Role of Dissolved Organic Nitrogen in the Soil Nitrogen Cycle of Forest Ecosystems

DISSERTATION

zur Erlangung des Doktorgrades (Dr. rer. nat.)
der Fakultät für Biologie, Chemie und Geowissenschaften
der Universität Bayreuth

vorgelegt von

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Bayreuth, im Juni 2010

Die vorliegende Arbeit wurde in der Zeit von April 2007 bis Juni 2010 am Lehrstuhl für Bodenökologie der Universität Bayreuth unter der Betreuung von Prof. Dr. Egbert Matzner angefertigt.

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Tag der Einreichung der Dissertation: 22.06.2010

Tag des wissenschaftlichen Kolloquiums: 10.12.2010

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“Adopt the pace of nature: her secret is patience.”
Ralph Waldo Emerson (1803-1882)

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List of Abbreviations

a.s.l.	Above sea level
DIN	Dissolved inorganic nitrogen
DOC	Dissolved organic carbon
DOM	Dissolved organic matter
DON	Dissolved organic nitrogen
EEM	Excitation-emission-matrix
EON	Extractable organic nitrogen
PARAFAC	Parallel factor (analysis)
PE	Polyethylene
PON	Particulate organic nitrogen
PVC	Polyvinyl chloride
SOM	Soil organic matter
SON	Soluble organic nitrogen
$SUVA_{280}$	Specific UV absorption at 280 nm
TDN	Total dissolved nitrogen
TN	Total nitrogen
TOC	Total organic carbon
TON	Total organic nitrogen
WEON	Water-extractable organic nitrogen

Summary

In the last years, dissolved organic N (DON) has been shown to be a crucial part of the soil N cycle in forest ecosystems. Despite this, information on its dynamics, sources and fate is still lacking. Especially data from (sub)tropical forest ecosystems are scarce. Therefore, this study investigated (i) the magnitude and drivers of DON fluxes in a subtropical montane forest, (ii) the biodegradability of DON from forest floors, (iii) the abiotic formation mechanism for DON in forest floors as postulated by the Ferrous Wheel Hypothesis and (iv) the link between DON and dissolved organic C (DOC) dynamics.

In a field study (2005-2008), average DON fluxes in forest floor percolates and seepage (60 cm) of a subtropical montainous cypress forest (16 and 8 kg N ha^{-1} yr^{-1}, respectively) were similar to fluxes in other (sub)tropical ecosystems, and dominated total N fluxes. Dissolved organic N concentrations in the soil were independent of the water flux (meaning that no dilution effect was visible). This implies that first, the pool size of potentially soluble DON is variable and second, that this pool is hard to deplete. In contrast, the linear relationship between soil organic solute and water fluxes was positive, showing that precipitation is an important driver for DON losses in this ecosystem. Although this has also been reported from temperate ecosystems, this relationship did not hold when analyzing the combined data from various (sub)tropical and temperate forest ecosystems.

The biodegradability of DON was highest in inoculated spruce-Oi water extracts in a 21-day incubation experiment, while in extracts from beech-Oi and Oa horizons, DON concentrations only slightly decreased. Dissolved organic N was recalcitrant in spruce-Oa and cypress-Oa extracts, indicating that this DON could add to the formation of stable soil N pools. As various additions of NO_3^- never influenced DON biodegradation, it is concluded that microbes do not necessarily prefer mineral N over DON as substrate. Mineralization was always more important than microbial uptake in samples without NO_3^- additions, and denitrification only played a minor role in spruce-Oi samples (as indicated by a negative balance of all N species after 21 days). Fluorescence excitation-emission spectroscopy and subsequent parallel factor analysis identified four groups of fluorophores in the extracts. The initial concentration of two of these so-called factors was correlated with DON biodegradation, but protein-like fluorescence (which has been suggested as a proxy for dissolved organic matter biodegradation) was shown to be independent of DON biodegradation due to similar excitation-emission-maxima of recalcitrant compounds. Therefore, these factors might not always be suitable to predict DON biodegradation.

The abiotic reaction of NO_2^- with DOC (as postulated by the last step of the Ferrous Wheel Hypothesis) was tested in a second incubation experiment in extracts with varying

DOC concentrations and qualities and NO_2^- additions under oxic conditions. Concentrations of added NO_2^- never decreased within 60 min, indicating, that no DON formation from added NO_2^- took place. The results show, that the last step of the Ferrous Wheel Hypothesis (which has been suggested to be fast) is unlikely to occur in forest floors.

Dissolved organic N and C fluxes were both highly dependent on precipitation at the cypress site, suggesting a strong link between these two classes of compounds. This assumption was supported by the first incubation experiment, where both DON and DOC biodegradation were not influenced by NO_3^- additions. Moreover, DOC dynamics closely resembled DON dynamics, which suggests that DON biodegradation could be driven by microbial C demand. Therefore, the often used separation of DON and DOC into functionally different compound classes is not always warranted.

In conclusion, this study emphasized the need to include DON in biogeochemical N studies of both temperate and (sub)tropical ecosystems, and provided new and important insights regarding DON biodegradation, possible DON sources in forest floors and the link between DON and DOC dynamics in forest ecosystems.

Zusammenfassung

In den letzten Jahren konnte gezeigt werden, dass gelöster organischer Stickstoff (DON) eine wichtige Rolle im N-Kreislauf von Waldböden spielt. Trotz dieser Erkenntnis gibt es bis heute nur wenig Informationen über die Dynamik, die Quellen und die Senken von DON. Besonders bzgl. (sub)tropischer Waldökosysteme ist die Datenlage nur spärlich. Aus diesem Grund untersuchte diese Studie (i) die Größenordnung und die Einflussfaktoren von DON-Flüssen in einem subtropischen montanen Bergregenwald, (ii) die Bioabbaubarkeit von DON in organische Auflagen von Waldböden, (iii) die abiotische Bildung von DON nach der Ferrous Wheel Hypothesis und (iv) den Zusammenhang zwischen der Dynamik von DON und gelöstem organischen Kohlenstoff (DOC).

Die Flüsse von DON in Perkolaten der Humusauflage bzw. im Sickerwasser (60 cm) eines subtropischen montanen *Chamaecyparis*-Waldes (16 bzw. 8 kg N $\mathrm{ha^{-1}}$ $\mathrm{a^{-1}}$, gemessen von 2005-2008) waren vergleichbar mit Flüssen, die in anderen (sub)tropischen Ökosystemen gemessen wurden. In diesem Freilandexperiment war der Anteil der DON-Flüsse an den Gesamt-N-Flüssen höher als der Anteil der anorganischen N-Flüsse. Die Konzentrationen von DON im Boden waren nicht mit dem Wasserfluss korreliert (d.h. kein Verdünnungseffekt konnte beobachtet werden). Das impliziert zum einen, dass der Pool des potentiell löslichen DON von variabler Größe ist, und zum anderen, dass dieser Pool nahezu unerschöpflich ist. Der Zusammenhang zwischen den Flüssen der gelösten organischen Stoffe im Boden und dem Wasserfluss war linear und positiv, was bedeutet, dass der Niederschlag in diesem Ökosystem einen starken Einfluss auf die DON-Flüsse im Boden hat. Obwohl dieser Zusammenhang auch aus temperaten Ökosystemen bekannt ist, war der lineare Zusammenhang zwischen DON-Flüssen und der Niederschlagsmenge nicht mehr gegeben, als Daten aus temperaten und (sub)tropischen Ökosystemen zusammen analysiert wurden.

Die Bioabbaubarkeit von DON war in Wasserextrakten von Fichte-L höher als in Extrakten von Buche-L und Oh. In letzteren änderten sich die DON Konzentrationen während eines 21-tägigen Inkubationsexperiments nur wenig. In Extrakten von Fichte-Oh und Zypresse-Oh war DON nicht abbaubar, was impliziert, dass dieser Stickstoff zur Bildung von stabilen N-Pools im Boden beitragen könnte. Die Bioabbaubarkeit von DON änderte sich in keinem der Extrakte nach Zugabe von verschiedenen Nitratkonzentrationen, was bedeutet, dass Mikroorganismen mineralischen Stickstoff nicht unbedingt bevorzugen. In Extrakten ohne Nitratzugabe spielte Mineralisation immer eine größere Rolle als die mikrobielle Aufnahme. Denitrifikation trat nur in Extrakten von Fichte-L in geringem Maß auf (abgeleitet über eine Bilanz aller N-Spezies nach 21 Tagen).

Mit Hilfe von Fluoreszenzspektroskopie und anschließender paralleler Faktorenanalyse wurden vier Gruppen von Fluorophoren in den Extrakten identifiziert. Die Ausgangskonzentration von zwei dieser Gruppen (Faktoren) war mit der Bioabbaubarkeit von DON korreliert, aber die Fluoreszenz protein-ähnlicher Komponenten zeigte im Gegensatz zu anderen Studien keinen Zusammenhang mit der DON-Bioabbaubarkeit. Diese Beobachtung wurde dadurch erklärt, dass schwer abbaubare Verbindungen ähnliche Absorptions- und Emissionsmaxima haben, wie in Einzelkomponentenmessungen gezeigt werden konnte. Deshalb ist festzuhalten, dass diese Faktoren allein nicht unbedingt geeignet sind, um Rückschlüsse auf die Bioabbaubarkeit von DON zu ziehen.

Die abiotische Reaktion von Nitrit mit DOC, wie im letzten Schritt der Ferrous Wheel Hypothese postuliert, wurde in einem zweiten Inkubationsexperiment in Extrakten mit verschiedenen DOC-Konzentrationen und -Qualitäten und unterschiedlichen Nitritzugaben unter oxischen Bedingungen getestet. Die Nitritkonzentration änderte sich in keinem der Extrakte innerhalb von 60 min. Die Wahrscheinlichkeit, dass die letzte Reaktion der Ferrous Wheel Hypothese (die laut Literatur schnell ablaufen sollte) in Humusauflagen von Waldböden tatsächlich eine Rolle spielt, ist daher eher gering.

Die Beobachtung, dass im *Chamaecyparis*-Wald sowohl die DON- als die DOC-Flüsse stark abhängig vom Niederschlag waren, zeigt, dass es einen engen Zusammenhang zwischen diesen beiden Komponentenklassen gibt. Dies wurde durch die Ergebnisse des ersten Inkubationsexperimentes bestätigt, da weder die DON- noch die DOC-Bioabbaubarkeit durch NO_3^--Zugaben beeinflusst wurde. Beide Komponentenklassen zeigten eine ähnliche zeitliche Abbaudynamik, was darauf hindeutet, dass die DON-Bioabbaubarkeit eng mit dem mikrobiellen Kohlenstoffbedarf zusammenhängen könnte. Diese Ergebnisse legen nahe, dass die in der Literatur häufig auftretende Trennung von DON und DOC in funktionell verschiedene Komponenten nicht immer gerechtfertigt ist.

Die Befunde dieser Studie unterstreichen, dass DON in Studien zur Biogeochemie von temperaten und (sub)tropischen Waldökosystemen berücksichtigt werden muss. Außerdem gewähren die Ergebnisse neue und wichtige Einblicke in die Bereiche Bioabbaubarkeit von DON, DON-Quellen in Humusauflagen von Waldböden und den Zusammenhang zwischen DON- und DOC-Dynamik in Waldökosystemen.

CHAPTER 1

Synthesis: Role of dissolved organic nitrogen in the soil nitrogen cycle of forest ecosystems

1.1 Introduction

1.1.1 General introduction

In soils, 90% of N is present in organic forms, which are dominated by solid N compounds (Stevenson, 1982). This solid organic N is not available to plants and microbes (Jones et al., 2005a). Nutrients stored in solid soil organic matter (SOM) become available to the soil microbial community and plants as they are processed into smaller units (dissolved organic forms) by the soil microbial community (Schimel and Bennett, 2004; Jones et al., 2005a; van Hees et al., 2005). Dissolved organic nitrogen (DON) is thought to be a major constituent of terrestrial and marine N cycles (Antia et al., 1991; Chapin, 1995; Näsholm et al., 2000; Neff and Hooper, 2002) and needs to be included in ecosystem budgets and N cycling studies, as many studies confirm that DON is the major N form in stream water and/or forest floor percolates (Yavitt and Fahey, 1986; Qualls and Haines, 1991; Hedin et al., 1995; Northup et al., 1995; Currie et al., 1996; Campbell et al., 2000; Perakis and Hedin, 2002).

However, the importance of DON in the soil N cycle has not always been recognized. Especially in agriculture information on mineral N was long desired due to its importance in crop nutrition, the environmental impact of NO_3^- leaching and N_2O emissions (Bhogal et al., 2000). Moreover, in the last decades, global N cycles have been dramatically altered by anthropogenic activities such as industrial combustion processes and fertilizer application (Vitousek et al., 1997; Gruber and Galloway, 2008). Increasing atmospheric N deposition caused higher nitrification rates and higher leaching losses of NO_3^-, therefore, the traditional N cycling model, which is inorganic N-centered, was reinforced (Aber et al., 1998; Fenn et al., 1998; Gundersen et al., 1998). As a consequence, biogeochemical studies have focused on the dynamics of dissolved inorganic N (DIN, especially NH_4^+ and NO_3^-) and processes such as soil N mineralization, nitrification, plant uptake of DIN and NO_3^- losses (e.g. Aber et al., 1998; van Breemen et al., 2002). Previously N-limited regions (low atmospheric N deposition of <4 kg ha^{-1} yr^{-1} Holland et al., 1999) have received chronic and increasing loads of N inputs due to industrialisation (Mayewski et al., 1990; Bredemeier et al., 1998; Holland et al., 1999). These inputs continue until today.

In particular, (sub)tropical regions will receive increasing inputs of N (e.g. from fertilizer inputs or fossil fuel consumption) over the next decades (Galloway et al., 1994, 2008), but until now studies on DON dynamics from these regions in particular and the influence of changing ecosystem N status on key processes in the soil N cycle (e.g. biodegradation) in general are scarce.

Recently, more studies have concentrated on DON in soils, probably due to various reasons. First, the development of new analytical techniques made the determination of DON in soils faster and easier (Campbell et al., 2000). Second, various plants take up DON in N-limited ecosystems (e.g. Kielland, 1994; Näsholm et al., 1998), challenging the inorganic-N centered model of the N cycle. The third reason for the increasing interest in DON lies within new results regarding the composition of total dissolved N (TDN) export from forest ecosystems. While the dominance of DON over DIN in N losses from forest ecosystems has been reported from unpolluted forest (Perakis and Hedin, 2002), a number of studies recently confirmed, that not only NO_3^- but also DON can account for the majority of TDN losses in ecosystems receiving high atmospheric inputs of anthropogenically derived N (McDowell et al., 2004; Pellerin et al., 2006; Brookshire et al., 2007).

Until now, the connection between DON export from forested watersheds and N loadings remains unclear. Dissolved organic N losses have been found to be independent of N loading rates (Lovett et al., 2000; Perakis and Hedin, 2002; Pellerin et al., 2006), while other studies reported a positive relationship between DON fluxes in soil solution and N loading rates in long-term forest fertilization experiments (McDowell et al., 2004; Pregitzer et al., 2004). In the USA, Brookshire et al. (2007) also found that DON outputs from a temperate forest watershed increased with atmospheric N deposition (ranging from 5-45 $\mathrm{kg\,N\,ha^{-1}\ yr^{-1}}$). In contrast, this finding has not been supported by data from European sites (Gundersen et al., 1998; Raastad and Mulder, 1999; Sjöberg et al., 2003; Park and Matzner, 2006). These opposing findings suggest, that not only the N status of an ecosystem can explain N losses, but that other unknown controls of DON export from forest ecosystems exist.

Fang et al. (2009) listed several mechanisms that could lead to DON losses under N-saturated conditions. First, increasing dissolved organic carbon (DOC) losses have sometimes been reported from N fertilized forests (Pregitzer et al., 2004). Therefore, a concurrent increase of DON is likely, as DON export has been shown to follow DOC losses in a strict stochiometric relationship (Hedin et al., 1995; Rastetter et al., 2005; Brookshire et al., 2007). If organic matter decomposition is low, DOC losses will increase as compared to C losses in the form of CO_2, therefore, DON export is directly controlled by

SOM dynamics rather than by biological N demand (Brookshire et al., 2007). Second, increasing N mineralization could trigger DON production in the critical depolymerization step before ammonification (Schimel and Bennett, 2004). Third, the role of abiotic DIN incorporation might play an important role under high atmospheric N deposition (Aber et al., 1998; Berntson and Aber, 2000; Dail et al., 2001). Whether DON is dominant over DIN due to a potentially large DON source in soils (Jones and Kielland, 2002) or due to the lacking utilization of DON by plants and microorganisms (Jones et al., 2005a), remains an open question. In contrast, factors, that could explain the dominance of DIN over DON in ecosystems are soil C/N ratios (Hood et al., 2003) and N supply from biological N_2 fixation by plants (Fastie, 1995; Schwendenmann and Veldkamp, 2005). Despite the growing interest in DON in both temperate and (sub)tropical ecosystems, information on DON dynamics in the soil of forest ecosystems under high and low anthropogenic N deposition is still scarce.

1.1.2 Dissolved organic nitrogen in the soil nitrogen cycle

In the soil, N can be present in solid, gaseous or dissolved forms. Solid forms include N bound to SOM and N associated with the soil exchange complex, while gaseous forms are e.g. NH_3 and N_2O. Dissolved forms of N may include organic or inorganic N forms (mainly NH_4^+ and NO_3^-). Large proportions of dissolved nutrients in the soil are present as organic forms (e.g. Kalbitz et al., 2000; McDowell, 2003), particularily in forest ecosystems (Michalzik et al., 2001).

Although the dissolved form only contributes little to total soil N (0.1-3%, Haynes, 2005), it plays a crucial role in the soil N cycle because it is mobile and may provide nutrients and energy for microorganisms (Brooks et al., 1999). Dissolved organic matter (DOM) in general also contributes to nutrient and contaminant transport (Zsolnay, 2003) and is important for soil structure and formation (Stevenson, 1994; Lundström et al., 1995).

In the soil solution, DON participates in a variety of processes (Fig. 1.1). Dissolved organic N can be substrate for microbial growth and product of microbial activity (exudation or cell lysis) and compared to the size of the mineral N and soluble organic N (SON) pools there is a considerably large flux of N through the microbial biomass (Murphy et al., 2000). Uptake by microbes results in an increase in particulate organic N (PON). Other possible pathways in the soil solution are heterotrophic nitrification to NO_3^- or direct mineralization to NH_4^+. To assess the processes which are connected to DON dynamics, it is necessary to consider all other N species in solution as the dynamics of DIN, PON and DON are tightly coupled (Fig. 1.1).

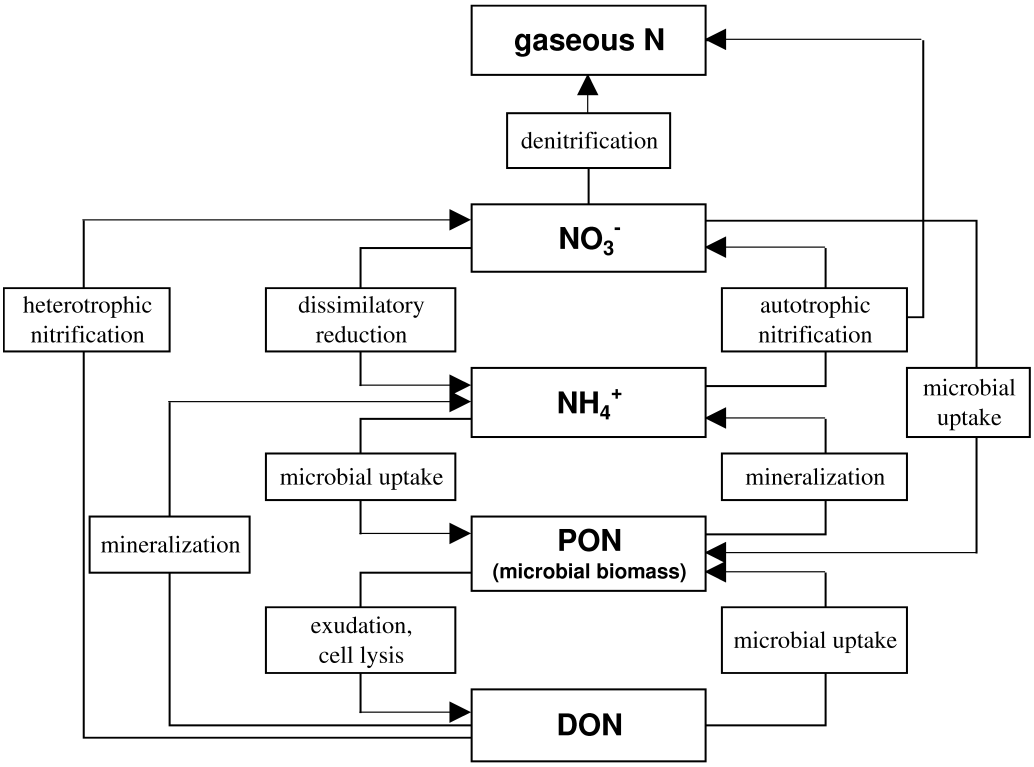


Fig. 1.1: Schematic representation of the processes in the soil N cycle (Fig. 3.1 from Chapter 3).

1.1.3 Composition of dissolved organic nitrogen

Dissolved organic N is part of DOM, which is operationally defined as molecules that pass a 0.45 μm filter (Thurman, 1985). There is no commonly used and accepted distinction between DON that is derived in situ and laboratory extractions of soil material with various extractants. Dissolved organic N is often defined as the organic N in solution that is measured by leaching methods or suction cups (Murphy et al., 2000; Zhong and Makeschin, 2003; Chen et al., 2005), while water-extractable organic nitrogen (WEON) or SON is sometimes used for describing laboratory extractions of soil with water (Zsolnay, 2003; Ros et al., 2009). Recently, the term extractable organic N (EON) was proposed to be used instead of SON (Xiang et al., 2008; Ros et al., 2009) to emphasize that the solutions are obtained by extraction procedures. The reason for the need to distiguish between these forms of soluble organic N lies within the different chemical composition of these solutes. Various extractants (Ros et al., 2009) as well as field methods (reviewed in Weihermüller et al., 2007) yield solutes with differing chemical composition. However, the terms WEON, SON, EON and DON are not used consistently among studies. If it is clearly stated, whether soluble organic material has been derived from the field or extracted in the lab this discrimination is not essential. In this study, DON is used as term for both field- and laboratory-derived solutes.

Dissolved organic C, which is also part of DOM, cannot be distinguished chemically from DON, as e.g. amino acids contain both C and N in their chemical structure. Therefore, DOC can serve as a rough proxy for DON (Neff et al., 2003). Many studies have found, that DOC and DON behave similar in soils (Qualls and Haines, 1992; Cleveland et al., 2004), while a substantial temporal and spatial variation of DOC/DON ratios in soils has also been reported (Michalzik and Matzner, 1999; Prechtel et al., 2000). This indicates that findings on DOC are not necessarily true for DON. Depending on the chemical composition, DOC and DON can behave differently in soils and findings on DOC for one ecosystems cannot be easily transferred to DON.

In contrast DIN, DON is not a single compound or a single class of compounds, but a structurally complex mixture of materials with very different chemical properties (Neff et al., 2003). Dissolved organic matter consists of a small labile and a large more stable pool (e.g. Qualls and Haines, 1992; Gregorich et al., 2003). The latter is of high molecular weight recalcitrant nature (DiTomaso et al., 1992; Bush, 1993; Yu et al., 2002), but the exact chemical composition of both DON and DOC remains unknown. Only 44% of DOC and 47% of DON in soil solution under temperate coniferous plantations could be characterized chemically in terms of total free amino-acid N, protein N and total phenolic C (Jones et al., 2008).

Due to the fact, that the exact chemical determination of compounds present in DOM is difficult, DOM has sometimes been described on the basis of fractionation techniques separating DOM into two operationally defined fractions, the so-called hydrophilic and hydrophobic fraction (Aiken and Leenheer, 1993). The following is a list of substances that may occur in the various fractions (Thurman et al., 1978; Leenheer, 1981; Thurman, 1985). The hydrophobic acid fraction includes the humic substances along with the amino acids and phosphate esters intimately bound to them, while the hydrophilic acids may consist of humic-like substances with lower molecular size and higher COOH:C ratios, oxidized carbohydrates with carboxylic acid groups, low molecular size carboxylic acids, and sugar phosphates. Hydrophobic neutrals may include lipids and some pigments, while hydrophilic neutrals may consist of simple and complex carbohydrates. The phenols (i.e., weak hydrophilic acids) include tannins and flavonoids without carboxylic acid groups. Finally, the base fraction is comprised of free amino acids and free proteins.

The most abundant fractions in soils are hydrophobic and hydrophilic acids (Hongve et al., 2000). Qualls and Haines (1991) found that in Oi horizons, DON was mostly in the hydrophobic and hydrophilic acid fraction, together with hydrophilic neutrals, phenols and bases. In Oa horizons, DON was mainly present as hydrophobic acids , hydrophilic acids and hydrophilic neutrals. Studies, that tried to investigate the exact chemical composition of DON found that a large proportion (up to 78%) of DON consists of amino acids, the majority of which is present as proteins (Westerhoff and Mash, 2002; Yu et al., 2002). Only a small proportion (less than 5%) of total amino acids in DON is in the free form (Westerhoff and Mash, 2002; Yu et al., 2002). Other compounds include amides (20%) and heterocyclic compounds such as pyroles, pyrrolidines, pyridines, pyrines and pyrazoles (25%) (Westerhoff and Mash, 2002).

The chemical composition of DON in forest soils can vary with tree species and degree of decomposition. Litter from different plant species differs not only in the composition but also in concentrations of water extractable compounds (Kuiters and Denneman, 1987; Hobbie, 1996; Gallet and Keller, 1999; Suominen et al., 2003; Wardle et al., 2003; Don and Kalbitz, 2005). In leaf litter, there are more easily degradable hydrophilic compounds such as sugars, amino acids and aliphatic acids (Hongve et al., 2000), while coniferous trees have higher concentrations of tannins and other phenolics (Gallet and Lebreton, 1995; Kraus et al., 2004). These chemical differences lead to differences in decomposition rates among different substrates. During biodegradation, concentrations of water-soluble compounds decrease, while concentrations of slowly decomposable compounds increase (Berg, 2000; McTiernan et al., 2003). As the layers of the forest floor represent different stages of decomposition, concentrations in water-extractable compounds also decrease with depth in organic layers (Qualls and Haines, 1992; Fröberg et al., 2003).

There have been contradicting results regarding the importance of forest floor layers for DOC production. While some studies found that the Oi layer is most important for DOC leaching (Qualls and Haines, 1992; Park et al., 2002), other studies reported that the Oe and Oa layer are significantly involved in the production of DOC (Solinger et al., 2001; Fröberg et al., 2007; Hilli et al., 2008). Don and Kalbitz (2005) found that in needle litter, DOC production increased after mass loss exceeded 20%, probably due to a larger contribution of lignin-derived compounds. Until now, there is no study that explicitly investigated the potential of the different forest floor layers for DON production.

1.1.4 Amounts and drivers of dissolved organic nitrogen in forest soils

For water-extractable C, concentrations in the soil are determined by C degradation and leaching on the one hand and C release by decomposition on the other hand (Michalzik and Matzner, 1999). Assumably, the same is true for DON. The largest DON concentrations usually occur in throughfall and in forest floor percolates (Michalzik et al., 2001) and usually decrease with soil depth (Möller et al., 2005; Schwendenmann and Veldkamp, 2005; Jones et al., 2008) due to biotic and abiotic processes. Some studies postulated that biotic processes are more important (Schwendenmann and Veldkamp, 2005), while other studies stressed the relevance of abiotic processes (Kaiser and Zech, 2000). In organic horizons, biotically driven processes such as microbial decomposition probably dominate, while abiotic processes such as sorption are relevant in the mineral soil. Both actual and potential DOM formation might be governed by biotic as well as abiotic processes (Kalbitz et al., 2000).

There is considerable variation in DON and DOC concentrations among studies. From temperate forest floors, DON concentrations ranging from 0.4-4.45 $\mathrm{mg\,N\,L^{-1}}$ and DOC concentrations in the range of 20-90 $\mathrm{mg\,C\,L^{-1}}$ have been reported by Michalzik et al. (2001). In the mineral soil, DON and DOC concentrations were lower, amounting to 0.2-1.1 $\mathrm{mg\,N\,L^{-1}}$ and 2-35 $\mathrm{mg\,C\,L^{-1}}$ (Michalzik et al., 2001). Other studies from Greece, Sweden and the UK found considerably higher soil concentrations (DON: 18 ± 3 $\mathrm{mg\,N\,L^{-1}}$ and DOC: 309 ± 80 $\mathrm{mg\,C\,L^{-1}}$) (van Hees et al., 2002; Christou et al., 2005). Another study also reported comparably high DON and DOC concentrations (12 $\mathrm{mg\,N\,L^{-1}}$ and 300 $\mathrm{mg\,C\,L^{-1}}$) and concluded that different soil solution extration methods might be responsible for these large variations among studies (Jones et al., 2008) as extraction methods are known to influence solute concentrations in the soil (Murphy et al., 2000). Moreover, NH_4^+ can interfere with low molecular weight DON (e.g. urea, amino acids) during analysis (Beier et al., 1992; Husted et al., 2000; Tiensing et al., 2001).

Studies on DON and DOC concentrations from (sub)tropical forest ecosystems are not as numerous as from temperate forest ecosystems. While many studies report DOC and DON concentrations in throughfall (e.g. Liu and Sheu, 2003; Guo et al., 2005; Heartsill-Scalley et al., 2007), data on these concentrations in forest floor percolates and soil solutions is scarce. Reported DOC concentrations in forest floor leachates ranged from 21-43 $\mathrm{mg\,C\,L^{-1}}$ and from 1-27 $\mathrm{mg\,C\,L^{-1}}$ in the soil solution in tropical forests in Ecuador and Tanzania (Wilcke et al., 2001; Goller et al., 2006; Schrumpf et al., 2006). Dissolved organic N concentrations were 0.7-1.4 $\mathrm{mg\,N\,L^{-1}}$ in forest floor leachates of (sub)tropical forests in Jamaica and Ecuador (Hafkenscheid, 2000; Goller et al., 2006). In soil solution, DON concentrations ranged from 1-3.2 mg N L^{-1} in (sub)tropical forests in Thailand and China (Möller et al., 2005; Fang et al., 2009). The highest DON fluxes occur parallel to highest DON concentrations in throughfall and forest floor percolates (Michalzik et al., 2001). In sandy soils, fluxes of DOC and DON are usually higher than in more heavily textured soils (Dosskey and Bertsch, 1994; Campbell et al., 2000). Mean annual fluxes of of DOC in forest floor percolates of temperate forest ecosystems can amount to more than 900 kg C ha^{-1} yr^{-1} (Kleja et al., 2008), but usually these fluxes range from 100-400 kg C ha^{-1} yr^{-1} and less than 1-18 kg N ha^{-1} yr^{-1} in forest floor percolates (Michalzik et al., 2001). In the mineral soil, DOC and DON fluxes are lower (10-200 kg C ha^{-1} yr^{-1} and 0.1-9.4 kg N ha^{-1} yr^{-1}) (Michalzik et al., 2001). Only few studies investigated DOC and DON fluxes in forest floor percolates of (sub)tropical ecosystems, probably due to the fact that the forest floor is often thin, caused by favourable decomposition conditions. Dissolved organic C and N fluxes in forest floor percolates were 277-962 kg C ha^{-1} yr^{-1} in Costa Rica and Taiwan (Schwendenmann and Veldkamp, 2005; Chang et al., 2007) and 8.2-17.6 kg N ha^{-1} yr^{-1} in Chile and Jamaica (Hafkenscheid, 2000; Oyarzún et al., 2004), while fluxes in seepage were lower, amounting to 43-490 kg C ha^{-1} yr^{-1} in Puerto Rico and Taiwan (McDowell et al., 1998; Chang et al., 2007) and 1-16.9 kg N ha^{-1} yr^{-1} in Costa Rica and China (Schwendenmann and Veldkamp, 2005; Fang et al., 2009).

These findings show, that fluxes of DON and DOC can be highly variable among ecosystems. Several possible drivers have been investigated to be able to predict ecosystem DON and DOC fluxes from ecosystem characteristics. Although plants play a crucial role for DON production in soils, vegetation did not seem to influence DON fluxes and concentrations when comparing coniferous and hardwood sites (Michalzik et al., 2001), although species type is known to affect the soil C/N ratio and DIN (Lovett et al., 2004). In contrast to litter quality, litter quantity had a strong positive effect on DOM fluxes from forest floors (Kalbitz et al., 2000; Park et al., 2002).

One major driver that explains the variation of DON and DOC fluxes under field conditions is the flux of water. Strong positive relationships between precipitation and DON fluxes in forest floor percolates have been reported by Michalzik et al. (2001). Concerning the relationship between DON and DOC concentrations and water fluxes, concentrations were observed to increase with increasing discharge (Jardine et al., 1990; Boyer et al., 1997; Hagedorn et al., 2000; Buffam et al., 2001; Solinger et al., 2001; Goller et al., 2006), probably due to limited contact of organic solutes with possible sorption sites (Luxmoore et al., 1990; Riise, 1999). Other studies found concentrations to decrease with increasing water fluxes (McDowell and Wood, 1984; Easthouse et al., 1992). Tipping et al. (1999)

concluded, that the export of DOC only increases with increasing water fluxes in soils with large pools of potential DOM. Potential DOM is defined as organic material, which can possibly enter the soil solution, depending on its interaction with the solid soil phase (Tipping, 2002). A positive relationship between DOC and DON concentrations in forest floor percolates and water fluxes should therefore point to large pools of potential DOM, while a negative relationship should point to small pools of potential DOM (dilution effect) that can be depleted fast.

Other environmental controls over DOM dynamics include e.g. drying-wetting events (Christ and David, 1996; Hentschel et al., 2007; Borken and Matzner, 2009), however, in continuosly wet regions of the (sub)tropics this process is probably less important. The same is true for freeze-thaw cycles (Zsolnay, 1996). Results on the effect of temperature on DOM release are inconsistent, but in the field other factors are probably more important (Kalbitz et al., 2000). The DOC flux from the forest floor has also been reported to increase with the stock of C in the O horizon of boreal and temperate forests (Fröberg et al., 2006), and positive relationships between soil organic matter stocks and DOC and DON fluxes have also been reported from Hawaii (Neff et al., 2000). However, this has not been confirmed on a global scale when including data from tropical forests (Fujii et al., 2009).

1.1.5 Sources of dissolved organic nitrogen in forest soils

In general, the sources of DON are poorly quantified (Kalbitz et al., 2000; Neff et al., 2003) and can be highly variable, depending on the location in soil profile (Yano et al., 2004). In addition to several biotic and abiotic formation mechanisms in soils, DON can also enter the soil by lateral flow from adjacent areas (Casper et al., 2003), by dry and wet deposition (Mopper and Zika, 1987) and by vegetation stemflow and throughfall (Michalzik and Matzner, 1999).

Biotic sources

Large proportions of DON and DOC in the soil are derived from above- and below-ground litter (Park et al., 2002; Yano et al., 2005). Moreover, plants add to DON in soils by exuding e.g. amino acids from roots (Farrar et al., 2003). Recently, the role of below-ground litter and rhizodeposition in DON formation has been emphasized (Nguyen, 2003; Yano et al., 2005).

Another important biotic source of DON in soils are microbes, which can contribute to DON directly by microbial turnover (Seely and Lajtha, 1997). Indirect microbial sources include production of extracellular enzymes and subsequent decomposition of proteinaceous material (Seely and Lajtha, 1997; Neff et al., 2003) and the release of microbial metabolites (Guggenberger et al., 1994; Huang et al., 1998). Dissolved organic N in soils can also originate from animal inputs such as faeces and urine (Bristow et al., 1992).

Abiotic sources

Recently, abiotic formation mechanisms of DON in forest soils have received increasing attention due to several findings. Abiotic N immobilization has been suggested to be an important N retention process in soils under high N deposition. This is based on the findings that soils, rather than plants, are the dominant long-term sink for applied N (Gundersen et al., 1998) and that the abiotic reaction of NO_3^- in soils has often been observed (Davidson et al., 1991; Berntson and Aber, 2000; Dail et al., 2001; Perakis and Hedin, 2001; Compton and Boone, 2002; Corre et al., 2007; Huygens et al., 2007; Sotta et al., 2008). In addition, the retention of anthropogenically derived NO_3^- happens without any response of the biotic factors (e.g. increased microbial biomass, accelerated litter decay, positive plant growth response; Colman et al., 2008).

Moreover, in a study with sterilized soils, NH_4^+ was primarily immobilized biotically, while NO_2^- was mainly immobilized via an unkown abiotic pathway (Fitzhugh et al., 2003). The abiotic reaction of NO_2^- with model compounds such as phenols, which are assumed contribute to organic matter in forest soils, has already been reported before (Azhar et al., 1989).

Stevenson (1994) mentioned three possible mechanisms for the chemical reaction of NO_2^-: the reaction with amino acids (van Slyke reaction), with amines, and with humic substances (at low soil pH). Free amino acids in soils represent only 10% of soil DON (Westerhoff and Mash, 2002; Yu et al., 2002) and nitrosamines are only of transitory existence. Therefore, only the reaction of NO_2^- with humic substances at low soil pH (as found in forest floors) is considered to play an important role in forest soils.

Various tracer studies have shown that NO_2^- added to humic substances is either fixed by organic matter or converted to N gases (Führ and Bremner, 1964a; Smith and Chalk, 1980). The main mechanism in reaction of NO_2^- with humic substances is assumed to be nitrosation (Thorn and Mikita, 2000).

The reaction of NO_2^- in soils increases with decreasing pH and increasing SOM content, NO_2^- level, time and temperature of incubation (Führ and Bremner, 1964a,b; Nelson and Bremner, 1969). Dail et al. (2001) confirmed that abiotic N immobilization was positively correlated with DOC concentrations and never occured in the mineral soil, but in horizons rich in organic matter. They concluded that after reduction of NO_3^- to NO_2^-

by biotic or abiotic processes, an abiotic reaction of NO_2^- with soil organic matter could take place.

Based on the findings of Dail et al. (2001), a new hypothesis (Ferrous Wheel Hypothesis) was published in 2003 by Davidson et al. (2003), which attempted to offer a possible explanation of abiotic NO_3^- immobilization via NO_2^- in forest floors. The Ferrous Wheel Hypothesis states, that metals such as Fe(II) or Mn(II) compounds will reduce NO_3^- in the forest floor to NO_2^-, which subsequently will react with DOC to newly formed DON compounds. This hypothesis was the first to state, that DOC and not SOM is the key acceptor for NO_2^-. Until now, most studies have been carried out in solid soil samples, where chemical or physical sterilization techniques probably led to artefacts (Wolf and Skipper, 1994). The Oa layer of forest soils meets all the requirements for abiotic immobilization of NO_3^- as stated in the hypothesis (some iron or manganese minerals, which are mixed into the forest floor by bioturbation, anoxic microzones, which promote the reduction of NO_3^- and the presence of adequate amounts of DOC).

1.1.6 Fate of dissolved organic nitrogen in forest soils

Many plant species with and without mycorrhizal associations are able to take up DON directly (Kielland, 1994; Chapin, 1995; Raab et al., 1996; Näsholm et al., 1998; Öhlund and Näsholm, 2004), especially in highly N-limited ecosystems. The absorption rate of amino acids, which contribute to DON, can be as fast or even faster than that of DIN (Kielland, 1994; Persson et al., 2003). Therefore, plants in such ecosystems do not need to rely on the mineralization of DON to DIN by microbes to meet their N demand. This finding also challenges the traditionally inorganic N-centered view of the terrestrial N cyle (Schimel and Bennett, 2004) , but whether DON represents a significant N source for trees in temperate forests is still unknown. Jones et al. (2005b) concluded that root uptake of amino acids will only be of minor importance in soils with high mineralization rates or with high anthropogenic N inputs and Finzi and Berthrong (2005) showed that temperate tree species took up amino acid N, NH_4^+ and NO_3^- in proportion to their presence in the soil.

Plants and microbes compete for DON and at low added concentrations, microorganisms effectively outcompete plants for amino acids in the soil (Bardgett et al., 2003) while at high soil concentrations plants probably become more competitive (Jones et al., 2005b). The extent, to which biotic uptake relies on DON should vary with the degree of N-limitation and DOM quality (Kaushal and Lewis, 2003; Neff et al., 2003).

The ability to take up amino acids is omnipresent in microorganisms (Holden, 1962; Anraku, 1980). Soil organisms use DON as substrate (Zsolnay and Steindl, 1991; Qualls and Haines, 1992; Nelson et al., 1994) but at the same time, DON is the product of microbial activity. This fact makes it difficult to quantify gross mineralization in soils. Although the biodegradation of DON by microorganisms is an important process in soils, it has rarely been investigated for the entire class of compounds of DON (Neff et al., 2003), but rather for single compounds that are known to be part of DON, such as amino acids or amino sugars (Jones, 1999; Vinolas et al., 2001; Roberts et al., 2007). In contrast, more information is available on the biodegradability of DOC (e.g. Yano et al., 2000; Kalbitz et al., 2003; Marschner and Kalbitz, 2003; Qualls, 2005; Schwesig et al., 2003; Don and Kalbitz, 2005), which can serve as a rough proxy for DON (Neff et al., 2003). This assumption is supported by laboratory studies that found DON not to decay faster than DOC (Qualls and Haines, 1992; Cleveland et al., 2004; Kiikkilä et al., 2005). However, in agricultural soils and aquatic systems, the biodegradation of DON was often greater than that of DOC (Gregorich et al., 2003; Wiegner and Seitzinger, 2004; Kaushal and Lewis, 2005; Petrone et al., 2009), which in turn supports the idea, that findings on DOC cannot be transferred to DON.

For forest soils, there are only few results concerning DON biodegradation. Kiikkilä et al. (2005) showed that DON biodegradation was related to tree species and was higher in water extracts of coniferous than decidious species. They also added NH_4Cl to their samples to investigate the effect of mineral N in solution, but as their samples were preincubated, conclusions regarding the actual degradability of DON are difficult to draw.

There are several measures for DOM biodegradability, e.g. the difference in concentration before and after incubation (e.g. Kiikkilä et al., 2005), CO_2 production during the incubation time in the headspace of incubation flasks (for DOC; e.g. Kalbitz et al., 2003). In this study, the term biodegradation is referred to as the sum of mineralization (breakdown of organic compounds to obtain energy and nutrients) and microbial uptake. While biodegradable DON is defined as the quantity of DON that is actually utilized by microorganisms, bioavailability only describes the ingestion and retention of organic compounds (Marschner and Kalbitz, 2003).

3D fluorescence spectroscopy and subsequent parallel factor (PARAFAC) analysis are new tools that were sucessfully used to identify major components (factors) in excitation-emission-matrices (EEMs) of DOM samples (e.g. Stedmon et al., 2003; Ohno and Bro, 2006; Fellman et al., 2008). Especially the group of protein-like fluorophores has been suggested to serve as proxy for DOM biodegradability (Fellman et al., 2008; Balcarczyk et al., 2009).

When assessing the fate of DON in soils, sorption processes also need to be considered. Both the sorption to mineral surfaces (Jardine et al., 1989; Qualls and Haines, 1992;

Kaiser and Zech, 2000) and to organic surfaces (Qualls, 2000; Guggenberger and Kaiser, 2003) may contribute to DON removal from the soil solution. Finally, DON can also be ultimately lost from the soil profile by leaching with percolating water (Qualls and Haines, 1991; Hedin et al., 1995; Currie et al., 1996; Perakis and Hedin, 2002).

1.1.7 Objectives of this study

In the last years, research on DON has mainly focused on analyzing the chemical composition of DON and quantifying the amounts and drivers of DON fluxes in temperate forest ecosystems. In contrast, the magnitude and influencing factors of DON fluxes in (sub)tropical ecosystems have received less attention, although large amounts of precipitation due to frequent storms might strongly influence DOC and DON losses from these ecosystems. Moreover, possible sources and sinks of DON in soils of forest ecosystems and the effect of changing ecosystem N status have rarely been investigated.

To adress these uncertainties, we conducted one field and two laboratory studies, and hypothesized that:

1. DOC and DON fluxes are large in the soil of a subtropical mountainous forest ecosystem in Taiwan,

2. precipitation plays a crucial role for soil DOC and DON fluxes in this ecosystem,

3. initial litter extract chemistry and the N status of an ecosystem (as indicated by DIN concentrations in solution) influence DON biodegradation,

4. DOC and DON dynamics are linked, and that

5. the abiotic formation of DON by the reaction of NO_2^- and DOC (Ferrous Wheel Hypothesis) is a possible source of DON in forest soils.

1.2 Material and methods

1.2.1 Sites

This study comprises one field study from a *Chamaecyparis obtusa* var. *formosana* forest in Taiwan (Chapter 2) and two laboratory studies (Chapters 3 and 4) with samples from the same cypress forest in Taiwan, a Norway spruce and a European beech stand in Germany.

The *Chamaecyparis obtusa* var. *formosana* site is located in the Chi-Lan Mountains in northern Taiwan (24°35'N, 121°24'E) at an altitude of 1,400-1,800 m a.s.l.. Mean annual air temperature is 13°C and annual precipitation ranges from 2,000 to more than 5,000 mm depending on the number and strength of storms (Chang et al., 2007). The dominant tree species is cypress (*Chamaecyparis obtusa* var. *formosana*) together with *Illicium philippinense* (Merr.) and *Rhododendron formosanum* (Hemsl.), which comprise the bulk of understory vegetation (Chang et al., 2007). The soil is a poorly developed Lithic Leptosol (FAO, 1998) with a very high content of coarse material (about 90 vol% are >2 mm) and a pH($CaCl_2$) of 2.8-3.2 in the mineral soil. The forest floor has a thickness of 7-10 cm and is poorly stratified due to intensive rooting. The C/N ratio of the total forest floor is 17 and the pH(H_2O) is 3.5 (Rees et al., 2006). Details on this site can be found in Rees et al. (2006) and Chang et al. (2007).

The Norway spruce site is located in the Fichtelgebirge, Germany (50°09´N, 11°52´E) at 785 m a.s.l.. The average annual temperature is about 5°C and average annual precipitation amounts to roughly 1,150 mm (Gerstberger et al., 2004). The dominant tree species is Norway spruce (*Picea abies* (L.) Karst.), while ground vegetation is mainly composed of acidophilic understory plant species such as *Deschampsia flexuosa* (L.) Drejer, *Calamagrostis villosa* (Chaix) J.F.Gmelin and *Vaccinium myrtillus* L. (Gerstberger et al., 2004). The main soil type at the site has been classified as Haplic Podzols (FAO, 1998), which has a pH($CaCl_2$) of 2.9-4.3 in the mineral soil. The mor type forest floor is about 8.5 cm thick and well stratified with Oa, Oe and Oi layers. The pH(H_2O) in the Oi and Oa layer is 4.5 and 3.5, respectively. Total organic C/total organic N (TOC/TON) ratios for the Oi layer amount to 24.8 and 22.6 in the Oa layer (Kalbitz, 2001). Details on this site can be found in Kalbitz (2001), Gerstberger et al. (2004) and Matzner et al. (2004).

The European beech site is located in the Steigerwald, Germany (49°52´N, 10°28´E) at 440 m a.s.l.. The average annual precipitation is about 750 mm with a high interannual variation and the average annual temperature is about 7.9°C (Gerstberger et al., 2004). The vegetation is mostly composed of European beech (*Fagus sylvatica* L., about 75%) and sessile oak (*Quercus petraea* (Matt.) Liebl, about 25%). Ground vegetation is sparse and dominated by moderately acidophilic species such as *Deschampsia flexuosa* (L.) Drejer, *Luzula luzuloides* (Lam.) Dandy & Wilm, *Oxalis acetosella* L. and *Calamagrostis arundinaceae* (L.) Roth (Gerstberger et al., 2004). The soils are mainly Dystric Cambisols (FAO, 1998) with a pH($CaCl_2$) of 3.2-4.2 in the mineral soil. The mor type forest floor is thin (about 3 cm) and Oe and Oa layer are hard to separate. The pH(H_2O) is 5.3 in the Oi layer and 4.1 in the Oa layer and TOC/TON ratios amount to 21.8 for the

Oi layer and 17.8 for the Oa layer (Kalbitz, 2001). Details on this site can be found in Kalbitz (2001) and Gerstberger et al. (2004).

1.2.2 Field measurements and incubation experiments

At the Chi-Lan Mountain site (field study, Chapter 2), bulk precipitation, throughfall, forest floor percolates and soil water seepage (60 cm) were sampled biweekly from January 2005 to December 2008. Bulk precipitation was collected in triplicates and twelve throughfall samples were aggregated to three replicates by volume-weighted mixture of each four collector solutions. Forest floor percolates were sampled by free draining lysimeters (January 2005-March 2007) and by PVC lysimeters with PE membranes (pore size 3 μm) of about 500 cm^2 (April 2007-December 2008) established in 4 replicates at the border of the O and A horizon. At 10 min intervals, suction was applied to the membrane of the PVC lysimeters to collect the sample into a PE bottle and to avoid water logging. Soil water seepage was collected by eight ceramic suction cups installed at about 60 cm depth. Each sampling device for seepage water was comprised of two suction cups, therefore, seepage water was collected in four replicates per sampling date. The suction cups were evacuated at about -0.2 bar.

For both incubation studies (Chapters 3 and 4) extracts were obtained by extracting Oi and Oa material from the three sites with distilled water (300 g soil horizon material and 3 L water). After incubation for 24 h at 5°C, the suspensions were first filtered through a ceramic plate with a pore diameter of approximately 1 μm (cleaned with bidistilled water) to remove large litter parts. Then, the samples were filtered through 0.45 μm cellulose acetate filters (Schleicher and Schüll OE 67) to exclude most of the microorganisms in solution. In Chapter 4, sterility was a precondition for the incubation experiment, therefore 0.2 μm cellulose acetate filters (Schleicher and Schüll OE 67) were used. As sterility was only required during a comparably short incubation time of 60 min, sterile filtration through 0.2 μm was considered to be sufficient for establishing sterility.

In a first experiment (Chapter 3), inoculum (obtained from mixed Oa material) was added to the samples and three replicates of each water extract (except for cypress-Oi) were incubated under oxic conditions for 21 days at 20°C. Additionally, NO_3^- was added as $NaNO_3$ to another set of inoculated samples in concentrations of 3, 5 and 10 mg NO_3^--N L^{-1} for beech-Oi samples, and 0.75, 1.5 and 3 mg NO_3^--N L^{-1} for all other solutions, to obtain similar DOC/TDN ratios. Samples were taken after 0, 1, 3, 5, 7, 10, 14 and 21 days and shaken gently by hand at each sampling date to mix the samples and to ensure aeration. Filtered and unfiltered subsamples were analyzed. Controls with pure water instead of DOM were treated in the same way to determine C and N losses from the inoculum (no changes occured during these incubations).

In addition we calculated a balance over all N species in solution over the whole incubation time. Mineralization and uptake by the microbial biomass lead to declining DON and NO_3^- concentrations) should equally be reflected by increasing NH_4^+ and PON concentrations, assuming no gaseous N losses. The balance was calculated as sum of change in NO_3^- and DON concentrations plus the sum of change in NH_4^+ and PON concentrations. Therefore, positive values mean N_2 fixation, while negative values mean gaseous N losses. To account for error propagation, only results which were more than $0.4\,\mathrm{mg\,N\,L^{-1}}$ different from zero were regarded to be significant.

In a second experiment (Chapter 4), DOC concentrations of litter extracts were adjusted to 15 and $60\,\mathrm{mg\,C\,L^{-1}}$ in three replicates for each litter extract and NO_2^- was added as $NaNO_2$ to the samples in concentrations of 2 and $20\,\mathrm{mg\,N\,L^{-1}}$. One set of samples was incubated without NO_2^- addition. After incubation under oxic conditions at room temperature (22°C), samples were taken after 0, 15, 30, 45, and 60 min.

1.2.3 Analytical methods

Elemental analysis

The DOM solutions from the field study (Chapter 2) were analyzed for total organic C (high temperature combustion, Elementar, High TOC II), NH_4^+, NO_3^- and TDN (flow injection, Lachat, QuickChem 8000 series). Dissolved organic N was calculated as TDN-(NO_3^-+NH_4^+).

The filtered samples from the first incubation experiment (Chapter 3) were analyzed for DOC and TDN (thermo-catalytic, multi N/C 2100, Analytik Jena), NO_3^- (Dionex DX 500 ion chromatograph) and NH_4^+ (FIA-LAB flow injector, MLE Dresden). The unfiltered samples were only analyzed for total N (TN). Particulate organic N was calculated as difference in TN between filtered and unfiltered samples and used as proxy for microbial biomass. The litter extracts and samples for the second incubation experiment (Chapter 4) were analyzed for DOC (thermo-catalytic, Analytik Jena) and NO_2^- (colorimetric, flow injection, Skalar Sanplus Analyzer).

UV/Vis and fluorescence spectroscopy

For the first incubation study (Chapter 3), specific UV absorbance at 280 nm ($SUVA_{280}$) was recorded (UV-1800, Shimadzu). Fluorescence excitation-emission-matrix spectroscopy (Coble et al., 1990) was used to obtain excitation-emission-matrices (EEMs) of all incubated samples and selected reference compounds (tyrosine, phenylalanine, phenol, 3-hydroxy-benzaldehyd and gallic acid) (Varian Cary Eclipse fluorescence spectrophotometer, excitation range: 240 to 450 nm, emission range: 300 to 600 nm, 5 nm increments). Absorbance spectra were obtained with a Varian Cary 50 Bio UV-visible spectrophotometer. Samples were acidified to pH 2 and diluted with bidistilled water to a maximum absorbance of 0.1 cm^{-1} before fluorescence spectroscopy, as fluorescence depends on pH and concentration (Laane, 1982; Zsolnay et al., 1999).

For processing, several steps were conducted. First, we corrected EEMs for instrument bias according to the manufacturer instructions and second for the inner filter effect, using the absorbance spectra (McKnight et al., 2001). Third, EEMs were normalized by dividing with the integral of the Raman scatter peaks and corrected water spectra were subtracted to obtain spectra in Raman units ((R.u.), Stedmon et al., 2003). Fourth, Rayleigh scatter peaks were removed and interpolated to minimize errors (Bahram et al., 2006).

1.2.4 Statistics, calculations and modelling

For the first incubation study (Chapter 3), fluorescence EEMs (except those of reference compounds) were analyzed using PARAFAC analysis (Carroll and Chang, 1970; Harshman, 1970; Bro, 1998). Modeling was conducted using the N-way Toolbox (version 2.10.) for MATLAB (The MathWorks) (Andersson and Bro, 2000). Details on the method can be found in Stedmon and Bro (2008). We applied non-negativity constraints to allow only chemically relevant results (i.e. only positive values). The model was tested for various groups of fluorophores (1-7 factors). The appropriate number of factors was identified by using core consistency diagnostics (Bro and Kiers, 2003) and plotting of residuals. The dataset could be revolved into four contributing factors which was confirmed by a split-half validation on the model (Stedmon et al., 2003).

All statistical tests and analyses were performed with SysStat and SigmaPlot (versions 3.5 and 10.0, respectively, Systat Software, Inc., Chicago). Graphs were plotted in SigmaPlot and Excel. This thesis and all tables have been designed in LaTeX.

1.3 Results and discussion

Soil fluxes of DON in the subtropical montane *Chamaecyparis obtusa* var. *formosana* forest were similar to fluxes reported from other (sub)tropical ecosystems, but DOC fluxes exceeded published values by far (compare Chapter 2). For both DON and DOC, fluxes in forest floor percolates (16 kg N ha^{-1} yr^{-1} and 962 kg C ha^{-1} yr^{-1}) were higher than in seepage (8 kg N ha^{-1} yr^{-1} and 478 kg C ha^{-1} yr^{-1}) (Table 1.1). The magnitude of these fluxes could be attributed to Oa material accumulation due to low decomposition rates at the site (Rees et al., 2006) as Oa horizons can significantly contribute to DOC release from the forest floor (Fröberg et al., 2003; Müller et al., 2009). Other explanations include the contribution of below-ground litter (Yano et al., 2005) and bryophytes to DON and DOC production. Moreover, DOM production can increase with increasing decomposition in samples with large lignin degradation (Kalbitz et al., 2006). The influence of none of these factors has been investigated at this site yet.

Table 1.1: Annual and mean annual fluxes of water, NO_3^-, NH_4^+, DON and DOC in throughfall, forest floor percolates and seepage (60 cm) (Table 2.1 from Chapter 2).

Year	**Water**	**NO_3^-**	**NH_4^+**	**DON**	**DOC**
	[mm]	——— [kg N ha^{-1} yr^{-1}] ———			[kg C ha^{-1} yr^{-1}]
Throughfall					
2005	4,587	2.3	1.5	1.8	140
2006	3,503	1.8	2.2	2.8	93
2007	4,560	4.6	2.3	4.7	97
2008	4,026	2.5	1.6	4.4	94
mean	4,169 ± 257	2.8 ± 0.6	1.9 ± 0.2	3.4 ± 0.7	106 ± 11
Forest floor percolates					
2005	4,533	2.9	0.9	12.6	894
2006	3,448	0.6	0.8	12.7	745
2007	4,506	3.2	2.1	21.0	1135
2008	3,972	1.5	2.1	16.4	1073
mean	4,115 ± 257	2.1 ± 0.6	1.5 ± 0.4	15.7 ± 2.0	9612 ± 89
Seepage (60 cm)					
2005	4,427	1.6	0.6	5.7	561
2006	3,343	1.3	0.7	5.3	411
2007	4,400	3.3	2.6	7.3	512
2008	3,866	2.8	1.3	7.7	477
mean	4,009 ± 257	2.3 ± 0.5	1.3 ± 0.5	6.5 ± 0.6	490 ± 32

In the soil, DON fluxes were always higher than DIN fluxes. Similar findings have been reported from other (sub)tropical ecosystems (e.g. Hafkenscheid, 2000), except when N_2-fixing plants were present (Schwendenmann and Veldkamp, 2005) or when atmospheric N put was large (Fang et al., 2008). At our site inorganic N input was low (averaging 4 kg N ha^{-1} yr^{-1}), which supports the finding that DON is dominant over DIN in N-limited forests with low anthropogenic N inputs (Qualls, 2000; Perakis and Hedin, 2002; Park and Matzner, 2006) and negligible proportions of N_2-fixing species.

Concentrations of DOC and DON in forest floor percolates and seepage were not related to bulk precipitation. Instead concentrations were highly variable at similar amounts of precipitation, contradicting the often observed "‘dilution effect"’. This means, that the pool of DOC and DON in the forest floor at this site is variable in size, e.g. due to varying decomposition conditions before rainfall. The influence of drying-wetting events on DOC and DON concentrations as observed by Goller et al. (2006) for a montane forest in Ecuador is unlikely at our site as the soil is permanently wet. Moreover, the potentially soluble DOM pool is probably quickly replenished by high storm-related litter inputs, as suggested by increasing DOC concentrations after litter addition in a litter manipulation experiment at this site (Chang et al., 2007).

Fluxes of DOC and DON in forest floor percolates and seepage were linearily related to bulk preciptation (r always higher than 0.84 with n=100 and p<0.01, Spearman rank correlation) (Fig. 1.2). This relationship was also reported by Michalzik et al. (2001) for bulk precipitation and DOC and DON fluxes in forest floor percolates of temperate forests. However, this relationship did not hold after including additional data on these fluxes as most DOC and DON fluxes from (sub)tropical ecosystems were lower than could be expected from the linear correlation. One possible explanation for this phenomenon could be that better climatic conditions and therefore higher decomposition rates might enforce the need to efficiently recycle nutrients in (sub)tropical ecosystems, before they are leached from the soil (Richards et al., 1996).

Organic solute concentrations in the soil at the *Chamaecyparis obtusa* var. *formosana* site could have been affected by the change in lysimeter type (Reynolds et al., 2004; Buckingham et al., 2008), but soil heterogeneity also can influence organic solute concentrations (Hendershot and Courchesne, 1991; Neff and Asner, 2001). Therefore it is not possible, which of these two factor influenced organic solute concentrations most. Dissolved organic C and N concentrations were significantly higher in forest floor percolates than in seepage (60 cm), while the difference in DIN concentrations between these compartments was considerably smaller. This decrease of organic solute concentrations with depth could be due to sorption (e.g. Kaiser and Zech, 1998) and decomposition processes (e.g. Yano et al., 2000). At our site, possible sorption sites are limited due to a shallow and partly water-saturated soil, but DON and DOC in inoculated extracts from Oa horizons were recalcitrant against microbil decay during 21 days in an incubation experiment (Fig. 1.3). Therefore, after these organic solutes are transported in the mineral soil, their primary fate must be rather sorption than biodegradation due to their largely refractory nature. Significant amounts of DOC and DON remain in the mineral soil (as indicated by the decrease in flux magnitude between forest floor percolates and seepage), therefore we postulate, that DON and DOC from the forest floor potentially add to stable organic soil pools at this site.

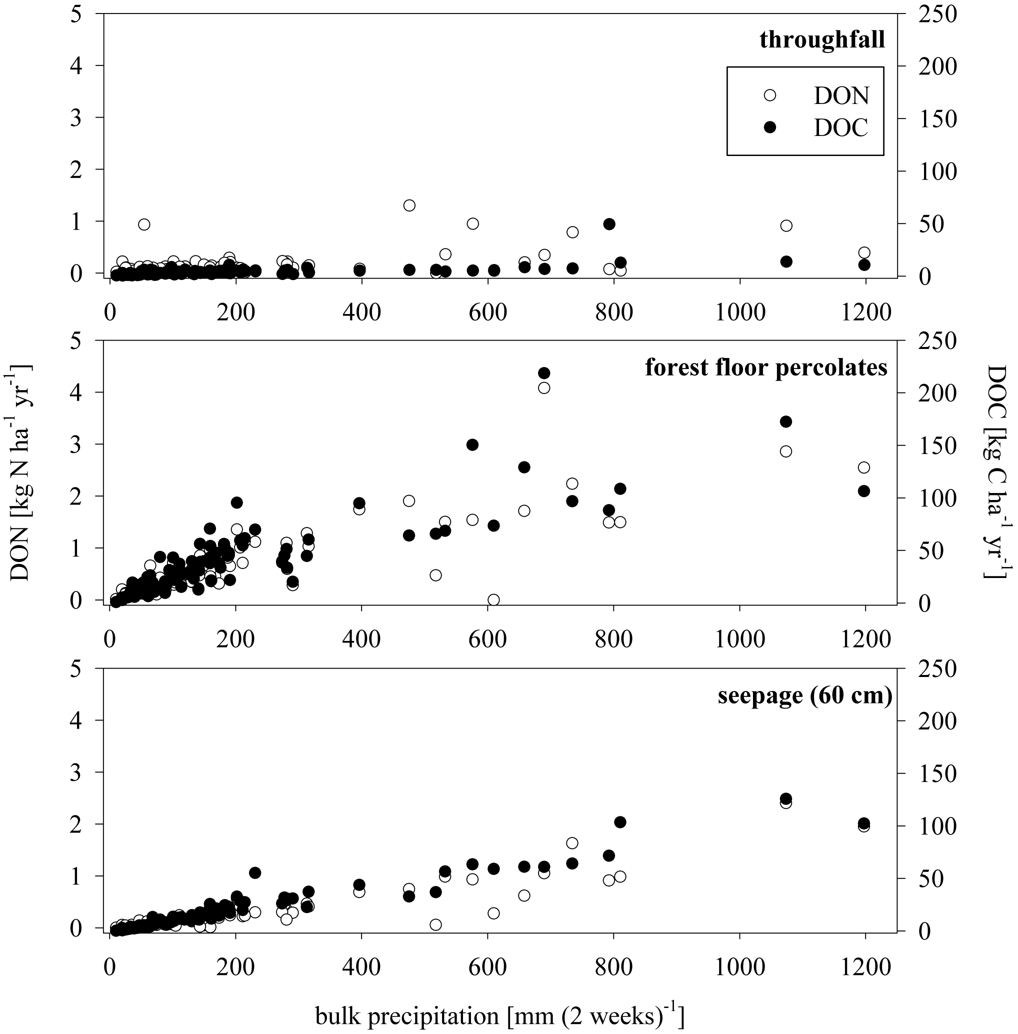
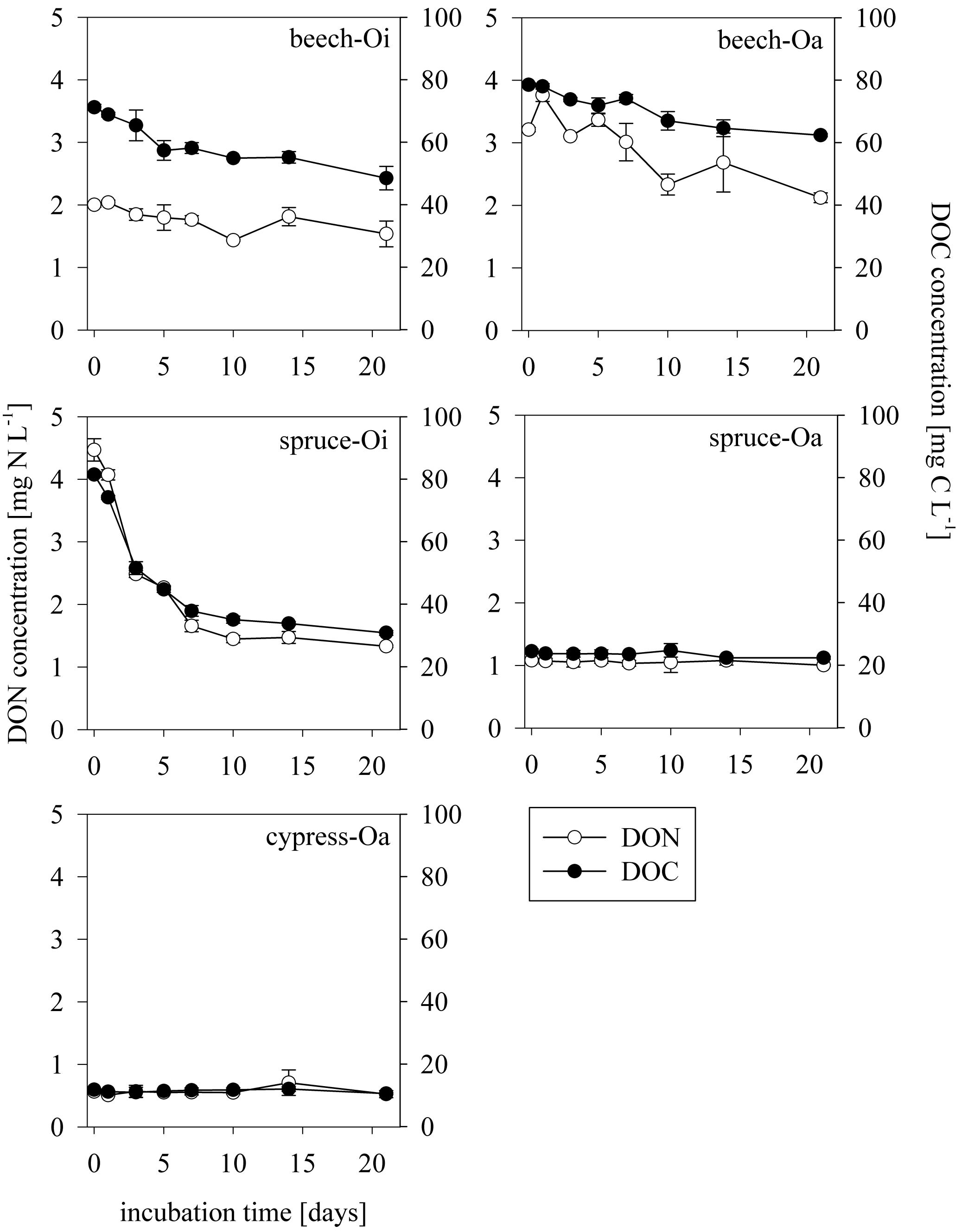


Fig. 1.2: Relationship between bulk precipitation and DOC and DON fluxes in throughfall, forest floor percolates and seepage (60 cm) (Fig. 2.3 from Chapter 2).

In the same experiment (compare Chapter 3), we also incubated inoculated extracts from Oi and Oa horizons of beech and spruce. During the incubation time, DON and DOC concentrations declined most in spruce-Oi samples (Fig. 1.3), which was in line with very low $SUVA_{280}$ values indicating low concentrations of aromatic structures and with exceptionally high PON concentrations as compared to other samples. There was no biodegradation in spruce-Oa extracts, and biodegradation in beech samples was low.

Due to the large biodegradation in spruce-Oi samples, O_2 consumption by the microbial decomposer community was high. This probably led to temporarily anoxic conditions which is corroborated by a negative balance of total N which points to denitrification processes. Higher substrate availability may increase biodegradability, but although there



Error bars represent one standard deviation (*n*=3)

Fig. 1.3: Changes in concentrations of DON (*left Y-axis*) and DOC (*right Y-axis*) in water extracts of beech-Oi, beech-Oa, spruce-Oi, spruce-Oa and cypress-Oa soil horizons during a 21-day incubation period (no NO_3^- addition) (Fig. 3.2 from Chapter 3).

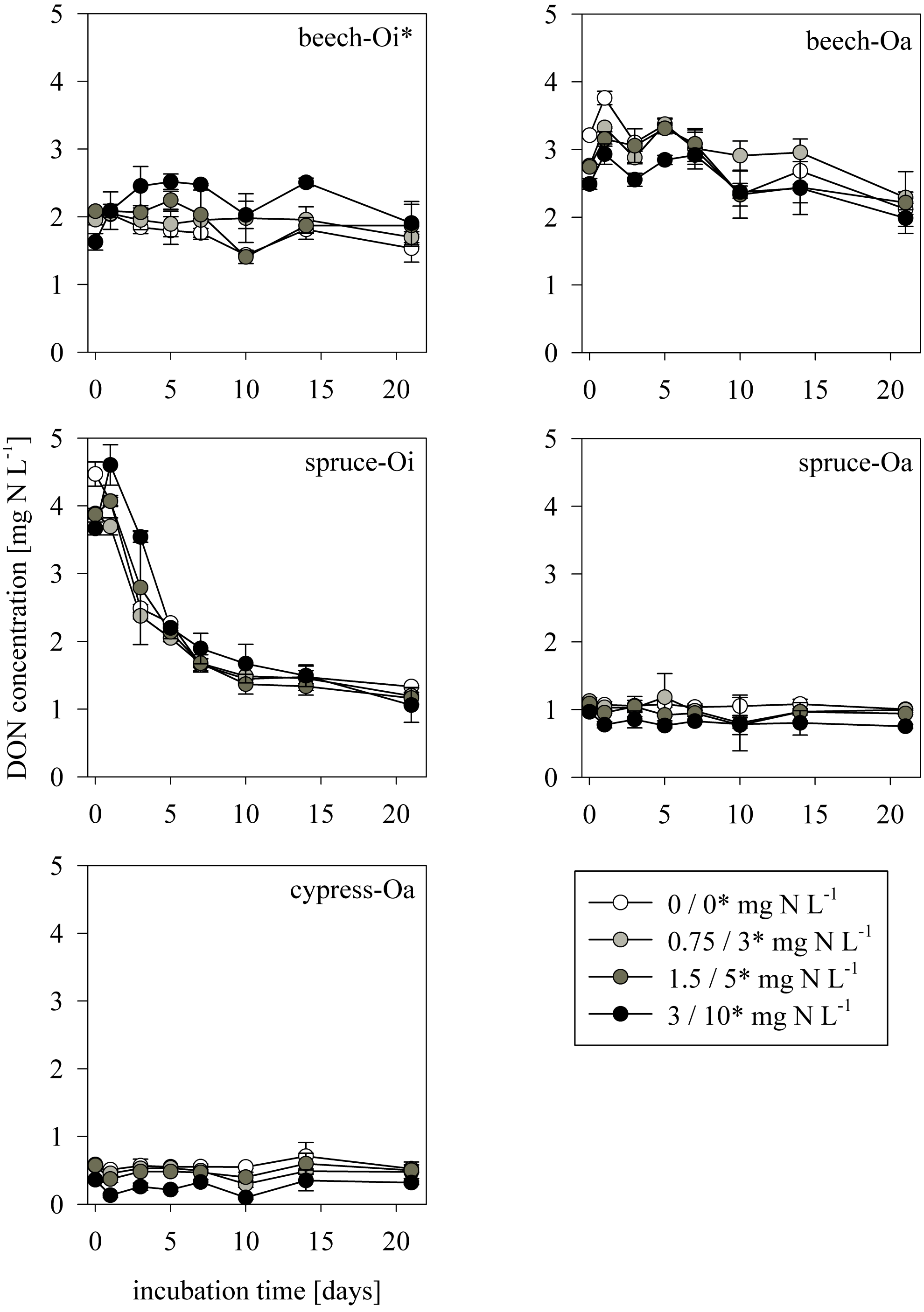
was a strong correlation between DOC and DON concentrations and biodegradability, concentration effects were not tested systematically in this study. The DOC/DON ratio of the water extracts was not related to DON and DOC biodegradation.

Protein-like compounds (e.g. tyrosine) identified by PARAFAC analysis have been reported to possibly serve as proxies for DOM biodegradation (e.g. Balcarczyk et al., 2009). We showed that other reclacitrant compounds (e.g. phenols) also show fluorescence emission exitation patterns similar to these easily degrable compounds. However, fulvic-acid type components and another unidentified group of fluorophores were significantly correlated with both DOC and DON biodegradation.

After 21 days of incubation, the main process responsible for the decrease in DON concentrations was mineralization. However, after five days microbial uptake was most important, emphasizing the need to consider incubation times when comparing data on DON biodegradation from different studies.

Temporal DON and DOC biodegradation were similar in our study (Fig. 1.3). While other studies found DON to decay faster than DOC in samples agricultural or aquatic environments (Gregorich et al., 2003; Kaushal and Lewis, 2005; Petrone et al., 2009), our results support the findings from forest floor extracts and in-situ obtained solutions where DON did not decay faster than DOC (Qualls and Haines, 1992; Cleveland et al., 2004; Kiikkilä et al., 2005). While this discrepancy in decay rates has been assigned to DOC and DON being concentrated in different factions of DOM (humic and non-humic, respectively; Petrone et al., 2009), Qualls and Haines (1992) postulated that not a biochemical need for N but rather DOC mineralization drives DON hydrolysis.

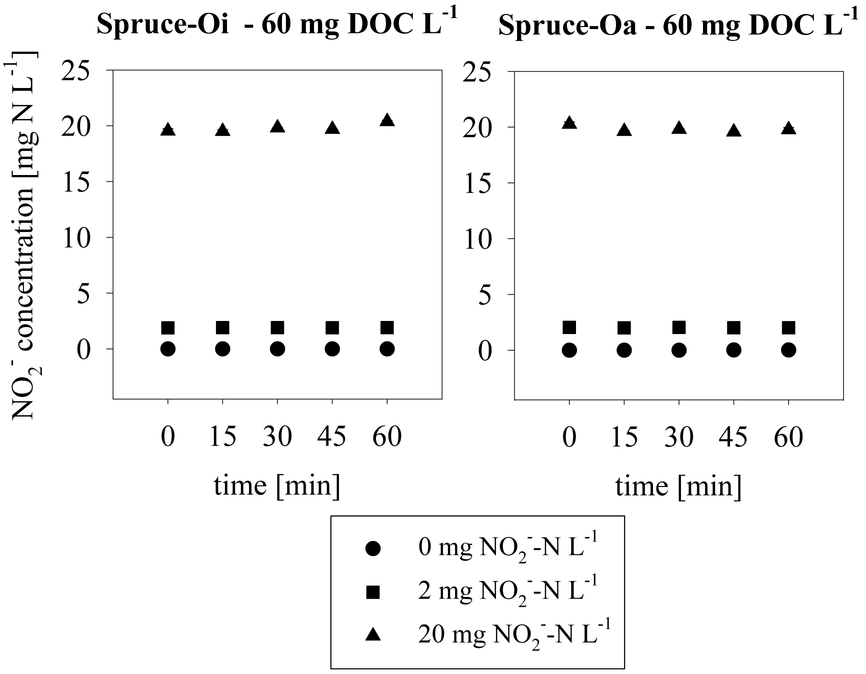
It is important to know, how processes in soil solutions are affected by N additions in order to understand how ecosystems will react to high anthropogenic N deposition. Therefore, we investigated the influence of the ecosystem N status (as simulated by adding different concentrations of NO_3^-) on biodegradation. Additions of NO_3^- did not have any effect on DON and DOC biodegradation in any of the samples (Fig. 1.4, data for DOC not shown). Mineral N was not preferentially taken up by microorganisms, instead NO_3^- concentrations remained constant in all samples, except in spruce-Oi extracts. Here, NO_3^- concentrations always decreased to zero within 3 to 5 days, mainly due to denitrification (as indicated by a negative balance of total N). Therefore, the labile fraction of DON in spruce-Oi samples must have been large enough to meet the microbial N demand. We also assume, that NO_3^- in the samples was not newly formed during incubation, but that it was present as NO_3^- originally added to the samples at all sampling times, as a formation of NO_3^- in the exact same magnitude of NO_3^- consumption is rather unlikely.



NO_3^- additions with asterisks (*) belong to beech-Oi samples. Error bars represent one standard deviation (*n*=3)

Fig. 1.4: Change of DON concentrations in water extracts of beech-Oi, beech-Oa, spruce-Oi, spruce-Oa and cypress-Oa soil horizons during a 21-day incubation period with NO_3^- addition (Fig. 3.5 from Chapter 3).

We showed, that the N status of samples had no effect on biodegradation, which is an important biotically driven process in forest ecosystems. In a second incubation experiment, the N status of samples (as indicated by different DOC/DON ratios of samples and the fluxes of DIN and DON at the respective sites) also did not influence an abiotically driven process in forest ecosystems (compare Chapter 4). In fact, NO_2^- concentrations remained constant in all samples (Fig. 1.5), indicating, that no abiotic DON formation at all took place from added NO_2^-. Both organic C and NO_2^- concentrations have been reported to increase the incorporation of NO_2^- into SOM. However, although DOC and NO_2^- concentrations in this study were higher or in the range of concentrations that can be found in the field (Michalzik et al., 2001; Venterea et al., 2003), NO_2^- concentrations never changed in any of the samples during the incubation time. Even if our solutions were not totally sterile, microbes potentially present in solutions did not influence NO_2^- concentrations.



Error bars represent one standard error of the mean (*n*=3)

Fig. 1.5: Temporal course of NO_2^- concentrations in water extracts from forest floors (Fig. 4.1 from Chapter 4).

Moreover, neither the high incubation temperature (as compared to field conditions) nor the wide range in litter extract quality (e.g. DOC/DON ratio, pH) stimulated the postulated reaction. We assume that reaction time in our study (60 min) was not too short for the reaction to occur, as it was chosen to be longer than in other studies that reported an abiotic reaction of NO_2^- with model compounds within 30 min (Davidson et al., 2003).

1.4 Conclusions

Two drivers that have been reported to influence DON dynamics in temperate ecosystems are precipitation and the N status of a ecosystem. We showed, that precipitation also plays a crucial role for DON and DOC losses in a subtropical montane *Chamaecyparis obtusa* var. *formosana* forest as indicated by a strong relationship between very large soil fluxes and bulk precipitation. However, a depletion of the potentially soluble DOM pool at this site is rather unlikely as these pools can be quickly replenished. One interesting finding was, that precipitation and DOC and DON fluxes in forest floor percolates were not related anymore on a global scale. More research on soil DON and DOC fluxes from different ecosystems is needed to understand the relationship of these fluxes with the amount of bulk precipitation.

Results concerning the effect of ecosystem N status on DON dynamics are contradicting. Data from our study site in Taiwan supported the assumption, that under low atmospheric N deposition, DON losses are high. The N status of the samples in our incubation experiment on DON biodegradation (as simulated by varying NO_3^- additions and different initial DIN concentrations) never influenced DON biodegradation. We conclude, that other factors, such as precipitation (at the *Chamaecyparis obtusa* var. *formosana* site) or the chemical composition of DON (in the first incubation experiment) are more important. More research on the exact chemical composition of DON is needed to make safe predictions on its biodegradability and behaviour in the ecosystem.

One recent example for an attempt to elucidate the chemical composition of DON are fluorescence spectroscopy and subsequent PARAFAC analysis. We showed, that although two PARAFAC factors were correlated with DON and DOC biodegradation, protein-like fluorophores were not related to biodegradation of DOC and DON. This factor has been assumed to serve as a good proxy for biodegradation, but our results show, that other very recalcitrant compounds show similar EEMs as protein-like fluorophores. Therefore, we conclude that information obtained from fluorescence spectroscopy and PARAFAC analysis can add to our understanding of the chemical nature of DON but that the single factors can probably not be used to safely predict biodegradation.

Recently, Colman et al. (2007) suggested that possible iron interference with NO_3^- measurements might have led to false assumptions regarding the abiotic NO_3^- incorporation into organic matter, which in turn challenges the Ferrous Wheel Hypothesis. Moreover, weaknesses of other steps of the Ferrous Wheel Hypothesis (e.g. slow kinetics of Fe^{2+} reduction of NO_3^- or wrong assumptions on possible catalysts in the soil) have been addressed by Colman et al. (2008). Artefatcs induced by the sterilization procedure could also promote wrong conclusions regarding NO_3^- incorporation in sterilized soil samples. Our results add to these considerations, indicating, that the Ferrous Wheel Hypothesis is unlikely to serve as explanation for the observed fast NO_3^- disappearance in sterilized soils. We conclude, that other intermediates than NO_2^- (e.g. radicals) or the presence of a solid phase (organic or mineral) could be the prerequisite for a sucessful N incorporation into organic matter.

By definition, DON and DOC are both part of DOM. However, both classes of compounds are often regarded to behave differently in soils, although DON and DOC cannot be separated structurally. Temporal DON and DOC dynamics were similar in a biodegradation experiment and did not respond differently to NO_3^- additions. Moreover, the fluxes of both classes of compounds were similarily driven by precipitation at the *Chamaecyparis obtusa* var. *formosana* site. Therefore, we conclude that the operational separation of DON and DOC into functionally different compounds, which is often employed in the literature, is not always useful.

This study shed light on key processes in the soil N cycle and emphasized the need to include DON in biogeochemical N studies in subtropical as well as temperate forest ecosystem. Our results concerning the biodegradability of DON were surprising, calling for more research in this field, as biodegradation is a major fate of DON in many soils. Moreover, we showed that the abiotic reaction of NO_2^- and DOC is most likely not an important DON formation mechanism under field-like conditions and that the Ferrous Wheel Hypothesis needs revision.

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CHAPTER 2

High precipitation causes large fluxes of dissolved organic carbon and nitrogen in a subtropical montane *Chamaecyparis* forest in Taiwan

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Published in *Biogeochemistry* (2010), doi:10.1007/s10533-010-9470-1
Received 29 October 2009, revised version accepted 14 May 2010

Abstract

Fluxes of dissolved organic carbon (DOC) and nitrogen (DON) may play an important role for losses of C and N from the soils of forest ecosystems, especially under conditions of high precipitation. We studied DOC and DON fluxes and concentrations in relation to precipitation intensity in a subtropical montane *Chamaecyparis obtusa* var. *formosana* forest in Taiwan. Our objective was, to quantify DOC and DON fluxes and to understand the role of high precipitation for DOC and DON export in this ecosystem. From 2005 to 2008 we sampled bulk precipitation, throughfall, forest floor percolates and seepage (60 cm) and analyzed DOC, DON and mineral N concentrations. Average DOC fluxes in the soil were extremely high (962 and 478 kg C ha^{-1} yr^{-1} in forest floor percolates and seepage, respectively) while DON fluxes were similar to other (sub)tropical ecosystems (16 and 8 kg N ha^{-1} yr^{-1}, respectively). Total N fluxes in the soil were dominated by DON. Dissolved organic C and N concentrations in forest floor percolates were independent of the water flux. No dilution effect was visible. Instead, the pool size of potentially soluble DOC and DON was variable as indicated by different DOC and DON concentrations in forest floor percolates at similar precipitation amounts. Therefore, we hypothesized, that these pools are not likely to be depleted in the long term. The relationship between water fluxes in bulk precipitation and DOC and DON fluxes in forest floor percolates was positive (DOC: r=0.908, DON: r=0.842, respectively, Spearman rank correlation). We concluded, that precipitation is an important driver for DOC and DON losses from this subtropical montane forest and that these DOC losses play an important role in the soil C cycle of this ecosystem. Moreover, we found that the linear relationship between bulk precipitation and DOC and DON fluxes in forest floor percolates of temperate ecosystems does not hold when incorporating additional data on these fluxes from (sub)tropical ecosystems.

Keywords: Dissolved organic carbon; Dissolved organic nitrogen; Fluxes; Forest floor; Precipitation; Subtropical montane forest

2.1 Introduction

Dissolved organic carbon (DOC) and dissolved organic nitrogen (DON) often play a prominent role in the C and N cycle of forest soils. Both DOC and DON represent measures for dissolved organic matter (DOM), which is operationally defined as molecules that are smaller than 0.45 μm (Thurman, 1985). However, DOC and DON do not behave similar in soils and should to be investigated separately (Michalzik et al., 2001). The concentrations and fluxes of DOC and DON in forest ecosystems often vary substantially which can be seen from the temporal dynamics and spatial patterns of the DOC/DON ratio (Michalzik and Matzner, 1999; Prechtel et al., 2000).

Most of the research on the soluble organic forms of C and N has been conducted in temperate ecosystems (Kalbitz et al., 2000; Michalzik et al., 2001; Perakis and Hedin, 2002; Aitkenhead-Peterson et al., 2005; Park and Matzner, 2006). In (sub)tropical ecosystems, DOC and DON concentrations and fluxes have received less attention, especially in forest floor percolates, although the forest floor is known to be an important source of DOM (Kalbitz et al., 2000). Of these studies, only two investigated both DOC and DON fluxes and concentrations in throughfall, forest floor percolates and soil water seepage under (sub)tropical conditions (Schwendenmann and Veldkamp, 2005; Chang et al., 2007). Other papers on DOC and DON in (sub)tropical ecosystems only focused on throughfall or seepage data (Tobón et al., 2004a; Schrumpf et al., 2006; Heartsill-Scalley et al., 2007; Fang et al., 2009), concentrations instead of fluxes (Wilcke et al., 2001; Guo et al., 2005; Möller et al., 2005; Goller et al., 2006; Zimmermann et al., 2007), or either DOC or DON (McDowell, 1998; Hafkenscheid, 2000; Liu and Sheu, 2003; Oyarzún et al., 2004; Tobón et al., 2004b; Fang et al., 2008; Fujii et al., 2009).

In (sub)tropical regions, DOC fluxes in throughfall ranged from 97 kg C ha^{-1} yr^{-1} to 232 kg C ha^{-1} yr^{-1} (Schwendenmann and Veldkamp, 2005; Fujii et al., 2009) and DON fluxes from 5.5 kg N ha^{-1} yr^{-1} to 20.1 kg N ha^{-1} yr^{-1} (Oyarzún et al., 2004; Fang et al., 2008). Chang et al. (2007) observed very high fluxes of DOC and DON in forest floor percolates (1,400 and 16.5 kg ha^{-1} 15 months^{-1}, respectively) and even high fluxes in seepage (653 and 4.6 kg ha^{-1} 15 months^{-1}, respectively) in a high precipitation mountain forest in Taiwan. Organic N fluxes in the soil exceeded those of mineral N by far. This finding is supported by other studies that reported a dominance of DON over mineral N in N-limited forest ecosystems (Qualls, 2000; McDowell, 2001; Perakis and Hedin, 2002). In contrast, Schwendenmann and Veldkamp (2005) found, that the flux of mineral N with litter leachates was extremely high (200 kg ha^{-1} yr^{-1}) as compared to the DON flux (13 kg ha^{-1} yr^{-1}). They concluded that a high proportion of N_2-fixing legumes at their site in Costa Rica may have been responsible for the high inorganic N fluxes.

The flux of water is a major control of the dynamics of DON and DOC in temperate forest soils (Michalzik et al., 2001; Qualls et al., 2002). Therefore, it can be assumed that in (sub)tropical regions, fluxes of DOC and DON should be high, as these ecosystems are often subject to high amounts of precipitation due to frequent heavy storms (hurricanes, cyclones, typhoons). Two contrasting scenarios concerning the relationship between DOC and DON concentrations and water fluxes have been reported. On the one hand, concentrations of DOM increased with increasing discharge (Jardine et al., 1990; Easthouse et al., 1992; Dosskey and Bertsch, 1994; Boyer et al., 1997; Hagedorn et al., 2000; Buffam et al., 2001), due to limited contact of organic solutes with possible sorption sites (Luxmoore et al., 1990; Edwards et al., 1993; Riise, 1999). This positive relationship has not only been reported for runoff from catchments or seepage from the mineral soil, but also for forest floor leachates of temperate and tropical forests (e.g. Michalzik et al., 2001; Solinger et al., 2001; Goller et al., 2006). On the other hand, some studies found a dilution effect on DOC concentrations due to increasing water fluxes (McDowell and Wood, 1984; Easthouse et al., 1992). Tipping et al. (1999) concluded, that the export of DOC only increases with increasing water fluxes in soils with large pools of potential DOM. Potential DOM is defined as organic material, which can possibly enter the soil solution, depending on its interaction with the solid soil phase (Tipping, 2002). One important source for potential DOM is the forest floor (Qualls, 2000; Park and Matzner, 2003). In (sub)tropical ecosystems with high amounts of precipitation, a positive relationship between DOC and DON concentrations in forest floor percolates and water fluxes should therefore point to large pools of potential DOM. These pools are hard to deplete, even under these conditions of high precipitation. On the other hand, a negative relationship between DOC and DON concentrations in forest floor percolates and water fluxes should point to small pools of potential DOM (dilution effect), that can be depleted fast.

Our objective was, to quantify DOC and DON fluxes in relation to precipitation intensity and to understand the role of high precipitation for DOC and DON export in this poorly studied subtropical ecosystem over four years.

2.2 Material and methods

2.2.1 Site description

The Chi-Lan Mountain site is located in northern Taiwan (24°35' N, 121°24' E). Altitude varies from 1,400 to 1,800 m above sea level with a mean annual air temperature of 13°C and annual precipitation from 2,000 to more than 5,000 mm depending on the number and strength of storms (Chang et al., 2007). Fog is very frequent and plays an important role for nutrient and water input. The average relative humidity of the atmosphere is 90% (Chang et al., 2007).

Vegetation is dominated by *Chamaecyparis obtusa* var. *formosana* , planted about 50 years ago, with a density of 1,820 trees ha^{-1} and 82% of the total basal area. Understory vegetation comprises species such as *Illicium philippinense* (Merr.) and *Rhododendron formosanum* (Hemsl.). The total annual litterfall of *Chamaecyparis obtusa* var. *formosana* amounts to 5,722 kg dry mass ha^{-1} yr^{-1} (Chang et al., 2007). The aboveground biomass of this species is 140 t dry mass ha^{-1} (12% leaves, 12% twigs, 76% stems) (Chang, unpublished data).

The soil is a poorly developed Lithic Leptosol (FAO, 1998) with a loamy texture, developed from metamorphic slate and quartzite of a former landslide. Due to a very high content (about 90 vol%) of coarse material (>2 mm), there is no surface runoff, even during very high precipitation events (Chang et al., 2007). Because of intensive rooting the stratification of the well developed forest floor is poor. Thickness of the forest floor varies between 7 and 10 cm and C and N contents amount to 340 g kg^{-1} and 20 g kg^{-1}, respectively. The C/N ratio of the total forest floor is 17 and the pH(H_2O) is 3.5. The pH($CaCl_2$) of the mineral soil varies between 2.8 (A horizon) and 3.2 (40 cm depth). The soil is very poor in nutrient cations (Ca^{2+}, Mg^{2+}, K^{+}), which are largely restricted to the O and A horizon. The soil is permanently moist due to continuously high precipitation over the year (Chang et al., 2007). Soil C stocks in the complete profile amount to 27.3 t C ha^{-1} , of which 32% is stored in the O horizon and 48% in the A horizon (Chang et al., 2008).

2.2.2 Sample collection and analysis

Samples from throughfall, forest floor percolates and seepage (60 cm) were taken once every two weeks for four years (January 2005-December 2008). Bulk precipitation was collected in three samplers of 20 cm diameter in an open field. Throughfall was collected with twelve 20 L PE bottles and 20 cm diameter funnels, which were installed in a systematic grid. Samples were then aggregated to three replicates by volume-weighted mixture of each four collector solutions. Forest floor percolates were sampled by free draining lysimeters (January 2005-March 2007) and by PVC lysimeters with PE membranes (pore size 3 μm) of about 500 cm^2 (April 2007-December 2008) established in four replicates at the border of the O and A horizon. At 10 minutes intervals, suction was applied to the membrane of the PVC lysimeters to collect the sample into a PE bottle and to avoid water logging.

Soil water seepage was collected by eight ceramic suction cups installed at about 60 cm depth. Each sampling device for seepage water was comprised of two suction cups, therefore, seepage water was collected in four replicates per sampling date. The suction cups were evacuated at about -0.2 bar.

Before analysis, all samples were filtered (0.45 μm) and then analyzed for DOC, DON and mineral N. NH_4^+, NO_3^- and TDN (total dissolved N) were determined using a flow injection analyzer (Lachat, QuikChem 8000 series, USA). Dissolved organic N was calculated as TDN-(NO_3^-+NH_4^+). Dissolved organic C was analyzed using high temperature combustion (Elementar, High TOC II, Germany).

2.2.3 Calculations

For 5% of all sampling dates, no data for bulk precipitation from the field samplers was available. For these dates, we used data from an additional rain gauge, installed in some distance from our sampling site. Values obtained from the rain gauge were comparable to values from our site ($r=0.99$, $p<0.01$, $n=96$, Pearson correlation). If the amount of precipitation exceeded the uptake capacity of throughfall samplers, throughfall fluxes were calculated using a regression equation derived from the relation of bulk precipitation and throughfall (at 5% of all sampling dates). For the regression equation, we used throughfall fluxes and bulk precipitation data since 20 November, 2002 ($r=0.98$, $p<0.01$, $n=147$, Pearson correlation). The calculation of solute fluxes in bulk precipitation and throughfall was done by multiplying measured water fluxes by measured concentrations.

Soil water fluxes were calculated using the transpiration of the stand and the distribution of fine roots in the soil. Transpiration was measured by using the sap flow method

(Chang et al., 2007) and amounts to about 160 mm yr^{-1}. Evaporation was assumed to be negligible (Chang et al., 2007). Compared to annual precipitation amounts, transpiration rates were very low and were assumed to be evenly distributed over the year. Therefore, we calculated the transpiration loss from the forest floor for each sampling date by dividing the total transpiration by the number of sampling dates per year. Forest floor percolate fluxes of water for each sampling date were calculated using the fine root distribution in the forest floor. As 34% of fine roots are located in the forest floor (Chang et al., 2007), transpiration losses amount to 34% and 66% from the forest floor and the mineral soil, respectively. Therefore, we subtracted 34% of transpiration at each sampling date from respective throughfall fluxes to obtain forest floor percolate fluxes of water. Seepage fluxes of water were calculated by subtracting 66% of transpiration at each sampling date from respective forest floor water fluxes.

2.2.4 Statistical analyses

Concentrations and fluxes are presented as arithmetic means with standard errors if not stated otherwise. Normality was tested with the Kolmogorov-Smirnov-test, and correlation analysis was carried out using Spearman rank or Pearson correlation, depending on whether the data were normally distributed. To meet the requirements for linear regression analysis, data were log-transformed. Mann-Whitney Rank Sum tests or t-tests were carried out at $p<0.001$. Significant effects were determined at $p<0.05$ unless stated otherwise. All statistical analyses were performed with SigmaStat (version 3.5, Systat Software, Inc., Chicago, IL, USA).

2.3 Results

Fig. 2.1 gives an overview over the bulk precipitation at each sampling date. Mean annual precipitation (years 2005-2008) amounted to 4,815 mm (±617 mm, standard deviation), the mean annual water flux in throughfall, forest floor percolates and seepage to 4,169 mm, 4,115 mm and 4,009 mm, respectively (Table 2.1).

Concentrations of NH_4^+ and NO_3^- in all solutions were very low during the sampling period (Table 2.2). While concentrations of inorganic N decreased with passage through the ecosystem, DOC and DON behaved differently. In throughfall, DOC and DON concentrations were low, increased clearly in forest floor percolates and decreased again in seepage (Table 2.2). In throughfall, DON concentrations were similar to NO_3^- concentrations, but in forest floor percolates, DON concentrations were considerably higher than inorganic N concentrations. In seepage, inorganic N concentrations were still lower than

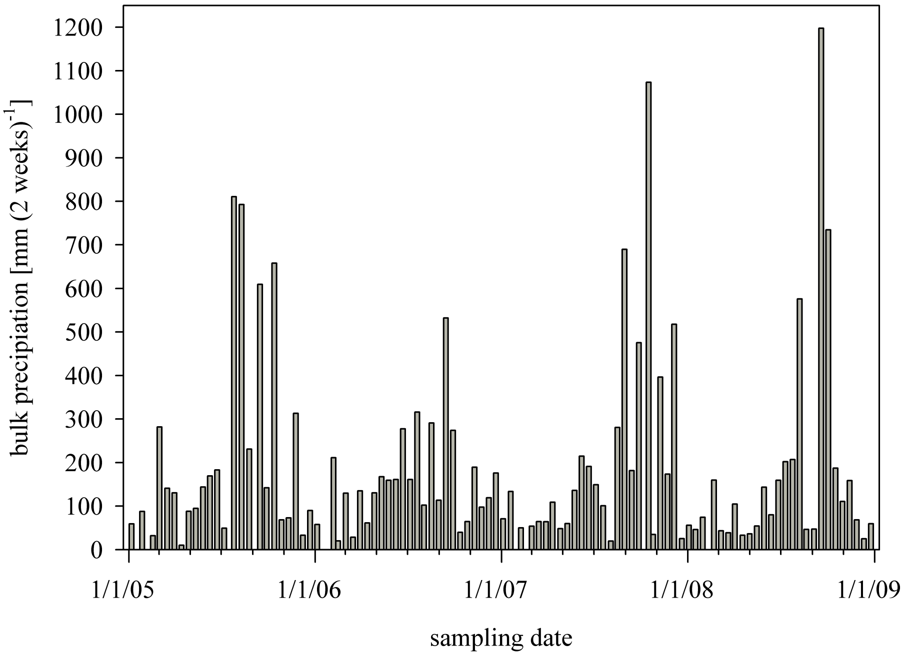


Fig. 2.1: Bulk precipitation at the study area from January 2005 until December 2008.

Table 2.1: Annual and mean annual fluxes of water, NO_3^-, NH_4^+, DON and DOC in throughfall, forest floor percolates and seepage (60 cm).

Year	**Water**	**NO_3^-**	**NH_4^+**	**DON**	**DOC**
	[mm]		[kg N ha^{-1} yr^{-1}]		[kg C ha^{-1} yr^{-1}]
Throughfall					
2005	4,587	2.3	1.5	1.8	140
2006	3,503	1.8	2.2	2.8	93
2007	4,560	4.6	2.3	4.7	97
2008	4,026	2.5	1.6	4.4	94
mean	4,169 ± 257	2.8 ± 0.6	1.9 ± 0.2	3.4 ± 0.7	106 ± 11
Forest floor percolates					
2005	4,533	2.9	0.9	12.6	894
2006	3,448	0.6	0.8	12.7	745
2007	4,506	3.2	2.1	21.0	1135
2008	3,972	1.5	2.1	16.4	1073
mean	4,115 ± 257	2.1 ± 0.6	1.5 ± 0.4	15.7 ± 2.0	9612 ± 89
Seepage (60 cm)					
2005	4,427	1.6	0.6	5.7	561
2006	3,343	1.3	0.7	5.3	411
2007	4,400	3.3	2.6	7.3	512
2008	3,866	2.8	1.3	7.7	477
mean	4,009 ± 257	2.3 ± 0.5	1.3 ± 0.5	6.5 ± 0.6	490 ± 32

DON concentrations, but the difference was not as prominent as in forest floor percolates (Table 2.2).

Table 2.2: Mean annual concentrations of NO_3^-, NH_4^+, DON and DOC in throughfall, forest floor percolates and seepage (60 cm).

	NO_3^-	**NH_4^+**	**DON**	**DOC**
		[mg N L^{-1}]		[mg C L^{-1}]
Throughfall	0.13 ± 0.03	0.08 ± 0.01	0.13 ± 0.03	3.9 ± 0.2
Forest floor percolates	0.06 ± 0.01	0.06 ± 0.02	0.46 ± 0.06	28.4 ± 3.5
Seepage (60 cm)	0.06 ± 0.02	0.04 ± 0.01	0.15 ± 0.01	12.3 ± 0.3

Mean annual DOC and DON fluxes were highest in forest floor percolates and seepage (Table 2.1) with exceptionally high DOC fluxes in forest floor percolates. Mineral N fluxes were similar to DON fluxes in throughfall, but significantly lower than DON fluxes in forest floor percolates and seepage. Again, the difference between DON and inorganic N fluxes was most prominent in forest floor percolates (Table 2.1).

There was no clear relationship between DOC and DON concentrations in throughfall, forest floor percolates and seepage and bulk precipitation per two week interval (Fig. 2.2). Most of the bulk precipitation per two weeks interval was in the range of 10-200 mm (2 weeks)$^{-1}$. Within this range, DOC and DON concentrations in forest floor percolates were highly variable at similar amounts of bulk precipitation.

On the contrary, there was a linear relationship between bulk precipitation per two weeks interval and DOC and DON fluxes in forest floor percolates (Fig. 2.3). Here, water fluxes explained 79.9% of DON fluxes and 82.3% of DOC fluxes (linear regression on log-transformed data). The relationship between DOC and DON fluxes in forest floor percolates and bulk precipitation per two weeks interval was significant (DOC: $r=0.908$, DON: $r=0.842$, $n=100$, $p<0.01$, Spearman rank correlation). The same was true when correlating organic solute fluxes in seepage and bulk precipitation per two weeks interval (DOC: $r=0.980$, DON: $r=0.866$, $n=100$, $p<0.01$, Spearman rank correlation).

2.4 Discussion

Solute concentrations in forest floor percolates can be influenced by lysimeter type. At our site, the lysimeter type was changed from zero-tension to suction-operated lysimeters in April 2007. For DOC, differences between zero-tensions and suction-operated lysimeters have been reported, but the results were not consistent among studies, probably due to

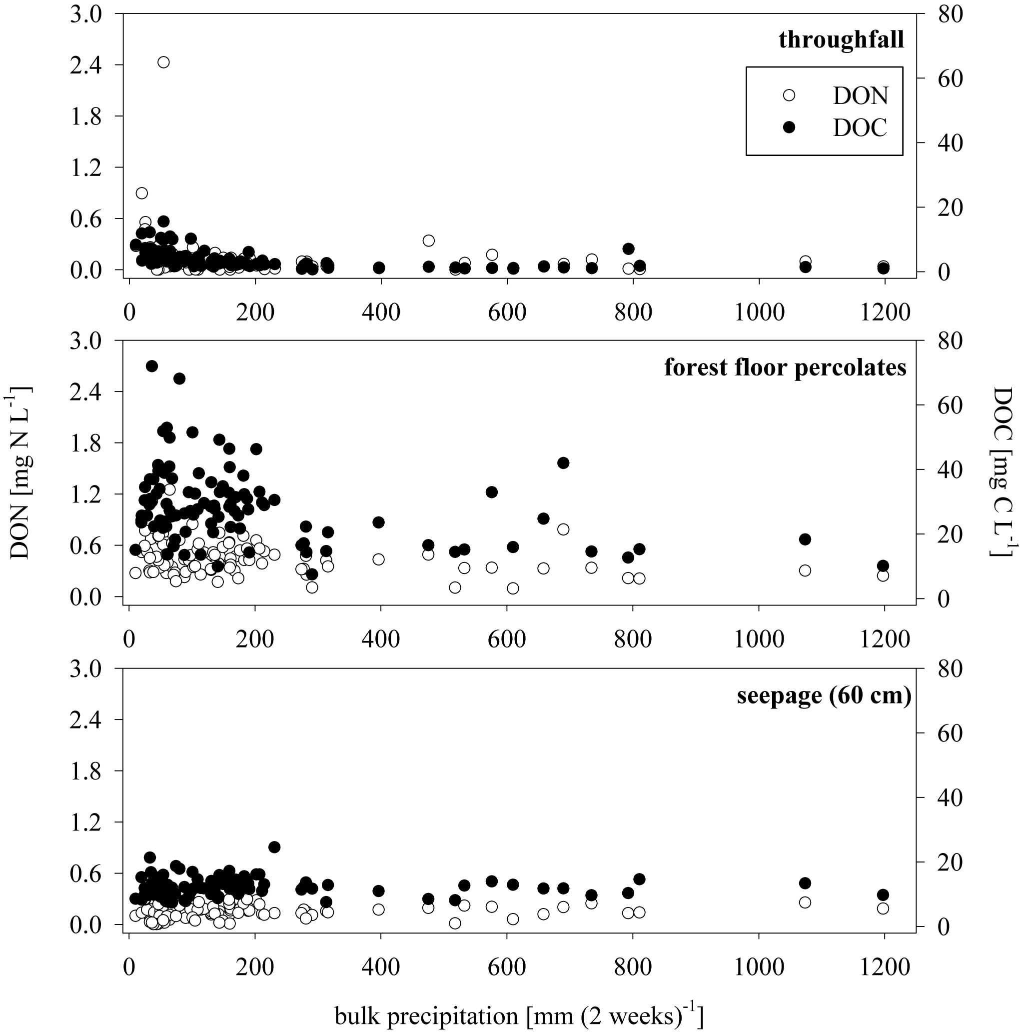


Fig. 2.2: Relationship between bulk precipitation and DOC and DON concentrations in throughfall, forest floor percolates and seepage (60 cm).

differences installation depth an other factors (Reynolds et al., 2004; Buckingham et al., 2008). In our study, both DOC and DON concentrations increased significantly after installing new lysimeters (DOC: Mann-Whitney Rank Sum Test, DON: t-test; n=56 before installation of new lysimeters, n=44 after installation of new lysimeters). The effect on fluxes was moderate: average DOC fluxes increased from 819 kg C ha^{-1} yr^{-1} (2005-2006) to 962 kg C ha^{-1} yr^{-1} (2005-2008) while average DON fluxes increased from 12.6 kg N ha^{-1} yr^{-1} (2005-2006) to 15.7 kg N ha^{-1} yr^{-1} (2005-2008). The observed increase might not only be due to the change of lysimeter type but also due to the large heterogeneity of the soil which makes it difficult to assess actual differences between lysimeter types in the field (Hendershot and Courchesne, 1991; Neff and Asner, 2001). Our re-

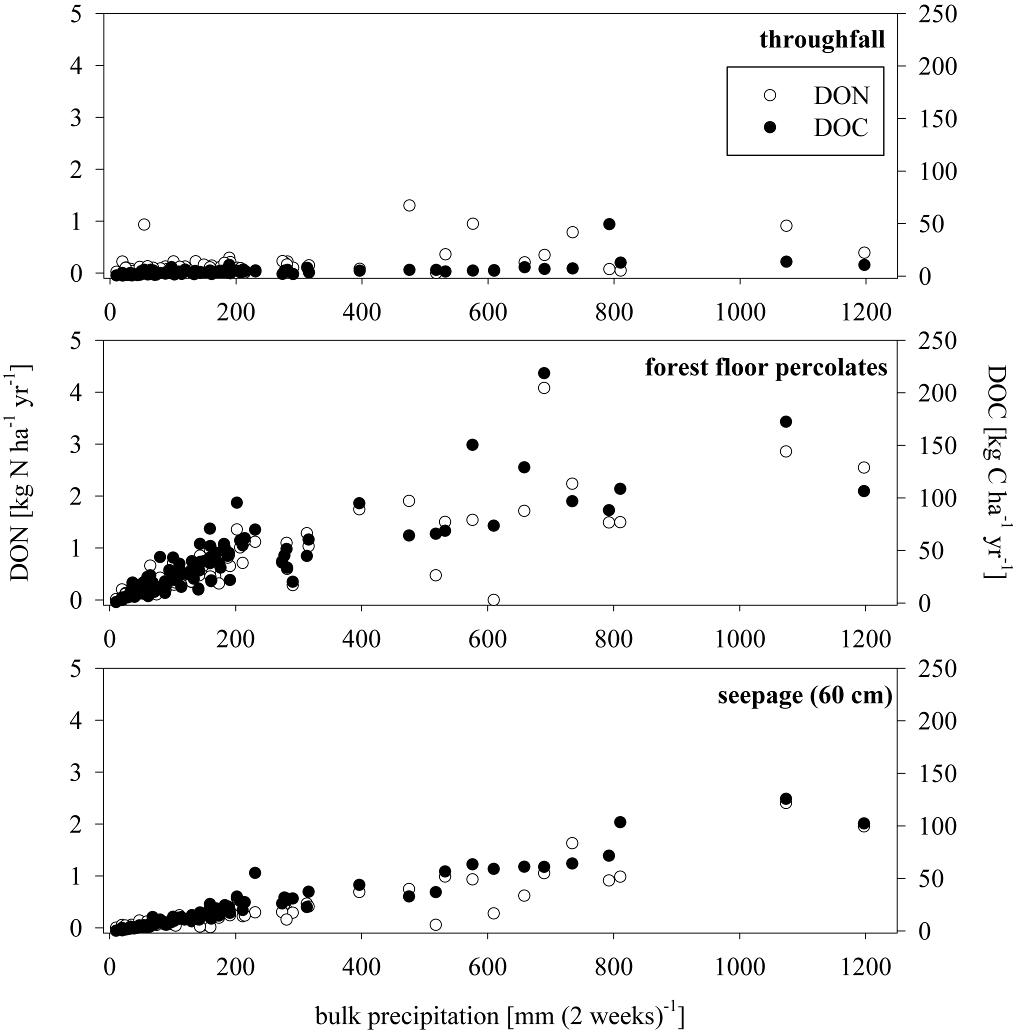


Fig. 2.3: Relationship between bulk precipitation and DOC and DON fluxes in throughfall, forest floor percolates and seepage (60 cm).

sults corroborate the finding, that there is a general source of uncertainty associated to flux measurements using lysimeters, as different lysimeter types collect different pools of water (Lajtha et al., 1999).

Mean annual fluxes of DOC and DON in throughfall and DON fluxes in forest floor percolates were well within the range of values reported in the literature, while DOC fluxes in forest floor percolates and seepage at our site were exceptionally high (Table 2.1). The magnitude of DOC and DON fluxes might be explained by several different factors. First, fresh litter contributes significantly to DOC production in forest floors (Kalbitz et al., 2000) and the input of litter is large at our site due to frequent typhoons (Rees et al., 2006). However, decomposition rates are low at our site due to the poor litter quality of

Chamaecyparis leaves (Rees et al., 2006) and due to very low pH values. The subsequent accumulation of Oa material can also lead to high DOC fluxes from the forest floor, as Oa material can play an even more important role in DOC release from the forest floor than fresh litter (Fröberg et al., 2003; Müller et al., 2009).

Second, rooting is very intensive at our study site with a total mass of living fine roots of 2,074 kg ha^{-1} in the forest floor (Chang et al., 2007). Yano et al. (2005) showed in a laboratory study that belowground litter can strongly add to DOC and DON production as root litter produced 10 times more water extractable DON than needle litter and twice as much DOC. This finding suggests, that the decay of fine roots might significantly contribute to DON fluxes under field conditions.

Third, bryophytes are abundant in the understory vegetation at our site. Research on leaching of DOC and DON from bryophytes and the decomposability of bryophyte species is lacking, therefore, it still has to be investigated if bryophytes significantly contribute to DOC and DON fluxes in forest ecosystems.

Fourth, Kalbitz et al. (2006) reported increasing DOM production (DOC is the main constituent of DOM) during the later phase of litter decomposition in samples with relatively large lignin degradation. They concluded that lignin can be an important source for DOM from decomposing litter. However, the lignin content of fresh *Chamaecyparis obtusa* var. *formosana* litter is low, amounting to 13.8% (Rees et al., 2006), but the actual role of lignin in the DOC production of *Chamaecyparis obtusa* var. *formosana* litter has not been investigated yet.

When assessing the role of DOC export from the forest floor in the C cycle of this ecosystem, sources and sinks of DOC in the forest floor need to be considered. Input of C to the forest floor at our site can occur via throughfall, above- and belowground litter and root exudates. The total annual aboveground litterfall at the Chi-Lan Mountain site amounts to 3,059 kg C ha^{-1} yr^{-1} (Chang et al., 2007), while throughfall input of DOC is 105.8 kg ha^{-1} yr^{-1}. No data on root litter and root exudate input of C is available for our site. Losses of DOC from the forest floor at our site will mainly occur via mineralization or leaching. Mineralization to CO_2 is the most important fate of organic matter supplied to the O horizon of forest soils, while export to the mineral soil as DOC is less important (McDowell and Likens, 1988; Zech and Guggenberger, 1996). In this study, losses of DOC from the forest floor were very high (962 kg C ha^{-1} yr^{-1}), while soil respiration amounts to 17.6 kg C ha^{-1} yr^{-1}. Therefore, we conclude, that the high losses of DOC from the forest floor play an important role in the soil C cycle of this forest ecosystem.

The passage of organic solutes through the mineral soil often leads to a decrease in DOC and DON concentrations in seepage as compared to the forest floor, as DOC and

DON are adsorbed onto mineral surfaces (Guggenberger et al., 1998; Kaiser and Zech, 1998, 2000) or mineralized (Yano et al., 2000; Kalbitz et al., 2003; Marschner and Kalbitz, 2003). The decrease in DOC and DON concentrations between forest floor percolates and seepage (Table 2.2) was not as prominent as compared to the results of Schwendenmann and Veldkamp (2005) as both sorption and decomposition are probably less important at our site. At the Chi-Lan Mountain site, the soil is very shallow and stony and also partly water-saturated. Consequently, the availability of iron oxides as sorption sites for DOC and DON is limited. In anaerobic soils, clay minerals can contribute significantly to sorption, if available iron oxide pools are small (Fiedler and Kalbitz, 2003). The clay content at our site is 13% in the first 5 cm of the mineral soil and increases to 20% below 5 cm.

As DOC and DON in water extracts from litter of the Chi-Lan Mountain site were recalcitrant to microbial decay in a laboratory experiment (Schmidt, unpublished data), decomposition probably only plays a minor role in these soils. Therefore, we suspect that at our site, sorption of DOC and DON is more important than decomposition. Both iron oxides and clay minerals may contribute to sorption, depending on the redox state of the soil.

Dissolved organic N played an important role for N losses from this ecosystems as compared to mineral N. The contribution of DON to total N fluxes was 42% in throughfall, 81% in forest floor percolates and 65% in seepage (60 cm) (Table 2.1). Hafkenscheid (2000) found similar contributions in a montane rain forest in Jamaica, where DON fluxes amounted to 67-79% of total N fluxes in litter percolates and to 54-71% in the Ah horizon, while Schwendenmann and Veldkamp (2005) reported a lower contribution of DON fluxes to total N fluxes in a tropical wet forest ecosystem in Costa Rica. At their site, mineral N fluxes were very high due to the high proportion of N_2-fixing legumes. Fang et al. (2008) also found, that the contribution of DON fluxes to total N fluxes was low in three subtropical forests in China. At their site, the total N flux in precipitation was very high (50 kg N ha^{-1} yr^{-1}) and mineral N fluxes in throughfall amounted to 23-32 kg N ha^{-1} yr^{-1}. As revealed by small throughfall fluxes of mineral N (averaging 4 kg N ha^{-1} yr^{-1}), N deposition was low at our site. This corroborates the finding, that DON is dominant over mineral N in N-limited forest ecosystems receiving low anthropogenic N inputs (Qualls, 2000; Perakis and Hedin, 2002; Park and Matzner, 2006).

Michalzik et al. (2001) correlated published data on a broad range of annual precipitation (500-1,800 mm) and DOC and DON fluxes in the forest floor percolates of temperate forest ecosystems and found a strong positive linear relationship. We collected additional data on these parameters from (sub)tropical forest ecosystems (see Table 2.3).

Moreover, additional data on DOC and DON fluxes in forest floor percolates of twelve temperate ecosystems were included (Table 2.4). Three of these studies were not included in Michalzik et al. (2001) although they were published before (Qualls and Haines, 1991; Zech and Guggenberger, 1996; Markewitz and Richter, 1998), while the other nine studies have been published afterwards (Solinger et al., 2001; McDowell et al., 2004; Yano et al., 2004; Fröberg et al., 2005, 2006; Fujii et al., 2008; Jones et al., 2008; Kleja et al., 2008; Rosenqvist et al., 2010). Although some studies were conducted at the same forests in Sweden, we included all of them, as they measured fluxes in different years. In addition, we included annual fluxes from our study, as annual precipitation was highly variable depending on the number and strength of typhoons (4,014-5,312 mm).

Annual DOC fluxes in forest floor percolates from our site fitted the linear correlation found by Michalzik et al. (2001) (Fig. 2.4). The same was true for additional data from temperate forest ecosystems (Table 2.4, Fig. 2.4). On the contrary, most of the DOC fluxes reported from other (sub)tropical ecosystems (Table 2.3) were lower than one could expect from the linear relationship reported by Michalzik et al. (2001) (Fig. 2.4). One explanation for this phenomenon could be that higher temperatures in (sub)tropical regions enhance decomposition and forest floors are often thinner than in temperate forest ecosystems. As soils in (sub)tropical ecosystems are often poor in nutrients and deeply weathered, the nutrients that enter the forest floor with litterfall, throughfall and stemflow need to be retained efficiently. Therefore, the forest floor is virtually "leak-proof" (Richards et al., 1996) and losses of DOC and DON will be minimized. Also, a high proportion of the nutrient stock is held in aerial tissues (Richards et al., 1996). These nutrients will not enter the forest floor and therefore will not be subject to leaching losses.

Mean annual DON fluxes from temperate forest ecosystems (Table 2.4) also fitted the linear relationship found by Michalzik et al. (2001) (Fig. 2.4). Again, additional DON flux data from (sub)tropical ecosystems was lower than one could expect from the observed linear relationship (Fig. 2.4). In contrast to DOC fluxes, DON fluxes from our site did not fit the linear correlation (Fig. 2.4). For DON, there seems to be a precipitation threshold above which DON fluxes no longer increase. This phenomenon might also be explained by the efficient nutrient retention mechanisms of (sub)tropical ecosystems (Richards et al., 1996). As N is a very important nutrient in forest ecosystems, the retention mechanisms for organic N might be even better developed than for DOC, e.g. via direct uptake of DON by plants in the forest floor. For example, ectomycorrhizal associations, which can enhance nitrogen uptake (Högberg, 1989) can be found in many tropical plant families (Richards et al., 1996).

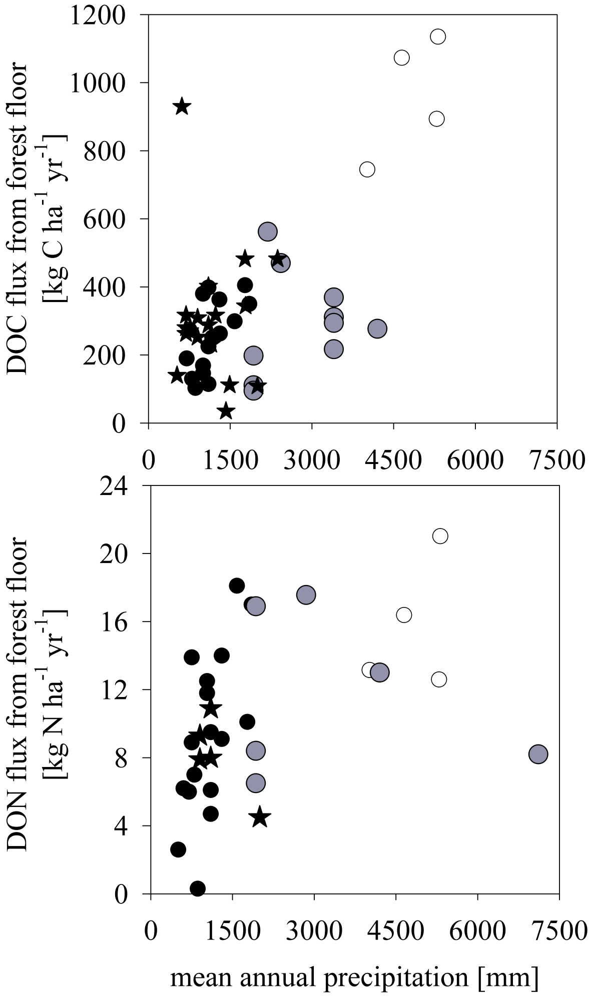
Table 2.3: Fluxes of DOC and DON in throughfall, forest floor percolates and seepage (60 cm) in (sub)tropical forest ecosystems.

Location	**Precipitation**	**Throughfall fluxes**		**Forest floor percolate fluxes**		**Seepage fluxes**		**References**
		DON	DOC	DON	DOC	DON	DOC	
	[mm]	[kg ha^{-1} yr^{-1}]						
Chi-Lan Mountains (Taiwan)	4,815	3.4	105	15.7	962	6.5 (60 cm)	490 (60 cm)	this study
Luquillo Mountains (Puerto Rico)	3,482 [a]	–	127	–	–	–	92 (40 cm) 43 (80 cm)	McDowell 1998
Blue Mountains (Jamaica)	2,850	–	–	17.6	–	1.0	–	Hafkenscheid, 2000
Guandaushi forest (Taiwan)	2,300-2,700 (three sites)	–	167-231	–	–	–	–	Liu and Sheu, 2003
Valley Antillanca (Chile)	7,111	5.5	–	8.2	–	–	–	Oyarzún et al., 2004
Peña Roja (Colombia)	3,400 (four sites)	–	148-190	–	–	–	–	Tobón et al., 2004a
Araracuara (Colombia)	3,400	–	–	–	217-369	–	–	Tobón et al., 2004b
La Selva Biological Station (Costa Rica)	4,200	9.0	232	13	277	3.0 (20 cm) 2.0 (40 cm) 1.0 (80 cm)	95 (20 cm) 64 (40 cm) 44 (80 cm)	Schwendenmann and Veldkamp, 2005
Kilimanjaro (Tanzania)	1,840 (three sites)	7.3-9.2	120-200	–	–	–	–	Schrumpf et al., 2006
Luquillo Mountains (Puerto Rico)	3,482	8.8	132	–	–	–	–	Heartsill-Scalley et al., 2007
Dinghushan Reserve (China)	1,927 (three sites)	14.6-20.1	–	–	–	6.5-16.9 (20 cm)	95-198 (20 cm)	Fang et al., 2008, 2009
East Kalimantan (Indonesia)	2,187/2,427 (two sites)	–	182/97	–	470/562	–	54/54 (30 cm)	Fujii et al., 2009

[a] taken from Heartsill-Scalley et al. 2007.

Table 2.4: Fluxes of DOC and DON in forest floor percolates in temperate forests in publications not included in Michalzik et al., 2001.

Location	**Precipitation**	**Forest floor percolate fluxes**		**References**
		DON	**DOC**	
	[mm]	——— [kg ha^{-1} yr^{-1}] ———		
Coweeta Hydrologic Laboratory (USA)	1,770	–	482	Qualls et al., 1991
Fichtelgebirge (Germany)	1,150	–	231	Zech and Guggenberger, 1996
Calhoun Experimental Forest (USA)	1,230	–	317	Markewitz and Richter, 1998
Steigerwald (Germany)	752	–	271	Solinger et al., 2001
Harvard Forest (USA)	1,100 (two sites)	8.0/10.9	288/402	McDowell, 2004
H.J. Andrews Experimental Forest (USA)	2,370	–	482	Yano et al., 2004
Asa (Sweden)	688	–	317	Fröberg et al., 2005
Asa, Knottåsen, Flakaliden (Sweden)	688	–	263	Fröberg et al., 2006
Mt. Yatsugatake, Tango Peninsula, Mt. Yoshida (Japan)	1,422-1,782 (three sites)	–	35-344	Fujii et al., 2008
North Wales (United Kingdom)	2,000	4.5	109	Jones et al., 2008
Asa, Knottåsen, Flakaliden (Sweden)	523-688 (three sites)	–	140-930	Kleja et al., 2008
Tönnersjöheden (Sweden)	800-1,000 (two sites)	7.9/9.3	252/309	Rosenqvist et al., 2010



Black dots are data from temperate forest ecosystems published by Michalzik et al., 2001, *black stars* are data from temperate forest ecosystems not included in Michalzik et al., 2001 (Table 2.4), *grey dots* are data from (sub)tropical ecosystems (Table 2.3), *white dots* are annual values from this study

Fig. 2.4: Fluxes of DOC (*top*) and DON (*bottom*) from forest floor percolates and mean annual precipitation.

One objective of this study was to understand the role of high precipitation for DOC and DON export in this ecosystem. We found, that the relationship between DOC and DON concentrations in forest floor percolates and water fluxes was weak (Fig. 2.2), while DOC and DON fluxes in forest floor percolates were governed by the flux of water to a large extent (Fig. 2.3). No dilution effect was visible as concentrations did not decrease with increasing water fluxes. At the same time, there was also no positive relationship between DOC and DON concentrations and water fluxes, which would indicate a large pool of potentially soluble DOM. Other studies also reported, that there was no correlation between DOC and DON concentrations in forest floor percolates and water fluxes (Guggenberger and Zech, 1994a; Michalzik et al., 1998; Michalzik and Matzner, 1999; Hilli et al., 2008). This implies, that a depletion of the pool of potentially soluble DOM is rather unlikely. However, our sampling frequency could have been too low to detect a relationship as the response of DOC and DON concentrations will occur within hours or days after rainfall (Michalzik et al., 1998).

Another possible explanation for the independence of DOC and DON concentrations from the flux of water might be, that at some times the pool of potentially soluble DOM was larger than at other times, leading to different concentrations of DOC and DON in forest floor percolates at similar precipitation amounts. The pool size of potentially soluble DOM is not constant, as it is governed by different factors. For example, decomposition conditions before rainfall can influence the amount of potentially soluble DOM that is available during rainfall, as microorganisms decompose soil organic matter to DOM (Guggenberger and Zech, 1994b; Park et al., 2002), that can be lost from the forest floor. A strong increase in DOC and DON concentrations due to rewetting the soil after a dry period as reported by Goller et al. (2006) for a montane forest in Ecuador, is not likely to occur under the conditions at the our site. Although the importance of drying-wetting events on the release of soluble organic compounds is well known (Christ and David, 1996; Hentschel et al., 2007; Borken and Matzner, 2009), this process is not likely to play an significant role at our site, as the soil is permanently moist due to high continuous precipitation and frequent fog (Chang et al., 2008).

Also the amount of substrate available for decomposition might need to be considered when assessing the depletion risk of the potentially soluble DOM pool in the forest floor. The Chi-Lan mountain site is subject to frequent typhoons which cause large additions of fresh litter to the forest floor. Chang et al. (2007) showed in a litter manipulation experiment, that DOC concentrations increased after litter addition at our site. This implies, that the pool of potentially soluble DOM is probably quickly replenished e.g. by DOM released by microorganisms during decomposition.

2.5 Conclusions

Precipitation plays a crucial role for DOC and DON losses in this forest ecosystems as fluxes are directly related to the amount of precipitation. These losses are important for the soil C and N cycle of this ecosystem. We conclude, that a depletion of potentially soluble DOM pools in the forest floor is unlikely. If these pools are depleted during a storm, they will probably quickly be replenished e.g. by DOM released by microorganisms during decomposition. More research on DOC and especially DON fluxes from the forest floor of (sub)tropical ecosystems is needed, to better understand the relationship of these fluxes and the amount of bulk precipitation on a global scale.

Acknowledgements

This study was funded by the Deutsche Forschungsgemeinschaft, Germany and the National Science Council, Taiwan. We are grateful to the lab staff and students of the Taiwan Forestry Research Institute (TFRI, Taipei, Taiwan) and the National Dong Hwa University (NDHU, Hualien, Taiwan) for field sampling and analysis. We thank two anonymous reviewers and the associate editor for helpful comments and suggestions on the manuscript.

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CHAPTER 3

Microbial uptake and mineralization of dissolved organic nitrogen from forest floors

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Submitted to *Soil Biology and Biochemistry*
Received 13 June 2010

Abstract

Dissolved organic nitrogen (DON) plays a key role in the N cycle of many ecosystems, as DON availability and biodegradation are important for processes such as plant growth and microbial metabolism. Although the biodegradation of dissolved organic carbon (DOC) has been investigated in detail, DON biodegradation as the sum of mineralization and microbial uptake is only poorly understood. Our hypotheses were that (i) DON biodegradation depends on litter origin and therefore on its initial chemistry (ii) DOC and DON biodegradation are similar and that (iii) mineral N availability is an important driver of DON biodegradation. Dissolved organic N biodegradation was determined in water extracts of spruce-Oi, spruce-Oa, beech-Oi, beech-Oa and cypress-Oa horizons. Nitrogen was added as NO_3^- with 0, 0.75, 1.5, 3 mg N L^{-1} in all samples except beech-Oi (0, 3, 5, 10 mg N L^{-1}). After inoculation and incubation at 20°C, subsamples were taken after 0, 1, 3, 5, 7, 10, 14 and 21 days. Dissolved organic C, DON, total dissolved nitrogen, NO_3^-, NH_4^+, particulate organic N (as a proxy for microbial biomass and therefore for microbial uptake), specific UV absorption at 280 nm ($SUVA_{280}$) and fluorescence excitation-emission-matrices (EEMs) were determined. In addition, a balance of total N after 21 days was calculated. Dissolved organic C and N concentrations declined most in spruce-Oi samples (by more than 50% and 70%, respectively). Moderate decreases were observed in beech samples, while there was no change in spruce-Oa and cypress-Oa samples. In all samples, mineralization was more important than microbial uptake. A negative balance of all N species after 21 days suggested, that denitrification only played a small role in spruce-Oi samples. Dissolved organic C and N biodegradation was significantly correlated with initial DOC and DON concentrations in the samples ($p<0.01$), but not with the initial DOC/DON ratio. Evaluation of EEMs by PARAFAC analysis revealed four factors (fluorophore groups). The initial concentrations of two fluorophore groups were significantly correlated with DOC and DON biodegradation ($p<0.05$ and $p<0.01$, respectively). Temporal patterns of DOC and DON biodegradation were similar in all samples and DOC and DON biodegradation were significantly correlated. In spruce-Oi and beech-Oa samples, DON biodegradation was higher than DOC biodegradation, while in all other samples DOC and DON biodegradation were similar. NO_3^- additions had no effect on DON and DOC biodegradation and microbial uptake, regardless of addition magnitude and chemical composition of our samples. Mineralization only increased in Oi samples with the highest NO_3^- addition. We conclude that microorganisms will not necessarily prefer mineral N over DON for meeting their N demand and that DON biodegradation could also be driven by microbial C demand. In addition, the recalcitrance of DON from Oa horizons suggests that this DON might be important for the formation of stable N pools in forest soils. Finally, the similar time course of DOC and DON biodegradation suggests, that a functional separation of these classes of compounds is not always warranted.

Keywords: Biodegradation; Dissolved organic carbon; Dissolved organic nitrogen; Microbial uptake; Mineralization; Nitrate; PARAFAC

3.1 Introduction

Dissolved organic nitrogen (DON) plays a prominent role in the N cycle of forest soils, especially under conditions of low N deposition (e.g. Perakis and Hedin, 2002). It often represents the dominant form of N in solution (e.g. van Breemen, 2002; Park and Matzner, 2006). Dissolved organic N is part of dissolved organic matter (DOM), which is defined as substances that pass a 0.45 μm filter (Thurman, 1985). In the last twenty years, research has mainly focused on the dynamics of dissolved organic carbon (DOC), while the dynamics of DON remain poorly understood in comparison.

Dissolved organic N mainly consists of free amino acids, peptides and proteins, amides, heterocyclic compounds, nucleic acid bases and amino sugars, and the majority of DON is hydrophilic (Kroeff and Pietrzyk, 1978; Qualls and Haines, 1991; Westerhoff and Mash, 2002; Yu et al., 2002). In Oa horizons, DON can become relatively enriched in hydrophobic compounds (Yu et al., 2002), which are mainly comprised of polyphenol complexes and/or complexes of amino compounds with humic substances (Qualls and Haines, 1991).

There are various measures for the biodegradability of DOM. For DOC, biodegradability has often been measured as the difference in concentration before and after incubation (e.g. Kiikkilä et al., 2005) or the production of CO_2 over time in the headspace of incubation flasks (Kalbitz et al., 2003). Here, biodegradation of DOM is referred to as the sum of mineralization (biodegradation of organic compounds as a source of energy and nutrients) and microbial uptake (formation of particulate organic matter, POM). The so-called biodegradable DOC and DON refers to the quantity of DOC and DON, that is actually utilized by microorganisms, while the term bioavailability only refers to ingestion and retention of organic compounds (Marschner and Kalbitz, 2003).

As DON is both a substrate for microbial growth and a product of microbial activity, it is difficult to quantify gross mineralization of DON; measuring changes of DON over time will only yield a measure of net DON mineralization. Processes other than mineralization may influence DON dynamics in solution during incubation (Fig. 3.1). For example, direct microbial uptake of DON (e.g. amino acids) results in an increase of particulate organic nitrogen (PON). If DON in solution is decomposed by microbes, NH_4^+ concentrations will increase (mineralization). In turn, NH_4^+ can be oxidized to NO_3^- by nitrification, but NH_4^+ can also be incorporated into microbial biomass (increase in PON). Subsequently, DON can be produced again, if microorganisms exude N-containing organic compounds or if cell lysis occurs. To assess net mineralization and to understand the processes involved in DON biodegradation it is necessary to measure not only DON, but also all other N species in solution at all sampling times.

Several studies have shown that DOC and DON do not behave similarly in soils and should be investigated separately (Michalzik et al., 2001). On the other hand, Cleveland et al. (2004) showed in a lab incubation experiment with live leaves and senescent litter from tropical and temperate species that DON biodegradation dynamics closely resembled DOC dynamics. Similar results were obtained by Qualls and Haines (1992) for solutions obtained from forest floors.

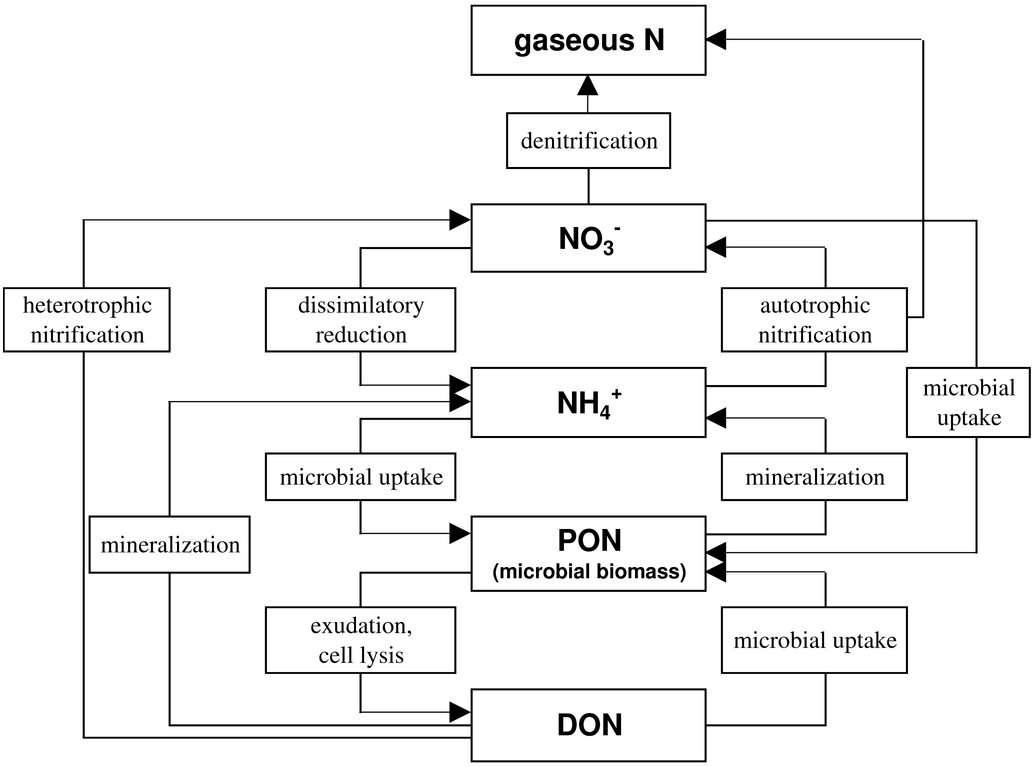


Fig. 3.1: Scheme of the processes involved in DON biodegradation

While the biodegradation of DOC derived from forest soils has been investigated in several studies (e.g. Yano et al., 2000; Kalbitz et al., 2003; Marschner and Kalbitz, 2003; Schwesig et al., 2003; Don and Kalbitz, 2005; Qualls, 2005), the stability of soil-derived DON against microbial decay is poorly understood (Neff et al., 2003). In precipitation and throughfall, DON concentrations were stable during a four week field incubation study in Germany (Michalzik et al., 1997), although Neff et al. (2003) concluded that DON in precipitation should be relatively available to microorganisms. Studies concerning the biodegradability of DON in forest floor material or litter leachates are rare (e.g. Qualls and Haines, 1992; Cleveland et al., 2004). For example, Kiikkilä et al. (2005) showed that the biodegradation of DON was tree species dependent and highest in water extracts of Scots pine and Norway spruce followed by silver birch, but no measurements on net mineralization of DON are available from this study. Some studies also investigated the biodegradation of certain compounds, that are part of DON by definition, such as amino acids or amino sugars (Jones, 1999; Vinolas et al., 2001; Roberts et al., 2007), but results for any particular compound may not be representative of those for the entire class of DON.

There are also only a few studies that investigate the influence of mineral N availability on DON biodegradation, although a large availability of mineral N may decrease the need of microorganisms to degrade DON. Gregorich et al. (2003) showed in a fertilization experiment that the addition of mineral N (NH_4^+ and NO_3^-) to maize-cropped soils did not have any effect on DON biodegradation in water extracts of these soils. In a nitrogen deposition experiment, Yano et al. (2000) found an increase in biodegradable DOC concentrations in field-derived solutions after NH_4NO_3 additions to a hardwood stand. In contrast, there is no study that deals with the influence of mineral N availability on DON biodegradation in forest floors. Kiikkilä et al. (2005) used water extracts from forest floors to assess DON biodegradation under the influence of mineral N, but their samples were already incubated for 15 days before addition of NH_4Cl. This pre-incubation likely changed the chemical composition of DON, and thus conclusions regarding the actual degradability of DON are difficult to draw.

For DOC, commonly used methods to elucidate decomposability are UV and fluorescence emission spectroscopy (Kalbitz et al., 2003; McDowell et al., 2006). Recently, 3D fluorescence spectroscopy and subsequent parallel factor analysis (PARAFAC) of DOM samples have proven valuable for identifying major components (factors) in fluorescence excitation-emission-matrices (EEMs) of DOM samples. Although only a small portion of organic matter acts as a fluorophore (about 1%, Leenheer and Croue, 2003), these fluorophores allow for a general chemical characterization of the organic matter present in a sample (Ohno et al., 2009). Some commonly found components and their corresponding excitation and emission maxima have been summarized by Fellman et al. (2008). The authors list various humic-like and fulvic-like fluorophores as well as tryptophan-like and tyrosine-like components. The exact chemical composition of these components is still largely unknown (Fellman et al., 2009a) but nevertheless, these factors can help to elucidate chemical differences among samples.

This study was conducted to achieve a better understanding of DON biodegradation dynamics and to clarify the relationship between DON biodegradation and litter origin (and therefore different chemical characteristics), DOC biodegradation and mineral N availability. We hypothesized that (i) DON biodegradation depends on the initial chemistry of the litter extracts, (ii) DOC and DON biodegradation are similar and that (iii) NO_3^- availability is an important driver of DON biodegradation. Moreover, we investigated whether 3D fluorescence spectroscopy and subsequent parallel factor analysis (PARAFAC) of our samples can help to predict DOC and DON biodegradation.

3.2 Material and methods

3.2.1 Sampling site

Soil samples were collected from the Oi and Oa horizons of three long-term ecological research sites: the Chi-Lan Mountain forest ecosystem in Northern Taiwan (cypress, *Chamaecyparis obtusa* var. *formosana* , only Oa material), the Steinkreuz site (European beech, *Fagus sylvatica* L.) and the Coulissenhieb site (Norway spruce, *Picea abies* (L.) Karst.), both in Germany. Details concerning climate, soils and vegetation can be found in Schmidt et al. (2010) and Gerstberger et al. (2004).

3.2.2 Sample preparation

Dissolved organic matter was prepared by adding 3 L of water to 300 g fresh weight of litter horizon material. The suspensions were then stored at 5°C, stirred three times during the extraction time (24 h) to ensure thorough mixing of water and litter and then filtered by suction through a ceramic plate (pore diameter about 1 μm) to exclude large litter parts and make subsequent filtration easier. The ceramic plate was first cleaned with distilled water and conditioned with some of the suspension, which was discarded after filtration. The solutions were then filtered through 0.45 μm cellulose acetate filters (Whatman, OE 67), which were pre-washed with 2 x 150 mL of pure water. This filtration step excluded most of the microorganisms in solutions. Samples of all Oa horizons, which were assumed to contain a diverse microbial community, were used to prepare a mixed inoculum (Marschner and Kalbitz, 2003). Pure water was added to the mixed Oa material at a ratio of 1:10. After adding 4 mM $CaCl_2$ solution the suspension was shaken for 30 minutes, incubated for 24 h at 20°C and subsequently filtered through pre-washed 5 μm filters (Millipore SMWP).

3.2.3 Incubation, analysis and calculations

Inoculum was added to each DOM solution (ratio 1:100, Marschner and Kalbitz, 2003) and mineral N was added as $NaNO_3$. The additions were 0, 3, 5 and 10 mg $NO_3{}^-$-N L^{-1} for beech-Oi, and 0, 0.75, 1.5 and 3 mg $NO_3{}^-$-N L^{-1} for all other solutions, to obtain similar DOC/TDN (total dissolved nitrogen) ratios. Controls (one control per $NO_3{}^-$ addition per horizon) with pure water instead of DOM solutions were treated in the same way to assess C and N losses from the inoculum (no changes occurred during these incubations). All samples were incubated in triplicates in closed PE bottles at 20°C in the dark for 21 days. At day 0 (t_0) and 21 (t_{21}) the pH in solution was measured (WTW, pH315i). Subsamples were taken after 0, 1, 3, 5, 7, 10, 14 and 21 days. Before sampling, solutions were gently shaken to homogenize and aerate the samples. In filtered subsamples (Minisart, 0.45 μm, cellulose acetate, prewashed), TDN, DOC (multi N/C 2100 (thermo-catalytic), Analytik Jena, detection limit TDN: $0.15\,\mathrm{mg\,N\,L^{-1}}$, NPOC: $0.66\,\mathrm{mg\,C\,L^{-1}}$), NO_3^- (Dionex DX 500 ion chromatograph, detection limit $0.29\,\mathrm{mg\,N\,L^{-1}}$) and NH_4^+ (FIA-LAB flow injector, MLE Dresden, detection limit: $0.25\,\mathrm{mg\,N\,L^{-1}}$) were determined. In addition, TN was also determined on unfiltered subsamples. Dissolved organic N was calculated from filtered subsamples as TDN-(NO_3^-+NH_4^+). Nitrite was assumed to be negligible. Particulate organic N was calculated as the difference in TN between filtered and unfiltered samples and used as proxy for microbial biomass.

3.2.4 Spectroscopic properties

Specific UV absorbance ($SUVA_{280}$) was measured with a UV spectrophotometer at 280 nm in samples which were diluted to $10\,\mathrm{mg\,C\,L^{-1}}$ to avoid concentration effects (UV-1800, Shimadzu). In addition, fluorescence excitation-emission-matrix spectroscopy (Coble et al., 1990) was used to qualitatively assess different DOM fractions. In preparation of the fluorescence measurements, samples were acidified to pH 2 with HCl, as fluorescence is pH dependent (Laane, 1982). Next, they were diluted with bidistilled water to a maximum absorbance of $0.1\,\mathrm{cm^{-1}}$ in the range of 240-600 nm to achieve an optically "thin" solution and to avoid concentration effects (Zsolnay et al., 1999). Absorbance spectra were obtained with a Varian Cary 50 Bio UV-visible spectrophotometer. Fluorescence excitation-emission-matrices (EEMs) were measured with a Varian Cary Eclipse fluorescence spectrophotometer with the excitation range set from 240 to 450 nm and the emission range set from 300 to 600 nm in 5 nm increments. For processing, EEMs were first corrected for instrument bias according to manufacturer instructions and second for the inner filter effect with the absorbance spectra (McKnight et al., 2001). Afterwards, they were normalized by dividing with the integral of the Raman scatter peaks (excitation wavelength 350 nm) and corrected water spectra were subtracted. This resulted in spectra that were in Raman units (R.u.) (Stedmon et al., 2003). Finally, Rayleigh scatter peaks were removed and interpolated to minimize errors (Bahram et al., 2006). The fluorescence excitation-emission-spectra of single reference compounds (tyrosine, phenylalanine, phenol, 3-hydroxy-benzaldehyd and gallic acid) were also measured. All fluorescence EEMs except those of reference compounds were then analyzed using parallel factor analysis (PARAFAC) (Carroll and Chang, 1970; Harshman, 1970; Bro, 1998). Modeling was conducted using the N-way Toolbox, version 2.10., for MATLAB (The MathWorks) (Andersson and Bro, 2000). Details on the method can be found in Stedmon and Bro (2008). As negative concentrations and fluorescence intensities are chemically impossible, we applied non-negativity constraints to the parameters to allow only chemically relevant results. The model was tested for various numbers of factors (1-7), i.e. groups of fluorophores. The appropriate number of factors was determined by using core consistency diagnostics (Bro and Kiers, 2003) and plotting of residuals. Although our core consistency diagnostic score was only 53% for the 4-factor model (and should be close to 100% for mathematically excellent models), the consideration of residuals confirmed that in our case, the dataset could be resolved into 4 contributing factors. Moreover, we did a split-half validation (Stedmon et al., 2003) on our model. To visualize excitation and emission maxima of the four factors we also plotted spectral loadings of excitation and emission against the respective wavelengths. In addition, we calculated the contribution of each fluorophore to the total pool of fluorophores.

3.2.5 Calculation of a nitrogen balance

To gain a better understanding of the N-transforming processes that took place in our samples, we calculated a balance of all N species over the whole incubation time. Declining DON and NO_3^- concentrations imply mineralization and uptake by microbial biomass, which in turn should be reflected in increasing NH_4^+ and PON concentrations. Therefore, with no gaseous N losses, the sum of the change in DON and NO_3^- concentrations as source terms should be equal to the sum of the change in NH_4^+ and PON concentrations as sink terms. The balance was calculated as the sum of source terms plus the sum of sink terms. Then, positive values mean N_2 fixation (additional N has entered the system), while negative values mean gaseous N losses from the system, which were not measured in our experiment). To account for error propagation, only results which were more than $0.4\,\mathrm{mg\,N\,L^{-1}}$ different from zero were regarded to be significant.

3.2.6 Statistical analyses

Concentrations are presented as arithmetic means with standard deviations if not stated otherwise. Normality was tested with the Kolmogorov Smirnov test, and correlation analysis was carried out using Spearman rank or Pearson correlation, depending on whether the data were normally distributed. Significant effects were determined at $p<0.05$ unless stated otherwise. The labile pools and mineralization rate constants of DOC and DON were determined using a nonlinear regression model for exponential decay (SigmaPlot, double, four parameters). Half-lives of the labile pools of DOC and DON were calculated as ln(2) divided by the mineralization rate constant of labile DOC and DON. All statistical analyses were performed with SigmaStat and Sigmaplot (version 3.5 and 10.0, respectively, Systat Software, Inc., Chicago, IL, USA).

3.3 Results

Our initial solutions differed in chemical properties such as pH, DON and DOC concentration, DOC/DON ratio, mineral N concentrations and spectroscopic properties (Table 3.1). Concentrations of NH_4^+ were highest in beech forest Oa and spruce forest Oi samples (up to 4.27 mg N L^{-1}), while concentrations in the other samples were considerably lower. Due to effective filtration, initial PON concentrations were negligible. Initial NO_3^- concentrations in all samples were close to zero, except for beech-Oa (Table 3.1). Spruce-Oi samples had the highest DOC and DON concentrations (Table 3.1), which decreased in the order beech-Oa > beech-Oi > spruce-Oa > cypress-Oa. The ratio of DOC/DON was lowest in spruce-Oi samples and highest in beech-Oi samples (Table 3.1). Spruce-Oi samples also had the lowest $SUVA_{280}$, while the $SUVA_{280}$ of all other samples was similar.

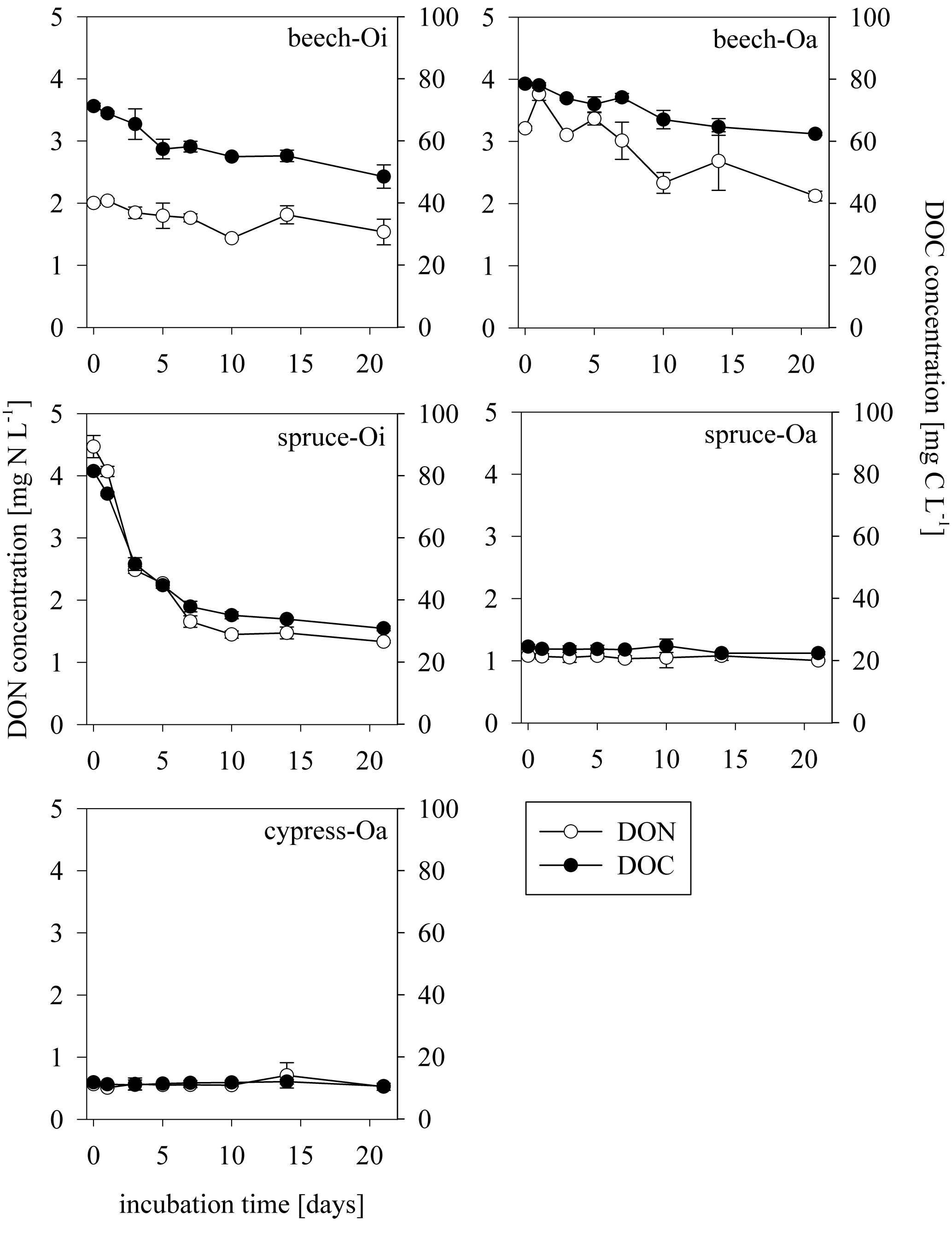
In our study, DOC biodegradation ranged from 6-63%, and was highest in extracts from Oi-horizons. The same was true for DON biodegradation (6-72%). In spruce-Oa and cypress-Oa samples, DOC and DON concentrations did not change during 21 days of incubation (Fig. 3.2). In contrast, DOC and DON concentrations in spruce-Oi samples declined rapidly by more than 50% and 70%, respectively, within seven days. After that time, no change in concentrations was observed. Dissolved organic C and N concentrations in both beech forest samples declined slightly during the incubations, but the decrease was smaller than observed for spruce-Oi samples. Dissolved organic N biodegradation was higher than DOC biodegradation in spruce-Oi and beech-Oa samples (Fig. 3.3), while DOC and DON biodegradation were similar in all other samples. DOC and DON biodegradation were significantly correlated during 21 days of incubation (Table 3.2).

After NO_3^- was added to the samples to investigate the influence of mineral N on DON biodegradation, NO_3^- concentrations only changed slightly in beech-Oi (addition of 10 mg N L^{-1}) and significantly in spruce-Oi (all additions), where no NO_3^- was detectable after five days (Fig. 3.4). NO_3^- additions, regardless of magnitude, had no effect on DON concentrations or dynamics in any of the solutions (Fig. 3.5). The same was true for DOC and PON (data not shown). NH_4^+ concentrations only increased at the highest NO_3^- addition in Oi samples (data not shown).

Table 3.1: Chemical characteristics (pH, DOC and DON concentrations, DOC/DON ratio, NO$_3^-$ and NH$_4^+$ concentrations and SUVA$_{280}$ of the five different water extracts from Oi and Oa horizons of beech, spruce and cypress.

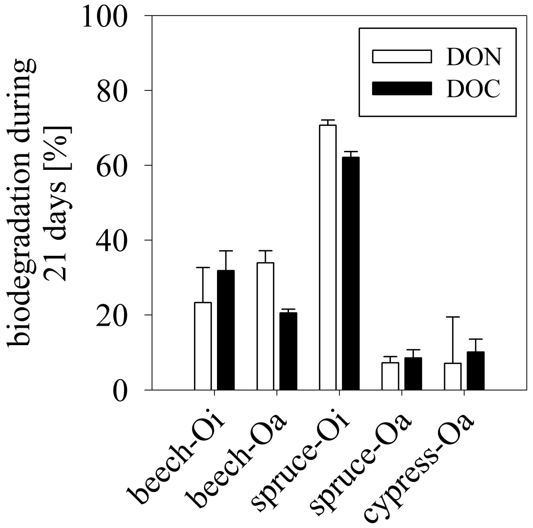
	pH	**DON** [mg N L^{-1}]	**DOC** [mg C L^{-1}]	**DOC/DON**	**NO$_3^-$**	**NH$_4^+$**	**SUVA$_{280}$** [L 10 mg C^{-1} cm^{-1}]
					[mg N L^{-1}]		
Beech-Oi	6.3	2.00 ±0.04	71.18 ±0.94	35.56 ±0.95	n.d.	0.74 ±0.14	0.26 ±0.01
Beech-Oa	5.8	3.21 ±0.04	78.53 ±0.66	24.46 ±0.10	0.31 ±0.04	4.27 ±0.06	0.28 ±0.01
Spruce-Oi	5.4	4.47 ±0.18	81.47 ±0.91	18.24 ±0.61	n.d.	3.28 ±0.03	0.09 ±0.00
Spruce-Oa	4.3	1.08 ±0.03	24.48 ±0.75	22.60 ±0.57	n.d.	0.60 ±0.01	0.33 ±0.01
Cypress-Oa	4.7	0.57 ±0.02	11.80 ±0.22	20.88 ±0.40	n.d.	1.06 ±0.03	0.26 ±0.03

Numbers in brackets are standard deviations (*n*=3), n.d.: not detectable



Error bars represent one standard deviation (*n*=3).

Fig. 3.2: Changes in concentrations of DON (*left Y-axis*) and DOC (*right Y-axis*) in water extracts of beech-Oi, beech-Oa, spruce-Oi, spruce-Oa and cypress-Oa soil horizons during a 21-day incubation period (no NO_3^- addition).



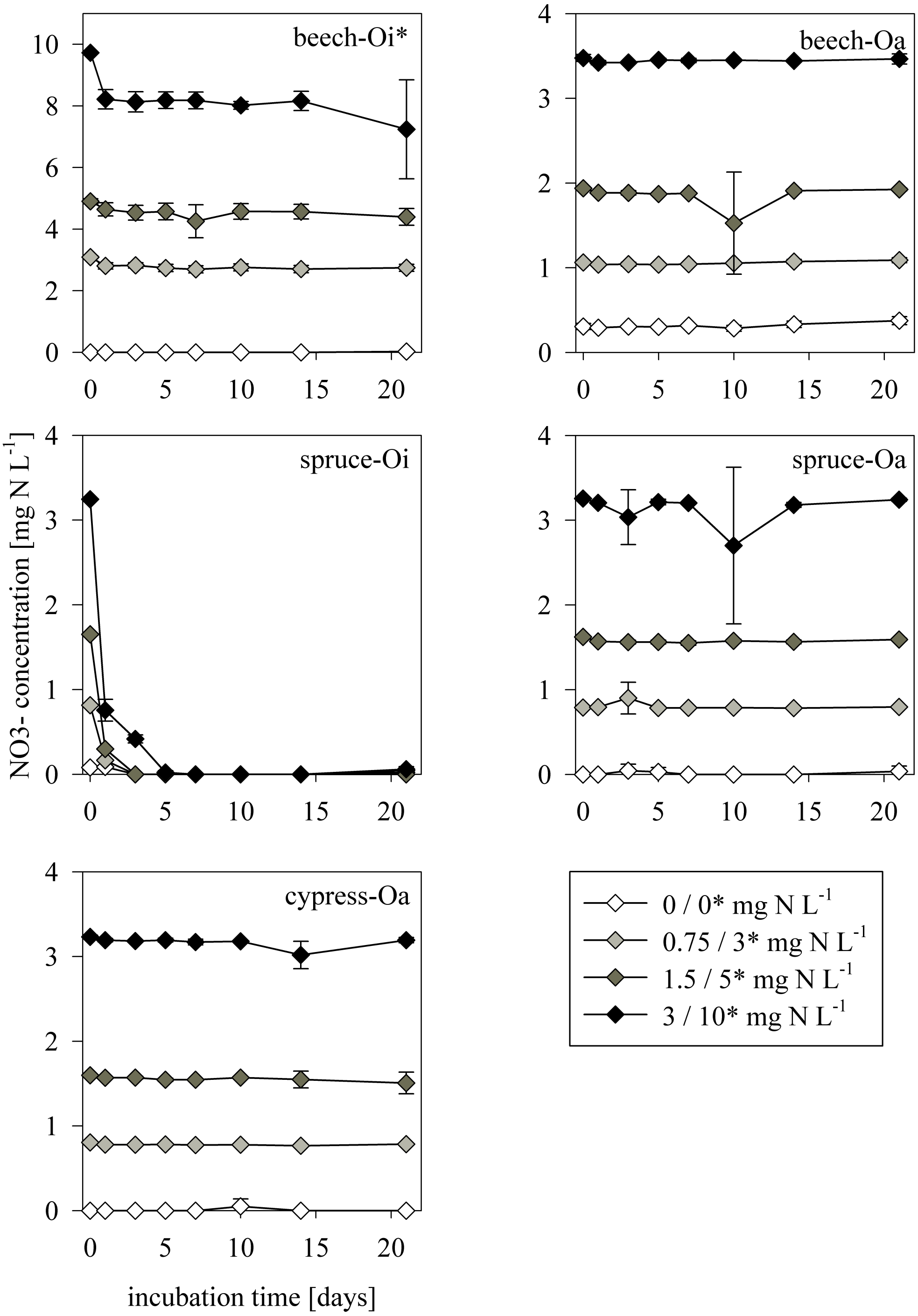
Error bars represent one standard deviation (*n*=3).

Fig. 3.3: DON and DOC biodegradation during a 21-day incubation period in all samples without NO_3^- addition.

Table 3.2: Spearman Rank correlation coefficients of initial concentration of chemical parameters with DOC and DON biodegradation in the five different water extracts from Oi and Oa horizons of beech, spruce and cypress.

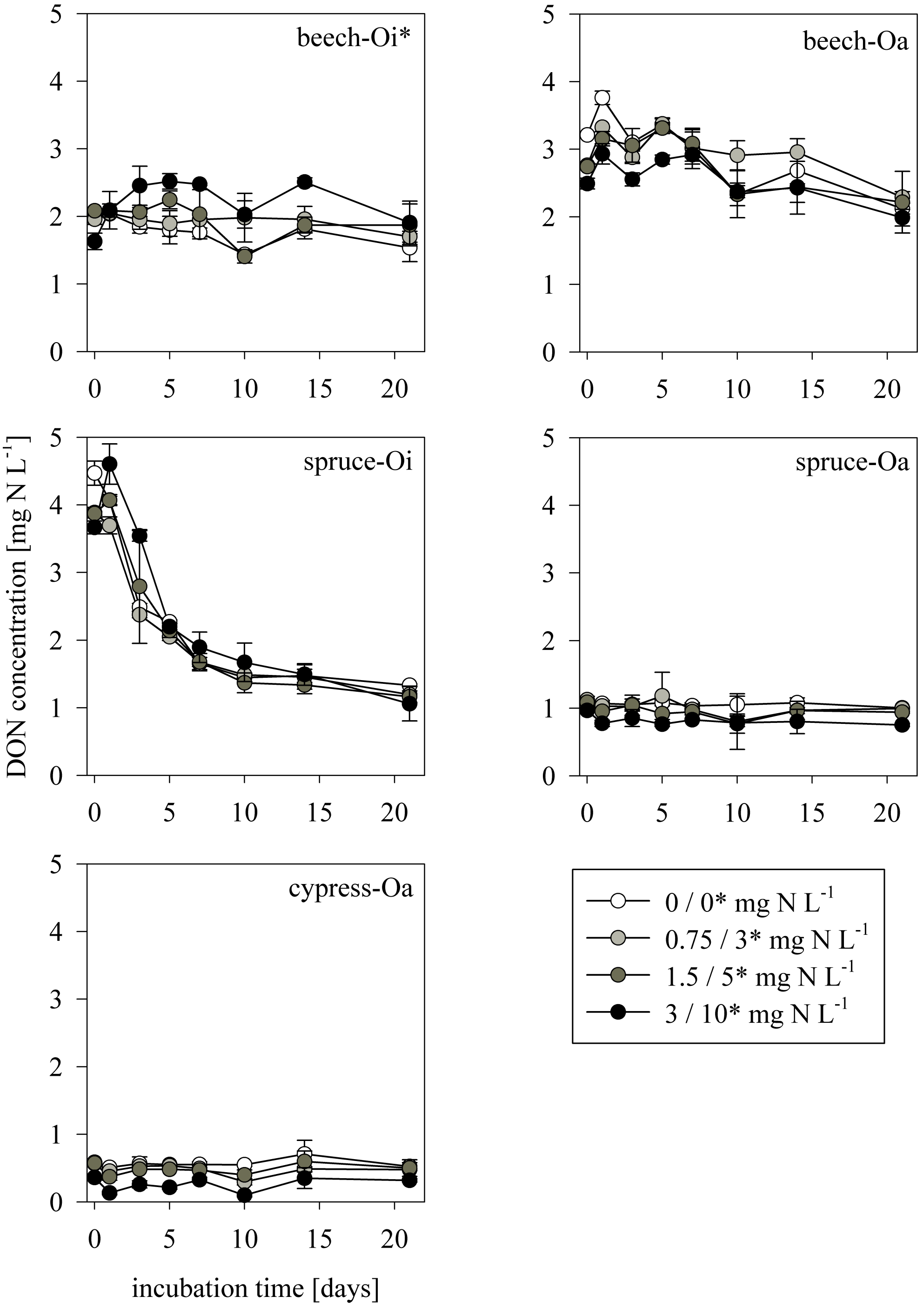
	Biodegradation of	
	DON	**DOC**
	[%]	
Initial DOC [mg C L^{-1}]	0.88**	0.79**
Initial DON [mg N L^{-1}]	0.87**	0.76**
Initial DOC/DON	-0.02	0.13
Initial SUVA$_{280}$ [L 10 mg C^{-1} cm^{-1}] (all samples)	-0.60*	-0.75**
Initial SUVA$_{280}$ [L 10 mg C^{-1} cm^{-1}] (without spruce-Oi)	-0.37	-0.60*
DOC biodegradation [%]	0.86**	

*=p<0.05, **=p<0.01, n=14, except for initial SUVA$_{280}$ without spruce-Oi, where n=12.



NO_3^- additions with asterisks (*) belong to beech-Oi samples. Error bars represent one standard deviation (*n*=3).

Fig. 3.4: Change of NO_3^- concentrations in water extracts of beech-Oi, beech-Oa, spruce-Oi, spruce-Oa and cypress-Oa soil horizons during the incubation time (with NO_3^- added to each extract).

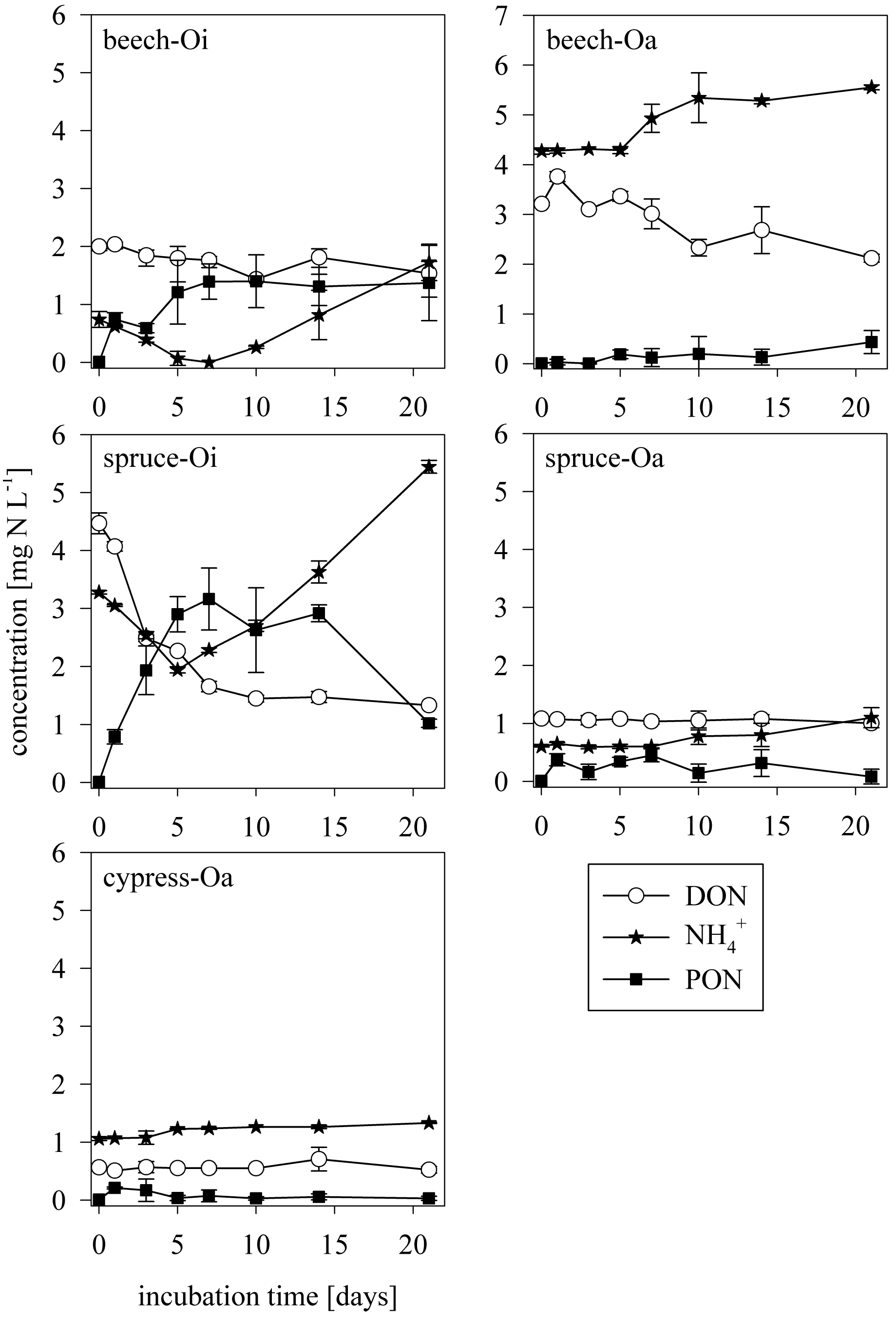


NO_3^- additions with asterisks (*) belong to beech-Oi samples. Error bars represent one standard deviation (*n*=3).

Fig. 3.5: Change of DON concentrations in water extracts of beech-Oi, beech-Oa, spruce-Oi, spruce-Oa and cypress-Oa soil horizons during a 21-day incubation period with NO_3^- addition.

Most of our parameters changed with incubation time. In both Oi-samples, NH_4^+ concentrations decreased until day 5 (spruce-Oi) and 7 (beech-Oi) and then increased to higher levels than initial concentrations during incubation time (Fig. 3.6). In beech-Oa samples, NH_4^+ concentrations increased from day 5 to day 10, but were constant before and afterwards. There was only a slight increase in NH_4^+ concentrations in spruce-Oa samples, while there was no change in cypress-Oa samples. Particulate organic matter concentrations increased clearly in both Oi samples until day 5 (Fig. 3.6). After day 5, PON concentrations were unchanged in beech-Oi samples, while in spruce-Oi samples, PON concentrations decreased again in the last phase of incubation. In Oa samples, PON concentrations did not change during the incubation. There was no increase in NO_3^- concentrations in any of the samples. Dissolved organic C and N concentrations did not change in spruce-Oa and cypress-Oa samples. While DOC and DON concentrations decreased clearly in spruce-Oi samples, the change was smaller in both beech samples (Fig. 3.2). The half-lives for the labile DOC and DON pool in spruce-Oi samples were 2.41 and 2.42 days, respectively (nonlinear regression model). During incubation, the DOC/DON ratio became wider in spruce-Oi samples and narrower in both beech extracts, but was unchanged in spruce-Oa and cypress-Oa samples.

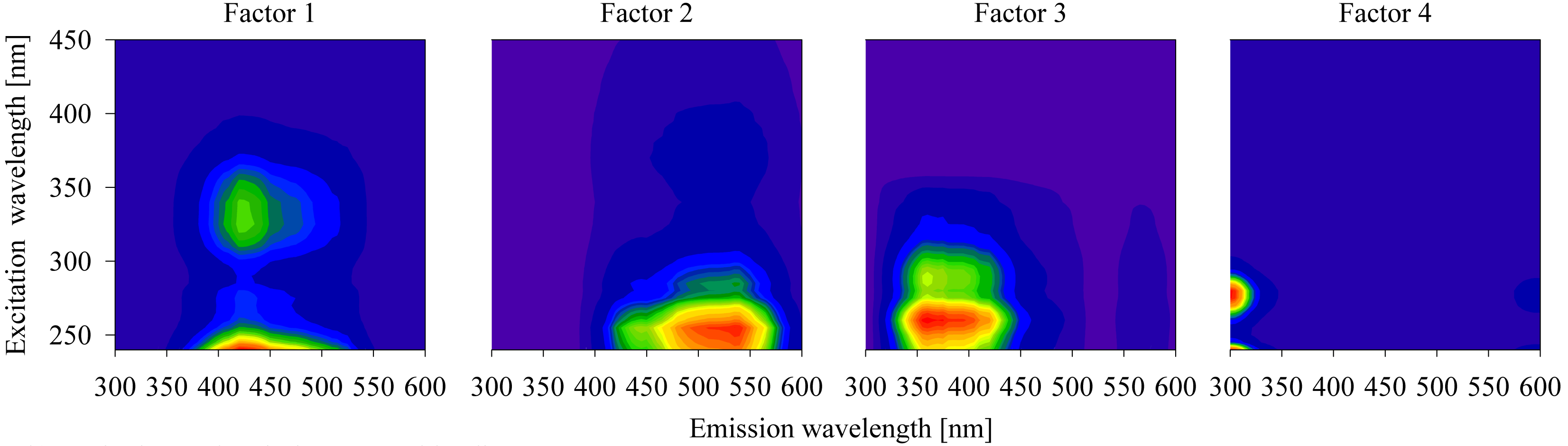
The PARAFAC analysis of EEMs from our samples separated the spectra into four factors (Fig. 3.7). Exact assignment of PARAFAC factors to chemical compounds is difficult (Ohno and Bro, 2006), nevertheless EEMs of some factors have successfully been related to classes of chemical compounds. For our first factor, maximum emission at 430 nm and maximum excitation at <260 nm and 325 nm point to fulvic acid-type components (Stedmon and Markager, 2005; Santin et al., 2009). Concerning the second factor, the very high spectral intensity at emission wavelengths of more than 500 nm and excitation wavelengths of less than 300 nm point to humic acids-type components (Yamashita and Jaffé, 2008; Santin et al., 2009). Semi-quinone-like fluorophores have also been reported to have similar excitation-emission maxima (Cory and McKnight, 2005). We did not find any class of compounds that has previously been assigned to the third factor identified in this study. Excitation-emission patterns of the fourth factor have been assigned to tyrosine-like components (Yamashita and Tanoue, 2003; Stedmon and Markager, 2005; Yamashita and Jaffé, 2008; Santin et al., 2009). On the other hand, several single reference compounds also showed excitation-emission-patterns similar to those of the fourth factor (Fig. 3.8). While some of these compounds are regarded to be easily degradable (e.g. phenylalanine) others are rather hard to degrade (e.g. phenol).



Error bars represent one standard deviation (*n*=3).

Fig. 3.6: Change of DON, NH_4^+ and PON concentrations in water extracts of beech-Oi, beech-Oa, spruce-Oi, spruce-Oa and cypress-Oa soil horizons during a 21-day incubation with no NO_3^- addition.

a) Excitation-emission matrices



b) Excitation and emission spectral loadings

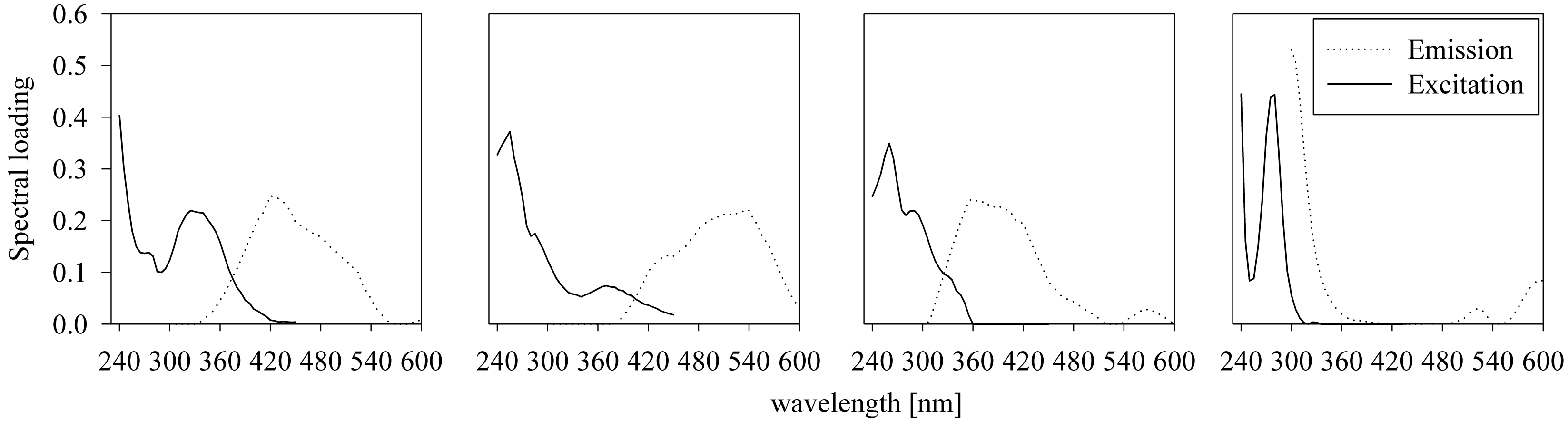


Fig. 3.7: EEM contour plots and loadings of the four factors identified by the PARAFAC model

In both Oi samples, SUVA_{280} increased with incubation time, while in Oa samples, SUVA_{280} did not show a clear trend (data not shown). The concentrations of none of the four fluorophores identified by PARAFAC changed during 21 days in spruce-Oa and cypress-Oa samples (Table 3.3). In beech-Oi and Oa samples, concentrations of the third fluorophore decreased most prominently, followed by the first and the fourth fluorophore. Concentrations of the second fluorophore remained constant in these samples. In spruce-Oi samples, only concentrations of the third and fourth factor decreased during incubation time.

In our study, DON biodegradation decreased with decreasing initial DON concentration (Table 3.2). The same was true for the relationship of initial DOC concentration and DOC biodegradation. Neither DOC nor DON biodegradation was correlated with the initial DOC/DON ratio of our solutions. There was a significant correlation of initial SUVA_{280} with DOC and DON biodegradation, but these high correlation coefficients were only due to very low initial SUVA_{280} values of spruce-Oi, as compared to all other samples (see Table 3.1). After omitting data for spruce-Oi samples from the data set, the correlation was still significant for DOC but no longer for DON.

Both DOC and DON biodegradation were significantly correlated with the initial concentrations of factor 1 and 3 (Table 3.4). The change in DOC/DON ratio only showed a significant negative correlation with the initial concentration of factor 3. The correlations with factor 2 and factor 4 concentrations were never significant. The initial relative contribution of factor 2 showed a significant negative correlation with DOC and DON biodegradation and a significant positive relationship with the change in DOC/DON ratio (Table 3.4). For factor 3, the correlation was significant and positive with DOC and DON biodegradation and significant and negative with the change in DOC and DON ratio. The correlations with factor 1 and 4 were never significant.

After 21 days of incubation without $\mathrm{NO_3}^-$ addition, the balance of total N was only even for extracts from Oa horizons (Table 3.5). In spruce-Oi samples, the balance was slightly negative (indicating denitrification), while it was positive for beech-Oi samples (indicating $\mathrm{N_2}$-fixation). The increase in $\mathrm{NH_4}^+$ was always larger than the increase in PON (Table 3.5). The increase in PON was negligible in Oa samples and similar in both Oi-samples. The change in $\mathrm{NH_4}^+$ concentrations was largest in spruce-Oi samples followed by both beech samples and the two other Oa-samples.

Nitrate additions did not influence the balance for Oa samples consistently (Table 3.5). For beech-Oi samples, the balance was even for all $\mathrm{NO_3}^-$ additions, while in spruce-Oi samples, the balance became more negative with increasing $\mathrm{NO_3}^-$ additions.

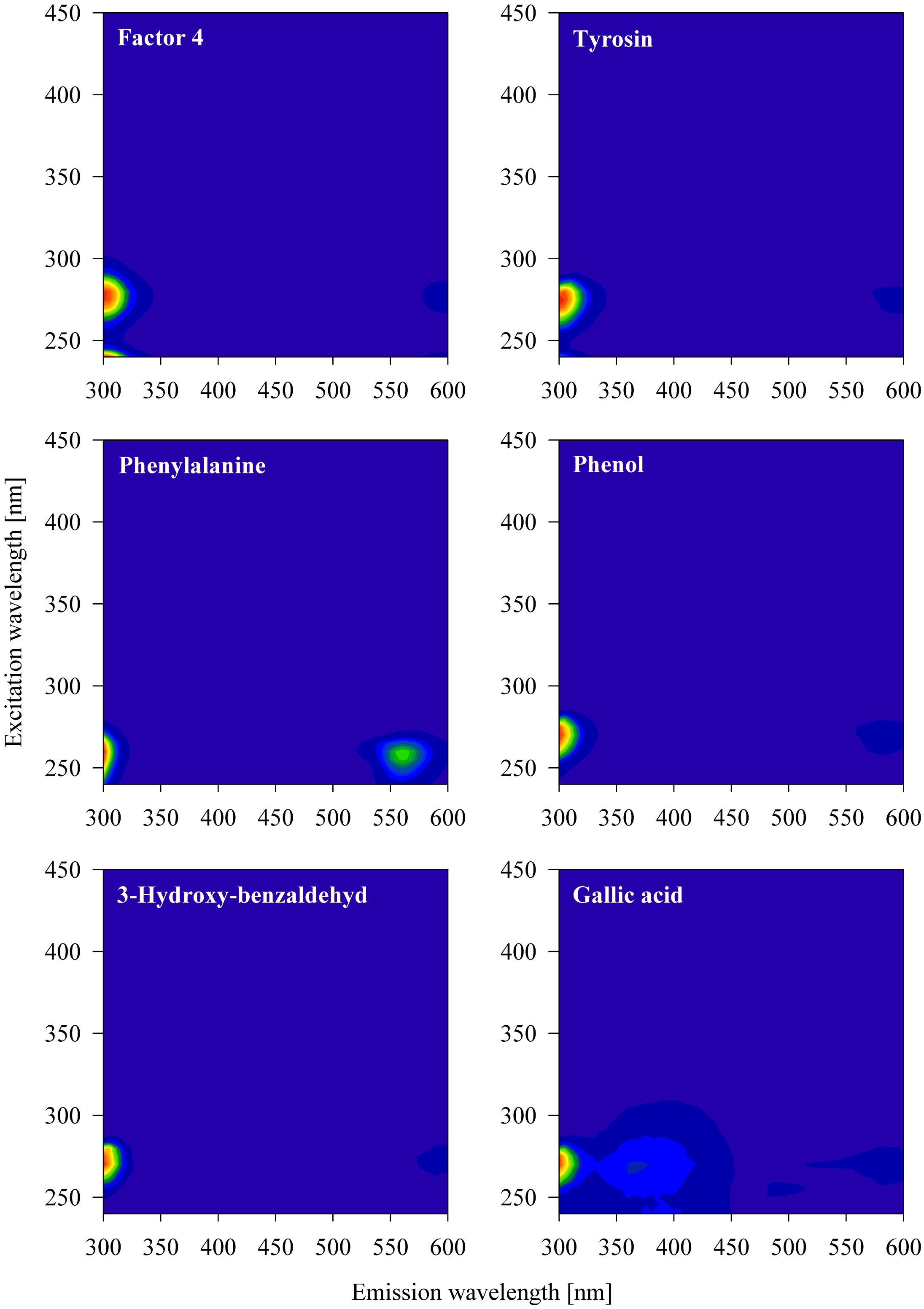


Fig. 3.8: EEM contour plots of the fourth factor identified by the PARAFAC model and single reference compounds with similar excitation-emission-maxima

Table 3.3: Concentrations of the four factors identified by the PARAFAC model before (t_0) and after (t_{21}) incubation in the five different water extracts from Oi and Oa soil horizons of beech, spruce and cypress.

	Factor 1		**Factor 2**		**Factor 3**		**Factor 4**	
	t_0	t_{21}	t_0	t_{21}	t_0	t_{21}	t_0	t_{21}
	[R.u.]							
Beech-Oi	6.93	6.00	4.76	4.65	2.59	1.71	1.48	1.33
	±0.14	±0.11	±0.41	±0.02	±0.22	±0.07	±0.03	±0.09
Beech-Oa	15.55	14.68	9.25	9.45	12.52	6.71	2.09	1.73
	±0.14	±0.23	±0.17	±0.07	±0.03	±0.11	±0.07	±0.16
Spruce-Oi	3.66	3.11	2.37	2.17	4.11	2.59	1.93	1.02
	±0.43	±0.03	±0.21	±0.02	±0.04	±0.05	±0.17	±0.02
Spruce-Oa	2.37	2.28	3.54	3.29	0.92	0.85	0.78	0.70
	±0.11	±0.02	±0.17	±0.03	±0.06	±0.03	±0.04	±0.03
Cypress-Oa	0.94	1.05	1.30	1.41	0.58	0.43	2.26	2.32
	±0.10	±0.07	±0.06	±0.05	±0.16	±0.01	±0.19	±0.14

PARAFAC factor concentrations are in Raman units [R.u.]. Numbers are means (*n*=3) with on standard deviation.

Table 3.4: Spearman Rank correlations of initial concentration of the four factors identified by the PARAFAC model with initial parameters and the change in parameters in the five different water extracts from Oi and Oa soil horizons of beech, spruce and cypress.

		Initial concentration [R.u.]			
		Factor 1	**Factor 2**	**Factor 3**	**Factor 4**
Change in concentration [%]	DON	0.66**	0.37	0.75**	0.37
	DOC	0.57*	0.27	0.61*	0.18
Change in ratio	DOC/DON	-0.28	0.10	-0.53*	-0.38

*=p<0.05, **=p<0.01, *n*=15, except for change in concentrations/ratio, where *n*=14.

Table 3.5: Balance and change of all N species during a 21-day incubation in all samples with all NO_3^- additions

	Change in [mg N L^{-1}]	**NO_3^- addition** [mg N L^{-1}]			
		0	0.75/3*	1.5/5*	3/10*
Beech-Oi	NO_3^-	0.02	-0.34	-0.51	-2.48
	DON	-0.47	-0.26	-0.21	0.28
	NH_4^+	0.99	1.23	1.17	1.68
	PON	0.66	-0.33	-0.12	0.72
	Balance of total N	**1.21**	**0.29**	**0.34**	**0.19**
Beech-Oa	NO_3^-	0.07	0.03	-0.01	-0.01
	DON	-1.09	-0.47	-0.53	-0.51
	NH_4^+	1.28	0.65	0.81	0.58
	PON	0.01	0.10	0.25	0.04
	Balance of total N	**0.27**	**0.30**	**0.52**	**0.11**
Spruce-Oi	NO_3^-	-0.06	-0.77	-1.65	-3.19
	DON	-3.14	-2.69	-2.71	-2.61
	NH_4^+	2.17	2.99	2.19	3.82
	PON	0.61	-0.25	1.48	0.27
	Balance of total N	**-0.41**	**-0.72**	**-0.70**	**-1.70**
Spruce-Oa	NO_3^-	0.04	0.01	-0.03	-0.01
	DON	-0.08	-0.13	-0.15	-0.21
	NH_4^+	0.50	-0.04	0.53	0.41
	PON	-0.18	-0.14	-0.20	0.06
	Balance of total N	**0.27**	**-0.30**	**0.15**	**0.24**
Cypress-Oa	NO_3^-	0.00	-0.02	-0.09	-0.04
	DON	-0.04	-0.11	-0.07	-0.04
	NH_4^+	0.27	0.75	0.32	0.30
	PON	-0.15	-0.02	-0.11	-0.09
	Balance of total N	**0.08**	**0.60**	**0.05**	**0.13**

NO_3^- additions with asterisks (*) belong to beech-Oi samples, values are mean of n=3

3.4 Discussion

3.4.1 Influence of initial litter extract chemistry on DON decomposition

Spruce-Oi samples had the lowest $SUVA_{280}$ (Table 3.1), which indicates low concentrations of aromatic structures corresponding to the large biodegradation observed in these samples. The negative relationship between $SUVA_{280}$ and DOC and DON biodegradation in our samples was only significant for DOC (Table 3.2), which was also reported in other studies (e.g. Kalbitz et al., 2003; Saadi et al., 2006). On the other hand, Marschner and Bredow (2002) reported no relationship between SUVA and biodegradable DOC. They concluded, that the portion of biodegradable DOC of non-aromatic compounds varies greatly, which might also hold true for biodegradable DON in our study.

The large biodegradation in spruce-Oi samples led to a large O_2 consumption by microbes. This in turn decreased the redox potential and led to denitrification as indicated by a negative balance, while the aeration performed at each sampling time was sufficient for all other samples to maintain oxic conditions. Compared to other solutions, PON concentrations were exceptionally high in spruce-Oi samples (Fig. 3.6), which corroborates this assumption. Moreover, during the later phases of incubation, flocculation was visible in spruce-Oi samples, which points to a strong growth of microorganisms.

Spruce-Oi extracts also had the highest initial DOC and DON concentration, which corresponded to the high biodegradation in these samples (see correlation coefficients in Table 3.2). Dissolved organic N biodegradability might depend on initial DOC and DON concentrations (higher substrate availability for microorganisms), but the effect of concentration was not tested systematically in this study.

Our results regarding the use of factors identified by PARAFAC analysis of EEMs for predicting DOC and DON biodegradation have to be interpreted with caution. The reported correlations of optical DOM characteristics with DOM quality suggest that these characteristics can be useful as proxies for bioavailability (Jaffé et al., 2008). Especially the abundance of protein-like fluorophores (factor 4 in our study) is thought to be a good indicator for the biodegradability of DOM, i.e. the labile DOM in natural environments (Fellman et al., 2008; Balcarczyk et al., 2009; Fellman et al., 2009b). In our study, initial factor 4 concentrations were not correlated with DOC and DON biodegradation (Table 3.4). This is probably due to the fact, that the EEM of the fourth factor can be assigned to a number of chemically different compounds that are very easily degradable (e.g. tyrosine) or very recalcitrant (e.g. phenol) (Fig. 3.8).

Only initial concentrations of factor 1 and 3 were significantly correlated with both DOC and DON biodegradation (Table 3.4). Changes in factor 1 and 3 concentrations were significantly correlated with DOC and DON biodegradation (r=0.74 and r=0.69, respectively (for factor 1), r=0.69 and r=0.83, respectively (for factor 3), Spearman Rank correlation with p<0.01 and n=14). The first factor has been identified as fulvic acid-type components (Stedmon and Markager, 2005; Santin et al., 2009), which are hydrophilic and therefore easy to degrade. We conclude, that the third group of fluorophores also should contain a high proportion of hydrophilic compounds. It is important to keep in mind that these factors do not represent certain chemical compounds, but rather mixtures of similar fluorophores (Stedmon et al., 2003).

After 21 days of incubation, biodegradation of DON in our samples was mainly explained by mineralization (Table 3.5), while microbial uptake was less important. After 5 days of incubation, this picture is quite different, with microbial uptake being the dominant process, while NH_4^+ decreased. In spruce-Oi samples, DON concentrations were constant after 5 days, but there was a constant decrease in DON concentrations in beech-Oa samples. These findings show that incubation time is a crucial factor when interpreting results and comparing data on DON biodegradation from different studies.

Of the other processes that can influence DON concentrations (Fig. 3.1), heterotrophic nitrification of DON to NO_3^- and subsequent dissimilatory reduction are assumed to be negligible. Except for spruce-Oi samples, NO_3^- concentrations always remained constant during the incubation time. These samples also had an equal or positive total N balance. This implies, that no denitrification took place (negative balance), which can be seen as evidence for the oxic state of our samples. Dissimilatory reduction of NO_3^- to NH_4^+ is sensitive to O_2 and only occurs under anoxic conditions. Moreover, we assume, that all other processes leading to a consumption or a formation of NO_3^- (in samples with equal or positive balance) were negligible, as the amount of NO_3^- formation and the amount of NO_3^- consumption would have to be equal to be consistent with constant NO_3^- concentrations.

3.4.2 Relationship between DOC and DON biodegradation

In our study, the temporal dynamics of DOC and DON biodegradation were similar (Fig. 3.2). The kinetics of biodegradation usually fit a double exponential model with a small labile and a large more stable pool (Qualls and Haines, 1992; Gregorich et al., 2003; Kalbitz et al., 2003). The half-lives calculated from the nonlinear regression model (two pools) for DOC and DON in spruce-Oi samples were 2.41 and 2.42 days for the labile pool, respectively. This is in accordance with reported half-lives for fresh spruce litter (4 days) and the labile DOC pool in a spruce-O horizon (2.6-5 days) (Kalbitz et al., 2003; Don and Kalbitz, 2005).

Dissolved organic N biodegradation was only higher than DOC biodegradation in beech-Oa and spruce-Oi samples. In studies from aquatic systems, the biodegradation of DON was often greater than of DOC (Wiegner and Seitzinger, 2004; Kaushal and Lewis, 2005; Petrone et al., 2009). One possible explanation could be, that DOC and DON may be concentrated in different fractions of DOM (humic and non-humic, respectively) (Petrone et al., 2009). In laboratory incubations of forest soil material (extracts and in-situ solutions), DON did not decay faster than DOC (Qualls and Haines, 1992; Cleveland et al., 2004; Kiikkilä et al., 2005), while in agricultural soils a faster biodegradation of DON as compared to DOC has been observed (Gregorich et al., 2003). On the other hand, Qualls and Haines (1992) postulated, that the hydrolysis of DON is closely linked to DOC mineralization and is not driven by a biochemical need of microorganisms for N. Cleveland et al. (2004) also showed, that within a N limited ecosystem, microbial biodegradation differed among litter leachates from two species which had different DOC/DON ratio. These findings suggest that in agricultural ecosystems, the N status may be an important factor for differences in DOC and DON biodegradation. In forest ecosystems (which are usually N limited as compared to agricultural systems), the chemical composition of individual plant species probably plays a more important role for differences in DOC and DON biodegradation.

3.4.3 Influence of NO_3^- additions on DON biodegradation

Based on the assumption that microorganisms can take up mineral and/or organic N forms, we also postulated, that addition of mineral nitrogen would decrease the biodegradation of DON due to a preferential uptake of mineral N forms. This hypothesis was not supported by our data, as DON biodegradation never decreased after NO_3^- addition. In fact, added NO_3^- proved to be quite stable during the incubation time, except in spruce-Oi samples, where added NO_3^- always disappeared within 3 to 5 days (Fig. 3.4).Theoretically, added NO_3^- could have been transformed but then the formation of new NO_3^- should happen in the same magnitude during the same time. This is very unlikely, therefore, we assume that NO_3^- measured at each sampling date was the NO_3^- that was originally added to the samples.

Moreover, we observed the highest biodegradation in samples with highest initial NH_4^+ concentrations (spruce Oi, Table 3.1). This is also in contrast to the assumption, that high mineral nitrogen concentrations would decrease the biodegradation of DON due to a preferential uptake of mineral N forms. However, the influence of NH_4^+ concentration on DON biodegradation was not tested systematically in this study.

Despite the large NO_3^- losses, DOC or DON biodegradation did not change in spruce-Oi sam-ples. This means, that no N limitation took place in our samples during the incubation time, as the easily decomposable fraction of DON was large enough to meet the microbial demand for N. The same conclusion was drawn by Gregorich et al. (2003) for samples from fertilized agricultural soils. However, denitrification increased with increasing NO_3^- additions in spruce-Oi samples, as indicated by a more negative total N balance.

3.5 Conclusion

We showed, that DON biodegradation in litter extracts with differing chemical quality, was highly variable. Especially the recalcitrant DON in extracts of coniferous Oa material might play an important role in building up stable organic N pools in forest soils. After being lost from Oa horizons, DON will be transported into the mineral soil, where sorption is its primary fate. Dissolved organic N fluxes from the mineral soil are usually smaller than DON fluxes in the forest floor, and therefore a significant portion of DON that is largely refractory, will remain in the mineral soil.

By definition, DOC and DON are both part of DOM. However, both classes of compounds are often regarded to behave differently in soils, although DOC and DON cannot be separated structurally. Our results show, that the separation of DOC and DON into functionally differ-ent compounds is not always useful, as DOC and DON biodegradation in all samples showed similar temporal dynamics.

The N status of the samples (as simulated by various NO_3^- additions) never influenced DON biodegradation, although our samples varied in initial chemical properties. We assumed that the supply of mineral N would lead to a decrease in DON biodegradation by microorganisms, however, in samples with DON biodegradation the amount of easily degradable DON was obviously large enough to supply the N need of microbes. Dissolved organic N biodegradation could also be driven by the C demand of microorganisms (meaning that DON is degraded as DOC is degraded) as the increase in microbial biomass N corresponded to DOC biodegradation.

The use of fluorescence spectroscopy and PARAFAC analysis for water extracts from forest floors is a promising tool that needs further development. Two factors were actually correlated with DOC and DON biodegradation, however, protein-like fluorophores, which are assumed to be good proxies for biodegradation, can also contain very recalcitrant compounds. The factors identified by PARAFAC analysis alone are probably not sufficient to safely predict biodegradation, as one group of fluorophore may contain two groups of substances with different degradability.

Acknowledgments

This study was funded by the Deutsche Forschungsgemeinschaft, Germany. The authors would like to thank Prof. Dr. Shih-Chieh Chang for providing samples from the Chi-Lan Mountain site, Taiwan. We are grateful to the members of the Central Analytical Department of the Bayreuth Center of Ecology and Environmental Research (BayCEER) for their help with the analysis of samples. We also thank the Institute of Soil Ecology, Helmholtz Zentrum München for providing the facilities for fluorescence excitation-emission-matrix spectroscopy and Prof. Dr. Walter Olbricht (University of Bayreuth) for statistical advice.

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CHAPTER 4

Abiotic reaction of nitrite with dissolved organic carbon? Testing the Ferrous Wheel Hypothesis

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Published in *Biogeochemistry* (2009), 93, 291-296
Received 30 July 2008, revised version accepted 23 January 2009

Abstract

The Ferrous Wheel Hypothesis (Davidson et al., 2003) postulates the abiotic formation of dissolved organic N (DON) in forest floors, by the fast reaction of NO_2^- with dissolved organic C (DOC). We investigated the abiotic reaction of NO_2^- with dissolved organic matter extracted from six different forest floors under oxic conditions. Solutions differed in DOC concentrations (15-60 mg L^{-1}), NO_2^- concentrations (0, 2, 20 mg NO_2^--N L^{-1}) and DOC/DON ratio (13.4 to 25.4). Concentrations of added NO_2^- never decreased within 60 min, therefore, no DON formation from added NO_2^- took place in any of the samples. Our results suggest that the reaction of NO_2^- with natural DOC in forest floors is rather unlikely.

Keywords: Abiotic nitrite immobilization; Dissolved organic carbon, Dissolved organic nitrogen; Ferrous Wheel Hypothesis

4.1 Introduction

The abiotic reaction of mineral N with soil organic matter (SOM) is defined as abiotic immobilization and is considered to be a major mechanism of N sequestration in soils. For example, Johnson et al. (2000) concluded that abiotic N immobilization accounts for 6-90% of total N immobilization in a variety of soils. Several studies have postulated abiotic N immobilization to explain the fast disappearance of added NO_3^- in soils (Davidson et al., 1991; Berntson and Aber, 2000; Dail et al., 2001; Perakis and Hedin, 2001; Compton and Boone, 2002; Corre et al., 2007; Huygens et al., 2007; Sotta et al., 2008). Fitzhugh et al. (2003) compared NH_4^+ , NO_3^- and NO_2^- immobilization in live and $HgCl_2$-sterilized soils and found that the main pathway for immobilization of NO_2^- was abiotic, while this process was of minor importance for NH_4^+ and NO_3^- . However, the added concentrations in their experiment (0.267 μmol NO_2^--N g^{-1} dry soil) caused a 300-1300-fold increase in the extractable NO_2^- pool when assuming that in northern hardwood forests NO_2^- concentrations usually range between 0.0002 and 0.0008 μmol $^{-1}$ (Venterea et al., 2003). In soils, NO_2^- concentrations are generally very low, mostly close to detection limits (Venterea et al., 2003; Müller et al., 2006).

Key factors determining the abiotic reaction of NO_2^- with SOM are organic matter content, soil pH, NO_2^- level, and temperature. The lower the pH and the higher the SOM content, NO_2^- level and temperature, the higher the immobilization of NO_2^- by SOM (Führ and Bremner, 1964a,b; Nelson and Bremner, 1969). Although NO_2^- immobilization also increases with time of incubation, the reaction of NO_2^- with SOM is fast (Fitzhugh et al., 2003), as over 50% of labeled $^{15}NO_2^-$ was recovered in SOM only 15 min following addition.

Another factor that might influence the abiotic NO_2^- immobilization to SOM is the N status of the ecosystem. Johnson et al. (2000) hypothesized, that abiotic N immobilization is less affected by N status than biotic immobilization. Nevertheless, it seems logical that more N can be immobilized by SOM if less binding places for N are occupied.

The Ferrous Wheel Hypothesis (Davidson et al., 2003) postulates the abiotic reaction of NO_2^- with dissolved organic carbon (DOC) instead of SOM, leading to formation of dissolved organic N (DON) and to N sequestration. The hypothesis tries to explain the fast removal of added NO_3^- in forest soils. It implies that NO_3^- in the forest floor of upland soils is first reduced by Fe(II) or Mn(II) to NO_2^- . The recovery of metals occurs as the oxidized metals are reduced again by SOM. The reaction of NO_2^- with DOC was postulated based on the fast reaction of NO_2^- with defined phenolic compounds (e.g. syringic acid, vanillic acid) used as surrogates for natural DOC (Davidson et al., 2003). The reaction of NO_2^- with defined phenolic compounds (α-naphthol) was also reported by Azhar et al. (1989).

The Ferrous Wheel Hypothesis explicitly states that DOC (not solid SOM) is the acceptor for NO_2^- . The advantage of working with dissolved (DOC) instead of solid SOM when investigating abiotic NO_2^- immobilization is obvious. To separate abiotic from biotic reactions, it is necessary to effectively sterilize soils with minimum alteration of soil chemical and physical properties (Wolf and Skipper, 1994). By definition, dissolved organic matter (DOM) is sterilized by filtration through 0.2 μm pore size filters to exclude microorganisms. This technique is fast, inexpensive and does not cause any physical or chemical alteration of the sample. The sterilization of the sample by addition of $HgCl_2$, which was suggested for soils (Wolf and Skipper, 1994) is not suitable since Hg would change the redox chemistry. Moreover, complexation of DOC with Hg will alter the structure of DOC.

To our knowledge, the abiotic reaction of NO_2^- with natural DOC has not been shown until now. Our aim was therefore to test whether an abiotic reaction of NO_2^- with natural DOC leads to fast DON formation at reasonable DOC and NO_2^- concentrations.

4.2 Material and methods

Samples were collected from the Oi and the Oa horizon of three long-term ecosystem research sites: the Chi-Lan Mountain forest ecosystem in Northern Taiwan (Cypress, *Chamaecyparis obtusa* var. *formosana*), the Steinkreuz site (European beech, *Fagus sylvatica* L.), and the Coulissenhieb site (Norway spruce, *Picea abies* (L.) Karst.), both in Germany. Details concerning climate, soils, and vegetation can be found in Rees et al. (2006) and Gerstberger et al. (2004). The soils were chosen to cover different pH, SOM, and DOC qualities (Table 4.1). Moreover, they represent different N saturation stages, as revealed by the DOC/DON ratios (Table 4.1) and the fluxes of DON and mineral N in the ecosystem (Table 4.2).

Dissolved organic C was prepared by adding 3 L of water to 300 g fresh weight of litter. The litter samples were previously frozen. The suspensions were then stored at 5°C and stirred three times. After 24 h the solutions were first filtered by suction through a ceramic plate (pore diameter about 1 μm). To exclude microorganisms, the solutions were then filtered through 0.2 μm cellulose acetate filters (Schleicher and Schüll OE 67), which were prewashed with 2x150 ml of pure water. Therefore, sterility of the solutions was assumed for the time of the measurement (60 min).

Dissolved organic C concentrations of all solutions were adjusted to 15 mg C L^{-1} and 60 mg C L^{-1}, and NO_2^- was added in concentrations of 2 mg N L^{-1} or 20 mg N L^{-1} as $NaNO_2$ at room temperature (22°C). If original DOC concentrations in the soil extracts were too low, solutions were freeze-dried and adjusted with distilled water to the desired concentration (for 15 mg C L^{-1}: Cypress Oi, Cypress Oa; for 60 mg C L^{-1}: Cypress Oi, Cypress Oa, Norway spruce Oi, Norway spruce Oa).

Table 4.1: Properties of the different forest floor horizons and their water extracts.

	pH ($CaCl_2$)	**CEC**$_{eff}$ [mmol$_c$ kg^{-1} soil]	**BS** [%]	**TOC** [g kg^{-1}]	**TON** [g kg^{-1}]	**TOC/TON** of forest floor	**DOC/DON** of extract	**pH** of extract
Beech-Oi	4.7	567.9	86.7	445	20.4	21.8	15.7	6.3
Beech-Oa	3.4	172.3	65.7	205	11.5	17.8	14.5	5.8
Spruce-Oi	3.6	245.8	22.7	478	19.3	24.8	13.4	5.4
Spruce-Oa	2.6	274.2	56.8	376	16.6	22.6	15.7	4.4
Cypress-Oi	2.8*	83.6*	55.6*	340*	20*	17*	25.4	4.7*
Cypress Oa							24.7	

CEC$_{eff}$: effective cation exchange capacity, BS: base saturation, TOC: total organic carbon, TON total organic N (Gerstberger et al., 2004; Rees et al., 2006; own data), * data only available for a mixed sample of Oi and Oa.

Table 4.2: Fluxes of NH_4^+, NO_3^-, DON and DOC in throughfall and in forest floor percolates (Oa) at the three experimental sites.

		European beech[a]	**Norway spruce**[b]	**Cypress**[c]
Throughfall	NH_4^+	7.0	9.2	2.5
[$kg\,ha^{-1}\,yr^{-1}$]	NO_3^-	6.2	11.5	1.4
	DON	3.9	3.4	2.4
	DOC	77.0	84.1	151.2
Forest floor percolates	NH_4^+	5.1	3.9	2.6
[$kg\,ha^{-1}\,yr^{-1}$]	NO_3^-	14.8	8.4	3.4
	DON	6.1	4.7	13.0
	DOC	176.0	114.7	1,137.6

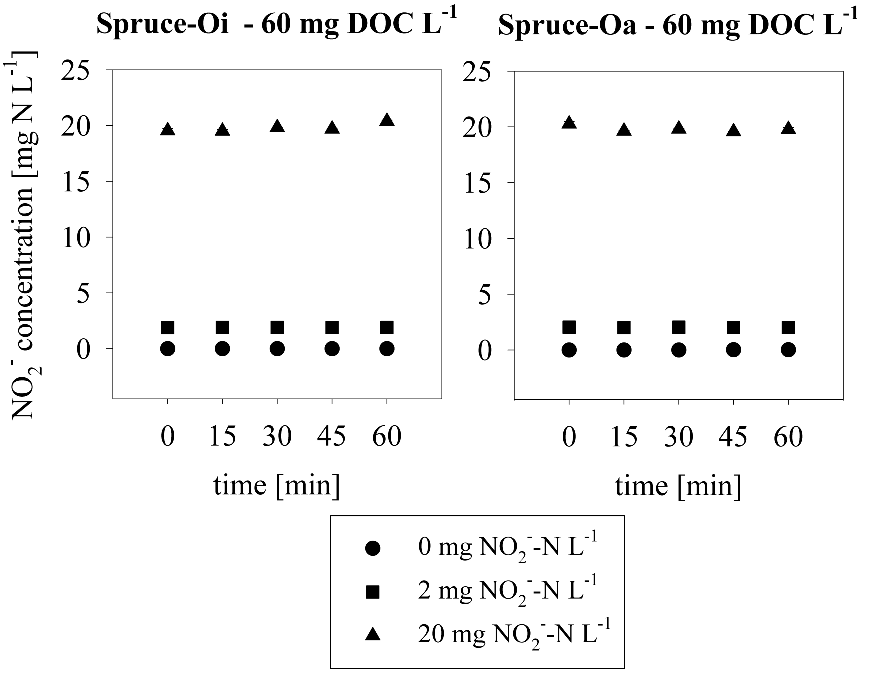
[a] Kalbitz et al., 2007 (years 1999-2005), [b] Michalzik and Matzner, 1999 (years 1995-1997), [c] Chang et al., 2007 (years 2003-2004), values recalculated from 15 to 12 months.

Each treatment had three replicates. Nitrite concentrations were determined after 0, 15, 30, 45 and 60 min with a segmented flow analyzer (Skalar Sanplus Analyzer) at 540 nm as highly coloured azo dye which is formed as NO_2^- is diazotized with sulfanilamide and coupled with α-naphthylethylenediamine dihydrochloride.

4.3 Results and discussion

During 60 min of reaction time, NO_2^- concentrations did not change in any of the DOM samples independent of DOC and NO_2^- concentrations (Fig. 4.1), data exemplarily shown for Norway spruce Oi and Oa with 60 mg C L^{-1}, other data not shown). Hence, a formation of DON by a fast, abiotic reaction of NO_2^- with DOM can be ruled out. Even if solutions were not totally sterile, microbes potentially present in solutions did not influence NO_2^- concentrations.

The fact that no abiotic reaction took place can be attributed to several factors. First, DOC concentrations might have been too small, as abiotic NO_2^- immobilization in soils was shown to increase with solid SOM concentrations (Nelson and Bremner, 1969; Führ and Bremner, 1964a,b). However, in our study even a 4-fold increase from 15 to 60 mg DOC L^{-1} did not trigger NO_2^- immobilization. Such DOC concentrations are commonly found in forest floor percolates under field conditions (Michalzik et al., 2001).



Error bars represent one standard error of the mean (*n*=3). If error bars are not visible, they are smaller than the size of the symbol.

Fig. 4.1: Temporal course of NO$_2^-$ concentrations in water extracts from forest floors.

Second, NO$_2^-$ concentrations might have been too small as the immobilization of NO$_2^-$ in soils increased with increasing NO$_2^-$ concentrations (Nelson and Bremner, 1969; Führ and Bremner, 1964a). But even at very high concentrations of NO$_2^-$ (20 mg N L^{-1} , no reaction took place. As NO$_2^-$ concentrations in soils are usually much less (Venterea et al., 2003; Müller et al., 2006), we do not expect a reaction to take place under field conditions.

It has been shown that the immobilization of NO$_2^-$ by SOM increases with increasing temperature (Nelson and Bremner, 1969). The temperature of the solutions (22°C) in our experiment exceeded the average soil temperatures commonly found in temperate forest soils by far. Hence, the reaction of NO$_2^-$ under field conditions appears to be unlikely if no reaction occurs under higher temperatures in the laboratory.

Our solutions differed in DOC/DON ratios but, although we expected to find differences in the reaction of NO$_2^-$ with DOC, none were observed. Also the N status of the ecosystem had no influence considering the variation of N fluxes in throughfall and forest floor percolates at the sites. Nitrite immobilization was found to be highest in acid soils (Nelson and Bremner, 1969), however, the differences in pH (4.4-6.3) did not lead to differences in NO_2^- immobilization in our study.

The reaction time might have been too short, but Davidson et al. (2003) showed that NO_2^- was highly reactive to defined phenolic compounds at pH 4 with significant decreases of added NO_2^- concentrations after 30 minutes. The authors used NO_2^- concentrations of $14\,mg\,NO_2^-$-N L^{-1} and equimolar additions of phenols ($12\,mg\,C\,L^{-1}$). Although in our study, NO_2^- concentrations (0, 2, $20\,mg\,NO_2^-$-N L^{-1}) and DOC concentrations (15 and $60\,mg\,C\,L^{-1}$) were very similar or higher, no reaction took place. Dissolved organic carbon is mostly comprised of humic substances with a much more complex chemical structure and higher N contents as compared to defined phenols. The DOC used in this experiment had aromatic C contents ranging only from 9 to 30% (Scheel et al., 2007). Thorn and Mikita (2000) demonstrated nitrosation reactions of humic substances with NO_2^- by NMR, but they used very high NO_2^- concentrations that do not occur in soils. In one case they showed nitrosation reactions at low NO_2^- concentrations but then the reaction time was very long (9 days) which does not support the idea of a fast reaction of NO_2^- with humic substances. Their approach also did not include a mass balance for added NO_2^- and a quantitative analysis of NO_2^- immobilization.

Nevertheless, a fast removal of added NO_3^- in sterilized soils has often been observed (e.g. Dail et al., 2001; Fitzhugh et al., 2003). Recently, Colman et al. (2007) challenged the observations on NO_3^- disappearance made by Dail et al. (2001), and pointed to possible iron interference during colorimetric determination of NO_3^- and subsequent underestimation of NO_3^- concentrations. This in turn would lead to an overestimation of abiotic NO_3^- immobilization. Davidson et al. (2008) tested the possible iron interference in the samples used by Dail et al. (2001) and concluded that iron concentrations were too low to lead to analytical errors.

Colman et al. (2007) might have failed to repeat the findings of Dail et al. (2001) because they likely destroyed anaerobic microsites in their samples (Davidson et al., 2008), which allow NO_3^- reduction to NO_2^-. In our study, NO_2^- was already available and the reduction of NO_3^- was not a limiting factor. However, anaerobic microsites could influence the redox status of soil organic matter which in turn could affect NO_2^- immobilization. It has been shown, that humic substances are redox-active (e.g. Bauer et al., 2007; Heitmann et al., 2007), but the effect of the redox status of DOM on reactions with reduced N compounds is unknown.

For explaining the fast disappearance of added NO_3^-, other intermediates of NO_3^- reduction or radicals might be considered as reactants. Another option is that the reaction requires the presence of a solid phase, such as solid organic matter or even a mineral surface as catalyst. Some observations of abiotic NO_3^- immobilization might also be due to artefacts, e.g. changes in SOM quality induced by sterilization or incomplete sterilization in general (lack of homogenization of sterilant and soil and subsequent formation of unsterile microsites).

According to our study, the postulated third step of the Ferrous Wheel Hypothesis, (reaction of NO_2^- with DOC) is not likely to occur under realistic concentrations of the reactants and under the experimental conditions used. Therefore, we conclude that the Ferrous Wheel Hypothesis needs revision and that other mechanisms of abiotic NO_3^- immobilization should be investigated.

Acknowledgements

The authors would like to thank Ludwig Haumaier for helpful assistance in the laboratory and the members of the Central Analytical Department of the Bayreuth Center of Ecology and Environmental Research (BayCEER) for their help with the analysis of samples. The authors also thank two anonymous reviewers for helpful comments on the manuscript. This work was financially supported by the Deutsche Forschungsgemeinschaft (DFG).

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Appendix

Own contributions of the candidate

Chapter 2 - High precipitation causes large fluxes of dissolved organic carbon and nitrogen in a subtropical montane *Chamaecyparis* forest in Taiwan

Schmidt, B. H. M., Wang, C.-P., Chang, S.-C., Matzner, E.
Published in *Biogeochemistry* (2010), doi:10.1007/s10533-010-9470-1

Experimental design: Shih-Chieh Chang, Chiao-Ping Wang and Egbert Matzner
Measurements: Shih-Chieh Chang and Chiao-Ping Wang
Data analysis: Bettina Schmidt
Graphs and Tables: Bettina Schmidt
Manuscript preparation: Bettina Schmidt (80%), Egbert Matzner (20%)

Chapter 3 - Microbial uptake and mineralization of dissolved organic nitrogen from forest floors

Schmidt, B. H. M., Kalbitz, K., Braun, S., Fuß, R., McDowell, W. H., Matzner, E.
Submitted to *Soil Biology and Biochemistry*

Experimental design: K. Kalbitz and W. H. McDowell
Measurements: S. Braun
Data analysis: S. Braun in cooperation with B. Schmidt (90%), R. Fuß (10%)
Graphs and Tables: B. Schmidt
Manuscript preparation: B. Schmidt (70%), E. Matzner and K. Kalbitz (20%), W. H. McDowell and R. Fuß (10%)

Chapter 4 - Abiotic reaction of nitrite with dissolved organic carbon? Testing the Ferrous Wheel Hypothesis

Schmidt, B. H. M., Matzner, E.
Published in *Biogeochemistry* (2009), 93, 291-296

Experimental design: B. Schmidt in cooperation with E. Matzner
Measurements: B. Schmidt
Data analysis: B. Schmidt
Graphs and Tables: B. Schmidt
Manuscript preparation: B. Schmidt (80%), E. Matzner (20%)

Publications

Schmidt, B. H. M., Matzner, E., 2009. Abiotic reaction of nitrite with dissolved organic carbon? Testing the Ferrous Wheel Hypothesis. Biogeochemistry 93, 291-296.

Schmidt, B. H. M., Wang, C.-P., Chang, S.-C., Matzner, E., 2010a. High precipitation causes large fluxes of dissolved organic carbon and nitrogen in a subtropical montane *Chamaecyparis* forest in Taiwan. Biogeochemistry, doi:10.1007/s10533-010-9470-1.

Schmidt, B. H. M., Kalbitz, K., Braun, S., Fuß, R., McDowell, W. H., Matzner, E., 2010b. Microbial uptake and mineralization of dissolved organic nitrogen from forest floors. Soil Biology and Biochemistry, submitted.

Acknowledgements

There are many people, who accompanied me during these three years and contributed to this success. A big thanks goes out to all of them. In particular, I would like to mention the following people:

- Prof. Dr. Egbert Matzner for being a great supervisor, for valuable comments and fruitful discussions, and for his neverending encouragement.
- Prof. Dr. Karsten Kalbitz for all discussions and support, be it personally or electronically: van harte bedankt.
- Chiao-Ping Wang and Min-Ze for not only being great hosts and friends, but also for scientific support and new perspectives on this thesis.
- Shih-Chieh Chang and his family for the wonderful time in Taiwan, the introduction to the Institute of Natural Resources, NDHU and the Chi-Lan Mountain site, and last but not least for helpful scientific advice.
- Ming-Jer, Shou-Pei, Tien-I and Hao-Kuei and all NDHU students, Günter Engling and his family and Chihong Wang for their warm welcome and their support in scientific and non-scientific questions regarding life and especially food in Taiwan.
- Roland Fuß for his time and support in München and for helping me to unravel the mysteries of PARAFAC analysis.
- Ludwig Haumaier for being the perfect roommate and for numerous tipps, tricks and discussions.
- Ingeborg Vogler, the lab staff of the Department of Soil Ecology and Environmental Microbiology, the members of the Central Analytical Department (ZAN) and all BayCEER technicians for their help with the analyses and their support and smiles.
- Sabine Braun for helping me to make a great step forward, for always having time for a cup of coffee, and for the ”undissolved mystery”.
- All PhD and diploma students, who were fellows and friends during my BayCEER time, for making the workplace a wonderful and very special ecosystem.
- Ulrich Zang and Fabian Stehn for providing answers to all questions concerning LaTeX that Google could not answer.

- Melanie Kappes and all my other friends for always being a shoulder to lean on.
- Henrik Prinz for making me forget the frustrations and showing me new perspectives for life after the thesis - you cannot imagine how much your support means to me.
- My family, but especially my parents and my sister for their neverending support in all my pursuits. And finally my grandfather for nourishing the seed of science he encountered in me.

Declaration / Erklärung

I hereby declare that this PhD thesis is entirely my own work, and that I did not use any other sources or auxiliary means other than those referenced. Moreover, I declare that no parts of this thesis have previously been submitted for the purpose of obtaining a PhD degree at any other scientific institution, and that I have not yet conclusively failed any doctoral examination procedure.

Hiermit erkläre ich, dass ich die vorliegende Arbeit selbständig verfasst und keine anderen als die angegeben Quellen und Hilfsmittel benutzt habe. Ich versichere weiterhin, dass ich diese Arbeit an keiner anderen wissenschaftlichen Einrichtung zum Zwecke der Promotion eingereicht habe, sowie, dass ich noch kein Promotionsverfahren endgültig nicht bestanden habe.

Bayreuth, den 05.02.2011

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