⁺ by flow injection analyzer (MLE, FIA-LAB) and NO₃⁻ by an ion chromatograph (DIONEX, DX500 Chromatography system). Dissolved organic nitrogen (DON) was calculated as difference between total N and mineral N. The fluxes were calculated by multiplying the concentrations in the percolates with the measured volume of the percolates for each column.

The aromaticity of DOC was estimated by specific UV absorbance at 280 nm (UVIKON 930, BIOTEK Instruments; (Chin et al., 1994)). Furthermore, emission fluorescence (SFM25, BIOTEK Instruments) was measured to calculate humification indices of DOC (Zsolnay et al., 1999).

Data were analysed using STATISTICA 6.0. Differences between the treatments were tested using the non-parametric Kruskal-Wallis test. When the Kruskal-Wallis Test was significant at $\alpha=0.05$, the Mann-Whitney U-Test was used as a post-hoc test. According to the sequential Bonferroni Method, the significance level was adjusted by α/k , $\alpha/k - 1$ to $\alpha/k - 5$, where k is the number of pairs ($n=6$) for each parameter. The lowest P value of the Mann-Whitney U-Test was compared with the lowest adjusted significance level (α/k) and the second lowest P value was compared with the second lowest significance level ($\alpha/k - 1$). The Tukey HSD test was performed to detect differences in cumulative DOC, DON, NH_4^+ and NO_3^- fluxes among the treatments, for each cycle and the sum of all cycles.

5.3. Results

Concentrations in percolates

The highest NH_4^+ concentrations in percolates from the O columns, comprising only the O layers, were observed in the control treatments. The concentrations there ranged from 12 to 20 mg $\text{NH}_4^+\text{-N l}^{-1}$ and were highest after the 3rd cycle (Table 5.2). In the frost treatments the concentrations were always lower than in the control, especially in the 2nd and 3rd cycle. Frost intensity had no effect on the NH_4^+ concentrations with the exception of the 3rd cycle where the -3°C treatment exceeded the -8°C and -13°C treatment. Concentrations of NO_3^- in the percolates from the O columns in general exceeded those of NH_4^+ . Similar to NH_4^+ , the NO_3^- concentrations were always highest in the controls. In contrast to NH_4^+ , there was a clear effect of frost intensity on NO_3^- concentrations: The -8°C and -13°C treatment caused the lowest NO_3^- concentrations in all three cycles.

The concentrations of DON were much less than those of mineral N (Table 5.2). No frost effect on DON release from the O layer was observed. The frost treatment substantially increased the concentrations of DOC in the percolates of the O columns in the 1st cycle in the -8°C and -13°C treatment. There, average concentrations were between 63 and 77 mg C l^{-1} while in the control and -3°C treatment, the range was 35-47 mg C l^{-1} . The frost effect disappeared in the 2nd and 3rd cycle where the differences between treatments were no longer statistically significant. The general increase of the UV absorption and humification indices of DOC from the 1st to the 4th sampling after the

frost periods indicated the release of less humified material at the beginning of the irrigation (Fig. 5.1) in all treatments. The spectroscopic properties of the DOC released from the frozen O columns were similar to the control or even had higher proportion of humified substances.

The concentrations of NH_4^+ in the percolates from the O+M columns, comprising O layers and mineral soil, were substantially lower than in the O columns. The concentrations range was from 0.2 to 4 mg l⁻¹ on average (Table 5.2). In contrast to the O columns, the frost increased the concentrations of NH_4 relative to the control. In the controls, the NH_4^+ concentrations decreased to near zero in the 2nd and 3rd cycle while concentrations tended to increase in the 2nd and 3rd cycle under frost influence. The frost effect was most obvious in the -8°C treatment. The concentrations of NO_3^- in the percolates of the O+M columns were in general comparable to those found in percolates from the O columns and exceeded the NH_4^+ concentrations by far (Table 5.2). The NO_3^- concentrations in the 2nd and 3rd cycle were generally higher than in the 1st cycle, and those of the control increased strongest. Like in the O columns, the NO_3^- concentrations of the frost treatments were generally lower than in the control, but the effect was less pronounced in comparison to the O columns. Nitrate concentrations in the percolates from the O-M columns were always lowest in the -13°C treatment. Like in the O columns, the -8°C and -13°C treatment resulted in higher DOC concentrations in the 1st and 2nd cycle, the latter being statistically significant. As expected, the concentration range of DOC (12-23 mg C l⁻¹) was much lower than in the percolates from the O columns.

Fluxes

The fluxes of N from the O columns during the experiment increased with time in the control from about 0.7 g NH_4^+ m⁻² and 1.4 g NO_3^- m⁻² after the 1st cycle to 1.5 g NH_4^+ m⁻² and 1.9 g NO_3^- m⁻² after the 3rd cycle (Fig. 5.2). With regard to frost effects, the fluxes largely reflect the picture evident from the concentrations. The NH_4^+ fluxes from the O columns were highest in the control and frost significantly reduced the fluxes in all three cycles. The same is true for NO_3^- , but here the -8°C and -13°C treatments caused a more negative effect than the -3°C treatment. The DON fluxes were relatively low and didn't exceed fluxes of 0.2 g N m⁻². After the 1st cycle, DOC fluxes from the O

Table 5.2 Average concentrations of NH_4^+ , NO_3^- , DON and DOC in the percolates of the O and O+M columns during three frost/thawing cycles. Different letters indicate significant differences at $\alpha=0.05$.

O columns												
mg l ⁻¹	Cycle 1				Cycle 2				Cycle 3			
	control	-3°C	-8°C	-13°C	control	-3°C	-8°C	-13°C	control	-3°C	-8°C	-13°C
DOC	46.8 ^a	34.8 ^b	77.3 ^c	63.5 ^c	53.8 ^a	42.4 ^a	60.8 ^a	50.7 ^a	51.8 ^{ab}	47.2 ^a	40.8 ^b	67.7 ^c
DON	1.9 ^{ac}	0.3 ^a	2.5 ^b	1.1 ^c	0.5 ^a	0.8 ^a	2.3 ^a	1.3 ^a	1.2 ^a	1.6 ^a	0.9 ^a	2.2 ^a
NH ₄ -N	11.8 ^a	7.6 ^b	8.1 ^b	9.1 ^a	16.8 ^a	11.0 ^b	8.5 ^c	9.0 ^c	20.4 ^a	13.9 ^b	8.5 ^c	11.0 ^d
NO ₃ -N	22.0 ^a	15.3 ^b	6.4 ^c	7.6 ^c	22.2 ^a	15.3 ^b	7.0 ^c	7.4 ^c	26.0 ^a	17.4 ^b	6.8 ^c	9.8 ^d
O+M columns												
mg l ⁻¹	Cycle 1				Cycle 2				Cycle 3			
	control	-3°C	-8°C	-13°C	control	-3°C	-8°C	-13°C	control	-3°C	-8°C	-13°C
DOC	13.0 ^a	12.7 ^a	19.0 ^b	16.2 ^b	16.2 ^a	17.8 ^{ab}	22.9 ^b	22.2 ^b	12.3 ^a	12.6 ^a	16.5 ^b	13.3 ^a
DON	0.7 ^{ab}	0.2 ^a	0.2 ^a	1.1 ^b	0.0 ^a	0.4 ^b	0.3 ^b	1.1 ^c	2.1 ^a	0.6 ^b	1.9 ^{ab}	1.9 ^a
NH ₄ -N	0.5 ^a	0.3 ^a	1.3 ^b	0.5 ^a	0.2 ^a	1.0 ^b	3.0 ^c	0.8 ^c	0.0 ^a	1.8 ^b	4.3 ^c	1.0 ^b
NO ₃ -N	8.3 ^a	7.1 ^{ab}	8.0 ^a	5.1 ^b	19.0 ^{ac}	13.3 ^b	18.0 ^c	12.0 ^c	22.4 ^a	16.0 ^b	16.3 ^b	14.2 ^b

columns were enlarged by about 1 to 2 g C m⁻² in the -8°C and -13°C treatment relative to the control and the -3°C treatment. The effect disappeared in the later cycles.

Fluxes of NH₄⁺ with percolates from the O+M columns were much lower as compared to the O columns. Most of the mineral N in percolates was in the form of NO₃⁻. Here, the frost caused a decrease of NO₃⁻ fluxes in the 2nd and 3rd cycle, independent of the frost temperature. Fluxes of DON from the O+M columns were not affected by the frost treatment, only in the 2nd cycle of the -13°C treatment, the DON fluxes exceeded those of the controls. DOC fluxes from the O+M columns were not affected by the frost treatment.

The net fluxes of mineral N (percolate flux - irrigation input) might be taken as a rough measure of net N mineralization. In case of the O columns the net fluxes over the three cycles reached 8.8 g m⁻² in the control, whereas in the frost treatments only 3.5-5.6 g N m⁻² were released from the columns (Table 5.3). The negative frost effect was obvious for both NO₃ and NH₄. Severe frost temperatures (-8°C and -13°C) had a stronger significant effect on NO₃⁻ and NH₄⁺ compared to mild temperatures (-3°C).

The observed frost effect on DOC fluxes in the 1st cycle was levelled out in the following cycles. The calculated net fluxes of the mineral soil (M in Table 5.3) revealed a strong retention of NH₄⁺ and NO₃⁻ in the mineral soil, the retention being highest in the control, which is mainly due to the lower inputs of N from the O layer in the frost treatments. The retention of DOC in the mineral soil was not influenced by frost treatments.

4. Discussion

The N net mineralization rates in the O columns, indicated by the net fluxes of total N, were rather high under control conditions. In total, 8.8 g N m⁻² were released from the O columns during the experimental period of 170 d. Given the low incubation temperature of +5°C this is somewhat surprising but emphasizes the relevance of N turnover at lower temperatures (Campbell et al., 2005) and the large potential for N mineralization in our soil at high water content.

Our initial hypothesis that soil frost induces higher rates of N mineralization after thawing was rejected by the experimental findings. In fact, N mineralization was always lower after soil frost as compared to the unfrozen control, confirming results from Lar-

sen et al. (2002), but contradicting studies with forest soils that observed an increase of mineralization (Nielsen et al., 2001; Grogan et al., 2004; Freppaz et al., 2007). Groffman et al. (2001) reported in situ N mineralization to be unaffected in a birch and a maple site after snow manipulation and moderate soil frost.

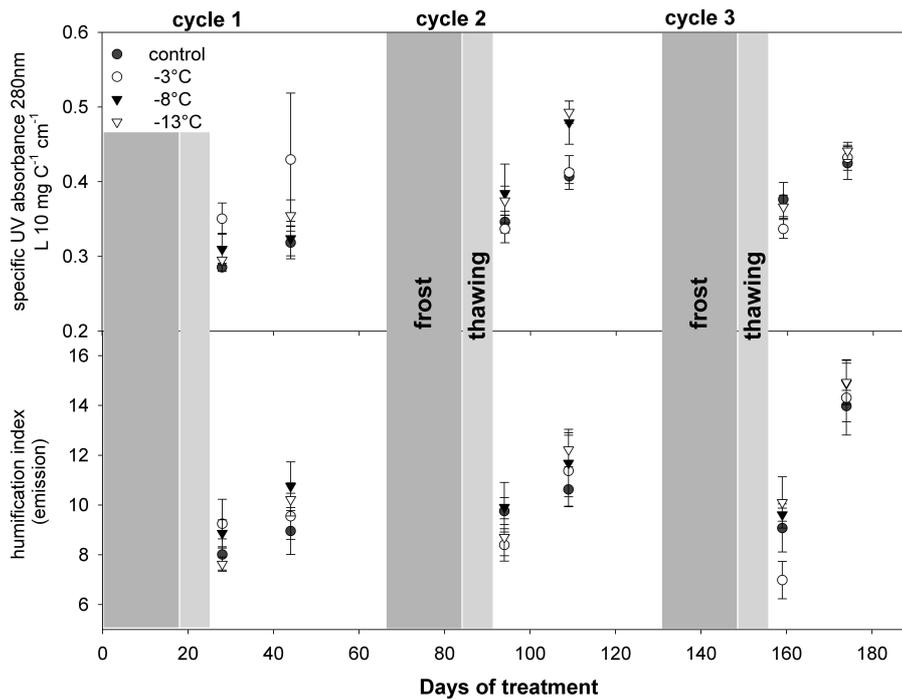


Figure 5.1 Mean specific absorbance at 280 nm and humification indices of DOC in soil solution of differently treated O columns after 20 mm and 80 mm irrigation in three freeze-thawing cycles. Error bars indicate the standard error of the mean (n=4).

There seems to be no general response of forest soils to frost events with respect to N mineralization. Different findings could be related to the mechanisms that explain higher rates of N mineralization after soil frost (Matzner and Borken, 2008) or to the experimental conditions (Henry, 2007). Mechanisms are hard to separate experimentally and have not been investigated in detail in most studies on soil frost effects. The degree of aggregation of particulate soil organic matter, providing occluded structures (Helfrich et al., 2006), differs at different sites. The frost might change the substrate availability by destruction of aggregates. Furthermore, the water content of the soil at freezing influences frost effects on aggregation (van Bochove et al., 2000) and on N turnover (Öquist et al. 2004) and might explain contradictions in the literature.

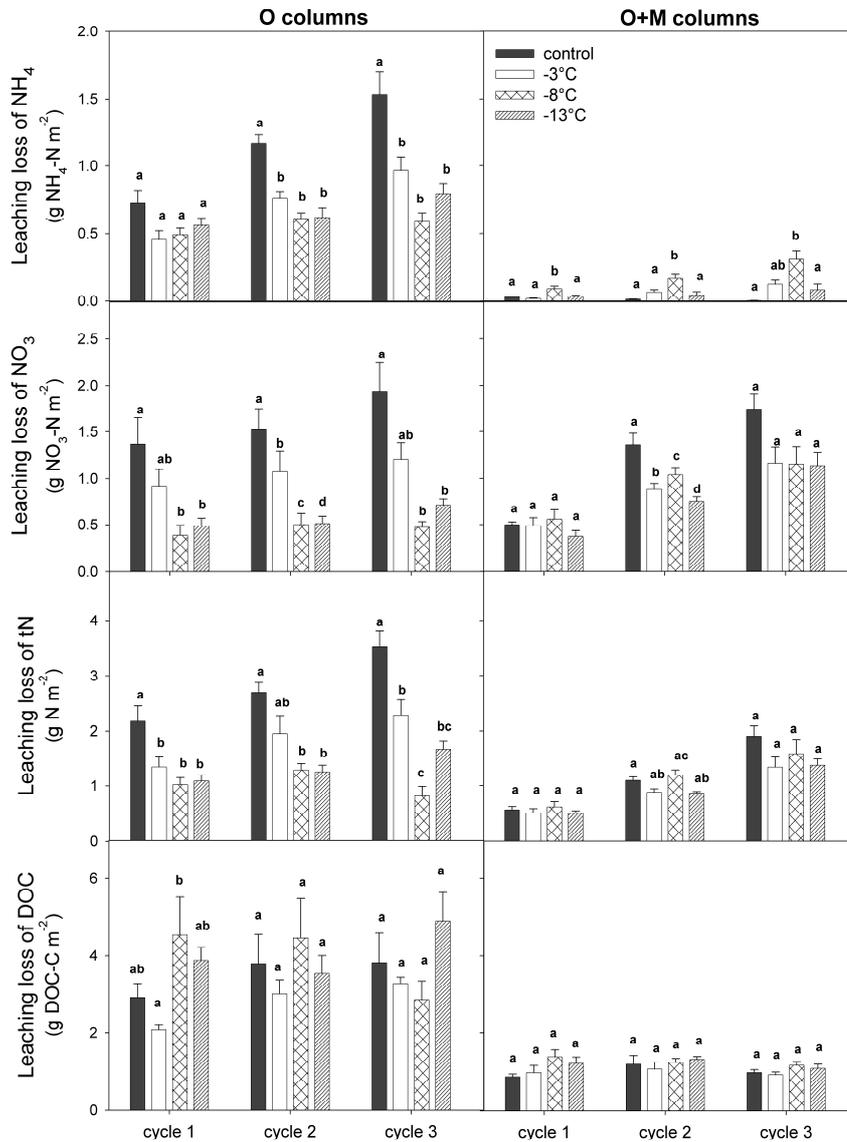


Figure 5.2 Leaching losses of NH₄⁺, NO₃⁻, DON and DOC of the O and O+M columns during three frosts/thawing cycles. Within each cycle, fluxes sharing the same letter are not significantly different ($\alpha=0.05$).

Since the microbial populations shift during the seasons the time of soil sampling might influence the sensitivity of soil processes to frost in experiments (Henry, 2007). In the control treatments, N mineralization occurred during the whole period of 170 d. The columns subjected to frost were frozen in total for 60 d and the soil temperatures were rather low in the first d of thawing. Thus, the rate of N mineralization in the frozen columns should be about 30 – 40 % less than under control conditions, if no other factors than temperature play a role and if N mineralization under frozen conditions is negligi-

ble. In fact, in the -3°C treatment, the total release of mineral N from the O columns was about 30 % less than the control (Table 5.2), while the release of N from the -8°C and -13°C columns was 53 % to 64 % less than the control. In an arctic soil, Schimel et al. (2004) found lower N mineralization rates after severe soil frost (-20°C) compared to moderate soil frost (-5°C), indicating damage to the microbial community at low temperatures, which seems also the case in our columns at low freezing temperatures.

Our second hypothesis, that decreasing frost temperatures enhance N mineralization was also rejected. At -8°C and -13°C , the net nitrification rate in the O horizon was obviously more negatively influenced than net ammonification, indicating a higher sensitivity of nitrifiers to severe frost. From long-term records of solute fluxes in a Norway spruce stand adjacent to the site used in our experiment, Callesen et al. (2007) reported increasing NO_3^- fluxes in the year following an exceptional soil frost (frost depth >35 cm soil depth). The findings from our laboratory experiments suggest that increased N mineralization after frost is rather unlikely to explain these NO_3^- losses. However, we also have to consider that our laboratory experiment only covered the initial response of the soil to frost cycles since the fluxes after the 3rd cycle were measured only for about 3 weeks and we cannot conclude on effects beyond this time period.

Dissolved organic N constituted only a small proportion of the total N (3- 11 %) in our percolates. The effects of freeze-thaw cycles on DON were also rather low and restricted to the mineral soil. Freppaz et al. (2007) found in alpine soils that DON replaced NO_3^- as a mobile form of N after freeze-thaw cycles, contradicting to our results. They used soil extract rather than percolates from undisturbed soil columns and the results are not fully comparable.

In case of DOC, both of our hypotheses were at least partly confirmed. In the 1st cycle, the -8°C and -13°C treatment caused an additional release of 1- 2 g C m^{-2} from the O columns in relation to the -3°C treatment and the control. While this was a strong single effect, its relevance for the DOC fluxes at a longer time scale remains low. The average annual DOC flux from the O layer to the mineral soil under temperate forest vegetation is in the range of 10- 40 g m^{-2} (Michalzik and Matzner, 1999). The additional DOC available after a freeze-thaw event might, however, be relevant for the burst of

Table 5.3: Cumulative mean net release of DOC, DON, NH_4^+ , NO_3^- and total N following three freeze-thawing cycles of the O and O+M columns. Potential net fluxes of the mineral soil (M) were calculated from the differences between O and O+M columns. Negative fluxes indicate a net sink of the mineral soil. Different letters indicate significant differences at $\alpha=0.05$.

g/m ²	O columns				O+M columns				(M)			
	control	-3°C	-8°C	-13°C	control	-3°C	-8°C	-13°C	control	-3°C	-8°C	-13°C
DOC	11.0 ^a	8.7 ^a	12.3 ^a	12.8 ^a	3.2 ^a	3.1 ^a	3.9 ^a	3.7 ^a	-7.8 ^a	-5.6 ^a	-8.4 ^a	-8.9 ^a
DON	0.3 ^a	0.2 ^a	0.4 ^a	0.3 ^a	0.2 ^{ab}	0.1 ^a	0.2 ^{ab}	0.3 ^b	0.1 ^a	-0.1 ^a	-0.2 ^a	-0.0 ^a
$\text{NH}_4\text{-N}$	3.5 ^a	2.2 ^b	1.7 ^c	2.0 ^{bc}	0.0 ^a	0.1 ^a	0.5 ^a	0.1 ^a	-3.5 ^a	-2.1 ^b	-1.2 ^c	-1.9 ^{bc}
$\text{NO}_3\text{-N}$	5.0 ^a	3.2 ^b	1.4 ^c	1.7 ^{bc}	3.8 ^a	2.6 ^a	2.8 ^a	2.3 ^a	-1.2 ^a	-0.7 ^a	1.4 ^a	0.6 ^a

CO₂ and N₂O emissions often observed after thawing of frozen soils (Sehy et al., 2004; Teepe and Ludwig, 2004; Morkved et al., 2006). The CO₂ emissions from the soil columns following the 1st freeze-thaw cycle were significantly higher for the -8°C and -13°C treatment as compared to the 2nd and 3rd cycle which might be partly due to the high availability of DOC after the severe frost (Goldberg et al., 2008)

The spectroscopic properties of the additionally released DOC in the -8°C and -13°C treatments remained unchanged in relation to other treatments or even point to more humified substances comprising the DOC. This is confirmed by higher proportions of lignin derived phenols in the DOC at -8°C and -13°C (Schmitt et al. 2008). The spectroscopic properties thus do not confirm the hypothesis that lysis of microbial biomass induced by frost was the major source of extra DOC in the O column percolates after thawing. Also low frost temperatures did not significantly influence the DOC/DON ratios of the O column percolates. The lysis of microorganisms after frost was also excluded by Grogan et al. (2004) and Lipson and Monson (1998) from studies on alpine and tundra soils. Thus, the additional DOC seems to result from frost effects on aggregates, exposing new surfaces to percolating water (van Bochove et al., 2000). This also explains why the effect only occurred in the 1st cycle.

5.5. Conclusion

In conclusion, our experiments did not confirm the hypothesis that soil frost increases N mineralization after thawing. On the contrary, N mineralization was reduced by the freeze-thaw cycles. Severe soil frost increased the release of DOC from the O horizon only in the 1st freeze-thaw cycle. The total amount of additionally released DOC was rather low in relation to the annual C fluxes, but may well influence other short term soil responses to frost like e.g. denitrification and soil respiration.

5.6. Acknowledgements

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Chapter 6

Effects of soil frost on nitrogen net mineralization, soil
solution chemistry and seepage losses in a temperate forest
soil

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Summary

Freezing and thawing may alter element turnover and solute fluxes in soils by changing physical and biological soil properties. We simulated soil frost in replicated snow removal plots in a mountainous Norway spruce stand in the Fichtelgebirge area, Germany, and investigated N net mineralization, solute concentrations and fluxes of dissolved organic carbon (DOC) and of mineral ions (NH_4^+ , NO_3^- , Na^+ , K^+ , Ca^{2+} , Mg^{2+}). At the snow removal plots the minimum soil temperature was -5°C at 5 cm depth, while the control plots were covered by snow and experienced no soil frost. The soil frost lasted for about 3 months and penetrated the soil to about 15 cm depth. In the 3 months after thawing, the in situ N net mineralization in the forest floor and upper mineral soil was not affected by soil frost. In late summer, NO_3^- concentrations increased in forest floor percolates and soil solutions at 20 cm soil depth in the snow removal plots relative to the control. The increase lasted for about 2- 4 months at a time of low seepage water fluxes. Soil frost did not affect DOC concentrations and radiocarbon signatures of DOC. No specific frost effect was observed for K^+ , Ca^{2+} and Mg^{2+} in soil solutions, however, the Na^+ concentrations in the upper mineral soil increased. In the 12 months following snowmelt, the solute fluxes of N, DOC, and mineral ions were not influenced by the previous soil frost at any depth. Our experiment did not support the hypothesis that moderate soil frost triggers solute losses of N, DOC, and mineral ions from temperate forest soils.

6.1. Introduction

The frequency and intensity of soil frost may change in the future due to global warming and by changes in the occurrence of insulating snow covers that prevent soil frost (IPCC, 2007). Freezing and thawing of soils might influence the rates of C and N turnover in soils, the effect being partly dependent on soil type, frost intensity and the frequency of freezing/thawing cycles as recently reviewed by Matzner and Borken (2008). Several studies have shown that N net mineralization at low soil temperatures in the winter period is not negligible at the annual time scale (Grogan et al., 2004; Campbell et al., 2005; Kielland et al., 2006; Monson et al., 2006; Miller et al., 2007). Nitrogen net mineralization often increased after thawing of frozen arable soils (De Luca et al., 1992; e.g.: van Bochove et al., 2000; Herrmann and Witter, 2002; Koponen et al., 2004). Ob-

servations are contradicting for soils under natural vegetation. Neilsen et al. (2001) reported an increase of N mineralization after thawing in the Oa horizon of a forest soil, while no effect was found in other soil horizons. Nitrogen net mineralization increased after frost in different alpine forest soils (Freppaz et al., 2007) and in an arctic soil (Grogan et al., 2004). In contrast, a number of studies in forest or arctic soils reported N mineralization to be unaffected or even lower after soil frost (Groffman et al., 2001; Larsen et al., 2002; Schimel et al., 2004; Hentschel et al., 2008). The reasons for the contradicting observations still need to be resolved. Methodical differences between the studies, e.g.: field vs. laboratory studies, different frost temperatures, sampling dates of soil, etc. seem to be involved (Henry, 2007).

Soil frost may contribute to the interannual variation of NO_3^- leaching with seepage and runoff in forest ecosystems. Mitchell et al. (1996) concluded that an increase of nitrate fluxes (by about $1.4\text{--}2.8 \text{ kg NO}_3^- \text{-N ha}^{-1} \text{ yr}^{-1}$) in runoff was due to an unusually cold preceding winter. The intensity of soil frost also explained the interannual variation of NO_3^- concentrations in Hubbard Brook streams. However, the relation of frost to NO_3^- fluxes was weak, the latter being mainly dominated by hydrological conditions (Fitzhugh et al., 2003). In a study on 16 Canadian watersheds, Watmough et al. (2004) found soil frost to be a driver of large NO_3^- concentrations in wetland-influenced streams, whereas the effect of frost did not show up in upland-draining streams. The amount of N leached after a strong frost event can be high. In a long-term study in a German Norway spruce site, NO_3^- fluxes with seepage increased (by about $13 \text{ kg N ha}^{-1} \text{ yr}^{-1}$) in the year after a severe soil frost (Callesen et al., 2007).

The main source of dissolved organic carbon (DOC) in forest soils is the forest floor, and the DOC infiltration into the mineral soil represents a significant contribution to the soil C cycle and to the C pool in deeper soil horizons (Michalzik et al., 2001; Kalbitz and Kaiser, 2008). In a laboratory study with forest floor, the fluxes of DOC increased following severe soil frost at $-8 \text{ }^\circ\text{C}$ and $-13 \text{ }^\circ\text{C}$, but not after mild frost at $-3 \text{ }^\circ\text{C}$. The effect occurred only after the first of three freeze/thaw cycles (Hentschel et al., 2008). The sources and mechanisms of DOC formation are still a matter of debate (Kalbitz et al., 2000). Often DOC infiltrating the mineral soil is attributed to the release from rather old soil organic matter (Hagedorn et al., 2004; Fröberg et al., 2007). Changes in the quality of DOC may help to identify different source pools of DOC as influenced by

soil frost. The ^{14}C signature of DOC was used to trace the sources of DOC in soils and streams (Schiff et al., 1997; Michalzik et al., 2003; Fröberg et al., 2007). Dynamics of DO^{14}C after soil frost have not been reported until now.

Potential changes of soil structure and in fluxes of N after soil frost may also affect the concentrations and fluxes of nutrient cations (Na^+ , K^+ , Mg^{2+} , Ca^{2+}) in soil solution. In many acidic forest soils, the pools of plant-available (exchangeable) nutrient cations are rather low (e.g. Feger, 1997) and additional losses might be critical for tree nutrition. Most of the studies about soil frost effects on solute fluxes have focussed on N, and no information about frost effects on fluxes of nutrient cations is available to date.

Overall, the effects of freezing and thawing of forest soils on the turnover and solute fluxes of C and mineral elements are far from being predictable, and there is a lack of field experiments on this subject. Hence, the overall goal of this experiment was to investigate the effects of soil frost on various soil processes (see also Muhr et al., 2008) and on fine root growth (Gaul et al., 2008). Our hypothesis was that freezing and thawing of the soil change the quality of DOC and increase N net mineralization as well as the solute fluxes of N, DOC, and mineral cations (Na^+ , K^+ , Ca^{2+} , Mg^{2+}).

6.2. Methods

Site description

The Coulissenhieb II site is a mature Norway spruce forest (*Picea abies*, L.) in the German Fichtelgebirge area, adjacent to the Coulissenhieb I site which is known from long-term biogeochemical studies (Matzner, 2004a,b). The mean annual precipitation is about 1160 mm and the mean annual air temperature is 5.3 °C. The soil has a sandy to loamy texture and is classified as Haplic Podzol according to the FAO soil classification (IUSS and WRB, 2006). The well stratified, mor-like forest floor has a thickness of 6-10 cm, comprising Oi, Oe and Oa horizons. The forest floor is almost completely covered by ground vegetation, mainly *Deschampsia flexuosa* and *Callamagrostis villosa*. To determine soil properties, 9 soil pits were sampled by horizon. The C and N contents of the soil decrease with increasing depth from 18% C in the Oa horizon to 0.4% C in the Bv horizon and from 1.0% N to < 0.05% N, respectively (Table 6.1). The C/N ratio of the Oa horizon as well as high atmospheric N deposition rates (Matzner et al., 2004)

Table 6.1 Chemical properties of the soil at the Coulissenhieb II site. (Mean values from 9 soil profiles, $\Delta^{14}\text{C}$: mean value from 3 soil profiles)

	Depth	pH	C	N	$\Delta^{14}\text{C}$	Ca^{2+}	Mg^{2+}	Na^+	K^+	Al^{3+}	H^+	CEC_{eff}
	(cm)	CaCl_2	— [%] —		(‰)	[mmol _c /kg]						
Oi	10	n.d.	46.6	1.7	13	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Oe	8	n.d.	38.8	1.9	61	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Oa	5	3.3	22.1	1.1	19	94.4	7.6	1.4	3.4	74.1	16.7	206
EA	-5	3.4	7.4	0.4	23	60.9	3.6	1.0	1.9	66.9	15.5	152
Bsh	-12	3.4	5.5	0.3	14	70.9	2.3	1.1	1.3	97.6	8.3	190
Bs	-18	3.7	3.4	0.2	63	30.1	1.1	0.9	1.0	87.8	3.0	126
Bv	-55	4.1	1.3	0.1	45	3.7	0.2	1.1	1.0	41.3	0.6	48
Bv/C	> 55	4.0	0.4	0.0	n.d.	2.7	0.2	3.0	1.1	35.3	0.5	43

n.d.= not detected

are typical for a highly N saturated ecosystem. The pH (in 1: 2.5 soil to water suspension) is between 4.0 in the Oa horizon and 4.5 in the Bv horizon. The base saturation is highest (54 %) in the Oa horizon and decreases to 12- 16 % in the subsoil, indicating the application of lime to the soil surface by past forest management. The ^{14}C signature of the bulk soil reveals relatively young C in the forest floor and an increasing mean residence time of soil organic matter with soil depth.

Snow removal experiment

The experimental plots (each 20 m \times 20 m) were established in the summer of 2005. Three plots were used as controls, and three plots for the snow removal experiment.

Weather conditions in the winter period of 2005/2006 were favourable for the experiment: Low air temperatures (daily mean <0 °C) lasted for several weeks, and a snow cover of 50–80 cm developed at the control plots. The forest floor of the snow removal plots was covered by glass fibre nets of 0.5 cm mesh size to avoid disturbance of the surface during snow removal. The nets were placed in December 2005 without removing shrubs and ground vegetation. From late December 2005 to early February 2006, snow was removed periodically after major snowfalls by shovelling. The snow removal started before soil frost occurred. After ceasing of the snow removal, snow was allowed to accumulate. The uppermost soil was frozen shortly after the beginning of the snow removal. Together with the nets, the thin ice layer of about 1 cm thickness provided a protective layer, and no visible disturbance of the forest floor occurred due to snow shovelling. After thawing in April 2006, the nets were removed from the soil surface. In total, the removed snow equalled 147 mm of throughfall. Soil temperatures were automatically recorded in 30 min intervals by sensors in each plot located at 5, 15, and 25 cm depth.

Soil solutions

Leachates from the forest floor were collected below the Oa horizon by three plate lysimeters per plot with a surface area of 176 cm² to which suction of -10 kPa was applied for 1 min every 5 min throughout the whole experimental period. The lysimeters were made of plastic bowls with a 50 μm pore-size polyethylene membrane on top. Three ceramic suction cups each at 20 cm soil depth and below the rooting zone at 90 cm soil depth were installed per plot and operated at a continuous suction of -25 kPa.

Soil solutions were collected biweekly, filtered with 0.4 μm cellulose-acetate filters and stored at +2 °C until chemical analysis.

Water extractable C and N in frozen soil

To determine initial changes in available soil C and N caused by soil frost, three cores (20 cm long, 7 cm diameter) of frozen soil were taken from each snow removal plot and from the unfrozen controls in March 2006. All cores were divided into forest floor and mineral soil, and the frozen cores were thawed in the laboratory within 2 h. Then, the water-extractable DOC, DON, NH_4^+ , and NO_3^- was determined by a 1 h extraction, shaking the soil at a wet soil/solution ratio of 1 : 6 (forest floor) and 1 : 3 (mineral soil) with deionized water. The suspensions were centrifuged ($5 \times 10^4 \text{ m s}^{-2}$, 15 min) and filtered through a 0.4 μm cellulose-acetate filter. Water extracts were stored at +2 °C until chemical analysis.

Nitrogen net mineralization

The N net mineralization in the postthawing period was determined by the in situ coring method (Adams et al., 1989). We focussed on the postthawing period because, according to the literature (Matzner and Borken, 2008), largest effects are reported after thawing. Six polyvinyl chloride tubes (6 cm diameter) were driven down to 20 cm soil depth at each plot. Three cores were taken immediately to determine the initial NH_4^+ and NO_3^- content of the soil. Three tubes with closed tops were left in the field for 28 days. Three successive in situ incubations were established from May 2006 to the end of July 2006. After field incubation the tubes were removed and the cores divided into the forest floor (average depth of 7 cm) and mineral soil (average depth of 13 cm). Concentrations of NH_4^+ and NO_3^- were determined by extraction of the soil with 1 M KCl at a soil/solution ratio of 1 : 10 (forest floor) and 1 : 3 (mineral soil).

Chemical analysis of soil solutions and extracts

DOC and total N in soil solutions and water extracts were determined by high temperature combustion and subsequent determination of CO_2 and NO_x (Elementar, high-TOC, Elementar Analysensysteme GmbH, Hanau, Germany), NH_4^+ by flow injection analyzer (MLE, FIA-LAB, Medizin und Labortechnik Engineering GmbH, Dresden, Germany) and NO_3^- by ion chromatography (DIONEX, DX500, Dionex Corporation, Sunnyvale, California, USA). DON was calculated as the difference between total N and inorganic N. An inductively coupled plasma optical emission spectroscope (ICP-OES, Jobin-

Yvon Horiba Group, JY2000, Varian Inc., Palo Alto, California, USA) was used to determine Na^+ , K^+ , Ca^{2+} , Mg^{2+} . The NH_4^+ and NO_3^- concentrations in the KCl extracts were determined by colorimetric methods using a segmented flow analyzer (Skalar Sanplus Analyzer, Skalar Analytical BV, Breda, the Netherlands).

Before ^{14}C analyses, soil solutions were pooled for the periods December 2005–January 2006, March–April 2006, August–September 2006, and October–December 2006 and then freeze-dried. Subsamples (~1 mg C) of the freeze-dried material and of bulk soil were combusted in sealed quartz tubes at 900 °C with CuO oxidiser and Ag powder. The resulting CO_2 was reduced to graphite using the zinc reduction method (Xu et al., 2007). The graphite targets were analysed by accelerated mass spectrometry (AMS) at the University of California, Irvine with a precision of $\pm 3\Delta\%$. Radiocarbon data are expressed as $\Delta^{14}\text{C}$ (‰ deviation from the $^{14}\text{C}/^{12}\text{C}$ ratio of oxalic acid standard in 1950). A $\Delta^{14}\text{C}$ signature of 0‰ refers to a standard (wood) from 1890. Positive $\Delta^{14}\text{C}$ indicate that the C was fixed from the atmosphere after 1890. Negative $\Delta^{14}\text{C}$ indicate that C was fixed before 1890, however, some negative $\Delta^{14}\text{C}$ signatures (up to -24%) were determined between 1890 and 1955 because of fossil fuel burning. The nuclear bomb tests in the 1950s and 1960s increased the atmospheric $^{14}\text{CO}_2$ signature up to $+1000\%$. For more details see Stuiver and Polach (1977).

Water fluxes and calculation of element fluxes with seepage

We calculated soil water fluxes at the snow removal and control plots with the simulation model WHNSIM (Huwe and van der Ploeg, 1991; Huwe and Totsche, 1995). The model is based on the Richards equation and calculates daily soil water movement at different depths as a function of precipitation, calculated evapotranspiration and soil hydraulic properties. Meteorological parameters were measured at a nearby tower (T. Foken, personal communication). Model calibration was based on soil water tensions measured by tensiometers at three different depths. The model comprises a tool to simulate snow accumulation and melting. The modelling of soil water fluxes started in autumn 2005. Here, we report the fluxes for the year following the snowmelt flush (1 May 2006–30 April 2007) because the water fluxes were similar at both treatments from 1 May on. Water fluxes were calculated for each treatment, but not for each plot within the treatments. Measurements of throughfall and soil water tensions gave no evidence for systematic hydrological differences between the plots of each treatment. Solute

fluxes were calculated for each plot based on the fortnightly solution concentrations which were multiplied with the cumulative water flux in that period.

Statistical analysis

The arithmetic means and standard errors of each treatment were calculated from the replicated plots ($n=3$). Analysis of variance (anova with repeated measures design) was used to examine the treatment effect on solution chemistry (NH_4^+-N , NO_3^--N , DON, DOC, Na^+ , K^+ , Ca^{2+} , Mg^{2+}) with the sampling date as main factor. Differences in the soil water extracts, soil KCl extracts and calculated solute fluxes of NH_4^+-N , NO_3^--N , DON, DOC, Na^+ , K^+ , Ca^{2+} , Mg^{2+} were tested with paired sample t-test. All statistical analyses were performed using statistica 6.0.

6.3. Results

Soil temperatures

Soil temperatures were not different between controls and snow removal plots before the treatment. The removal of the snow caused the soil temperatures to drop to a minimum of $-5\text{ }^\circ\text{C}$ at 5 cm depth in February 2007, while the control remained unfrozen

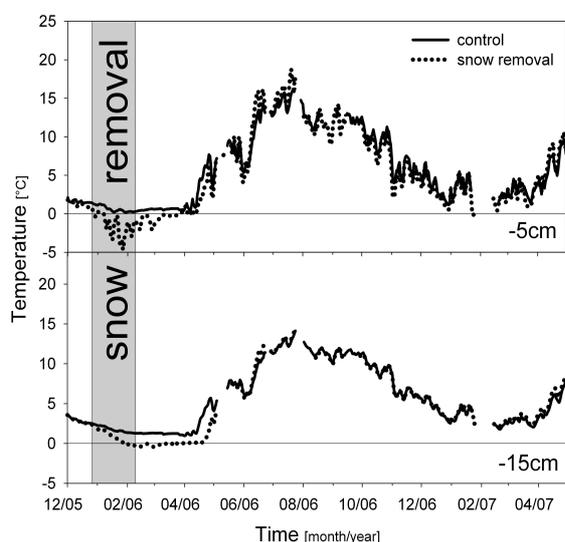


Figure 6.1 Soil temperatures at the control and snow removal plots.

(Fig. 6.1). The soil frost penetrated the soil down to about 15 cm depth. Thawing of the upper soil was completed in the mid of April, while soil temperatures at 15 cm depth exceeded $0\text{ }^\circ\text{C}$ by the end of April. The average soil temperature from 28 December- 1 April in the controls was $+0.6\text{ }^\circ\text{C}$ at 5 cm depth and $+1.1\text{ }^\circ\text{C}$ at 15 cm depth, while at the snow removal plots the average temperatures were $-1.0\text{ }^\circ\text{C}$ at 5 cm depth and $-0.1\text{ }^\circ\text{C}$ at 15 cm depth. From May 2006 on, the soil temperatures were similar at the control and snow removal plots. Soil temperatures at

25 cm depth were not different between treatment and control plots at any time (data not shown).

Water extractable C and N in the frozen soil

The water content of the frozen soil at the snow removal plots was higher than in the controls, because infiltrating water was frozen and accumulated in the upper soil. Hence, in Fig. 6.2 the water extractable amounts in kg ha^{-1} are given rather than the solute concentrations. In the forest floor, the amount of water extractable NH_4^+ was higher at the snow removal plots ($P=0.14$) while the amount of water-extractable NO_3^- was less ($P=0.06$) (Fig. 6.2). The total amount of inorganic N was not influenced by the treatment. In the mineral soil, the water-extractable NO_3^- at the snow removal plots was about half those of the control plots (not significant, $P=0.26$). Amounts of water-extractable DOC and DON in both horizons were not influenced by the treatment.

Nitrogen net mineralization

The in situ measurements of N net ammonification and net nitrification in the spring and summer of 2006 revealed no differences between the snow removal and the control plots, neither in the forest floor nor in the mineral soil (Fig. 6.3). Expressed in kg N ha^{-1} , N net mineralization rates in the forest floor slightly exceeded those in the upper mineral soil.

Solute concentrations

There was no effect of snow removal and soil frost on the concentrations of DOC in soil solutions in forest floor percolates and at 20 cm depth (Fig. 6.4). The concentrations of DOC in soil solutions were highest in the forest floor percolates and decreased with soil depth. At 90 cm depth, concentrations were higher at the snow removal plots for two sampling dates during the snow melt period, which is based only on a single sample collected at these dates.

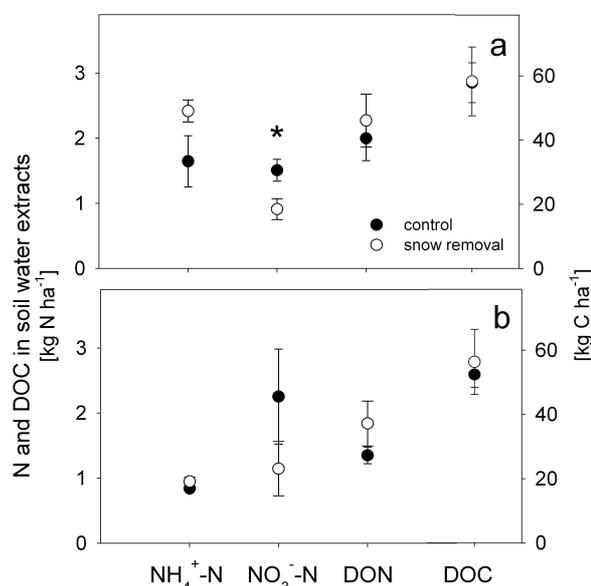


Figure 6.2 Water extractable NH_4^+ , NO_3^- , DON, DOC in frozen (snow removal) and unfrozen (control) soil in March 2006. (a) forest floor, (b) and mineral soil. Error bars represent standard errors ($n=3$).

The snow removal and soil frost had no effect on the ^{14}C signature of DOC in forest floor percolates. The average ^{14}C signature of DOC in forest floor percolates after snow melt was around 0 ‰ (Fig. 6.5). The solutions were collected at the transition from the Oa to the EA horizon. The DOC was on average older than the bulk of the Oa horizon (see Table 6.1). The samples collected during the pretreatment period comprised older DOC at both depths, which might be an artefact caused by the installation of samplers in the summer/autumn of 2005. The DOC in soil solutions from 90 cm depth were older than in forest floor percolates, which corresponds to the gradient observed in the soil organic matter in the soil profile (Table 6.1). The differences in DO^{14}C at 90 cm depth between control and snow removal plots were not statistically significant.

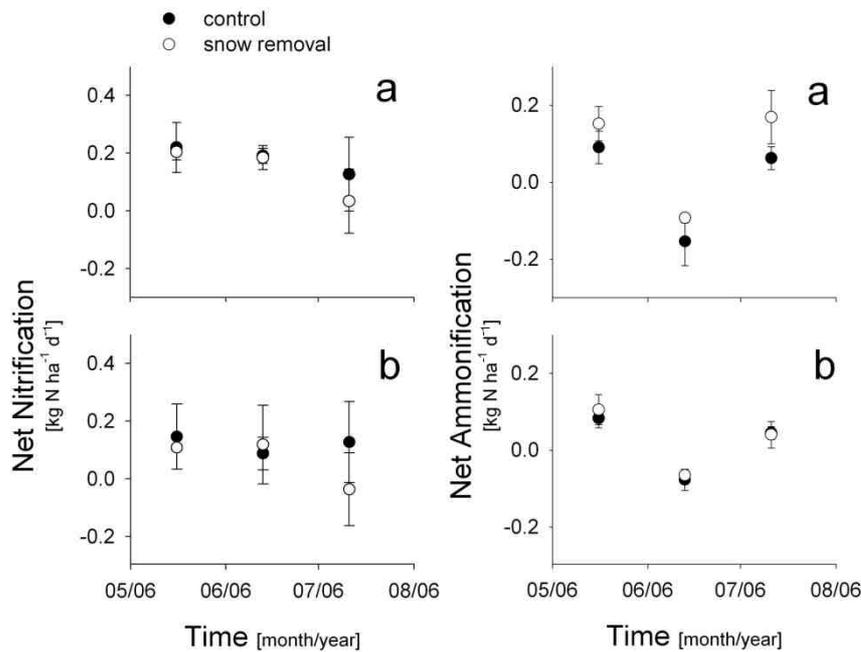


Figure 6.3 Net N ammonification and net nitrification after thawing in 2006. (a) forest floor, (b) and mineral soil (up to 13 cm depth). Error bars represent standard errors (n=3).

The NH_4^+ concentrations in the forest floor percolates were generally low in comparison with NO_3^- . Already before the treatment, the NH_4^+ concentrations were higher in the snow removal than in the control plots. The NH_4^+ concentrations in the forest floor percolates of the snow removal plots exceeded those of the controls from May to July 2006 (Fig. 6.4). This increase cannot be attributed to the frost effect considering the differ-

ences in the pretreatment period. From July on, the NH_4^+ concentrations were similar in both treatments. No differences were found for NH_4^+ in the deeper soil horizons where the concentrations were generally much lower than in the forest floor.

There was no immediate response of NO_3^- concentrations in soil solution after thawing. However, in late summer and autumn of 2006, NO_3^- concentrations increased in the percolates of the forest floor and in soil solutions at 20 cm soil depth at the snow removal plots (Fig. 6.4). The increase at 20 cm depth was statistically significant ($P=0.02$) at 12 dates (19 March –12 December 2006). In two consecutive samplings in August and September 2006, the concentrations in forest floor percolates of the snow removal plots were about twice those of the controls ($P=0.06$). At 90 cm depth, the concentrations of NO_3^- were lower in the snow removal plots before and shortly after snow removal. An increasing trend was observed at the snow removal plots, driving the NO_3^- concentrations at 90 cm depth in both treatments to similar values by the end of 2006. Differences between the treatments were not statistically significant.

The increase of NO_3^- concentrations in forest floor percolates and 20 cm soil depth at the snow removal plots in autumn 2006 was accompanied by increasing Ca^{2+} and Mg^{2+} concentrations (Fig. 6.6) as revealed by the charge balance of the solution (data not shown). No effect of snow removal and soil frost was found for K^+ in solution at any soil depth. Immediately after thawing, the Na^+ concentrations at 20 cm soil depth were about twice those of the controls, and the increase lasted until the end of 2006 ($P<0.06$). The effect occurred neither in forest floor percolates nor at 90 cm depth and was independent of the NO_3^- dynamics. Differences in Ca^{2+} and Mg^{2+} concentrations at 90 cm depth existed already before the treatment and should not be interpreted as treatment effect.

The increase of NO_3^- concentrations in forest floor percolates and 20 cm soil depth at the snow removal plots in autumn 2006 was followed by increasing Ca^{2+} and Mg^{2+} concentrations (Fig. 6.6). No effect of snow removal and soil frost was found for K^+ in solution at any soil depth. Immediately after thawing the Na^+ concentrations at 20 cm soil depth were about twice those of the controls and the increase lasted until the end of 2006 ($p < 0.06$). The effect occurred neither in forest floor percolates nor at 90 cm depth and was independent of the NO_3^- dynamic. Differences in Ca^{2+} and Mg^{2+} concentrations

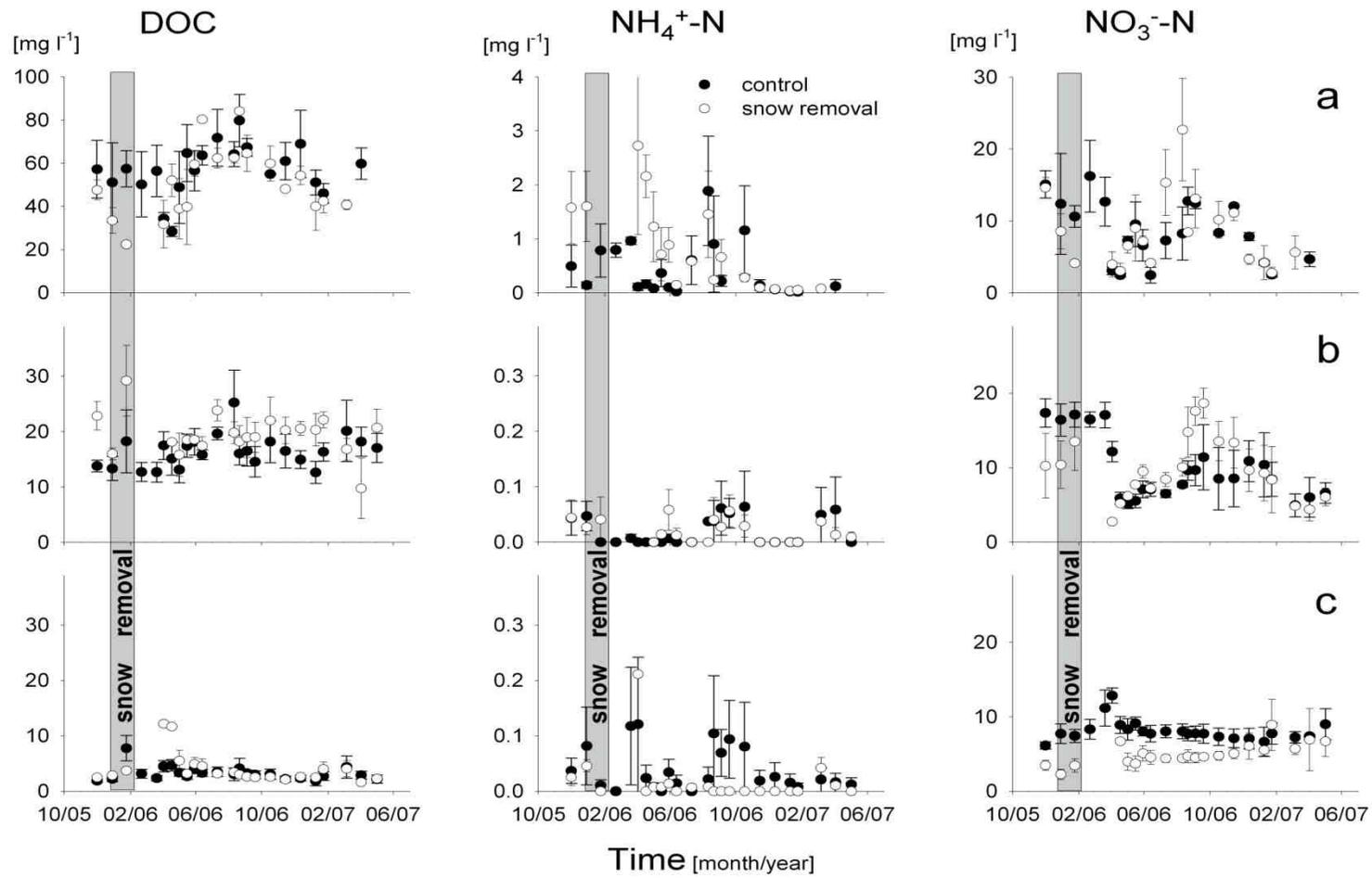


Figure 6.4 Concentrations of DOC, NH_4^+ and NO_3^- in forest floor percolates and in soil solutions. (a) forest floor, (b) 20 cm depth, (c) 90 cm depth. Error bars represent standard errors (n=3).

at 90 cm depth existed already before the treatment and should not be interpreted as treatment effect.

Seepage fluxes

In the 12 months following thawing and snow melt, the fluxes of inorganic N with seepage were rather high at our site. Seepage losses at 90 cm depth were in the same range as inorganic N fluxes in throughfall (Table 6.2). The soil frost and snow removal had no effect on the NO_3^- fluxes. The increased NO_3^- concentrations in autumn 2006 coincided with a period of low seepage fluxes, and did not induce large NO_3^- fluxes. The same is true for the effect of increased Ca^{2+} and Mg^{2+} concentrations. The fluxes of Na^+ at 20 cm soil depth increased in the snow removal plots relative to the control from 4.6 to 6.8 kg ha^{-1} ($P=0.05$), while no effect was observed at 90 cm depth. The annual DOC fluxes in forest floor percolates and at 20 cm soil depth were unaffected by soil frost. The higher fluxes at the snow removal plots in 90 cm are due to higher concentrations in May 2006 at only two sampling dates.

6.4. Discussion

Soil temperature regime and processes in the winter period

The soil frost in our experiment lasted for about 3 months with a minimum soil temperature at 5 cm depth of $-5\text{ }^\circ\text{C}$ and a frost depth of about 15 cm. We consider the frost intensity induced by the experiment as moderate compared with other studies (Hentschel et al., 2008; Matzner and Borke, 2008) and also to an extreme frost events at our site in the past (Callesen et al., 2007). The differences in soil temperatures between treatments and controls were rather small (from late December to 1 April on average $\Delta 1.6\text{ }^\circ\text{C}$ at 5 cm depth and $\Delta 1.2\text{ }^\circ\text{C}$ at 15 cm depth). The soil frost itself might have an additional effect on the N net mineralization because of low diffusion rates in the remaining unfrozen water films. Hence, lower temperatures and soil frost should have caused less N net mineralization at the snow removal plots in the winter. On the other hand, the thawing of frozen soil often resulted in a burst of N net mineralization. The cumulative effect of lower temperature and soil frost on the one hand and of the burst of mineralization after thawing on the other hand will determine the overall wintery N net mineralization (Matzner and Borke, 2008). Soil solution concentrations of NO_3^- in the winter and thawing period were often lower at the snow removal plots, but

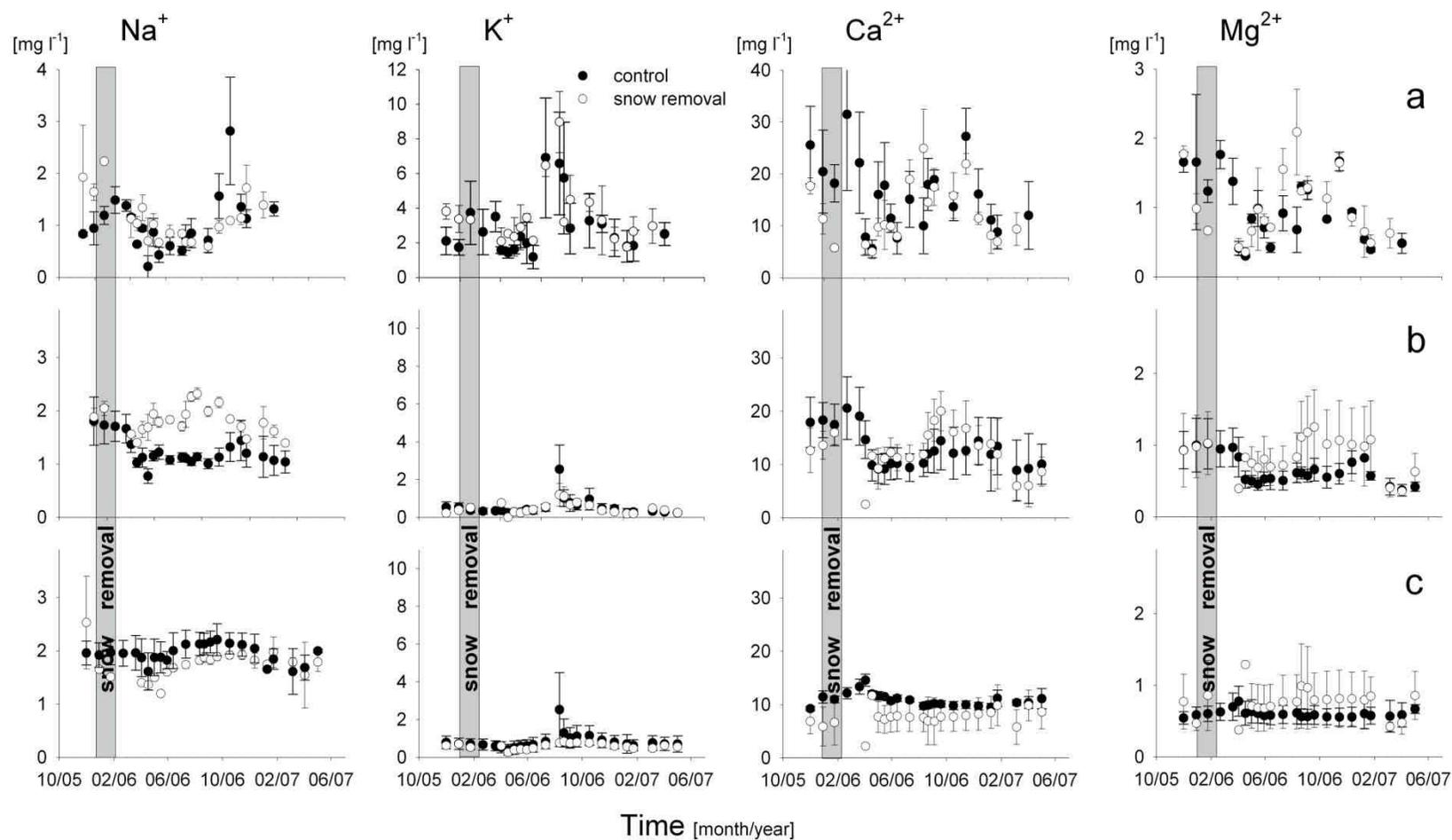


Figure 6.6 Concentrations of Na^+ , K^+ , Ca^{2+} and Mg^{2+} in soil solutions. (a) forest floor percolates, (b) 20 cm depth, (c) 90 cm depth. Error bars represent standard errors ($n=3$).

the differences occurred already before the experiment, hampering the interpretation of frost effects. Despite these restrictions, it is obvious that the net effect of lower temperatures, frost, and thawing did not cause significant changes in soil solution chemistry during the winter and thawing period as suggested by our initial hypothesis.

The burst of C and N mineralization after thawing was often attributed to the destruction of aggregates and the use of formerly occluded substrates by microorganisms (Van

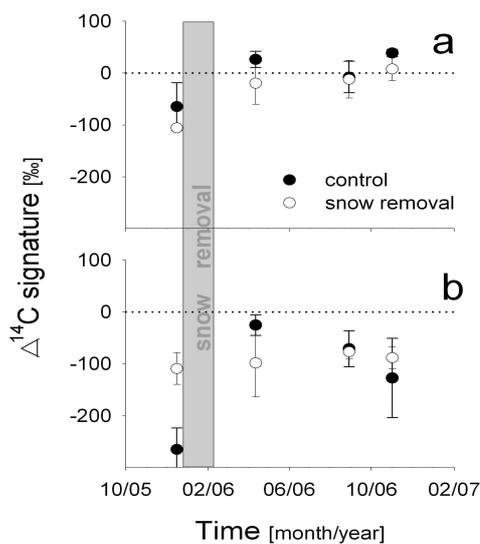


Figure 6.5 $\Delta^{14}\text{C}$ values of DOC from (a) forest floor percolates and (b) 90 cm depth. Error bars represent standard errors ($n=3$).

Bochove et al., 2000) or to lysis of microorganisms and the subsequent turnover of the microbial necromass (Herrmann and Witter, 2002). Our data on DOC and DON content of water extracts from frozen soil revealed neither a flush of DOC nor a change in the DOC/DON ratio which could be expected after the lysis of microbial cells or after a change in substrate availability. The observed differences of inorganic N content of the water extracts cannot be interpreted as differences in net mineralization rates between the treatments because the soil frost prevented vertical water transport and the removal of mineralized N with seepage.

Nitrogen net mineralization and fluxes in the post thawing period

In the 3 months following thawing, the in situ N net mineralization was not affected by the soil frost, corresponding to similar field measurements after soil frost reported by Groffman et al. (2001). Unfortunately we did not continue the measurements of in situ N net mineralization beyond July 2006. The time period of late summer and autumn of 2006 was characterized by rewetting of the soil after the summer drought. Soil respiration reached a maximum during this period. Muhr et al. (2008) differentiated heterotrophic and rhizosphere respiration by using the dynamics of the $^{14}\text{CO}_2$ signature of soil respiration. They pointed out a significant reduction of heterotrophic respiration at the snow removal plots. As a consequence, NO_3^- immobilization by growing heterotrophic microorganisms should have been less at the snow removal plots, amplifying net nitrifi-

cation and NO_3^- concentrations. Differences in denitrification rates between the treatments as a cause of increasing NO_3^- concentrations at the snow removal plots are unlikely because soil emissions of NO and N_2O were similar during that time (S. Goldberg, personal communication). Tierney et al. (2001) speculated that the increased NO_3^- availability in their soil frost experiment was due to reduced root uptake and to increased root turnover, despite similar root biomass in the treatments. In our experiment, differences in the NO_3^- uptake by roots seem unlikely, because the fine root biomass at this time of the year was similar in both plots and the root production was even higher at the snow removal plots (Gaul et al., 2008). Thus, based on indirect evidence, less NO_3^- immobilization by heterotrophic microorganisms at the snow removal plots likely explains the increase of NO_3^- in the late growing season. As discussed by Muhr et al. (2008) and Schmitt et al. (2008) this may be caused by changes in the microbial community due to the frost, namely by a decrease in fungal biomass.

The snow removal and the soil frost had no effect on NO_3^- fluxes in forest floor percolates and in seepage in the postthawing year (May 2006- April 2007). The increasing NO_3^- concentrations in autumn 2006 had only a minor effect on the leaching because of small water fluxes during that time. The NO_3^- concentrations in seepage at 90 cm depth were not significantly affected by the snow removal and the lower concentrations at the snow removal plots were already observed before the treatment.

In a Norway spruce stand adjacent to our site, the NO_3^- fluxes with seepage increased by about 13 kg N ha^{-1} after an exceptional soil frost in the winter of 1995/1996 (Callesen et al., 2007). The natural soil frost at that time, with soil frost down to 70 cm depth, was exceptional and much more severe than in our snow removal experiment which might explain the differences to our experiment. The conditions induced by our experiment are more frequently observed at the site (Callesen et al., 2007). A substantial increase of NO_3^- fluxes after deep soil frost was also reported by Brooks et al. (1998). In their snow removal experiment in two temperate forests, Fitzhugh et al. (2001) induced moderate soil frost. They observed an increase of total N fluxes in forest floor percolates. However, similar to our study, the seepage losses of N at deeper soil were almost unaffected. Given the contradicting findings in the literature, there are obviously site-specific conditions that play a role in the evolution of soil frost effects on solute fluxes. The intensity and duration of soil frost seem to be major factors.

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DOC fluxes

The concentrations and fluxes of DOC in forest floor percolates and in the mineral soil were unaffected by soil frost, similar to findings from the snow removal experiment of Fitzhugh et al. (2001). Our field observations correspond to results from a laboratory study with soil from our study site (Hentschel et al., 2008). There, an increase of DOC in forest floor percolates after thawing occurred only at frost temperatures of $-8 \text{ }^\circ\text{C}$ and $-13 \text{ }^\circ\text{C}$, but not in the $-3 \text{ }^\circ\text{C}$ treatment which is closest to the conditions of the field experiment. In our field study, the origin of DOC was not affected by frost as indicated by the ^{14}C signatures. Thus, there is no evidence for a DOC release from killed microorganisms, assuming that the microbial biomass comprises relatively young C. Also the release of DOC from the increased fine root necromass at the snow removal plots (Gaul et al., 2008) seems not relevant for the DOC dynamic in soil solutions. This should have resulted in a shift of the ^{14}C signature to younger C after frost.

The ^{14}C signature of DOC in forest floor percolates was lower than the one of the bulk Oa horizon, pointing to a preferential release of older C into the DOC fraction. The ^{14}C signature of DOC from 90 cm depth was more negative than in forest floor percolates and similar to the bulk soil organic matter in the Bv horizon (Table 6.1). This confirms the conclusion of Schiff et al. (1997) that the DOC in deeper soil horizons is

Table 6.2: Solute fluxes from 1st of May 2006 to 30st of April 2007

	Water flux (mm)	DOC	tN	NH ₄ ⁺ -N	NO ₃ ⁻ -N	Na ⁺	K ⁺	Ca ²⁺	Mg ²⁺
		(kg ha ⁻¹ 12months ⁻¹)							
<i>Throughfall</i>									
control	723	123.1	24.8	10.0	10.9	7.9	22.5	8.5	2.0
snow removal	723	123.1	24.8	10.0	10.9	7.9	22.5	8.5	2.0
<i>Forest floor perco-</i>									
control	440	236.3	32.5	0.9	25.9	5.5	9.7	55.8	3.0
snow removal	440	212.5	35.9	1.2	29.5	5.3	13.5	49.9	3.7
<i>20 cm soil depth</i>									
control	393	64.7	33.4	0.1	30.9	4.6	1.6	44.6	2.3
snow removal	393	75.2	38.9	0.1	36.7	6.8 *	1.4	48.4	3.6
<i>90 cm soil depth</i>									
control	319	8.6	26.5	0.0	26.1	6.3	1.9	35.4	1.9
snow removal	319	15.8	21.2	0.1	20.5	5.5	1.5	27.5	2.4

tN = total nitrogen

* Difference significant at P= 0.05

released from the soil solid phase at the specific depth rather than being transported from the forest floor.

Concentration and fluxes of mineral cations

The elevated NO_3^- concentrations in forest floor percolates and at 20 cm soil depth at the snow removal plots in autumn 2006 were accompanied mainly by increasing Ca^{2+} and Mg^{2+} concentrations as revealed by charge balance calculations. The increased concentrations of Ca^{2+} and Mg^{2+} thus seem to be due to cation exchange reactions caused by the proton load from excess nitrification (root uptake+immobilization<gross nitrification). No other specific release of mineral cations from the forest floor can be seen, that might be attributed to the frost effect. The strong seasonal dynamics of the K^+ concentrations in forest floor percolates is related to natural drying/rewetting of the forest floor in the summer of 2006. Only in case of Na^+ , there seems to be a frost effect on solution chemistry: From May to December 2006, the Na^+ concentrations and fluxes at 20 cm depth exceeded those of the control plots and the increase was not paralleled by Cl^- (data not shown). No such patterns were observed in the forest floor percolates and at 90 cm soil depth. Because the exchangeable Na pool is rather low and the dynamics of the Na^+ concentrations was not connected to similar dynamics of the major anions, we assume that the release of Na^+ originated from frost-induced weathering of silicates. The weathering of silicates is a major Na^+ input in our soils (Matzner et al., 2004b).

6.5. Conclusion

The soil frost induced by the snow removal did not increase the solute fluxes of N, DOC, and mineral ions, contradicting our initial hypothesis that soil frost enhances solute losses. In the 3 months after thawing, N net mineralization was not affected by the preceding soil frost. The increase of NO_3^- concentrations in the soil during the late growing season, which was likely caused by less microbial immobilization, did not result in increased NO_3^- leaching because of low water fluxes. DOC fluxes were unaffected by soil frost and the ^{14}C signature of DOC gave no evidence of changing DOC origin after freezing and thawing. Moderate intensity of soil frost, like in our experiment, seems to have little effect on the solute losses from temperate forest ecosystems.

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6.7. References

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Appendix

List of further publications

Reth S, **Hentschel K**, Drösler M, Falge E (2004) DenNit - Experimental analysis and modelling of soil N₂O efflux in response on changes of soil water content, soil temperature, soil pH, nutrient availability and the time after rain event. *Plant and Soil* 272: 349-363

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Hiermit erkläre ich, dass ich die vorliegende Arbeit selbstständig verfasst und keine anderen als die angegebenen Quellen und Hilfsmittel benutzt habe. Ich versichere weiterhin, dass ich nicht diese oder eine gleichartige Doktorprüfung an einer anderen Hochschule endgültig nicht bestanden habe.

Kerstin Schulze