Dissolved organic matter in differently managed forest ecosystems

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Table of Contents

List of Figures

List of Tables

List of Abbrevations

ALB Schwäbische Alb

ANOVA analysis of variance

BDOC biodegradable organic matter

C carbon

cDOM chromophoric DOM

CRAM carboxylic-rich alicyclic molecules, carboxylic-rich alicyclic molecules

Da dalton

DBE double bond equivalent

DOC dissolved organiccarbonr

DOM dissolved organic matter

EEMs Excitation-Emission-Matrices

EM emission

ESI electrospry ionization

EX excitation

ForMI forest management intensity index

FT-ICR MS fourier-transform ion cyclotron resonance mass spectroscopy

H hydrogen

HAI Hainich-Dün

HIX humification index

k minerilazation constant

LL litter leachate

O oxygen

O_a fermentation layers

O_i litter layer

PARAFAC parallel factor analysis
c-components PARAFAC components of the preservation experiment
C-components PARAFAC components of the DOM composition evaluation

PCA principal component analys

PE polyethylene

PERMANOVA permutational multivariate analysis of variance

PU polyurethane

RU raman unit

S sulfur

SCH Schorfheide-Chorin

SF stemflow

SOM soil organic matter

SPE solid phase extraction

SUB subsoil solution

$SUVA_{254}$ specific ultraviolet absorption at 254nm

TF throughfall

TOP topsoil solution

Abstract

Dissolved organic matter (DOM) represents one of the most dynamic fractions of organic matter. It plays an important role in the biogeochemical cycles of carbon and nutrients, which in turn is strongly affected by its chemical quality. This thesis aims to improve the understanding of DOM quality and its changes along the water flow path through forested Mid-European ecosystems, as they are influenced by forest management. I hypothesized that (i) the composition and (ii) the biodegradability of DOM changes systematically along the water flow path through different compartments (throughfall, stemflow, litter leachate, mineral topsoil and subsoil solution), whereby DOM quality as well as the direction and magnitude of its changes depend on forest management practice. These include vegetation cover and intensity of management activity. These hypotheses were tested using samples from forest plots of the German "Biodiversity Exploratories", which were located in three different regions of Germany (Schwäbische Alb, Hainich-Dün and Schorfheide-Chorin) and comprised age-class coniferous stands as well as age-class and unmanaged beech forests. The chemical and optical DOM properties were investigated with UV-vis and fluorescence spectroscopy as well as FT-ICR MS measurements, while DOM bioavailability was examined with an incubation experiment. These investigations were accompanied by an evaluation of the effects of sample preservation on dissolved organic carbon (DOC) concentration and DOM composition, which showed that freezing and thawing affected the DOC concentration, as well as spectral absorption and fluorescence DOM properties of all compartments. However, since all sample types were equally affected, it was possible to consider the under- or overestimations, caused by the storage protocol, when interpreting the results of DOM quality characterization.

Throughfall showed the highest amount of protein-related substances and contained the lowest amounts of aromatic compounds. Decreasing amounts of protein-related constituents and increasing shares of aromatic compounds were detected when following the water via stemflow to litter leachate. Results of the biodegradability experiment confirmed that the increase of aromaticity was due to microbial processing, i.e. the preferential degradation and assimilation of non-aromatic compounds. After percolating further downward and passing organic soil layers, DOM of subsoil solution showed decreasing DOC concentration and decreasing amounts of aromatic components, likely due to preferential adsorption of the latter. The remaining DOM was characterized by an accumulation of products of microbial lignin degradation and other refractory compounds.

DOM quality and DOC concentration differed between coniferous and beech forest stands for above ground samples. Following the water flow path below ground, DOM properties converged and vegetation-related differences disappeared. Apart from this tree-species effect, management categories like unmanaged and age-class beech forests, as well as established indices of forest management intensity had no statistically significant influence on DOM properties and DOC concentration.

This thesis confirmed that DOM composition and bioavailability of forested ecosystems changes systematically along the water flow path from the canopy through organic soil layers to the subsoil. While properties of DOM in throughfall and stemflow samples differ between dominant tree species (coniferous trees versus beech trees), these differences disappear along the flow path belowground most likely as a consequence of microbial processing and interactions with the mineral soil. Hence, a better quantitative understanding of microbial generation and processing as well as sorption of DOM fractions in different soils might be necessary to identify effects of a management intensity on DOM quality and quantity. The suitability of DOM as indicator of management effects on carbon cycling in forests is limited by the multitude of factors that affect its quantity and quality.

Zusammenfassung

Gelöste organische Substanz (DOM) repräsentiert eine der dynamischsten Fraktion von organischem Material. Sie spielt eine wichtige Rolle im biogeochemischen Kreislauf von Kohlenstoff und anderen Nährstoffen. Diese Rolle wird wiederum durch die DOM Qualität, also ihre chemische Zusammensetzung und Bioverfügbarkeit, beeinflusst. Ziel dieser Arbeit ist es, unser Verständnis über die Qualität von gelöster organischer Substanz und deren Änderung entlang des Wasserpfades in einem europäischen Waldökosystem zu vertiefen, sowie den Einfluss, den das Waldmanagement darauf ausübt, zu untersuchen. Es wurden die folgenden Hypothesen getestet: i) Die Zusammensetzung und ii) die Bioverfügbarkeit von DOM ändert sich systematisch entlang des Wasserweges durch unterschiedliche Schichten des Waldes, über Bestandsniederschlag und Stammabfluss, durch die Streuschicht hin durch mineralischen Ober- und Unterboden, wobei nicht nur die Qualität, sondern auch die Richtung und das Ausmaß der Änderung vom Waldmanagement abhängen. Dieses umfasst sowohl die Auswahl verschiedener Baumarten, als auch die Intensität der Bewirtschaftung. Die Proben zur Prüfung der Hypothesen wurden auf ausgewählten Waldflächen der „Exploratorien zur funktionellen Biodiversitätsforschung“ der DFG gewonnen. Diese befinden sich in drei Regionen Deutschlands und umfassen sowohl Altersklassenwälder als auch unbewirtschaftete Buchenwälder. Die chemischen und optischen Eigenschaften von DOM wurden mittels UV-vis - und Fluoreszenzsspektrometrie, sowie mit hochauflösender FT-ICR Massenspektrometrie untersucht, während zur Bestimmung der Bioverfügbarkeit ein Inkubationsversuch durchgeführt wurde. Begleitend zu der Charakterisierung wurde der Einfluss der Probenlagerung auf die DOM Zusammensetzung und Konzentration untersucht. Diese Methodenvalidierung zeigte, dass das Einfrieren der Proben die Konzentration sowie die Absorptions- und Fluoreszenzeigenschaften der DOM in allen untersuchten Proben beeinflusste. Da jedoch alle untersuchten Variablen unabhängig von der Waldschicht in gleicher Weise beeinflusst wurden, ist es möglich die Über- oder Unterschätzungen der einzelnen Parameter durch das Einfrieren bei der Interpretation von Änderungen der DOM-Qualität zu berücksichtigen.

Der Bestandsniederschlag zeigte den höchsten Anteil proteinhaltiger und den geringsten Anteil an aromatischer DOM. Entlang des Wasserfließpfades über den Stammabfluss in die Streuschicht nahm der Anteil der Ersteren ab und der Zweiteren zu. Der Inkubationsversuch mit inokulierten Lösungsproben zeigt, dass die Zunahme der Aromatizität des DOM auf einem präferenziellen biologischen Abbau nicht-aromatischer Verbindungen beruht. Nach dem Bodeneintritt und dem Durchfließen von organischen Bodenschichten, zeigte die Mineralbodenlösung wieder eine Abnahme von aromatischen DOM-Bestandteilen, was wahrscheinlich auf deren selektiver Sorption an mineralischen Bodenbestandteilen zurückzuführen ist. Die verbleibende DOM zeigte eine Anreicherung von Lignin-Abbauprodukten und anderen refraktären Bestandteilen.

Die DOM-Qualität und –Konzentration unterschied sich zwischen Nadel- und Buchenwäldern für oberirdische Probentypen. Im Boden verschwanden diese Unterschiede wieder. Statistisch signifikante Unterschiede zwischen der Zusammensetzung von DOM aus unterschiedlich bewirtschafteten Wäldern einer Baumart konnten nicht festgestellt werden.

Diese Arbeit bestätigt, dass sich Quantität und Qualität von DOM entlang des Wasserfließweges durch Waldökosysteme systematisch verändern. Während die Qualität und Konzentration von DOM in Bestandsniederschlag und Stammabfluss signifikant durch die Hauptbaumart (Nadelbaum versus Buche) beeinflusst wird, verschwinden diese Unterschiede auf dem Weg durch den Boden, wahrscheinlich aufgrund der mikrobiellen Umsetzung und der Interaktion von DOM mit dem Mineralboden. Ein besseres Verständnis der mikrobiellen Produktion und Umsetzung aber auch der Sorption von DOM in verschiedenen Böden scheint eine Voraussetzung zur Aufklärung des Effektes von Management auf DOM zu sein. Die Eignung von DOM als Indikator für Managementeffekte auf den Kohlenstoffkreislauf von Wäldern wird durch die Vielzahl von Faktoren begrenzt, die seine Konzentration und Zusammensetzung beeinflussen.

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1 Introduction

1.1.1 DOM sources and chemical composition

Dissolved organic matter (DOM) represents one of the most dynamic fractions of organic matter (Kaiser und Kalbitz 2012). As such, it plays an important role in the biogeochemical cycles of carbon and nutrients. DOM and associated nutrients undergo biological transformation, sportive stabilization, mobilization and transport in terrestrial ecosystems (Bolan et al. 2011). The functioning of DOM as a component of the carbon and nutrient cycles in ecosystems is strongly affected by its chemical properties. These properties, in turn, depend on the DOM sources, their respective mixing processes and natural transformation along the water flow path (Bolan et al. 2011). Following the water passing through a forest ecosystem, we find numerous sources of DOM. Rain water moves through the atmosphere, washes through forest canopies and understory vegetation, infiltrates and percolates the forest litter layer and the organic soil horizon and passes further downward through the mineral soil reaching groundwater tables and entering the aquifer.

Precipitation incorporates atmospheric dust and gases containing organic carbon (Aitkenhead-Peterson et al. 2002). After interception in the canopy, water is separated into throughfall (TF) and stemflow (SF) on its way to the forest floor, both gaining different DOM quality (Moore 2003; Inamdar et al. 2012; Levia et al. 2012; Levia und Germer 2015; Michalzik et al. 2016). DOM is released from leaves (Wickland et al. 2007), twigs and tree-stems (Levia und Germer 2015), but also from insects (Michalzik et al. 2016) and bacteria (Lindow und Brandl 2003) inhabiting the canopy and leaf surfaces. Solute composition and concentration of throughfall showed to be influenced by the presence or absence of epiphytic bryophyte in the canopy (Chuyong et al. 2004) as well as by bark inhabiting lichens (Levia 2002). It is generally observed that different plant species composition leads to differences in throughfall volume and solute inputs (Levia und Frost 2006). Important sources of DOM at the soil surface are deadwood and coarse woody debris (Kahl et al. 2012; Bantle et al. 2014; Magnússon et al. 2016) and decomposition of leaf litter (Cleveland et al. 2004; Klotzbücher et al. 2013). Various studies under laboratory and field conditions show differences in litter leachate (LL) dissolved organic matter (DOC) concentrations, DOM biodegradability and compositions for different tree species (Cleveland et al. 2004, 2004; Don und Kalbitz 2005, 2005; Cuss und Guéguen 2013, Klotzbücher et al. 2013, 2013). Changing the amount and origin of deadwood influences fungal community composition and thus wood decomposition and release of DOM and quality (Arnstadt et al. 2016). Degradation of vascular plants in general produces DOM that contains proteins, carbohydrates (mainly cellulose), some lipids concentrated in the roots and leaf cuticles, lignin, and other macromolecules of biological origin (Hatakka 2005; Killops und Killops 2005). According to Hur et al. (2009), other “humic-like” aromatic components in the DOM extracts from leaf litter may be formed by microbial utilization of labile components, such as simple carbohydrates and amino acids. DOM directly leached or washed from foliar surfaces

on the other hand, tends to be enriched in unsaturated aliphatics and sugars (Guggenberger et al. 1994; Michalzik et al. 2001; Kalbitz et al. 2007). Principal belowground sources of DOM are root exudates (Yano et al. 2000; Baetz und Martinoia 2014), microbial primary and secondary metabolites (Aitkenhead-Peterson et al. 2002; Högberg und Högberg 2002), as well as degradation products of soil organic matter (DOM as left-over of soil organic matter (SOM) degradation, e.g. (Gödde et al. 1996; Hagedorn et al. 2004). Root exudates are mostly composed of low molecular weight sugars, proteinaceous amino acids and organic acids (Rees et al. 2005). The composition of SOM-derived DOM in the upper soil profile has a signature influenced by the vegetation and is dominated by lignin-derived phenols and plant derived carbohydrates, while microbial-originated DOM predominated in subsoil (Kaiser et al. 2004; Ohno et al. 2010; Kaiser und Kalbitz 2012).

1.1.2 DOM bioavailability

Biological synthesis and mineralization of organic carbon are important mechanisms regulating DOM dynamics in the environment (Benner 2002; Bolan et al. 2011). Biodegradability of DOM is, beside others, controlled by intrinsic characteristics like molecular structure, functional group content or size of the molecules (Marschner und Kalbitz 2003). The rate of biodegradation in incubation experiments has been found to cover a wide range and to vary among sources. Qualls and Haines (1992) found 49% of biodegradable DOC (BDOC) in throughfall of deciduous forests. Even within DOM sources varying amounts of degradability were found. While Kalbitz et al. (2003) found 65 and 61% respectively of total DOC was degradable of samples from beech and spruce forests litter, Hogve et al. (2000) found 75% BDOC for deciduous but 0% for spruce litter. Incubation studies of soil solutions found 10-44% of DOC were microbial degradable (Jandl und Sletten 1999; Kalbitz et al. 2000; Yano et al. 2000).

It was found that biodegradation of DOM is closely related to its chemical properties. Correlations have been found between BDOC and specific absorbance as well as humic-like fluorescence (Kalbitz et al. 2003; Fellman et al. 2008b), indicating that especially aromatic structures are highly stable against degradation. In contrast, carbohydrates and protein-containing components were preferentially utilized during microbial degradation (Kalbitz et al. 2003; Fellman et al. 2008b).

1.1.3 Influence of forest management practice on DOM sources

Forest management practices can influence various sources of DOM and thereby its quality. In temperate forests, management practices range from coniferous and deciduous age-class forests resulting from clear cutting or shelterwood logging over selectively cut, uneven-aged forest to unmanaged forests (Fischer et al. 2010; Hessenmöller et al. 2011). Beside abiotic factors like temperature, light, wind speed and moisture (Chen et al. 1999), forest management influences biotic stand structural attributes (Hessenmöller et al. 2011; Ehbrecht et al. 2017). It has an effect on species richness and abundances of plants (Schmidt

2005; Boch et al. 2013a), lichens and bryophytes (Boch 2011; Boch et al. 2013b; Boch et al. 2013a). As another source of aboveground DOM, the amount of deadwood and coarse woody debris is influenced by forest management system (Hessenmöller et al. 2011; Blaser et al. 2013). Changes in aboveground forest structure variables can indirectly influence belowground DOM release by affecting soil temperature and moisture patterns and can lead to a complex mosaic of soil chemistry gradients (Levia und Frost 2006). Fungal community structure is affected by water availability, understory vegetation and litter cover (Burke et al. 2009; Wubet et al. 2012). In the mineral soil, the chemical composition of root exudates appears to be species specific and hence the microbial rhizosphere community associated with each plant species is different (van Dam und Bouwmeester 2016). In addition to the kind of DOM source, also DOM processing affects its chemical composition (Stubbins et al. 2017). During DOM transformation and mineralization by microorganisms, several classes of chemical compounds are preferentially oxidized to CO_2 (e.g. carbohydrates and carboxylic acids), while others passively accumulate as leftover, e.g. lignin, lipids and waxes (Kalbitz et al. 2003). Similarly, some fractions of DOM are sorbed more strongly by solid soil constituents than others, so that DOM quality is systematically changed as a consequence of sorption processes (e.g., (Kaiser et al. 1996).

1.1.4 Investigating DOM quality

Optical methods like UV-vis absorption and fluorescence spectroscopy used as single Excitation/Emission scans, synchronous scans and Excitation-Emission-Matrices (EEMs) in combination with different spectroscopic indices and/or parallel factor analysis (PARAFAC) are increasingly used to characterize chromophoric dissolved organic matter (cDOM) in various environmental systems. Due to its advantages like small sample quantities required, only small or no sample preparation, short measuring time and high precision it is applied to determine origin, dynamics, biogeochemical functions and fate of cDOM in a wide range of aquatic systems (e.g. Jaffé et al. 2004; Fellman et al. 2008a; Miller und McKnight 2010; Yamashita et al. 2010a; Graeber et al. 2012). Optical methods are used to monitor wastewater treatment processes and quality (Reynolds 2002; Hudson et al. 2008) and to monitor DOM during drinking water treatment (Matilainen et al. 2011). Extractable soil organic matter and pore water (Otero et al. 2007; Hur et al. 2014; Traversa et al. 2014) were investigated, as well as isolated humic substances from soil and litter (Kalbitz et al. 1999; D'Orazio und Senesi 2009). Inamdar et al. (2012) studied changes in DOM concentrations and quality along the water flow path in one forested Mid-Atlantic watershed and found pronounced differences between the DOM sources.

The applicability of optical methods for characterizing DOM and the comparability of results in multidisciplinary studies relies on the preservation of samples prior to their analysis. DOM properties depend on many physicochemical and biological boundary conditions, so that artifacts caused by sample storage or sample pre-treatment may be produced easily. For these reasons it is recommended to directly filter samples after collection and store them in the cold and dark prior to measurement as soon as possible (Santos et al. 2010; Spencer und

Coble 2014). However, immediate measurement is often not possible for practical reasons such as a large number of samples or remote or separated sampling sites, so that freezing of filtered DOM samples is often the selected storage method (Murphy et al. 2008; Yamashita et al. 2010b; Graeber et al. 2012). Freezing can affect the physicochemical composition of samples (Edwards und Cresser 1992), so that improved conservation techniques, which avoid or minimize potential artifacts of freezing, are required. The impact of sample preservation like freezing seems highly variable depending on sample and DOM characteristics (e.g. (Spencer et al. 2007; Fellman et al. 2008a; Conmy et al. 2009; Yamashita et al. 2010a; Peacock et al. 2015). While most studies focused on samples from marine or freshwater ecosystems, there is a lack of information on sample pre-treatment effects on cDOM properties of water samples from terrestrial ecosystems, especially soil solution.

Despite the benefits of fluorescence-based studies, they are unable to provide more detailed information on the molecular level of DOM. High-resolution Fourier-transform ion cyclotron resonance mass spectrometry (FT-ICR MS) coupled with electrospray ionization (ESI) is increasingly utilized to chemically characterize natural organic matter (e.g.(Ohno et al. 2014; Roth et al. 2015; Ide et al. 2017; Stubbins et al. 2017). The high mass resolution and accuracy of this method allows identifying several thousand compounds and their molecular formulae in a sample (Reemtsma 2009). A standard approach for comparing this huge amount of data between samples is through graphical van Krevelen diagrams (van Krevelen 1950). They feature a broad overview on the average molecular properties by plotting each formula based on their H:C and O:C ratios. Based on the position in the van Krevelen diagram it is possible to group the identified molecules into biochemical molecular classes that typically include proteins, lipids, carbohydrates, unsaturated hydrocarbons, lignins, condensed aromatics, and tannins (Sleighter und Hatcher 2007; Hockaday et al. 2009).

1.1.5 Objective and outline of this thesis

The aim of this thesis is to improve our understanding of chemical and optical DOM properties and their changes along the water flow path in forested Mid-European ecosystems as they are influenced by forest management practice. I first tested:

i. the influence of sample preservation (freezing) on DOC concentrations and DOM optical properties of different water samples (section 3.1[1]).

I further tested the hypotheses that:

ii. the composition (section 3.2) and
iii. the biodegradability (section 3.3) of DOM changes systematically along the water flow path from throughfall (TF), stemflow (SF), and litter leachate (LL) to mineral topsoil (TOP) and subsoil (SUB) solution, whereby DOM composition as well as direction and magnitude of its changes depend in forest management.

[1] Results are published: Biogeosciences, 13, 4697-4705, 2016. https://doi.org/10.5194/bg-13-4697-2016

2 Methods

2.1.1 Study sites

The study was conducted on experimental plots in the Schwäbische Alb (ALB), the Schorfheide-Chorin (SCH) and the Hainich-Dün (HAI) exploratories of the German "Biodiversity Exploratories", which were established as platform for large-scale and long-term functional biodiversity research (DFG Schwerpunktprogramm 1374, www.biodiversity-exploratories.de). The three regions are representative of large areas in Central Europe. Important climatic, geological and soil properties of the three regions are given in Table 2.1 (modified from Fischer et al. 2010).

Table 2.1: Environmental properties of the three exploratory sites Schwäbische Alb, Hainich-Dün and Schorfheide-Chorin

	Schwäbische Alb (ALB)	**Hainich-Dün (HAI)**	**Schorfheide-Chorin (SCH)**
Location	Southwest Germany (53° 2´ N, 13° 51` E)	Central Germany (51° 10' N, 10° 23' E)	Northeast Germany (53° 2' N, 13° 51' E)
Altitude	460-860 m a.s.l.	285 - 550 m a.s.l.	3-140 m a.s.l.
Mean anual temperature	6-7°C	6.5-8°C	8-8.5 °C
Mean anual precipitation	700-1000mm	500-800 mm	500-600 mm
Bedrock	Jurassic limestone	Triassic limestone with loess cover	Quartzitic glacial till
Main soil types	Leptosols, Cambisols	Luvisols, Stagnosols	Cambisols, Albeluvisols
Main tree species	*Fagus sylvatica* L. Picea abies (L.) H. Karst.	*Fagus sylvatica* L. Picea abies (L.) H. Karst. Fraxinus exelsior L.	*Fagus sylvatica* L. Pinus sylvestris L. Quercus spp.

For sample collection, I selected the very intensive plots of each exploratory, resulting in nine forests in the HAI named HEW1-HEW6 and HEW10-HEW12. In the SCH I excluded one beech/pine mixed plot selecting eight forests (SEW1-SEW3, SEW5-SEW9). And again nine forests in the ALB exploratory (AEW1-AEW9). All comprising three different management categories: i) unmanaged beech-dominated forests (*Fagus sylvatica* L., for at least 60 years), ii) beech-dominated age-class forests, and iii) coniferous age-class forests (spruce, *Picea abies* L., forests in the ALB and HAI, and pine, *Pinus sylvestris* L., forests in the SCH exploratory). Essential properties of the investigated forest ecosystems are given in Table 2.2, for detailed information see also Fischer et al. (2010).

As a measure for forest management intensity in this thesis, I used the forest management intensity indicator (ForMI) proposed by Kahl and Bauhus (2014). The ForMI is the sum of three management related factors: the proportion of harvested tree volume, the proportion of non-natural tree species, and the proportion of deadwood volume with saw-cuts to the total amount of deadwood (Kahl und Bauhus 2014).

Table 2.2: Detailed plot information for all three exploratories

Region	Plot ID	Main tree species	Management type	Stand density (n ha-1)	Mean dbh (cm)	Basal area (m^2 ha-1)	Forest management intensity (ForMI)	Soil type
Schwäbische Alb	AEW1	Spruce	age-class forest	752	26.35	44.11	1.805	Cambisol
	AEW2	Spruce	age-class forest	480	32.18	43.98	2.266	Leptosol
	AEW3	Spruce	age-class forest	568	30.17	44.54	2.353	Cambisol
	AEW4	Beech	age-class forest	2219	12.40	29.70	1.563	Cambisol
	AEW5	Beech	age-class forest	104	45.16	17.99	0.939	Cambisol
	AEW6	Beech	age-class forest	374	27.60	26.50	1.188	Cambisol
	AEW7	Beech	unmanaged	312	33.94	40.11	1.029	Leptosol
	AEW8	Beech	unmanaged	348	36.40	46.86	0.000	Cambisol
	AEW9	Beech	unmanaged	444	27.09	31.43	0.485	Leptosol
Hainich-Dün	HEW1	Spruce	age-class forest	320	40.23	28.28	1.869	Cambisol
	HEW2	Spruce	age-class forest	720	25.17	39.50	1.307	Luvisol
	HEW3	Spruce	age-class forest	564	29.84	42.40	2.198	Luvisol
	HEW4	Beech	age-class forest	thicket			1.890	Cambisol
	HEW5	Beech	age-class forest	488	24.68	28.28	0.956	Luvisol
	HEW6	Beech	age-class forest	300	38.73	39.50	0.747	Luvisol
	HEW10	Beech	unmanaged	360	29.04	35.37	0.064	Luvisol
	HEW11	Beech	unmanaged	564	22.81	39.32	0.518	Cambisol
	HEW12	Beech	unmanaged	260	35.03	36.36	0.000	Luvisol
Schorfheide-Chorin	SEW1	Pine	age-class forest	1440	17.29	36.40	1.900	Arenosol
	SEW2	Pine	age-class forest	1124	20.24	39.85	1.405	Arenosol
	SEW3	Pine	age-class forest	416	33.06	37.41	1.898	Arenosol
	SEW5	Beech	age-class forest	92	57.20	28.16	0.689	Arenosol
	SEW6	Beech	age-class forest	thicket with	shelterwood		1.402	Arenosol
	SEW7	Beech	unmanaged	172	50.96	39.25	0.086	Arenosol
	SEW8	Beech	unmanaged	168	49.55	39.82	0.187	Albeluvisol
	SEW9	Beech	unmanaged	284	40.70	43.38	0.646	Arenosol

2.1.2 Instrumentation

The infrastructure for the collection of samples along the water flow path through a forest ecosystem included samplers for TF, SF, LL, as well as top- and subsoil solution (TOP, SUB). With the exception of SF collectors on plots HEW1, HEW2 and HEW3 (spring 2010), all instrumentation was installed in summer and autumn 2009.

TF was sampled with 20 funnel-type collectors (diameter 0.12 m, polyethylene (PE)) per forest ecosystem, which were placed 0.3 m above the soil surface and arranged in two lines of 10 samplers equidistantly in a cross-shaped form in a 20m x 20m subplot. To minimize alterations of the TF samples, e.g., by evaporation, photochemical reactions, or growth of algae, the sampling bottles were wrapped with aluminum foil and the opening of the collection bottle was covered with a polyester mesh (mesh size=1.6 mm) and a table-tennis ball. The subplots where chosen to be representative for forest structure and vegetation composition. SF was sampled with sliced polyurethane (PU) hoses (diameter: 0.04 m) fixed around tree stems and sealed with a PU based glue to the bark of three representative trees per site, at approximately 1.5 m height. The polyurethane hose was connected with a PU or PE barrel via a PE tube. Forest floor litter leachate was collected with three zero-tension lysimeters per site (280 cm^2 sampling area) consisting of polyvinyl chloride plates covered with a PE net (mesh size 0.5 mm) connected with PE hoses to 2 L PE bottles stored in a box below ground. The lysimeters where placed horizontally under the organic layer inside a fenced subplot area to avoid animal disturbance.

I sampled soil solution with nylon membrane (0.45 µm) suction cups (ecoTech, Germany). Three suction cups per site were installed beneath the A horizon (TOP) at approximately 10 cm (ALB and SCH) and 15cm (HAI) depth. In the Schorfheide and Hainich another three suction cups were installed in the B horizon (SUB) in approximately 50 cm and 35cm depth respectively. Due to shallow soils in the ALB exploratory, sampling was restricted to topsoil solution. In 2012 we replaced the Schorfheide nylon suction cups with glass suction cups because they continuously failed to deliver soil solution. Suction cups were connected to 1 L PE bottles in an insulated aluminum box placed into a soil pit. Soil water was extracted by applying a vacuum of 50 kPa to the PE bottles with an electric pump after each sampling.

2.1.3 Sampling and sample preparation

The sampling for the investigations of this thesis took place in the framework of a fortnightly sampling campaign of above- and below-ground ecosystem solution samples during the vegetation periods (roughly from March to November) starting April 2010 and ending November 2016. The sampling scheme is shown in Figure 2.1.

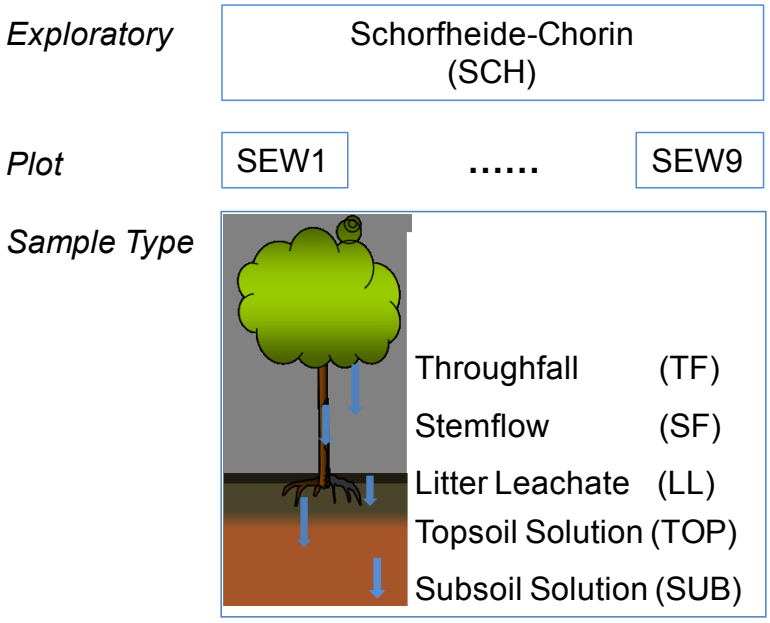

Figure 2.1: Exemplary sampling scheme the for Schorfheide-Chorin exploratory.

Partners in Karlsruhe (Chair of Geomorphology and Soil Science, Karlsruhe Institute of Technology) where responsible for the sampling campaign in the ALB, partners in Jena for the HAI plots (Department of Soil Science, University of Jena), while the Department of Soil Science, TU Berlin was responsible for the SCH plots. After recording sample volumes with graded cylinders and merging samples from individual samplers to volume-weighted composite samples per plot in the field, the samples were transported on ice to the laboratory and stored overnight at 5°C. Obviously contaminated samples (e.g. animal faces) were excluded. In the laboratory, we first measured pH (Knick, Germany) and electrical conductivity (WTW, Germany) in all samples prior to filtration through folded cellulose filters (Satorius, Germany, Grade: 292) on the next day. The filters were first washed with 100 mL deionized water and 10 mL of sample prior filtration of the remaining sample. All samples were stored frozen at -18°C until further analysis (except samples for the freezing experiment, see following section). Samples for DOM characterization, absorption and fluorescence measurement (Berlin), FT-ICR MS analysis (Forschungszentrum Jülich, Institute of Bio- and Geosciences) and for the incubation experiment (Jena) where transported to the corresponding laboratories in insolated polystyrene boxes without thawing.

The Samples for the evaluation of DOM properties in different ecosystem strata by means of fluorescence (section 3.2) where taken on several sampling dates between April 2011 and November 2013 at the SCH and HAI experimental plots. The Samples for additional FT-ICR MS analysis (section 3.2) where collected at the SCH plots in April and Mai 2015. The investigation of influence of sample preservation on optical properties of DOM (section 3.1) where conducted at samples collected in June 2014 at the SCH plots. Samples for assessing the biodegradability of DOM (section 3.2) where collected in October 2012 (summary see Table 2.3). Detailed sample compositions for all sections in the following.

Table 2.3: Summary of sample characteristics and sample number for all evaluations in this thesis

Part of thesis	Exploratory	Period of Sampling	Sample type	n
sample preservation	SCH	June 2014	TF, SF, LL, TOP, SUB	27
DOM characterization	SCH, HAI	fluorescence: 4/ 2011-11/ 2013	TF, SF, LL, TOP, SUB	84
	SCH	FTICR-MS: 4+5/2015	TF, SF, LL,SUB	8
DOM biodegradability	SCH, HAI, ALB	October 2012	TF, SF, LL	25

2.1.4 Testing the effects of sample preservation on optical DOM properties

Sampled plots per sample type are shown in Table 2.4. Prior to filtration through glass microfiber filters (~ 0.7 µm, Whatman GF/F), the filters were washed with 100 mL deionized water and 10 mL of sample before sample filtration. Each filtered sample was split in three aliquots for different preservation treatments: (i) no preservation (fresh) for which samples were stored at 5°C in the dark and DOC concentrations were measured 24 h after sampling while fluorescence as well as absorbance were measured within 48 h; (ii) preservation by freezing for which the samples were stored at −18°C for 4 weeks, and (iii) fast freezing with liquid nitrogen (N_2), for which 12 mL sample aliquots were filled in pre-rinsed 15 mL (5 mL sample) PP falcon tubes, dipped in liquid nitrogen for 30 s and then stored at −18°C for 42 days. Fresh samples and samples frozen at −18°C were stored in 20 mL PE scintillation vials (NeoLab) that were pre-rinsed with 5 mL sample before filling. Fluorescence, absorbance and DOC concentration from all frozen samples were measured after defrosting over night at 5°C in the dark. For all preparation steps and treatments control samples of ultrapure water (EVOQUA, Germany) were analyzed, showing no release of DOM (DOC concentration and DOM fluorescence) from laboratory equipment.

Table 2.4: Assessment of sample storage effects: information about sampled plots per sample type

	forest management	sampled plots per sample type				
		TF	SF	LL	TOP	SUB
Schorfheide-Chorin	beech unmanaged	SEW 9	SEW 9	SEW 9	SEW 9	SEW 9
	beech age-class	SEW5	SEW5	SEW5	SEW5	SEW5
		SEW6	SEW6	SEW6	SEW6	SEW6
	pine age-class	SEW1	SEW1	SEW1		SEW1
		SEW2	SEW2	SEW2		SEW2
		SEW3	SEW3	SEW3	SEW3	SEW3
	grassland	SEG5			SEG3	SEG3
					SEG5	
						SEG39

2.1.5 Assessment of DOM characteristics and changes along the water flow path

Frozen samples where thawed over night at 8°C and fluorescence and absorption measurement could be conducted without further preparations. An overview of selected plots and number of measured samples per sample type is shown in Table 2.5.

For further chemical characterization I chose TF, SF, LL and SUB samples from unmanaged beech and age-class pine forests of the SCH exploratory. To gain enough sample for the TF-ICR MS analysis I pooled samples from two forest sites per management category (Table 2.6). The filtered (0,45µm,) samples were desalted and concentrated using solid phase extraction (SPE, C18 hydra cartridges, Machery & Nagel, Düren, Germany) using methanol (≥99.98 %, Ultra C-MS grade; Carl Roth, Karlsruhe, Germany) as eluent. After SPE the eluent where vaporized at room temperature. Before FT-ICR MS measurements the samples where re-solved with methanol. I used ultrapure water (EVOQUA, Germany) as blank through the whole SPE routine, which showed no systematic signal from the solid phase and following storing steps.

Table 2.5: Selected plots and number of fluorescence measurements per plot and sample type used to assemble the mean dataset used for the characterization of cDOM changes along the water flow path

Schorfheide-Chorin	**SEW1 n**	**SEW2 n**	**SEW3 n**	**SEW4 n**	**SEW5 n**	**SEW6 n**	**SEW7 n**	**SEW8 n**	**SEW9 n**
TF	10	12	12	14	10	11	10	10	12
SF	10	13	9	14	13	13	11	15	11
LL	15	12	13	15	9	16	13	9	9
Top	1	0	2	7	3	3	4	3	3
Sub	6	2	2	7	1	7	0	4	3

Hainich-Dün	**HEW1 n**	**HEW2 n**	**HEW3 n**	**HEW4 n**	**HEW5 n**	**HEW6 n**	**HEW10 n**	**HEW11 n**	**HEW12 n**
TF	3	3	2	1	2	3	2	3	3
SF	1	2	2	0	3	3	3	2	1
LL	2	2	2	2	2	2	2	2	3
Top	2	2	1	0	2	2	2	2	2
Sub	2	2	2	0	2	2	0	2	2

Table 2.6: Pooled samples per forest management and sample type for characterizing the molecular composition of DOM using FT-ICR MS

	forest management	**plot composition per sample type**			
		TF	SF	LL	SUB
Schorfheide-Chorin	beech unmanaged	SEW8+9	SEW8+9	SEW8+9	SEW8+9
	pine age-class	SEW1+3	SEW1+3	SEW1+3	SEW1+3

2.1.6 Characterization of the biodegradability of DOM

We used TF, SF and LL samples from forests of three different management categories including unmanaged and managed beech forests and coniferous forests (spruce for ALB and HAI, pine for SCH forests). For each management category and sample type we pooled samples from two to three forests per exploratory gaining a total number of 25 samples (Detailed composition see Table 3.7).

Table 2.7: Pooled samples per forest management and sample type used for assessing the biodegradability of DOM

	forest management	plot composition per sample type		
		TF	SF	LL
Schwäbische Alb	beech unmanaged	AEW4-6	AEW4-6	AEW4-6
	beech age-class	AEW7-9	AEW7-9	
	spruce age-class	AEW1-3		AEW1-3
Hainich Dün	beech unmanaged	HWE10-12	HWE10-12	HWE10-12
	beech age-class	HEW5+6	HEW5+6	HEW5+6
	spruce age-class	HEW1-3	HEW1-3	HEW1+2
Schorfheide Chorin	beech unmanaged	SEW6-9	SEW6-9	SEW6-9
	beech age-class	SEW4+5	SEW4+5	SEW4+5
	pine age-class	SEW1-3	SEW1-3	SEW1-3

The Samples where filtered through a 0.2 µ m Vacuflo filter in a laminar flow box beside a Bunsen burner, to remove the majority of microbial biomass and to minimize microbial contamination. Afterwards 40ml of the filtrate was transferred to sterile 250ml suspension culture flasks (Greiner Bio-One, Frickenhausen/Germany). After adding 2 ml of bacterial inoculum the flasks where closed with a semi permeable caps. Each sample was set as triplicate for seven sample points 0, 3, 6, 10, 14, 20 and 28 days. The samples where incubated at 20°C in the dark. after incubation the samples where filtered with sterile 60ml Soft-Ject single use syringes (Henke-Sass, Wolf; Tuttlingen/Germany) equipped with nylon syringe filters pore size 0,45 µ m (Rotilabo, Carl Roth; Karlsruhe/Germany) and stored frozen till further analysis.

The bacteria inoculum was prepared by collecting and merging soil samples from forests of each exploratory. Sieved, field moist soil combined with unfiltered throughfall solution of all three exploratories in proportion 1:10, where shaken for 30min prior to centrifugation at 4400 rpm for 10min. The supernatant where stored at 8°C ahead incubation.

2.1.7 Chemical analyses

DOC concentration

I measured DOC concentrations on a TOC Analyzer. Analytical equipment differed between exploratory and corresponding laboratory (Table 2.8).

Table 2.8: Analytical equipment used to determine DOC concentrations and DOM properties in all experiments

parameter	exploratory	analytical equipment	specifications
DOC	Schwäbische Alb	TOC Analizer	VCPH, Shimadzu, Düsseldorf, Germany
	Hainich-Dün	TOC Analizer	VCPH, Shimadzu
	Schorfheide Chorin	TOC Analizer	VarioTOC cube, Elementar Analysensysteme GmbH, Hanau, Germany
DOM			
absorbance	all	UV-vis spectrometer	Lamda 20, Perkin Elmer, Waltham, USA
fluorescence	all	fluorescence spectrometer	F-4500 ,Hitachi, Tokio, Japan
mass spectra	all	FTICR-MS	ESI-LTQ-FT Ultra instrument , ThermoFisher Scientific, San Jose, USA)

UV-vis and fluorescence spectroscopy

UV-vis absorption spectra of DOM were recorded for wavelengths ranging from 200 nm to 600 nm using a Lambda 20 UV-vis spectrometer (Perkin Elmer, USA) and a 1 cm quartz cuvette. Measurements were baseline-corrected using ultra-pure water and all sample spectra were blank subtracted (ultra-pure water, EVOQUA, Warrendale, USA). Fluorescence, measured as EEMs, was recorded on a Hitachi F-4500 fluorescence spectrometer (Hitachi, Japan) directly after absorption measurement in the same cuvette. I used excitation wavelengths ranging from 240 nm to 450 nm (5 nm steps) and emission wavelengths ranging from 300 nm to 600 nm (2 nm steps) with a slit width of 5 nm and scan speed 12000 nm/min. We corrected our EEMs according to the protocol of Murphy (2010) with the fdomcorrect function in the drEEM toolbox (Murphy et al. 2013) using Matlab (Matlab). For the excitation and emission correction factors, we used the supplies provided by the manufacturer. Ultra-pure water fluorescence spectra were measured for blank correction and for converting EEMs to Raman units by normalizing them to the area under the Raman peak at 350 nm excitation wavelength. In order to use the toolbox-integrated inner filter correction by Lackowitz (2006), all aliquots were diluted with ultra-pure water to ensure an absorption at 254 nm <0,3 (Ohno 2002).

FTICR-MS

Ultra-high-resolution mass spectra were acquired using an ESI-LTQ-FT Ultra instrument (ThermoFisher Scientific, San Jose, CA, USA) equipped with a 7 T supra-conducting magnet (Oxford Instruments, Abingdon, UK). The mass spectrometer was used in negative mode, tuned daily and calibrated following a standard optimization procedure for almost all settings. Hence, the settings of the ion optics varied typically slightly from day to day. Samples were analyzed within three days as pure methanol solution without any pH modification or water addition. Typical standard conditions were: spray voltage 2.9 kV, capillary voltage -50 V, tube lens -93 V. Best performances was received under renunciation of sheath, auxiliary and sweep gas, respectively. The transfer capillary temperature was set to 275°C. Samples were introduced into the ESI source with a syringe pump at a rate of 5 µl min^{-1}. Mass spectra in profile mode were recorded in full scan from 200-1000 Da, measured at a resolution of 400.000 at m/z 400 Da (for complete separation of CHONS- from 13C, CHOS in even numbered peaks). Each individual mass spectrum contained 50 transients. The automatic gain control target in the ICR cell was set to 5*E5 (for nearly negligible interactions between the ions) to achieve deviations considerably below 1 ppm (supplier specification). Six spectra were averaged for improving the statistical robustness of the final spectra that were further processed. Mean deviation of the gained samples was approximately 0.4 ppm at m/z 400 Da, therefore all files were recalibrated before data processing (to prevent two possible assignments as CHO and CHOS2, respectively, for the same peak, which would lead to the exclusion of this mass from further consideration). Prior

and between some analyses, blanks were measured which showed no systematic signal. Molecular formulae were assigned using an in-house automated post-processing Scilab routine (Scilab Enterprises 2012), developed in the Agrosphere Institute (Research Center Jülich). For quality control, all peaks of at least two randomly selected masses (odd and subsequent even numbered, respectively) were characterized by hand for controlling of exactness of measured and recalculated peaks, respectively, as well as for setting proper constraints in the calculation program (maximal number of C, H, O, N and S, respectively).

2.1.8 Calculations of DOM quality indices and statistical analysis

DOM quality indices:

Using the absorbance spectra, we calculated specific ultraviolet absorbance ($SUVA_{254}$) as the absorbance at 254 nm divided by the concentration of DOC, reported in ($L\ mg^{-1}\ m^{-1}$). The $SUVA_{254}$ index reflects the bulk aromaticity of DOM (Weishaar, 2003). I calculated the humification index (HIX) from fluorescence EEMs according to (Ohno 2002). The HIX ranges from 0 to 1 and allows characterizing samples based on their degree of DOM humification.

Statistical analysis of fluorescence data:

To identify the underlying fluorescence components of the DOM, I used PARAFAC to mathematically decompose the trilinear data of the EEMs (Stedmon et al. 2003). All further preprocessing steps of EEMs, like smoothing of Rayleigh and Raman scatter and normalization, as well as the PARAFAC analysis were conducted with the drEEM toolbox (Murphy et al. 2013) in Matlab. To choose a fitting PARAFAC model, I visually checked the randomness of residuals and the component spectral loadings, split-half validated the model and generated the best fit by random initialization. For comparison in statistical analyses, I used the relative percentage distribution of the PARAFAC components (% of the sum of total fluorescence of all PARAFAC components).

In this thesis I validated two different PARAFAC models. One, 4 component model, by modeling the freezing experiment data (c-components) and one, containing 6 components, by modeling the DOM properties evaluation mean fluorescence data per plot and sample type from 2011 to 2013 (C-components).

Preservation experiment

I conducted a pair-wise (samples as strata) permutational multivariate analysis of variance (PERMANOVA) with DOC concentrations of the fresh samples as factor based on Euclidean distances in R (Oksanen et al. 2015, R core team 2015, 2015). The adonis function was used to assess the influence of sample preparation (fresh, frozen, fast-freezing) and of the initial DOC concentration on DOM variables. To investigate preservation effects on single variables we conducted linear mixed-effect models (sometimes called multi-level models, lme function, Linear and Nonlinear Mixed Effects Models package for R, Pinheiro et al. (2015))

with samples as random intercept on each of the DOM composition variables. These were used instead of simple linear models or analysis of variances (ANOVA), since I could not expect the same intercept for all samples due to different sample concentrations. To test the influence of the initial DOC concentration on single preservation treatments I performed Spearman Rank Order Correlation. To assess the influence of sample type (TF, SF, LL, Top or Sub) on the relative change of DOM composition due to fast-freezing with liquid nitrogen or freezing at −18°C in relation to the measurement of fresh, cooled samples, we used an ANOVA with the sample type as fixed factor (aov function in R). To remove sample concentration-related effects and to calculate relative changes, the differences between the two preservations (either fast-freezing or freezing at −18 ◦C) relative to the measurements of fresh samples were calculated for each sample before the ANOVA. This was only done for variables, for which I found strong, significant effects with the linear mixed-effect models.

Assessment of DOM characteristics and changes along the water flow path

Analysis of FT-ICR MS data: Once molecular formulae had been assigned, one possible way to visualize and characterize the large amount of information was the 2D van Krevelen plot (van Krevelen 1950). The elemental ratios of oxygen to carbon (O/C) and hydrogen to carbon (H/C) for each formula where plotted on the x- and y-axis, respectively. This can be done according to elemental composition (C, H, O, N and S) including all elements, or using only subclasses of formulas containing only CHO, CHON or CHOS compounds, respectively. All assigned formulas were roughly grouped according to major classes of biopolymers found in natural organic matter depending on the position in the van Krevelen diagram. Figure 3.2.4a shows the van Krevelen diagrams of the molecular formulae assigned, highlighting the H/C – O/C regions of lipids (H/C = 1.7 – 2.25, O/C = 0 – 0.22), proteins (H/C = 1.5 – 2.0, O/C = 0.2 – 0.5), amino sugars, (H/C = 1.5 – 1.75, O/C = 0.55 – 0.7), carbohydrates, (H/C = 1.5 – 2.0, O/C = 0.7 – 1.0), lignin, (H/C = 0.75 – 1.5, O/C = 0.2 – 0.6), tannins (H/C = 0.5 – 1.25, O/C = 0.6 – 0.95),and condensed carbohydrates (H/C = 0.2 – 0.75, O/C= 0 – 0.7), as reported by Sleighter and Hatcher (2007). The number of formulas in each functional group were then summed and normalized by the total number of assignable formulas for all functional groups to produce a relative abundance (as percent) for the six different classes of biopolymers (Tfaily et al. 2015). We conducted a cluster analysis with the standardized peak intensities of assigned formulas using Jaccard´s distances and Ward´s method (vegdist function and hclust function in R) according to Ide et al. (2017) and Stubbins et al (2017). Standardized peak intensities per sample were calculated as followed:

$$z = \frac{x - \mu}{\sigma}$$

Where, x is the measured peak intensity, μ is mean peak intensity within the sample, and σ is the standard deviation in peak intensity within the sample (Spencer und Coble 2014; Stubbins et al. 2017).

Correlation of PARAFAC and FT-ICR MS data: To link the modeled PARAFAC components with the biochemical information resulting from FT-ICR-MS measurements, we conducted a correlation analysis (Spearman Rank Order Correlation, stats package in R) between the relative abundances of PARAFAC components and the relative abundances of biopolymers extracted from van Krevelen plots.

Effect of tree species and management on DOM composition: PERMANOVA (n = 79, adonis function, vegan package, R) were used to assess the effect of sample type (TF, SF, LL, TOP, SUB), tree species (beech or coniferous), management intensity (ForMI) and their interactions on DOM composition (%L-PARAFAC components, $SUVA_{245}$). DOC concentration values were not included to separately investigate effects of the factors on DOM composition and DOC quantity. With the same DOM composition variables, a principal component analysis (PCA, rda function, vegan package) was conducted to visualize the PERMANOVA results. All DOM composition variables were scaled to one standard deviation. To test whether sample type, tree species or management intensity affected DOC concentration, a type II ANOVA (anova function, car package, R) with interaction was conducted (model Df = 19, residual Df = 59). DOC concentration was log transformed, after which normal distribution and homoscedacity of the residuals was given. Pairwise tests were conducted to assess effects of sample type for each of the PARAFAC components, separately for beech and coniferous trees. Moreover, for DOC concentration and $SUVA_{254}$, the effect of sample type was tested separately for beech managed, beech unmanaged and coniferous trees. Finally, for DOC concentration and $SUVA_{254}$, pairwise differences of tree species and management (beech managed, beech unmanaged and coniferous trees) were assessed separately for each of the sample types. If normal distribution of the residuals was given, pairwise t-tests with Holm-Bonferroni correction to correct for family-wise error rate were used (pairwise.t.test function, R), otherwise, Nemenyi-Damico-Wolfe-Dunn tests (Monte-Carlo test variant with 50000 iterations) were used (independence_test function, coin package, R).

Characterization of DOM biodegradability

In this study, I refer to biodegradable dissolved organic carbon (BDOC) as the DOC utilized by heterotrophic microbes via complete mineralization of C to obtain energy, and by incorporation of carbon into microbial biomass. To describe the degradation kinetic, I fitted a single exponential model. The rate of biodegradation is quantified by the mineralization constant (k). I applied the existing 6 component PARAFAC model from the long-term DOM evaluation on the EEMs from samples measured before and after 28 days of incubation.

In order to classify DOM samples according to their biodegradability, I performed principal component analysis (PCA function, FactoMineR package in R) including BDOC, k, DOC concentrations and %PARAFAC values. Wilcoxon rank sum tests as paired test (wilcox.test function, stats package in R) were used to evaluate the influence of incubation on DOC concentrations, optical indices ($SUVA_{254}$, HIX) and %PARAFAC values. I used Spearman Rank

Order correlation (cor.test function, stats package in R) to assess the relationships between all variables (%BDOC, k, $SUVA_{254}$, HIX, %PARAFAC values).

3 Results and discussions

3.1 Sample storage effects on DOC concentrations and DOM properties

3.1.1 Results

DOM concentrations

The samples covered a wide range of DOC concentrations (Figure 3.1a, b). Fresh TF samples showed the lowest concentrations ranging from 5 to 17 mg C L^{-1}, SF samples had the highest DOC concentrations ranging from 12 to 138 mg C L^{-1} (Figure 3.1b). High concentrations up to 75 mg C L^{-1} were also found for LL samples, but average values were smaller than for SF (Figure 3.1b). In the mineral soil, concentrations decreased from 13 to 124 mg C L^{-1} in topsoil samples to 9 to 47 mg C L^{-1} in subsoil samples. I found a significant treatment effect (linear mixed effect models (lme), $p < 0.05$) on DOC concentration when comparing the fresh and frozen samples (Figure 3.1c). In 24 of 27 samples DOC concentrations decreased after freezing at −18 ◦C and subsequent thawing, with an average change of −1.6 mg C L^{-1} or −6 % respectively. The maximum decrease that was found equaled −6 mg C L^{-1} and −25%, respectively. In contrast to freezing at −18 ◦C, fast-freezing with liquid nitrogen did not result in significant changes (lme, $p > 0.05$) of DOC concentrations (Figure 3.1c). This different behavior between normal freezing and fast-freezing was also found for the influence of the initial DOC concentration on changes of DOM properties. Only the −18°C treatment showed a significant correlation (Spearmans rank $r = -0.447$, $p = 0.0194$), indicating a larger decrease of DOC concentrations due to freezing for samples with higher initial DOC concentrations.

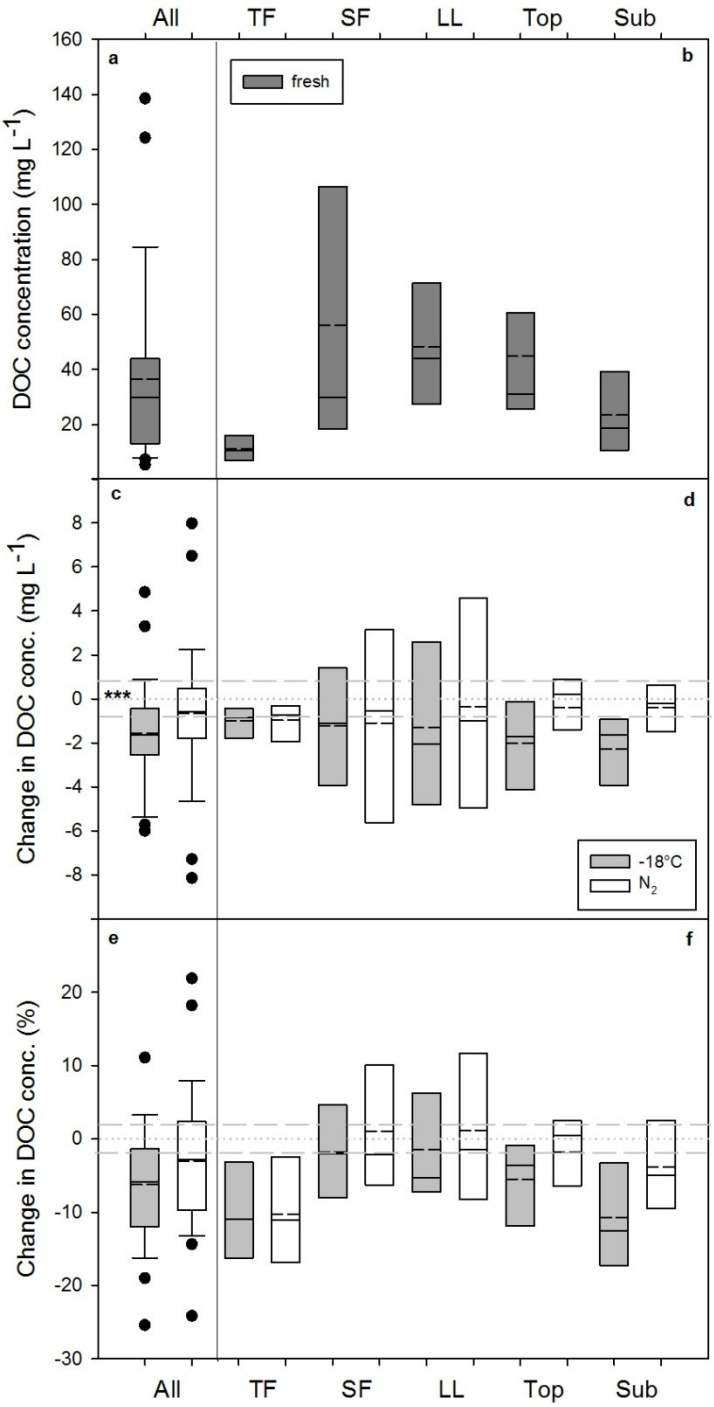

Figure 3.1: Absolute DOC concentrations (measured in fresh samples) and changes in DOC concentrations after freezing (−18 ◦C) and fast-freezing with liquid nitrogen; (a, c, e) all samples (n = 27); (b, d, f) ordered by sample type (throughfall (TF) n = 6, stemflow (SF) n = 5, litter leachate (LL) n = 5, top soil solution (Top) n = 6, sub-soil solution (Sub) n = 5); gray dashed line: analytical reproducibility; ∗∗∗ significant changes (linear mixed models (lme),$p < 0.05$); boxplots: solid line: median, dashed line: mean.

PARAFAC fluorescence components

The analysis of fluorescence spectra using PARAFAC resulted in four components (c1-c4) that were characterized according to the review of Fellman et al. (2010) (Table 3.1). c1 exhibited its main excitation maximum at < 250 nm, a secondary maximum at 340 nm and an emission maximum at 480 nm and was described as UVA humic-like fluorophore with a terrestrial source and a high molecular weight (Stedmon et al. 2003; Murphy et al. 2006; Fellman et al. 2010; Shutova et al. 2014). c2 had a maximum excitation at 335 nm and an emission maximum at 408 nm and was named also UVA humic-like, but associated with low molecular weight (Stedmon et al. 2003; Murphy et al. 2006; Fellman et al. 2010). c3 was defined by an excitation maximum at < 250 nm, a secondary maximum at 305 nm and an emission maximum at 438 nm. This component dominated fulvic acid fractions of humic substances (He et al. 2006; Santín et al. 2009). Finally, c4 was characterized by its excitation maximum at 280 nm and an emission maximum at 328 nm and was classified as tryptophan-like, as its fluorescence resembles free tryptophan. Therefore, this component was associated with free or bound proteins (Fellman et al. 2010). We found different distributions of PARAFAC components for different sample types (Figure 3.2). The contribution of %c1 to the total fluorescence increased from TF over SF to LL and then decreased again from LL to Sub (Figure 3.2), while %c2 showed just the opposite trend. In contrast, %c3 tended to increase from TF to Sub, whereas %c4 showed a decreasing trend (Figure 3.2).

Table 3.1: Characteristics of PARAFAC components based on Fellman et al. (2010). EX=excitation, EM=emission

Components	Ex_{max} (nm)	Em_{max} (nm)	description
c1	<250 (340)	480	humic-like, terrestrial
c2	355	408	humic-like
c3	<250 (305)	438	fulvic acid-type
c4	280	323	tryptophan-like

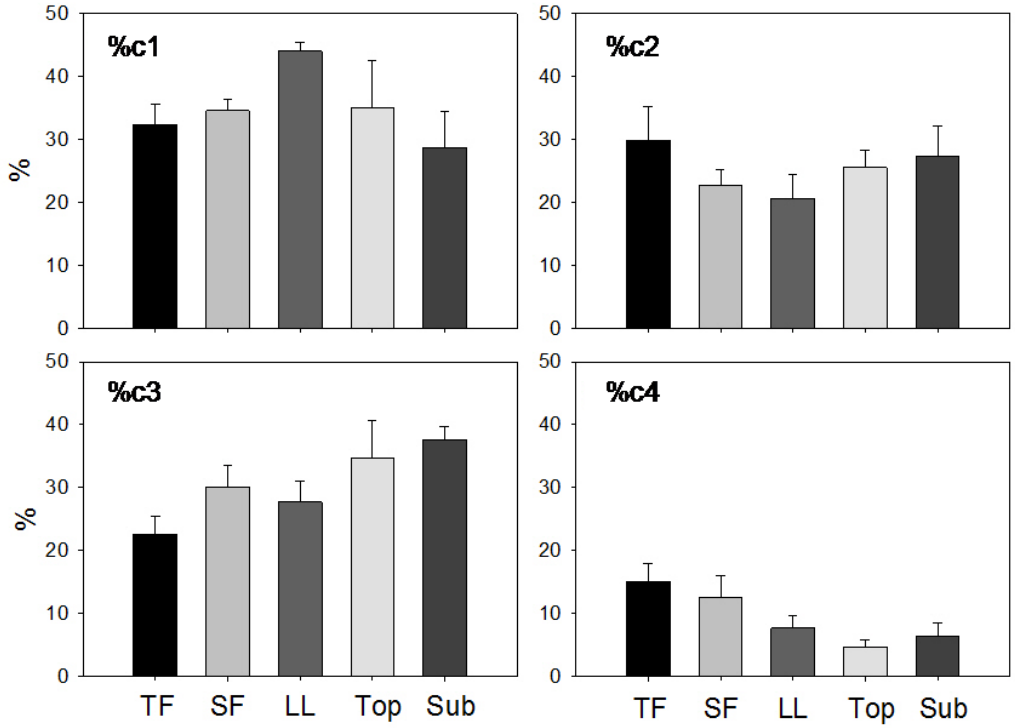

Figure 3.2: Mean distribution of PARAFAC components %c1–%c4 for different sample types

The conducted PERMANOVA was highly significant ($p < 0.001$), indicating that the preservation significantly affects the DOM composition. The interaction between treatment and initial DOC concentration of the fresh treatment explains a reasonable part of the variance ($R^2 = 0.14$) and is highly significant ($p < 0.001$). Therefore the original DOC concentration of the fresh sample well explains the variable strength of the treatment effect. Similar changes in component distribution were found as a consequence of freezing at −18°C and fast-freezing with liquid nitrogen (Figure 3.3). I observed a significant (lme, $p < 0.05$) decrease in all samples for the relative fraction of the humic-like components %c1 and %c2 after freezing at −18°C and fast-freezing compared to the fresh control samples (Figure 3.3a, b). The contribution of %c1 to the total fluorescence decreased on average by −3 % with maximum changes of −5% for freezing at −18°C and −6% for fast-freezing with liquid nitrogen. The average decrease of %c2 was −3% and the maximum −8% for both treatments. In contrast to %c1 and %c2, the share of %c3 to the total fluorescence intensity increased upon freezing (Figure 3.3e, f). All samples frozen at −18°C showed an increase in the relative intensity of the %c3 signal, with an average increase of +6% for both treatments. The maximum increase was 10% (freezing at −18 ◦C) and 12% (freezing with liquid N_2). No significant effects of sample preservation (lme, $p > 0.05$) were found for %c4, the protein-like component (Figure 3.3g, h).

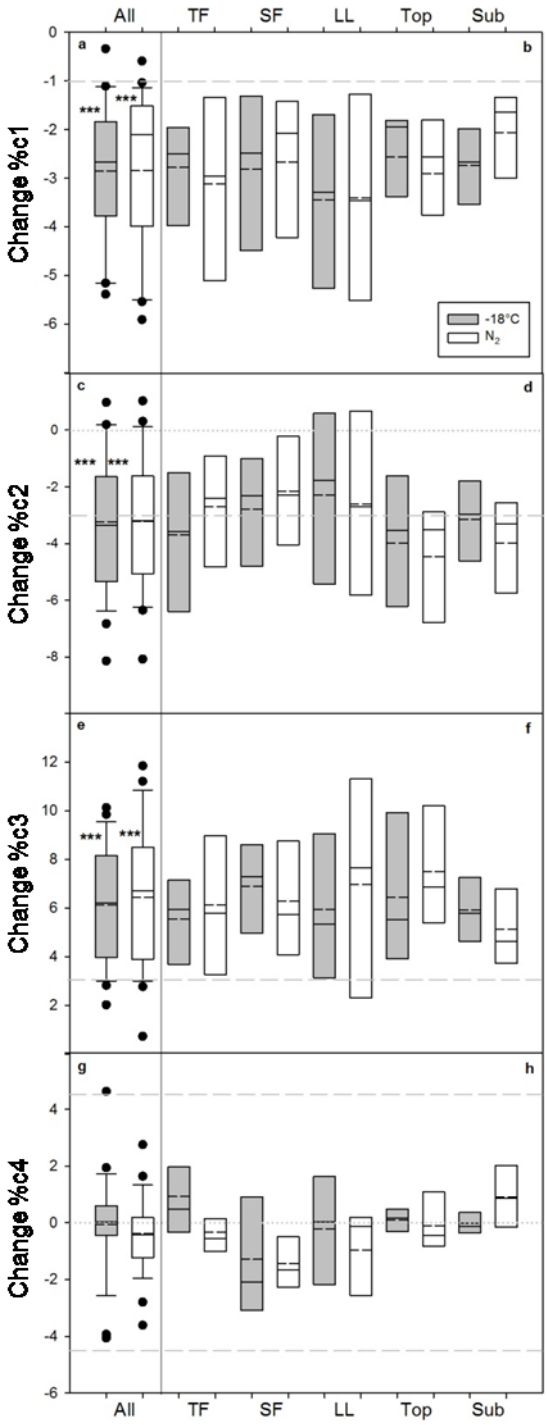

Figure 3.3: Changes of relative distribution of PARAFAC components after freezing (−18 ◦C) and fast-freezing with liquid nitrogen; (a, c, e, g) all samples (n = 27); (b, d, f, h) ordered by sample type (throughfall (TF) n = 6, stemflow (SF) n = 5, litter leachate (LL) n = 5, top soil solution (Top) n = 6, sub-soil solution (Sub) n = 5); gray dashed line: analytical reproducibility; ∗∗∗ significant changes (linear mixed models (lme), $p < 0.05$) ; boxplots: solid line: median, dashed line: mean

Aromaticity and humification index

I found $SUVA_{254}$-values ranging from 1.1 L mg^{-1}*m^{-1} up to 4.5 L*mg^{-1} m^{-1} for fresh samples (Figure 3.4a, b). Samples frozen at −18°C and fast-frozen samples showed a significant increase (lme, $p< 0.05$) of their $SUVA_{254}$ (Figure 3.4c). The average change was +0.4 L mg^{-1} m^{-1} equivalent to +20% for samples frozen at −18°C and +0.5 L mg^{-1} m^{-1} equivalent to +24% for samples that were fast-frozen with liquid nitrogen. The humification index of the freshly measured samples ranged from 0.806 to 0.931 in TF and SF samples and from 0.849 to 0.975 for Sub, Top and LL samples (Figure 3.5a, b). I found a significant decrease (lme, $p< 0.05$) of the HIX when comparing the freshly measured samples with the frozen and the fast-frozen samples (Figure 3.5c). The average change was −0.016 or −2 % for samples frozen at −18 ◦C and −0.020 or −2% for samples fast-frozen with liquid nitrogen. The maximum decrease was −0.128 or −15% for −18°C samples and −0.076 or −8% for liquid nitrogen samples (Figure 3.5c, d, e, f).

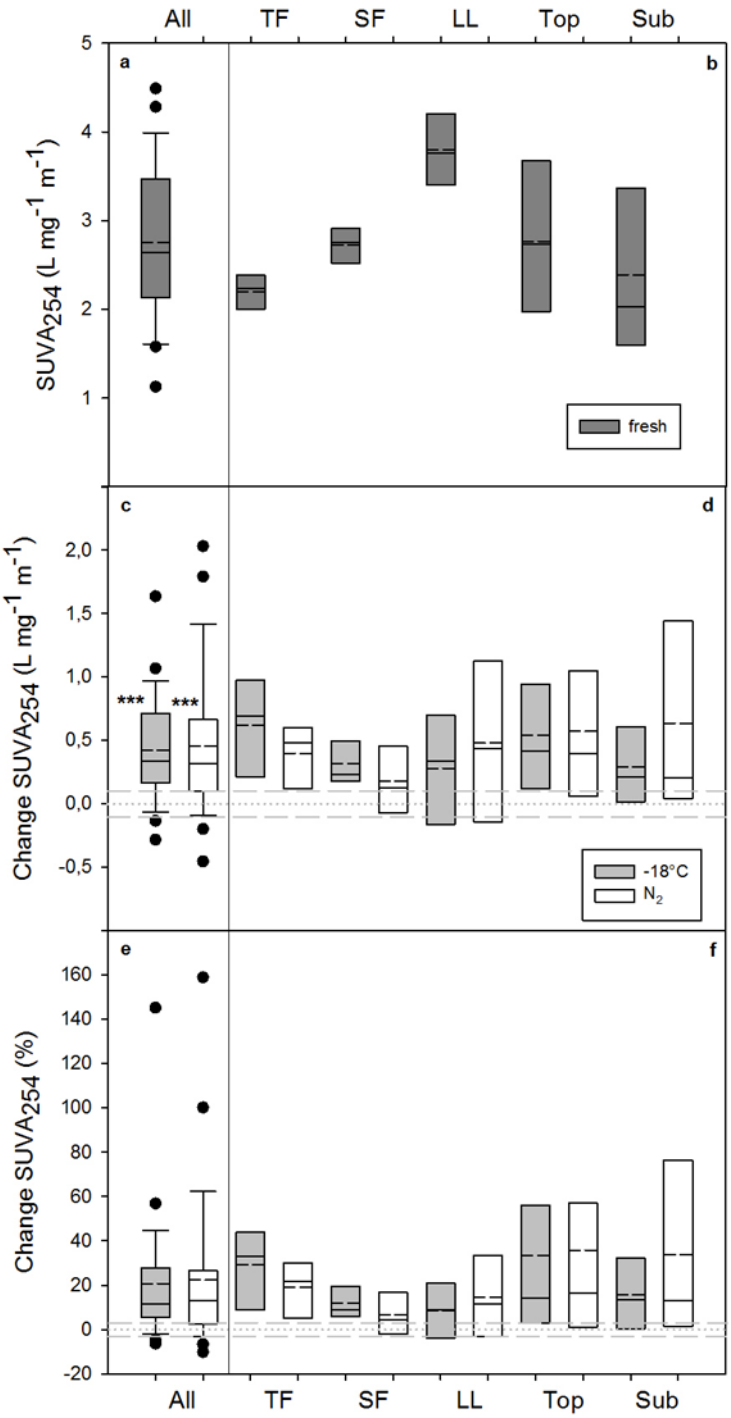

Figure 3.4: Absolute values (measured in fresh samples) and changes of $SUVA_{254}$ after freezing (−18 ◦C) and fast-freezing with liquid nitrogen; (a, c, e) all samples (n = 27); (b, d, f) ordered by sample type (throughfall (TF) n = 6, stemflow (SF) n = 5, litter leachate (LL) n = 5, top soil solution (Top) n = 6, sub-soil solution (Sub) n = 5); gray dashed line: analytical reproducibility; ∗∗∗ significant changes (linear mixed models (lme), $p < 0.05$); boxplots: solid line: median, dashed line: mean.

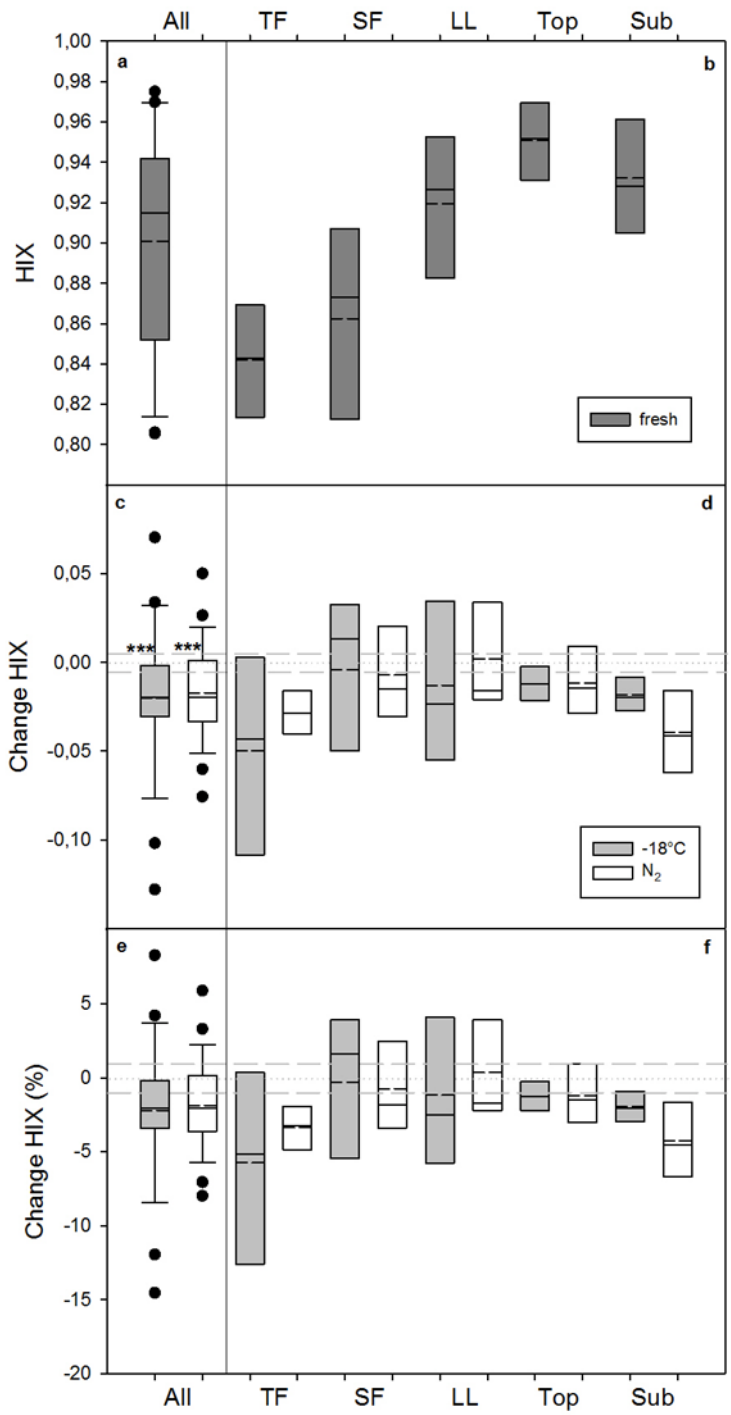

Figure 3.5: Absolute values (measured in fresh samples) and changes of HIX after freezing (−18 ◦C) and fast-freezing with liquid nitrogen; (a, c, e) all samples (n = 27); (b, d, f) ordered by sample type (throughfall (TF) n = 6, stemflow (SF) n = 5, litter leachate (LL) n = 5, top soil solution (Top) n = 6, sub-soil solution (Sub) n = 5); gray dashed line: analytical reproducibility; ∗∗∗ significant changes (linear mixed models (lme), $p < 0.05$); boxplots: solid line: median dashed line: mean.

3.1.2 Discussion

I found that freezing at −18 ◦C significantly reduced DOC concentrations across all sample types and that the effect is higher with higher initial DOC concentrations. This is in line with results of Fellman et al. (2008a) investigating the effect of freezing and thawing on Alaskan stream water samples. This loss of DOC concentration might be due to aggregation and irreversible particle formation (Giesy und Briese 1978) induced by partitioning and concentration effects during the freezing process (Belzile et al. 2002; Xue et al. 2015). Indeed, my results indicated that fast-freezing with liquid nitrogen can prevent significant reductions of bulk DOC for samples with a large range of DOM concentrations. In contrast to effects on DOC concentrations, I found similar significant effects of fast-freezing as well as freezing at −18°C on the chromophoric humic fraction of DOM (PARAFAC components, HIX and $SUVA_{254}$). The increase of aromaticity as indicated by higher $SUVA_{254}$ values indicates a stronger removal of non-aromatic DOM during freezing and thawing. On the other hand, the decrease in the HIX suggests a preferential removal of humified cDOM. One potential explanation for the fact that fast-freezing in liquid nitrogen resulted in significant changes of DOM fluorescence properties, but only small changes of bulk DOC concentrations, is that cDOM reacted stronger to freezing and thawing than the remaining DOM so that spectroscopic properties were affected, but bulk DOC concentrations were not. Fast freezing may have failed to prevent changes of cDOM composition because (i) cDOM changes occurred not only during the freezing process (−18 or −196°C in liquid nitrogen), but also in frozen state at −18°C in the freezer during storage or (ii) cDOM was affected by the thawing process that was identical for both freezing treatments. The former might be supported by a re-crystallization of ice crystals in frozen state (Luyet 1967; Meryman 2007). No significant changes of protein-like fluorescence (%c4) due to freezing and thawing were observed. This is in contrast to the results of Spencer et al. (2007) and Santos et al. (2010), which could be related to similar fluorescence characteristics, but different chemical composition of proteinaceous fluorescence material from aquatic sources and the solutions from terrestrial ecosystems tested in this study. In our experiment we used relative small sample volumes (fresh, −18 ◦C: 20 mL, N2: 12 mL) because we commonly keep the volume that is stored frozen as small as possible due to space limitations in deep freezers. I think that increasing the volume of samples that are subjected to freezing also increases the risk of artifacts, because of increasing concentration effects due to extended freezing time.

3.2 Changes of DOM properties along the water flow path in differently managed forest ecosystems

3.2.1 Results

Effect of tree species, sample type and management practice on DOC concentrations and $SUVA_{254}$

The mean DOC concentrations and $SUVA_{254}$ values varied between sample type and tree species (Figure 3.6a). The ANOVA showed a significant effect of sample type and tree species on DOC concentrations (ANOVA, $p < 0.001$), but no significant effect of forest-management intensity expressed as ForMI-index. TF samples from coniferous forests contained significantly larger DOC concentrations (17±3 $mg*L^{-1}$) than TF samples from beech forests (9±2 $mg*L^{-1}$, Figure 3.6a). DOC concentrations in SF of coniferous forests were significantly larger than concentrations in TF of coniferous forest ($p < 0.01$, Figure 3.6a). SF DOC concentrations of coniferous forests (90±35 $mg*L^{-1}$) also exceeded those in beech forests (22±14 $mg*L^{-1}$, $p<0.01$, Figure 3.6a). Also LL DOC concentrations of coniferous forests (55±12 $mg*L^{-1}$) were significantly larger than concentrations in LL samples of age-class beech forests (26±9 $mg*L^{-1}$, $p<0.01$, Fig. 3.6a). Mean DOC concentrations of TOP and SUB did not differ significantly between coniferous and beech forests (Nemenyi-Damico Wolfe-Dunn test, Figure 3.6a). I found no significant differences between DOC concentrations of managed versus unmanaged beech forests (Fig. 3.6a).

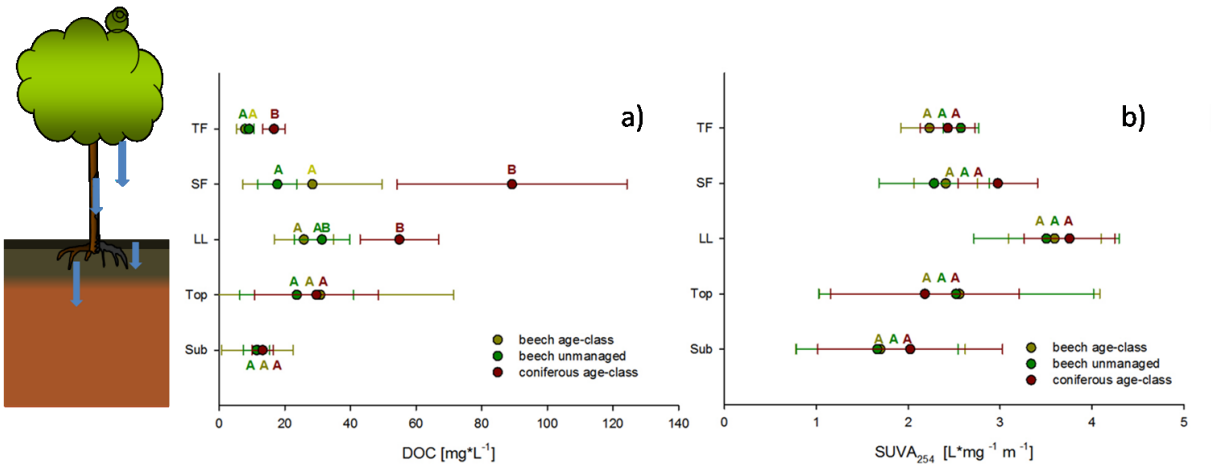

Figure 3.6: DOC concentrations (a) and $SUVA_{254}$ values (b) for all sample types (TF, SF, LL, TOP, SUB) and forest management practice (beech unmanaged, beech age-class, coniferous age-class). Dots indicated mean values; the range is the standard deviation. TF=Throughfall, SF=Stemflow, LL= Litter Leachate, TOP= Topsoil Solution, SUB= Subsoil solution. Letters indicating significant differences between management practice

Mean $SUVA_{254}$ values were similar for all sample types except LL independent of management practice. Mean values for TF, SF, TOP and SUB were in the range of 1.6–2.6 $L*mg^{-1}*m^{-1}$ with coniferous SF rising up to 2.9 $L*mg^{-1}*m^{-1}$. Significantly higher $SUVA_{254}$

values ($p<0.05$) for LL samples compared to TF, SF, LL, TOP and SUB were in the range of 3.5–3.7 $L*mg^{-1}*m^{-1}$ (Fig.3.6b).

Due to the insignificant differences between DOC concentrations and DOM $SUVA_{254}$ (Fig. 3.6) found in unmanaged and age-class beech forests, I distinguish only between deciduous forests on the one hand and coniferous forests on the other hand in the following.

FTICR-MS characterization of the molecular composition of DOM

The FT-ICR MS spectra revealed differences in the distribution and abundance of organic molecules of varying mass and composition between sample types and tree species (Figures 3.7 and 3.8).

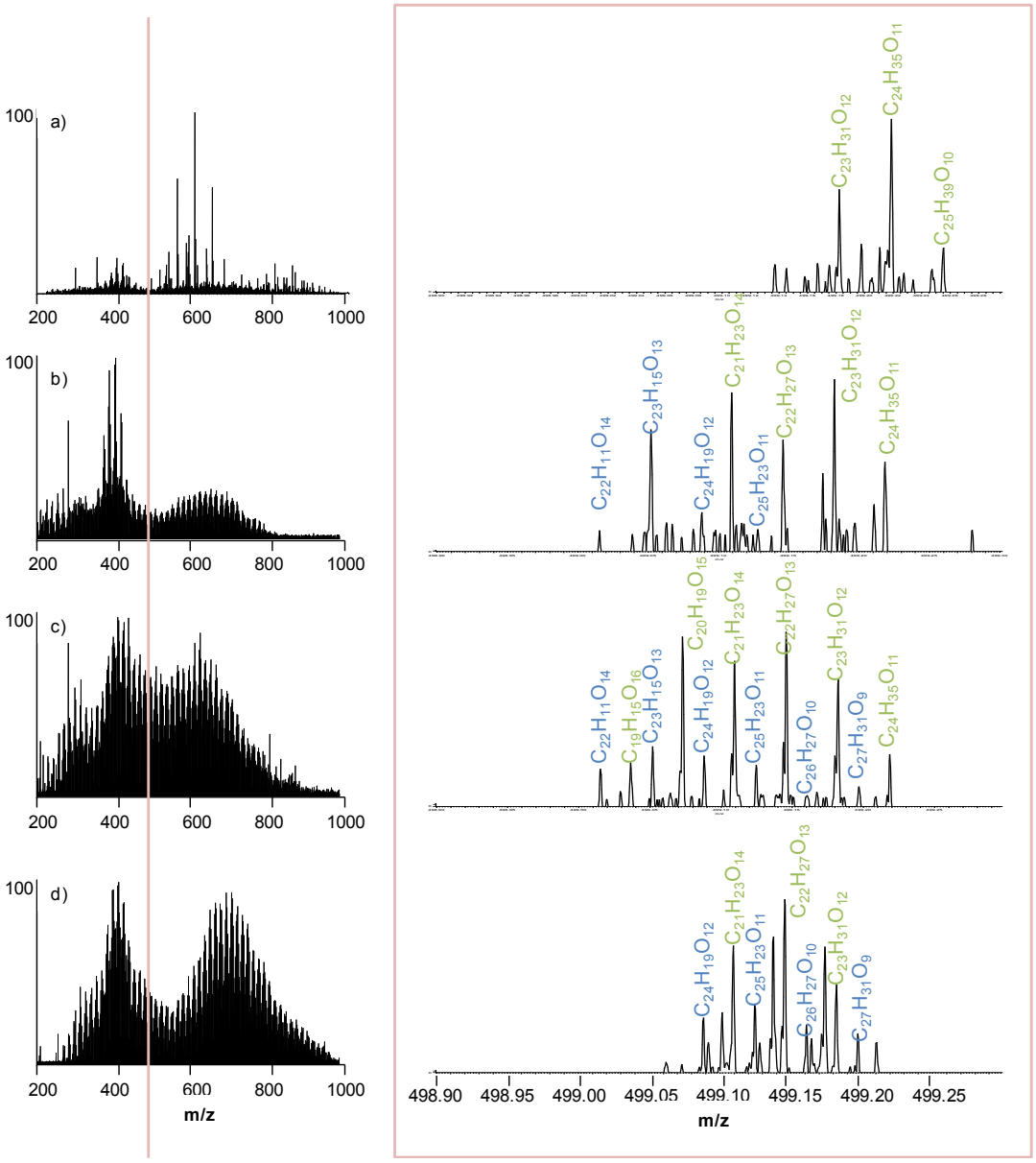

Figure 3.7: Raw electrospray ionization Fourier transformation ion cyclotron mass spectra (ESI FT-ICR MS) of unmanaged beech forest samples (left side) and detail for 499 m/z (right side). a=Throughfall, b=Stemflow, c= Litter Leachate, d= Subsoil solution

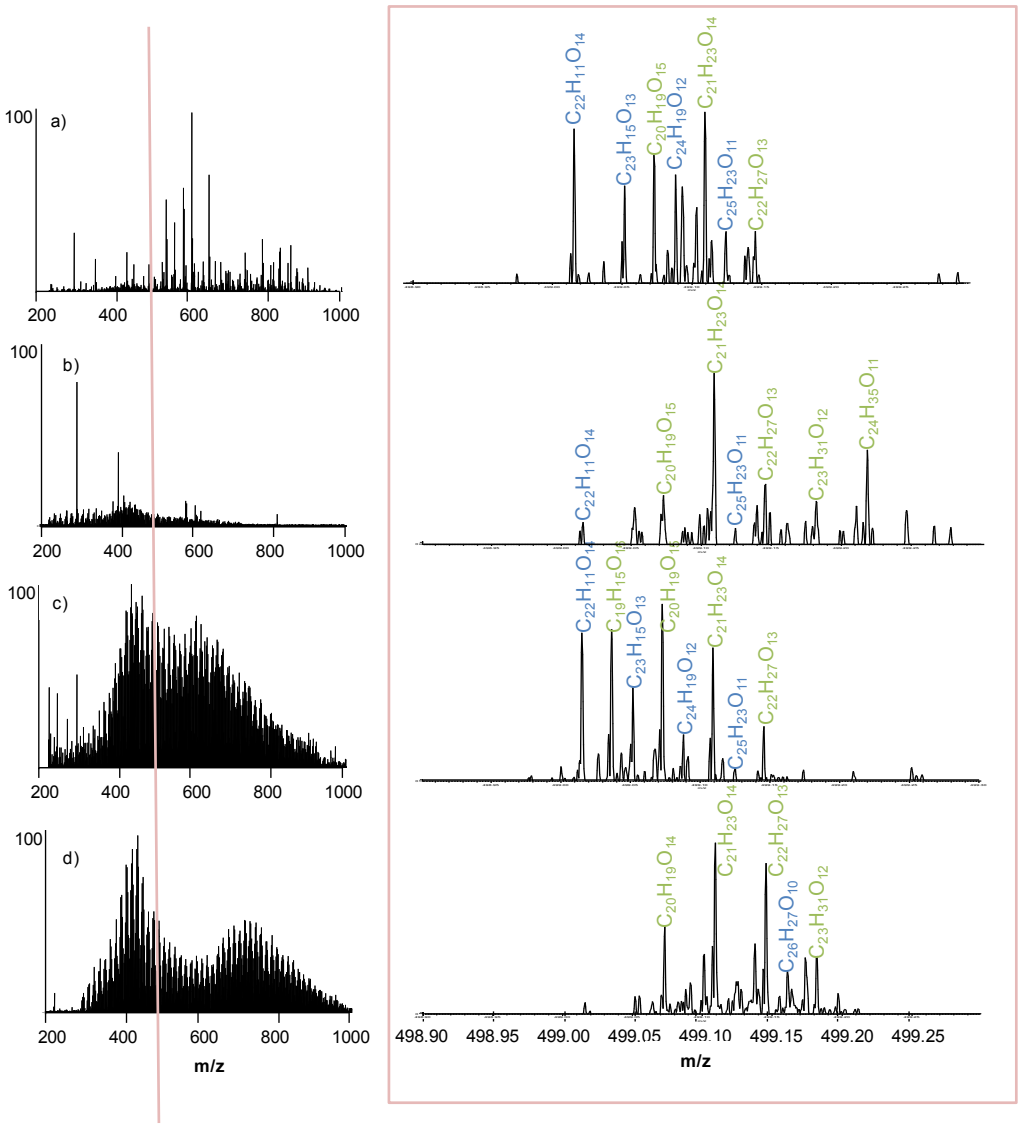

Figure 3.8: Raw electrospray ionization Fourier transformation ion cyclotron mass spectra (ESI FT-ICR MS) of age-class pine forest samples (left side) and detail for 499 m/z (right side). a=Throughfall, b=Stemflow, c= Litter Leachate, d= Subsoil solution

The numbers of assigned formulas in pine forest samples were similar for all sample types (Table 3.2). In contrast we found a slightly higher number of assigned formulas for LL and SUB beech forest samples compared to TF and SF samples. All pine forest samples contained slightly more CHO-only compounds (27.6–35.5%) compared with CHOS- (16.2–21%) and CHON-compounds (24–30%, Table 3.2). In contrast, beech forest samples showed an equal or slightly higher share of CHON-compounds compared to CHO-formulas (Table 3.2). For pine forest samples I found similar relative contributions of CHO and CHOS-formulas for TF and SUB as well as for SF and LL samples. The relative contribution for beech forest samples revealed no consistent pattern between sample types and elemental composition (Table 3.2). When comparing not only peak numbers, but taking the peak intensities into account, CHO was the series with the highest amount of high intensity peaks compared with CHOS,

CHOS and CHOSN for all sample and both tree types. One representative example is shown in Figure 3.9.

Table 3.2: Molecular signatures (FT-ICR MS) of pine and beech forest DOM. Number of assigned formulas and relative contribution (%). TF=Throughfall, SF=Stemflow, LL= Litter leachate, SUB= Subsoil solution.

	Sample type	**Formulars within each sample type**	
		Pine forest	Beech forest
Total Assigned Formulars	TF	8126	9878
	SF	8208	5435
	LL	8712	10112
	SUB	9522	13447
CHO	TF	2243 28%	2073 21%
	SF	2658 32%	1692 31%
	LL	3092 35%	2885 29%
	SUB	2747 29%	3449 26%
with S	TF	1689 21%	2170 22%
	SF	1348 16%	946 17%
	LL	1413 16%	1911 19%
	SUB	1995 21%	3058 23%
with N	TF	2194 27%	2720 28%
	SF	2459 30%	1679 31%
	LL	2361 27%	2616 26%
	SUB	2355 25%	3410 25%
with S+N	TF	2000 25%	2915 30%
	SF	1743 21%	1118 21%
	LL	1846 21%	2700 27%
	SUB	2425 25%	3530 26%

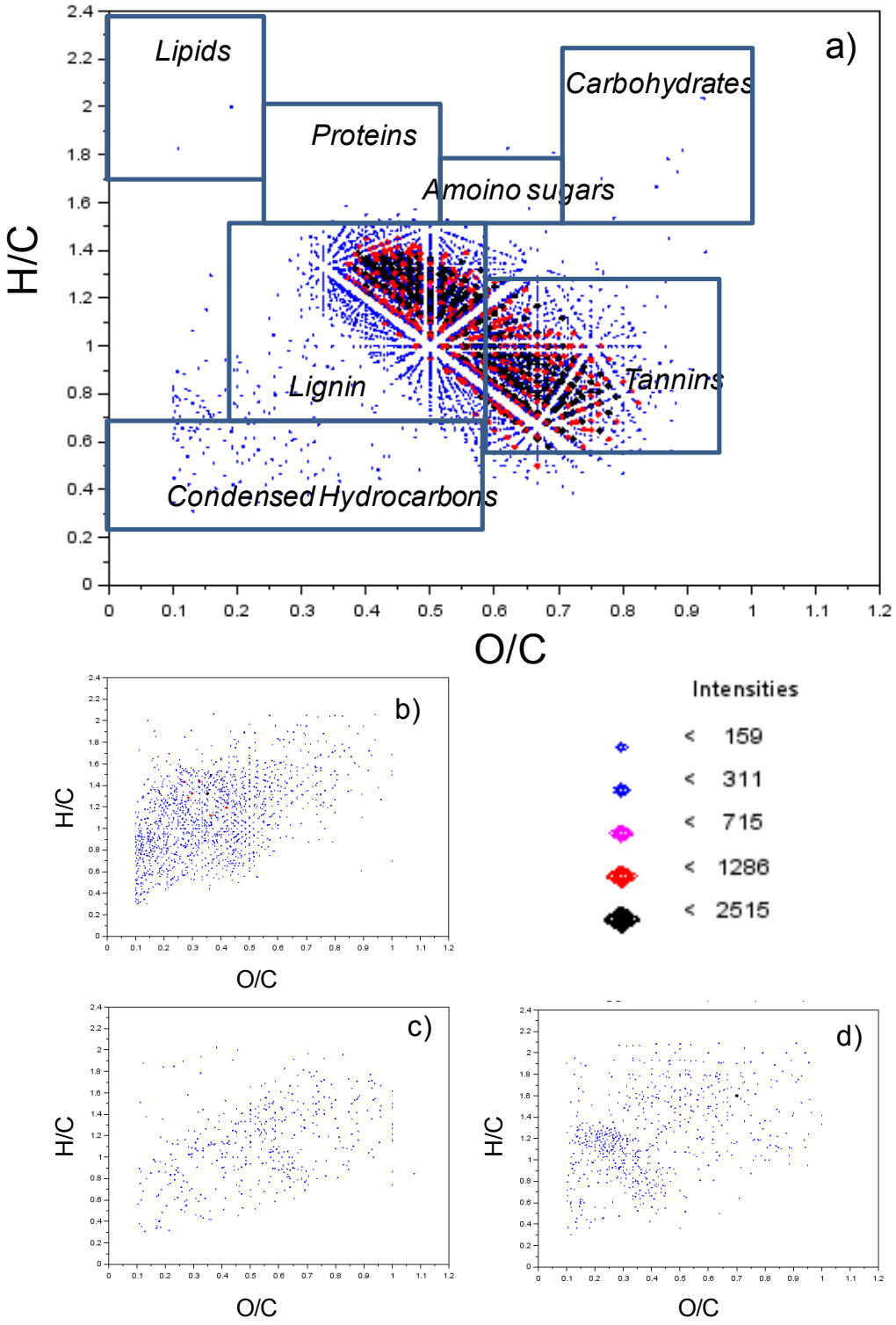

Figure 3.9: Van Krevelen plot of pine LL DOM. a=all CHO compounds, b= all CHOS compounds, c=all CHON compounds and d=all CHOSN compounds. Rectangles represent classes of biocompounds according to Sleighter and Hatcher (2007).

To further investigate the molecular diversity of DOM from pine and beech forests, I conducted a hierarchical cluster analysis. The resulting dendrogram for standardized intensity of all assigned formulas showed major molecular similarities for SUB samples of both vegetation types (Figure 3.10). In contrast, the distances between SF and LL solution of

pine forests and all aboveground samples from beech forests showed compositional differences (Figure 3.10).

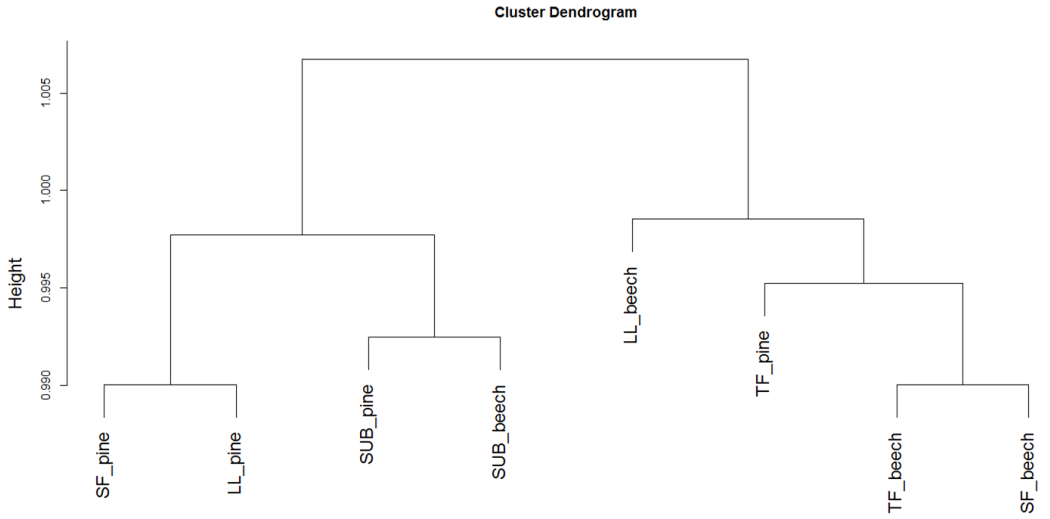

Figure 3.10: Cluster dendrogram of pine and beech forest DOM for standardized peak intensities of all molecular compounds identified

Elemental formulas of CHO compounds for all samples plotted as van Krevelen diagrams revealed distinct differences for all above ground sample types between pine and beech forests (Figure 3.11). While van Krevelen plots for all pine forest samples exhibit a distinct share of formulas with a H/C ratio of 1.2–1.6 and a O/C ratio of 0.3–0.6, there was a lack of them in the aboveground beech forest samples (Figure 3.11). The space DOM compositions of the different tree species covered in the van Krevelen diagrams, indicated by ellipsoids in the plots, became more conform when following the water downward (Figure 3.11). Plots of double bond equivalent (DBE) against H/C ratio revealed a higher share of compounds with low DBE (5–20) and medium H/C (1–1.5) ratio compounds for pine forest samples compared to beech forest samples. In contrast I found a higher share of compounds in the region of DBE 5–20 and H/C 0.4–0.8 for beech forest samples. Only few differences between mass spectra and resulting plots for both forests were found for subsoil solution samples (Figure 3.12)

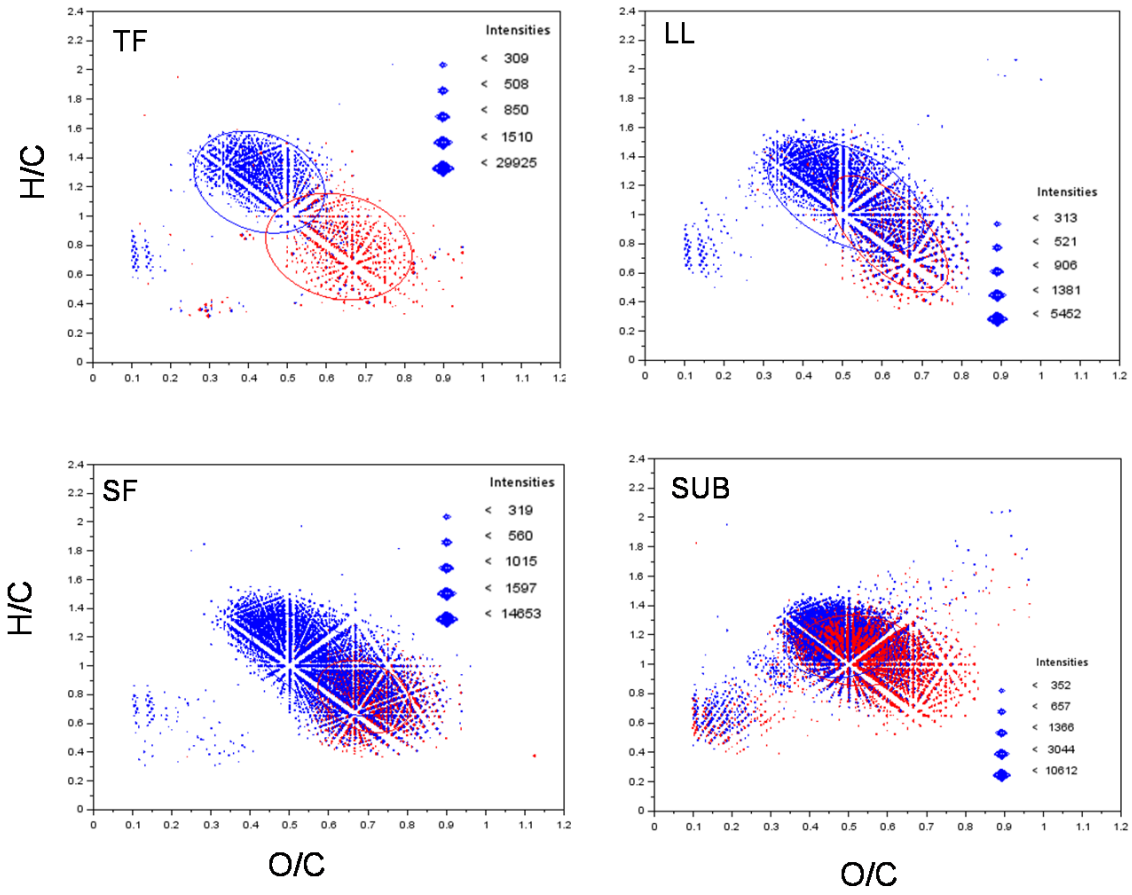

Figure 3.11: van Krevelen plots of CHO compounds for beech (red) and pine (blue) forests DOM samples. Ellipsoids indicate space covered by DOM samples. TF=Throughfall, SF=Stemflow, LL= Litter Leachate, SUB= Subsoil Solution

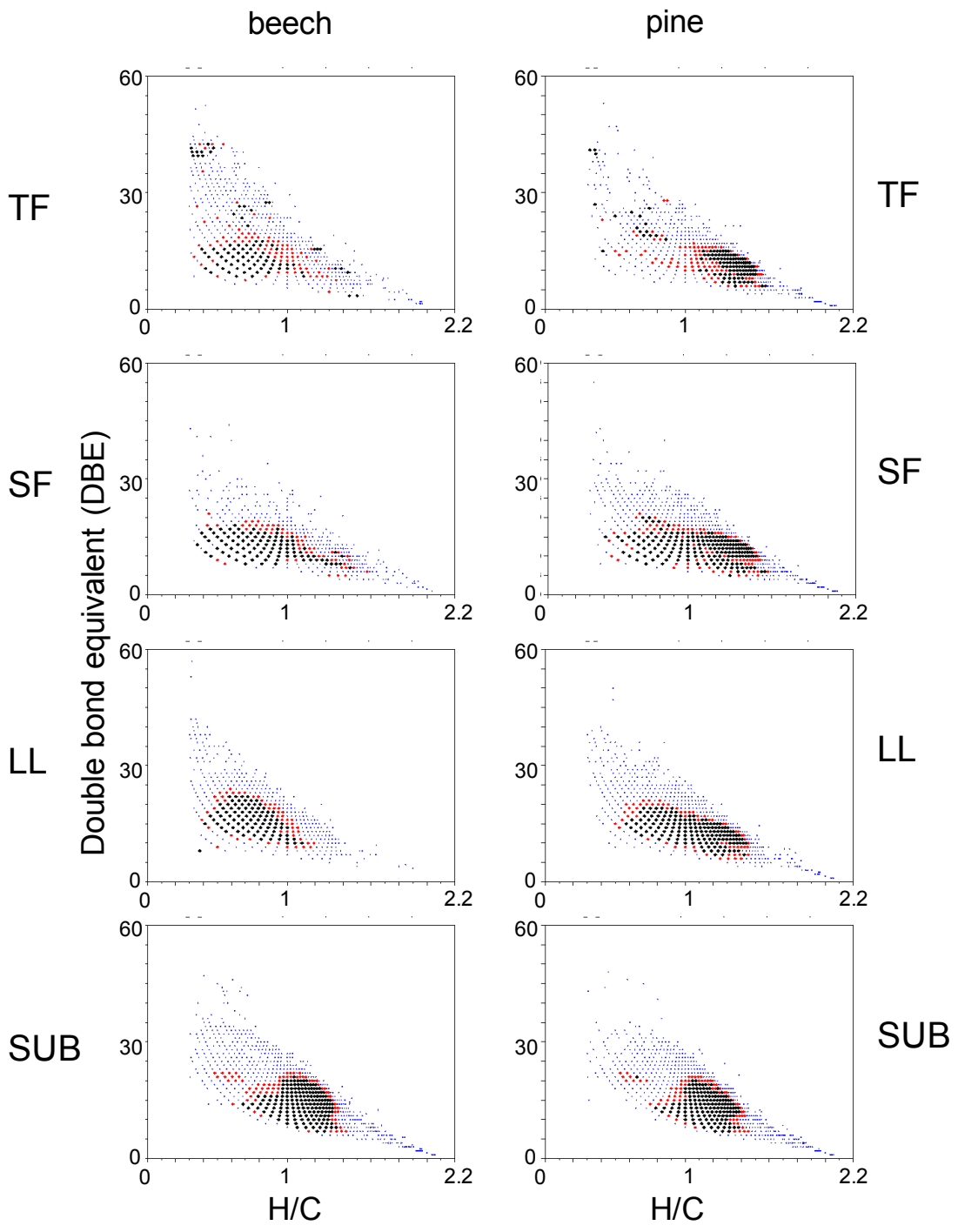

Figure 3.12: Double bond equivalent plots of beech and pine forests DOM samples. TF=Throughfall, SF=Stemflow, LL= Litter Leachate, SUB= Subsoil Solution. Colors indicating intensities (blue=low, red=medium, black=high)

Depending on their position in the van Krevelen diagram (thus their position in a plot of the ratios H/C against O/C ratios), I assigned molecular formulas to seven major biomolecular classes according to Sleighter and Hatcher (2007) see Figure 3.9 and Table 3.3. These are lipids (H/C = 1.7–2.25, O/C = 0–0.22), proteins (H/C = 1.5–2.0, O/C = 0.2–0.5), amino sugars, (H/C = 1.5–1.75, O/C = 0.55–0.7), carbohydtates, (H/C = 1.5–2.0, O/C = 0.7–1.0), lignin (H/C = 0.75–1.5, O/C = 0.2–0.6), tannins (H/C = 0.5–1.25, O/C = 0.6–0.95) and condensed

carbohydrates (H/C = 0.2–0.75, O/C= 0–0.7). Comparing their relative abundances between sample types and tree species, I found distinct differences between sample types and between beech and pine forests. While lignin-like formulas were the dominant molecules in all sample types of pine forest DOM (50–66%), I found almost balanced shares of lignin- and tannin-like molecules for TF (20–35%) and SF (39 –40%) of beech forest DOM. Following the water flow path further downward, we found a higher share of tannin-like compounds with higher O/C ratios for beech LL and reversed conditions for SUB samples. The other compounds like proteins, lipids, amino sugars, and carbohydrates hardly contributed to the total molecular composition. Only condensed hydrocarbons had additional, noticeable shares of molecule composition for pine and beech TF samples (15% and 36%, Table 3.3).

Table 3.3: Number of molecules assigned to major groups of biomoleculs according to Sleighter and Hatcher (2007) for all sample types of pine and beech forest DOM mass spectra

biopolymer class	Sample type	**Formulars within each sample type**	
		Pine forest	Beech forest
Lignin-like	TF	840 (53%)	194 (20%)
	SF	1173 (50%)	229 (39%)
	LL	1088 (59%)	108 (14%)
	SUB	2735 (66%)	2619 (63%)
Tannin-like	TF	96 (6%)	345 (35%)
	SF	309 (13%)	231 (40%)
	LL	503 (27%)	583 (77%)
	SUB	205 (5%)	512 (12%)
Protein-like	TF	74 (5%)	5 (1%)
	SF	98 (4%)	39 (7%)
	LL	24 (1%)	0 (0%)
	SUB	67 (2%)	48 (1%)
Amino Sugar-like	TF	17 (1%)	3 (0%)
	SF	35 (2%)	10 (2%)
	LL	8 (0%)	0 (0%)
	SUB	27 (1%)	16 (0%)
Lipid-like	TF	0 (0%)	2 (0%)
	SF	7 (0%)	1 (0%)
	LL	0 (0%)	0 (0%)
	SUB	5 (0%)	3 (0%)
Carbohydrate-like	TF	1 (0%)	1 (0%)
	SF	21 (1%)	2 (0%)
	LL	4 (0%)	0 (0%)
	SUB	53 (1%)	52 (1%)
Condensed Hydrocarbons	TF	235 (15%)	358 (36%)
	SF	45 (2%)	30 (5%)
	LL	21 (1%)	49 (6%)
	SUB	89 (2%)	145 (4%)

Cluster analysis with numbers of molecules assigned to major groups of biomolecules showed three distinct clusters. One included both subsoil samples, the second all remaining sample types of pine forests and the third the same for beech forests (Figure 3.13).

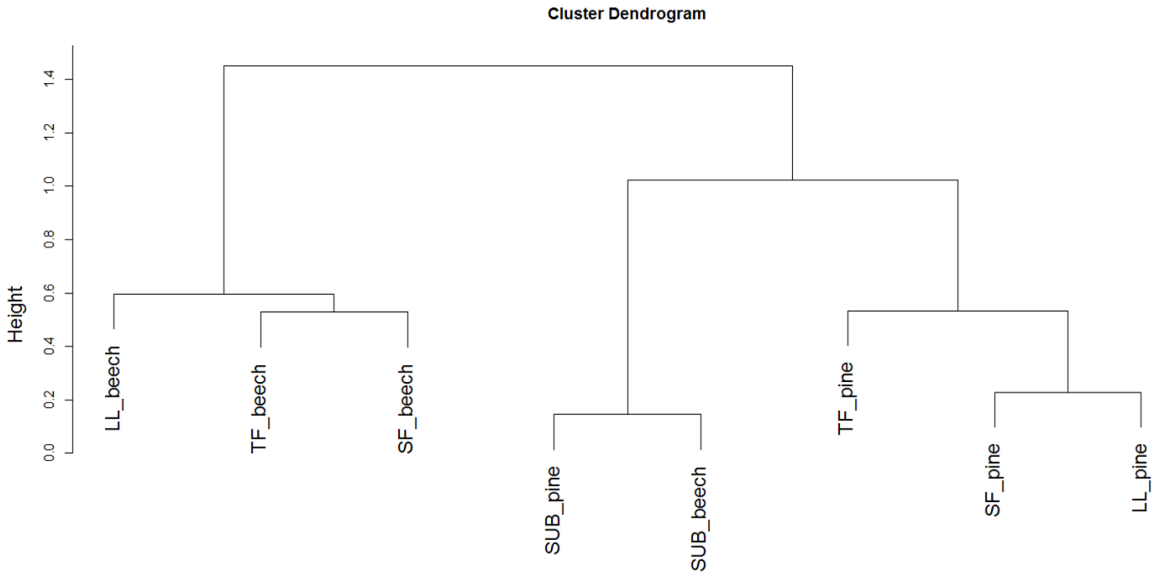

Figure 3.13: Cluster dendrogram for number of molecules assigned to bio compounds (tannin, lignin, lipids, proteins, amino sugars, and hydrocarbons) according to Sleighter and Hatcher (2007).

PARAFAC components - description and correlation with biochemical compounds

I validated a six-component PARAFAC model for describing the variation of the fluorescence of DOM. The components were referred as C1 to C6. Two fluorescence components (C1 and C6, Figure 3.14) had single excitation and emission maxima, whereas the other four components (C2–C5, Figure 3.14) showed two excitation maxima alongside one emission maximum. Component C1 was characterized by an excitation maximum <250 nm and an emission maximum at 436 nm. C2 showed two peaks of excitation maxima at 265 nm and 375 nm, having an emission maximum at 480 nm. C3 exhibited two excitation maxima, one at wavelengths <250 nm and the second at a wavelength of 315 nm, in combination with an emission maximum at 404 nm. C4 showed two excitation maxima at wavelengths <250 nm and at a wavelength of 325 nm, with an emission maximum at 446 nm. The fourth component with two excitation maxima (<250 nm and 350 nm) was C5, which showed an emission maximum at a wavelength of 428 nm. The fluorescence of component C6 was characterized with an excitation maximum at 280 nm and an emission maximum at 334 nm.

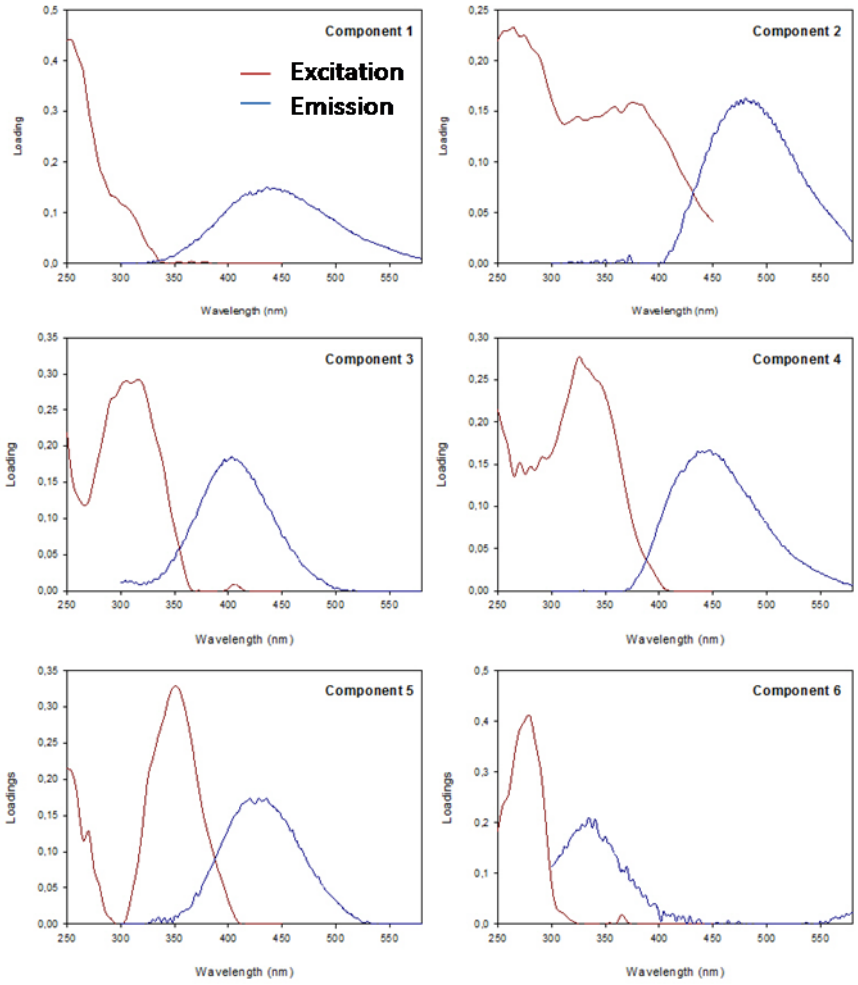

Figure 3.14: Excitation (red) and emission (blue) spectra of PARAFAC components (C1-C6)

I applied the validated 6 component PARAFAC model to the fluorescence spectra of the DOM samples that were also characterized using FT-ICR MS, in order to explore the molecular chemical background of the underlying fluorescence patterns. I found a significant positive correlation (Spearmans´ρ, $p<0.05$) between the relative contribution of fluorescence component C2 and the relative number of tannin molecules identified by mass spectrometry. Significant negative correlations were found between %C2 and the fraction of identified protein and amino sugar molecules (Table 3.4). The relative contribution of fluorescence component C3 to overall fluorescence significantly and positively correlated with the fraction of molecules assigned to the class of lignin biopolymers, while a significant negative correlation (Spearmans´ρ, $p<0.05$) was observed with the fraction of tannin molecules (Table 3.4). The contribution of PARAFAC component C6 to overall fluorescence positively correlated with the fraction of protein molecules and amino sugar molecules (Table 3.4).

Table 3.4: Correlation (Spearmans´ρ) between the percentage relative abundances of PARAFAC components (%C1-%C6) and the relative abundances of biopolymers extracted from FT-ICR MS van Krevelen plots. Significance level: * = p<0.05; ** = p< 0.01

	%C1	%C2	%C3	%C4	%C5	%C6
DOC	**-0.67***	**0.73***	-0.37	**0.71***	-0.15	-0.01
lipid-like	0.6	-0.39	0.07	0.15	-0.34	0.48
protein-like	0.43	**-0.72***	0.27	0.34	-0.53	**0.74***
amino sugar	0.45	**-0.64***	0.21	0.42	-0.56	**0.74***
lignin-like	0.05	-0.5	**0.80****	-0.41	0.31	0.18
tannin-like	-0.29	**0.72***	**-0.66***	0.26	0.01	-0.49

Distribution of PARAFAC components per sample type

While the relevance of fluorescence components C2 and C4 for overall fluorescence intensity increased with increasing DOC concentration of the undiluted original samples, the contribution of fluorescence component C1 decreased with increasing DOC concentration (Table 3.4).

With a mean share of 32–39%, component C1 dominated the overall fluorescence of both, DOM samples from beech forests as well as DOM samples from coniferous forests (Fig. 3.15). Significantly different shares of %C1 between deciduous and coniferous samples (Wilcoxon-test, p<0.05) was only found for LL samples.

The mean contribution of components C2 ranged from 12–23 % of total fluorescence and differed significantly between beech and coniferous forests, with samples from coniferous forests showing a larger share of C2 to total fluorescence than samples from deciduous forests (Wilcoxon-test, p<0.05). In contrast, the mean contribution of C3 (13–22%) was similar for both forest types. The relevance of C2 decreased from LL samples over TOP samples to SUB samples with increasing depth along the water flow path (Figure 3.15). Fluorescence component C3 showed an opposite trend to C2, with smallest contributions to total fluorescence in LL samples, increasing again over TOP to having its maximum contribution in SUB samples (Figure 3.15).

I found a decreasing mean contribution of fluorescence component C4 along the water flow path from 23±6% in beech and 21±1% in coniferous TF samples to 8±3%, respectively 4±3%, in SUB samples. The reversed trend was found for fluorescence component C5, whose mean share increased from 0–3% in TF and SF samples for both forest types to 16±4% for beech and to 18±4% for coniferous SUB samples. The mean share of protein-like component C6 of total fluorescence was largest in TF samples (beech forests: 12±6%, coniferous forests:

13±4%). This share decreased along the flow path to 4±1% in LL samples of beech forests and 3±1% in TOP samples of coniferous forests (Figure 3.15).

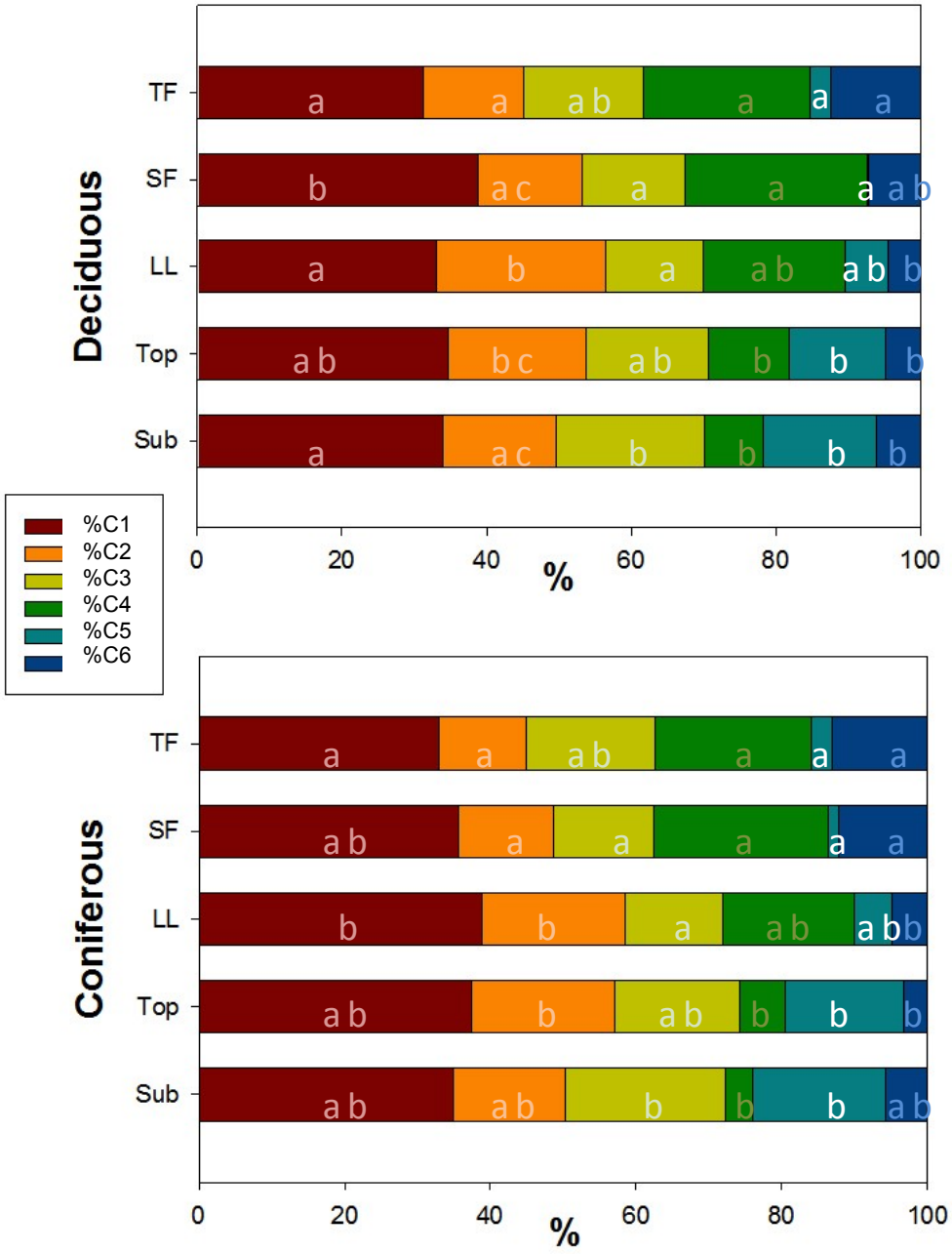

Figure 3.15: Mean distribution of PARAFAC components in different sample types of deciduous and coniferous forests. Letters (reading vertically) indicating differences between sample types with regard to PARAFAC components (Nemenyi-DamicoWolfe-Dunn test). TF = Throughfall, SF = Stemflow, LL = Litter Leachate, Top = Topsoil solution, Sub = Subsoil solution.

Effect of tree species, sample type and management on DOM composition

I found a significant effect of sample type on DOM composition variables (PERMANOVA, p = 0.001), which was slightly modulated by management intensity, indicated by a significant interaction between sample type and ForMI (PERMANOVA, p = 0.029). Near significant effects on DOM fluorescence characteristics were found for tree species (PERMANOVA, p = 0.06). When investigating the single sample types in detail, significant differences (Wilcoxon test, p<0.05) were found for above ground sample types between coniferous and beech forest stands especially for %C2 (tannin-like). Prominent differences disappeared when following the water underground, except for %C4 which showed the opposite behavior. No significant effects were found for management intensity alone (PERMANOVA, p =1).

A PCA illustrated the strong effect of sample type on DOM composition (Figure 3.16). The first two components identified by the PCA explained 88% of the total variance (PC1: 60%, PC2: 28%). TF and SF samples were closely grouped together and differentiated from TOP and SUB samples along PC1, based most strongly on their different contributions of C4 and C5 to overall fluorescence (Figure 3.16). LL samples also clustered nicely, and separated especially from TF and SUB samples along PC2, based predominantly on their larger SUVA and smaller contribution of C6 to overall fluorescence (Figure 3.16)

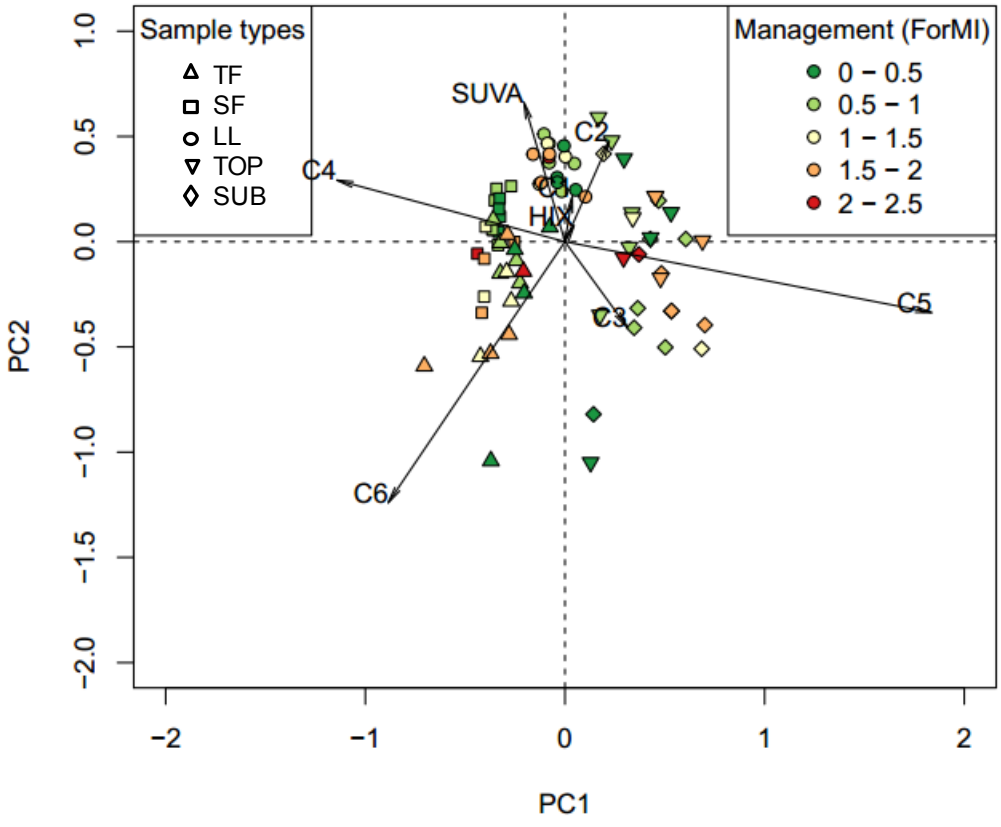

Figure 3.16: PCA plot of DOM composition variables

Because the environmental conditions and the types of conifers differed between the Hainich exploratory and the Schorfheide exploratory, I conducted a separate PERMANOVA and PCA including the exploratory as factor. The results showed a significant exploratory effect on DOM spectral properties and fluorescence (PERMANOVA. $p < 0.01$, Figure 3.17). This exploratory effect was strongest for the TOP and SUB samples. Also the TF samples of both exploratories plotted separately from each other, when the factor "exploratory" was included in the PCA (Figure 3.17), In contrast, the factor "exploratory" hardly affected the spectral properties and fluorescence of DOM in SF and LL samples (Figure 3.17).

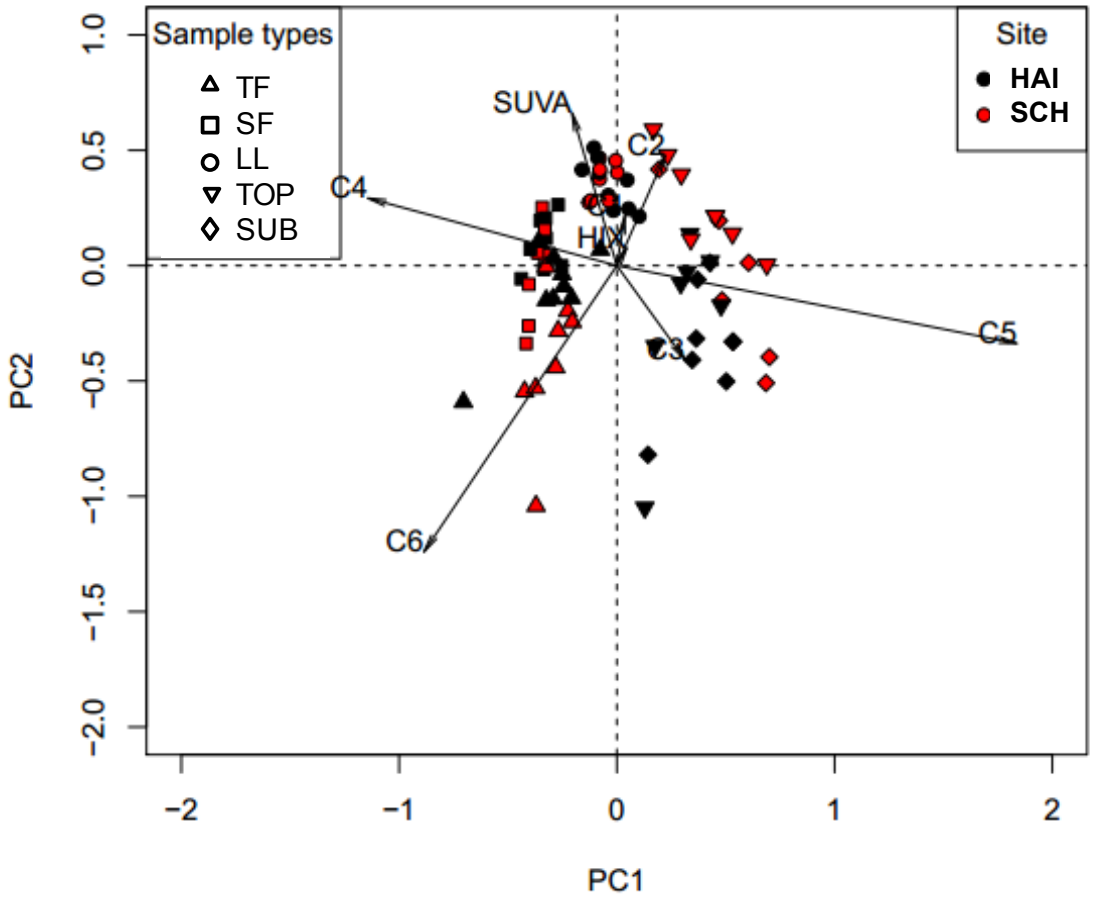

Figure 3.17: PCA plot of DOM composition variables including the exploratory as factor

The PCA plot (Figure 3.16) illustrated the clear sample type effect. It showed the influence of the protein-like PARAFAC component C6 on the TF samples as well as the relevance of $SUVA_{254}$ for LL and C4 for SF. In opposite direction to C4 and SF, I found the soil solution samples TOP and SUB differentiated from the other sample types especially by C5.

3.2.2 Discussion

Concentrations, optical and chemical properties of DOM

Mean DOC concentrations of solution samples for temperate deciduous and coniferous forests were consistent with concentrations reported in previous studies. For throughfall findings ranged between 2–35 mg*L^{-1} (Michalzik et al. 2001; Moore 2003; Stubbins et al. 2017), for stemflow between 12–95 mg*L^{-1} (Moore 2003; Levia et al. 2012; Stubbins et al. 2017) and for litter leachate between 14–90 mg*L^{-1} (Michalzik et al. 2001; Ide et al. 2017; Stubbins et al. 2017). Investigating soil solutions, others reported DOC concentrations between 7–43 mg*L^{-1} for topsoil (Moore 2003; Fellman et al. 2008b; Kindler et al. 2011; Ide et al. 2017) and 2–5 mg*L^{-1} for subsoil solutions (Michalzik et al. 2001; Peichl et al. 2007; Kindler et al. 2011). This pattern indicates that water is enriched in DOM during aboveground ecosystem passage and depleted while passing through soil horizons.

Also consistent with findings in other studies, $SUVA_{254}$ values of our DOM samples ranged between 1.8- 4.7 for TF (Peichl et al. 2007; Inamdar et al. 2012; Stubbins et al. 2017), between 1.9–11.2 for SF (Levia et al. 2012; Stubbins et al. 2017) and between 2.7–5.2 for LL (Peichl et al. 2007; Inamdar et al. 2012). This showed an increasing share of aromatic DOM compounds in water when passing through the aboveground forest ecosystem. Reported ranges for topsoil solutions (2.2–.9) and for subsoil samples (1.4–2.7) are consistent with our findings too (Peichl et al. 2007; Fellman et al. 2008b; Inamdar et al. 2012). This decrease of DOM $SUVA_{254}$ values during mineral soil passage could be related with preferential sorption of aromatic DOM fractions (Kaiser und Guggenberger 2000; Peichl et al. 2007).

ESI FT-ICR MS measurements of forest DOM samples using negative mode ionization generated spectra with thousands of m/z peaks, whose amount and distribution were comparable with previous studies of natural DOM samples (e.g. (Stenson et al. 2003; Sleighter et al. 2010; Tfaily et al. 2015). Due to the ultrahigh mass resolution of this kind of mass spectroscopy, it is possible to assign molecular formulas to a majority of detected masses. The molecular composition as well as the distribution of biocompounds (carbohydrates, lignin, tannin, protein, amino sugars, condensed hydrocarbons and lipids) assignable to molecular formulas of our forest DOM samples, are similar to those reported by others for TF, SF, LL and subsoil solution samples (Tfaily et al. 2015; Ide et al. 2017; Stubbins et al. 2017). Consistent with other studies of DOM samples from terrestrial ecosystems or influenced by them, CHO-only compounds were the main fraction of assigned molecules (D'Andrilli et al. 2013; Hertkorn et al. 2016; Stubbins et al. 2017).

Correlating molecular composition and optical properties

Identified PARAFAC components were often described by comparison with previously published PARAFAC models, either manually or by using tools like the OpenFluor database (Murphy et al. 2014). Additionally, we use the results of the spearman correlation between

FT-ICR MS data and PARAFAC results to suggest additional structural information of the PARAFAC components in our study.

OpenFluor found close matches for component C1 with components from studies in various environments characterized as “humic-like with terrestrial origin” (Santos et al. 2010; Yamashita et al. 2010b; Kothawala et al. 2012; Shutova et al. 2014; Dainard et al. 2015).

Studies by Stedmon et al. (2003) in a Danish estuary and by Lambert et al. (2016) with Congo River water found components with spectra matching our component C2. They described this component also as “humic-like with terrestrial origin”. The positive correlation with the number of m/z peaks assigned to tannin-like compounds based on their position in the van Krevelen plots (ρ = 0.75, Table 3.4) along with the high contribution of C2 to the fluorescence found in LL and TOP samples (Figure 3.15) indicated that component C2 contained plant-derived, tannin-like components.

Component C3 with its maximum excitation wavelengths of 250 nm and 300nm and its maximum emission wavelength of 400 nm resembled components previously described as “microbially altered humic material” (Murphy et al. 2011), which were, among others, found in humic substances from sediments, in fen and bog pore water as well as in lakes, streams and estuaries (Santín et al. 2009; Shutova et al. 2014; Tfaily et al. 2015; Osburn et al. 2016). C3 showed similar excitation and emission wavelength (λ_{ex}= 250, 300nm; λ_{em}= 400nm) published in studies investigating fluorescence of lignin from different sources (e.g. (Thruston, JR. 1970; Albinsson et al. 1999). We found a significant positive correlation of the contribution of C3 to total fluorescence with the number of m/z peaks assigned to lignin-like compounds detected using FT-ICR MS (ρ = 0.80, Table 3.4). Therefore, we suggest that the share of C3 to total fluorescence reflected the presence of lignin and lignin-derived degradation products in DOM.

As another “humic-like” component C4 was termed “C peak” by Coble et al. (1996), which matched fluorescence components found by Kothowala et al. (2012) studying Swedish lakes as well as by studies investigating river and lake water (Lambert et al. 2016; Osburn et al. 2016). The fifths humic-like component C5 only matched a component in the Open Fluor database that was reported by Lambert et al. (2016) studying Congo River water. It also falls into the EX/EM range of a component described as “humic-like C” by Coble et al. (2014) with sources referred also as “humic” and “terrestrial”.

The fluorescence of component C6 was similar to the fluorescence of tryptophan and was therefore described as “protein-like”, representing fluorescence of free amino acids and such bound in proteins. The component was included in numerous PARAFAC models of fluorescence of DOM from various environments (e.g. (Murphy et al. 2013; Yu et al. 2015). The positive correlation between the protein as well as amino sugar fraction of FT-ICR MS data and %C6 (rho = 0.74, Table 3.4) confirmed the assumption that protein-like fluorescence actually represented the fluorescence of proteins. This finding was also in line with results of Tfaily et al. (2015). However, phenolic compounds such as tannins and simple

phenols have also been shown to contribute to those regions of fluorescence (Goldberg und Weiner 1993; Maie et al. 2007; Hernes et al. 2009).

Considering the previous results from the preservation experiment (see section 3.1), we need to remember the difference influence of sample preservation on optical DOM properties. Subsequently, I projected the PARAFAC model on the fluorescence Data of the freezing experiment. Independent from sample type (TF, SF, LL, TOP and SUB) I found a significant (Wilcoxon paired test, $p<0.001$) increase of C1. The mean difference for maximum fluorescence intensity was +0.416 raman units (RU), which relate to an average increase of 12% compared to the mean maximum fluorescence intensity of C1 for all samples. I found a significant decreases for %C2 (average: -14%) und %C3 (average: -18%) between fresh and frozen samples, which were above the analytical reproducibility. I found no significant differences between differently stored samples for %C4, %C5 and %C6. This leads to a slight overestimation of C1 and an underestimation of C2 and C3. Considering the dimensions of changes and that all sample types are equally affected, I expect no significant influence when interpreting DOM quality trends in our samples.

Changes in DOM concentration and composition along the water flow path during ecosystem passage

My results showed distinct differences for DOC concentrations as well as for compositional DOM properties between forest ecosystem sample types. TF samples were enriched in DOC (9–17 $mg*L^{-1}$) compared to precipitation (2–5 mg*L-1) measured in the same exploratories during the same sampling period (data not shown). In consensus with other studies (Peichl et al. 2007; Inamdar et al. 2012), low values for optical DOM properties like $SUVA_{254}$ (Figure 3.6) and humic PARAFAC components C1 and C2 (Figure 3.15) indicated a less “humic-like” and less aromatic DOM composition for TF compared to the other aboveground sample types. According to this interpretation, we would expect low percentages of molecules assigned to the lignin, tannin and condensed hydrocarbons fractions gained by FT-ICR MS analysis of TF samples. However, this was only found for tannin-like not for lignin-like compounds or condensed hydrocarbons (Table 3.3).

In contrast, in TF I found elevated shares of condensed hydrocarbons in compared to the other samples types (Table 3.3). This is in agreement with findings of Stubbins et al. (2017) studying oak and cedar TF and SF samples. In line with Stubbins et al. (2017), I suggest that deposited combustion products caused the large fraction of condensed hydrocarbons in DOM washed off from leaf surfaces. Combustion products have been shown to contribute to the designated molecule fraction in the van Krevelen diagram (Kim et al. 2003; Kim et al. 2004). TF samples were also richest in N-containing compounds, such as free and bound proteins and amino sugares. This is shown by the highest relative contribution of component C6 (Figure 3.15) as well as of the protein associated fraction of FT-ICR MS molecules (Table 3.3) of all sample types. This observation is also in consensus with findings in other studies (Inamdar et al. 2012; Ide et al. 2017).

Litter leachate showed the highest portion of aromatic DOM compounds. This was indicated by the highest $SUVA_{254}$ values (Figure 3.6), the highest percentage of the tannin-associated PARAFAC component C2 (Figure 3.15), as well as the highest share of the tannin and lignin molecules (Table 3.3). This observation is coincident with studies of Peichl et al. (2007) and Inamdar et al. (2012). The release of lignin fragments and other aromatic biomolecules by degradation of litter (Killops und Killops 2005), preferential microbial utilization of labile components, such as simple carbohydrates and amino acids, originating from canopy and tree stem contact, may release humic-like aromatic components including lignin degradation products (Guggenberger et al. 1994; Hur et al. 2009).

While following the water passage downward into subsoil layers, the decreasing DOC concentrations and $SUVA_{245}$ values (Figure 3.6) as well as decreasing percentages of tannin-like compounds (Table 3.3) were in line with a preferential sorption of aromatic, polyphenolic DOM in mineral soils (Kaiser und Guggenberger 2000; Avneri-Katz et al. 2017). I had expected the fraction of molecules assigned to lignin in the van Krevelen plot to follow this behavior. That I found increasing amounts instead, might be due to increased solubility of lignin associated compounds, induced by microbial oxidation, which are still less sorbed on the mineral phase than the highly oxidized compounds associated with tannins. Additional evidence of the ongoing microbial processing is the increasing share of microbial derived PARAFAC component C3 (Figure 3.15). As further possible explanation for the accumulation of lignin associated compounds it was suggested by others, that the space covered by lignin molecules in the van Krevelen diagram should not only be linked to higher plant source material, but also to other types of compounds proposed to be refractory, including non-aromatic compounds like carboxylic-rich alicyclic molecules (CRAM) (Hertkorn et al. 2006; Stubbins et al. 2010; D'Andrilli et al. 2013).

Influence of tree species and management intensity on DOC concentrations and DOM properties

I found significant differences in DOC concentrations of all aboveground sample types between deciduous and coniferous forests (Figure 3.6). Higher DOC concentrations for coniferous stands compared to beech forests might partly be due to differences of tree physiology, like canopy and bark structure, and thus different water-vegetation contact times (Guggenberger et al. 1994). Cluster analysis of biomolecules according to molecular composition (Figure 3.13) showed the influence of tree species on aboveground DOM characteristics. Following the water downward, DOM properties assessed with FT-ICR MS of coniferous stands and beech forests from the same exploratory converged, so that both forest type subsoil samples grouped in one cluster, regardless of the covering tree species. The same observation was true for all but one fluorescence components, showing the disappearance of significant differences when investigating TOP and SUB samples.

Comparing soil solution samples among the experimental regions, I found a strong effect of exploratory on DOC concentrations and DOM properties (Figure 3.17), which could be explained by different soil types and climate in the two areas.

The compositional differences between aboveground DOM of both tree types were mainly related to differences in the fractions associated with aromatic compounds like lignin and tannin (Table 3.3). We found a higher share of lignin associated compounds for pine forest samples as revealed in the patterns of the van Krevelen and the DBE plots (Figure 3.11, Figure 3.12), which was in agreement with findings of Ide et al. (2017). Additionally, we found different lignin-tannin ratios for both tree species. While pine samples exhibited up to 10-fold higher shares of lignin-like than tannin-like compounds, the ratio was close to one or in favor for tannin-like fractions especially in LL beech samples (Table 3.3). Tannins are secondary plant metabolites and play a role in herbivore defense and additionally may affect ecosystem processes (Kraus et al. 2003). The higher amount of tannins in beech samples compared to pine was also reflected in significantly higher shares of PARAFAC component C2 for TF, SF and LL samples (Figure 3.15). This is in agreement with findings of Lorenz et al. (2004), finding higher amounts of tannins in beech leaf litter than in pine needles. A higher share of phenolic carbon in beech than spruce solution samples from the same plots than this study was found by Bischoff et al. (2015) too, when conducting 13C NMR analysis.

Besides the effect of different tree species, I found no statistically significant effect of management practice on DOM composition. There were no differences between managed and unmanaged beech forests as well as no influence of forest management intensity index (ForMI) on optical DOM composition variables and DOC concentrations. With the ForMI, I applied an index, which is only based on above ground vegetation related attributes (harvested tree volume, non-natural tree species, and deadwood volume with saw-cuts) to all sample types. It might be more constructive to use less broad and more specific proxies which are indirectly related to forest management, like lichens richness (Boch et al. 2013a). Another approach might be to correlate more particular sample type parameters with DOM composition variables. So that applying the ForMI only to TF composition might reveal expected relations with management. While for SF DOM this could be the richness of wood-inhabiting fungi, for soil DOM the composition of soil algae might be relevant. Both proved to be influenced by forest management practice (Purahong et al. 2014a; Hallmann 2015).

3.3 Characterization of the biodegradability of DOM

3.3.1 Results

DOM biodegradation - extent and kinetics

DOC concentrations of solutions before incubation ranged between 7 and 19 mg*L^{-1} for TF, between 10 and 109 mg*L^{-1} for SF and between 22 and 87 mg*L^{-1} for LL samples (Figure 3.18).

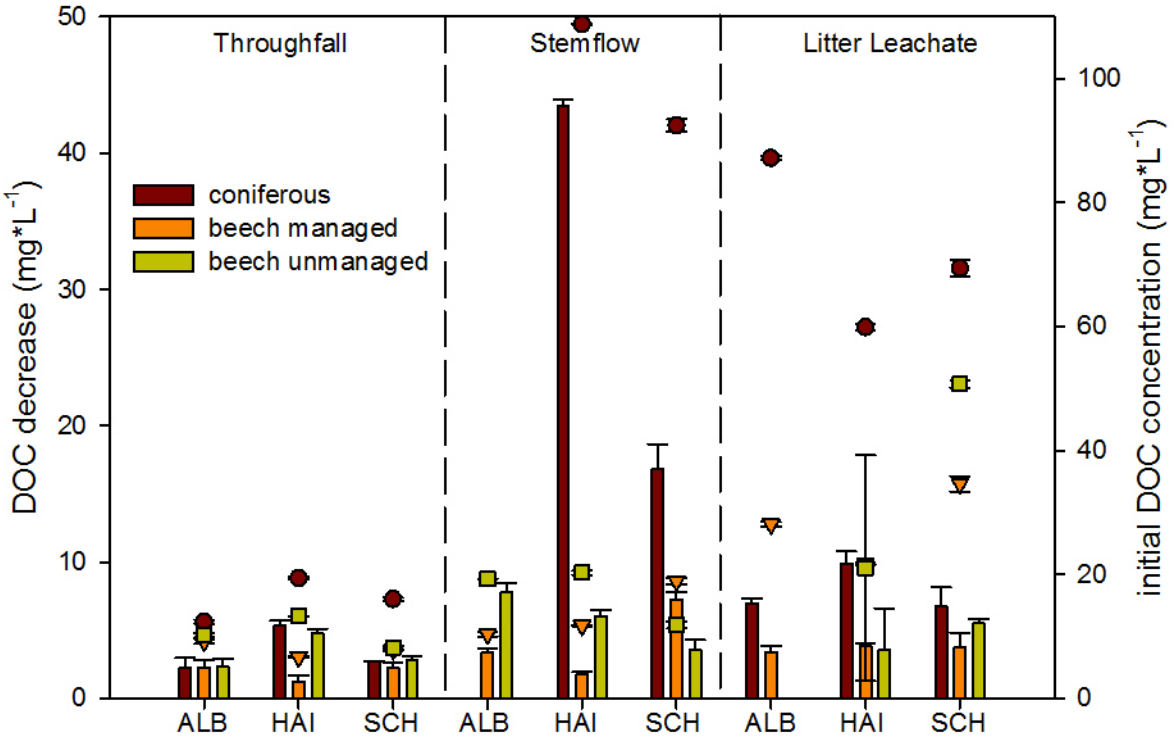

Figure 3.18: Initial DOC concentration (right axis, scatter plot) and absolute decrease of DOC concentration after 28 days of incubation (left axis, bar chart). Means and standard deviation of 3 replicates. ALB=Schwäbische Alb, HAI= Hainich-Dün, SCH=Schorfheide-Chorin.

I found a significant (Wilcoxon rank sum test, $p<0.001$) decrease of DOC concentrations with increasing time of incubation for all samples, except for the control samples. An example for the decrease of DOC concentrations over time is shown in Figure 3.19. The amount of assimilated and mineralized DOC (BDOC) after 28 days of incubation ranged from 8–40% of the initial DOC concentration (Figure 3.20). The DOC decrease could be adequately described using a single two parameter exponential model. Calculated degradation rate constants are shown in Figure 3.20. They were found significantly different from zero for all samples, except for the control samples. SF proved to be the group with the highest extend and rate of DOC degradation followed by TF with slightly lower values. In SF samples, 15–40% and in TF samples 17–35% of initial DOC was degraded within 28 days. With 8–18% of BDOC, LL

samples showed two times lower values of degradation and up to 10 times lower rate constants than SF and TF (Figure.3.20).

No significant differences for %BDOC and k were found between coniferous and beech forests.

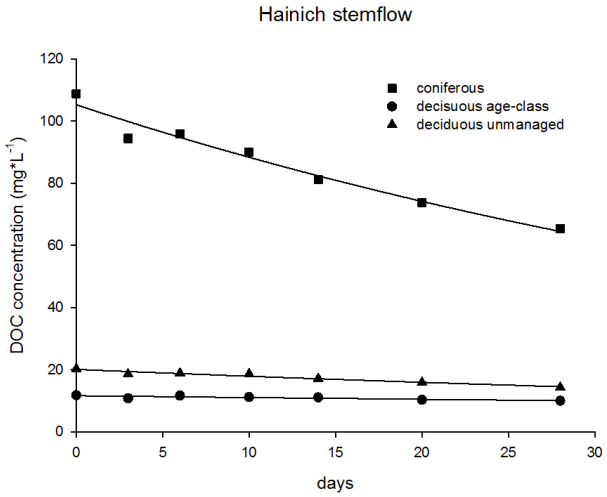

Figure 3.19: Dynamic of DOC concentration during 28 days of incubation. Black lines: fit of the single two parameter exponential model, error bars are smaller than the symbol size

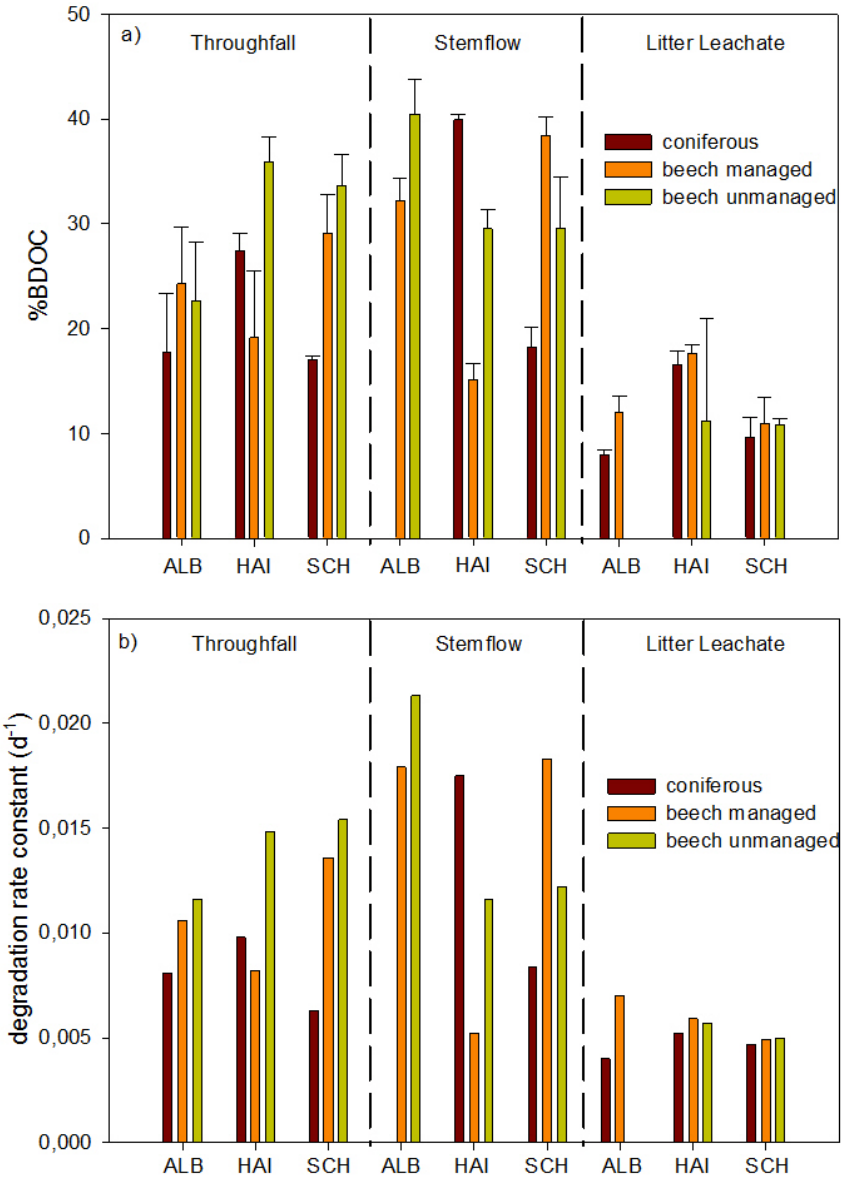

Figure 3.20: Assimilated and mineralized DOC (BDOC) after 28 days of incubation (a) and degradation rate constants (b) for all incubated DOM samples. ALB=Schwäbische Alb, HAI= Hainich Dün, SCH=Schorfheide Chorin

Spectroscopic characteristics and PARAFAC modeling

$SUVA_{254}$ values, which where highest for LL samples (3.1–5.0 $L*mg^{-1}*m^{-1}$) and similar for TF (2.0–3.2 $L*mg^{-1}*m^{-1}$) and SF (2.1–4.5 $L*mg^{-1}*m^{-1}$) samples, showed a significant increase over the time of incubation (Wilcoxon rank sum test, $p<0.001$) for all sample types. The mean increase was lowest for TF samples (0.5 $L*mg^{-1}*m^{-1}$) and similar for SF and LL (1.0 $L*mg^{-1}*m^{-1}$) (Figure 3.21). No significant changes were found for HIX values over the course of incubation (Figure 3.21).

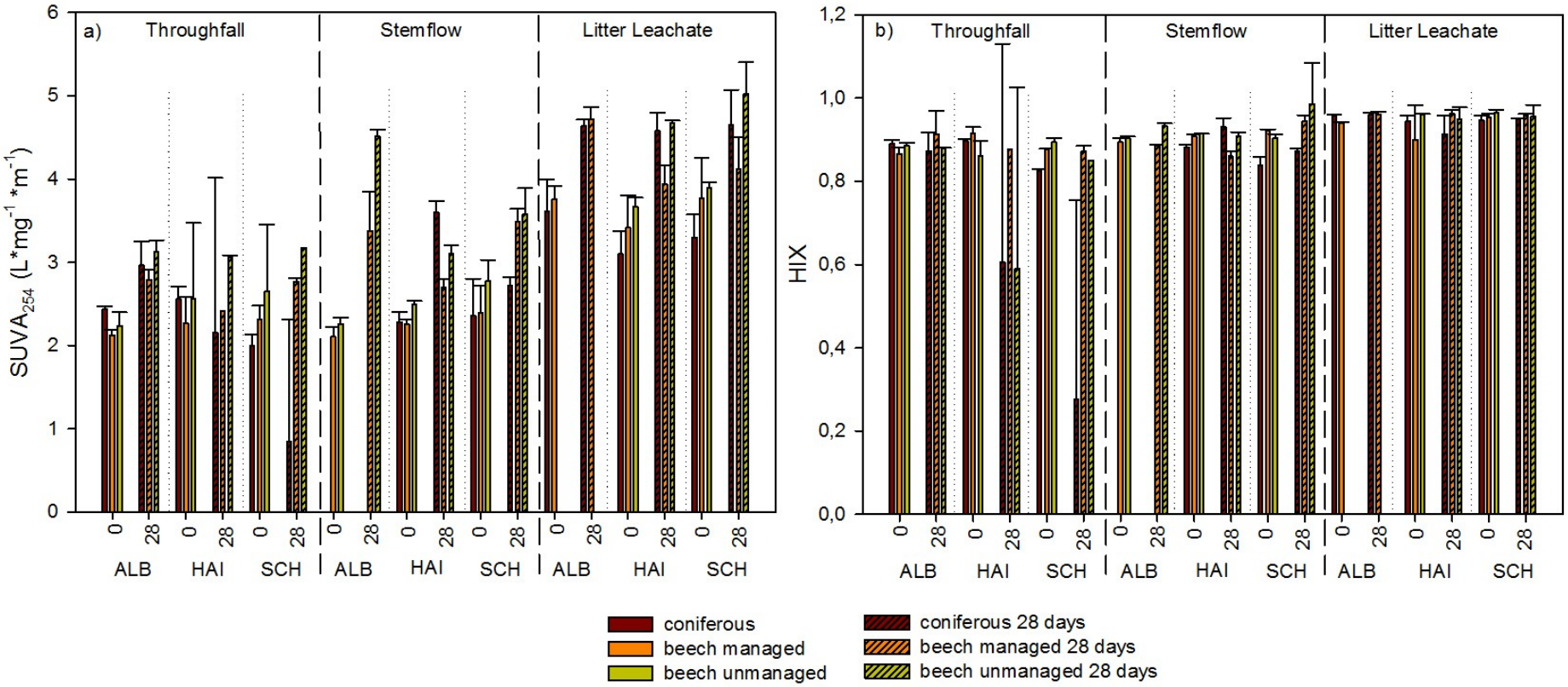

Figure3.21: $SUVA_{254}$ (a) and HIX (b) values before (0) and after 28 days of incubation (28) for all DOM samples. Means and standard deviation of 3 replicates. ALB=Schwäbische Alb, HAI= Hainich Dün, SCH=Schorfheide Chorin.

I found significant effects of incubation, forest management practice and sample type on DOM composition and DOC concentration (PERMANOVA, $p<0.01$). While incubation and forest management explained only small part of the variance ($R^2 = 0.05$ and $R^2 = 0.1$), sample type explained a larger portion ($R^2 = 0.5$). Wilcoxon tests revealed that differences for forest management practice existed only between forests with different tree species (coniferous vs. unmanaged beech, coniferous vs. age-class beech), but not between differently managed beech forests. Due to these findings I distinguish only between deciduous forests on the one hand and coniferous forests on the other in the following evaluation.

The PCA using chemical and optical properties (DOC, $SUVA_{254}$, HIX, %PARAFAC components) as well as degradation parameters %BDOC and k, illustrated the strong effect of sample type on DOM composition, DOC concentration and DOC degradation (Figure 3.22).

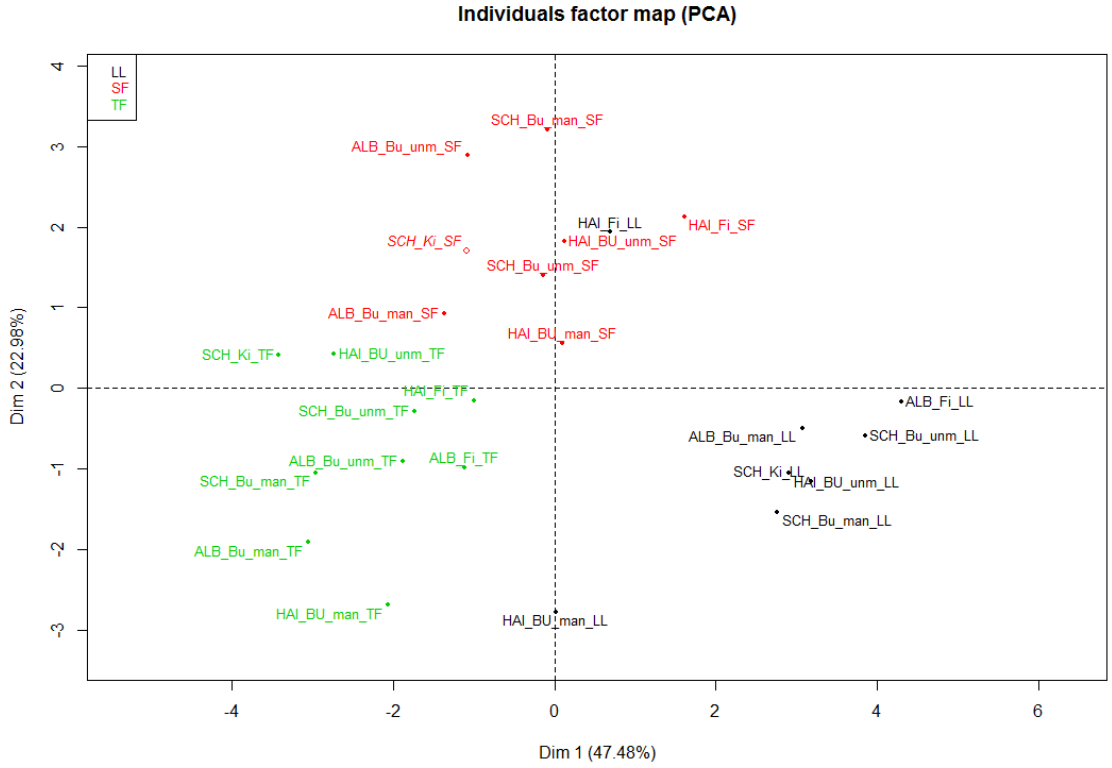

Figure 3.22: Individual plot of PCA with spectroscopic properties (DOC, SUVA, HIX, %PARAFAC components) as well as degradation parameters (%BDO, k). Green = Throughfall (TF); Black = Litter Leachate (LL); red = Stemflow (SF)

I applied the existing 6 component PARAFAC model of the evaluation of the chemical DOM composition (section 3.2) on the EEMs of samples measured before and after 28 days of incubation. The dominant component in all sample types was C1, with a mean relative contribution of 27–36% for deciduous and 30–37% for coniferous forest samples. I found a significant increase after 28 days of %C1 for TF and SF (Wilcoxon rank sum test, $p<0.01$), but not for LL samples. With mean shares of 14–21%, 15–20% and 17–22% for deciduous and 13–19%, 14–18% and 19–23% for coniferous forests, components C2, C3 and C4,

respectively, contributed similar amounts to the total fluorescence. I found a significant decrease of %C3 and %C4 during the incubation for TF samples only (Wilcoxon rank sum test, $p<0.01$). The component contributing the smallest percentage to total fluorescence was C5 with 0-11%. Component C6 showed the widest range of relative contribution among all samples with 2–22% (Figure 3.23). They showed no significant differences after 28 days of incubation for all sample types.

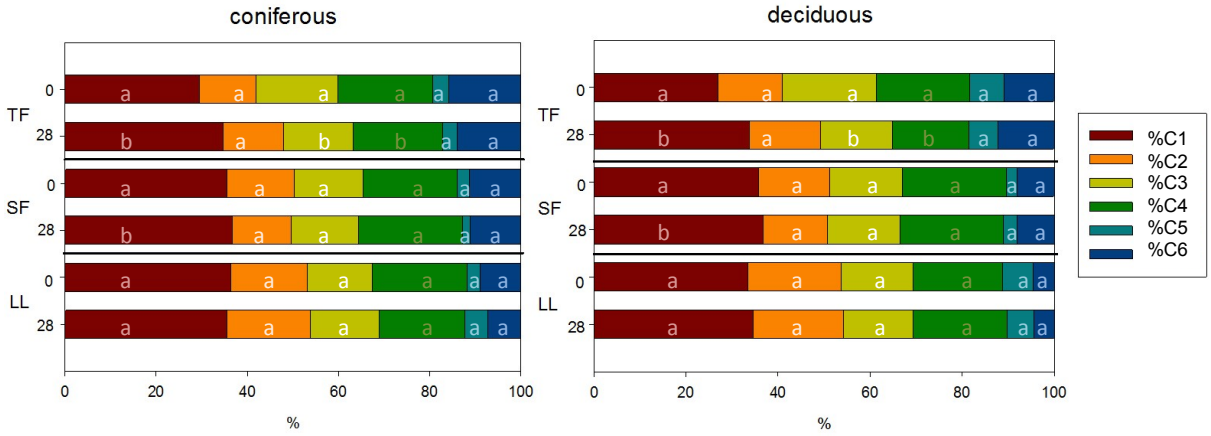

Figure 3.23: Mean distribution (n-coniferous=3, n-deciduous=6) of PARAFAC components before (0) and after 28 days of incubation (28). TF=throughfall; SF= stemflow; LL=litter leachate. Letters indicate significant differences (Wilcoxon rank sum test, $p<0.01$) before and after incubation

Differences between beech and coniferous stands were only found for lignin-like PARAFAC component C3 and for component C5.

I found a significant (Spearmans´ρ, $p<0.05$) negative correlation between %BDOC and $SUVA_{254}$ (Figure 3.24). Although $SUVA_{254}$ was positively correlated with PARAFAC components %C1 and %C2 (Figure 3.25), and negatively correlated with %C3 and %C6 (Figure 3.25), no correlations were found between BDOC and PARAFAC components. The same was true for %BDOC and HIX.

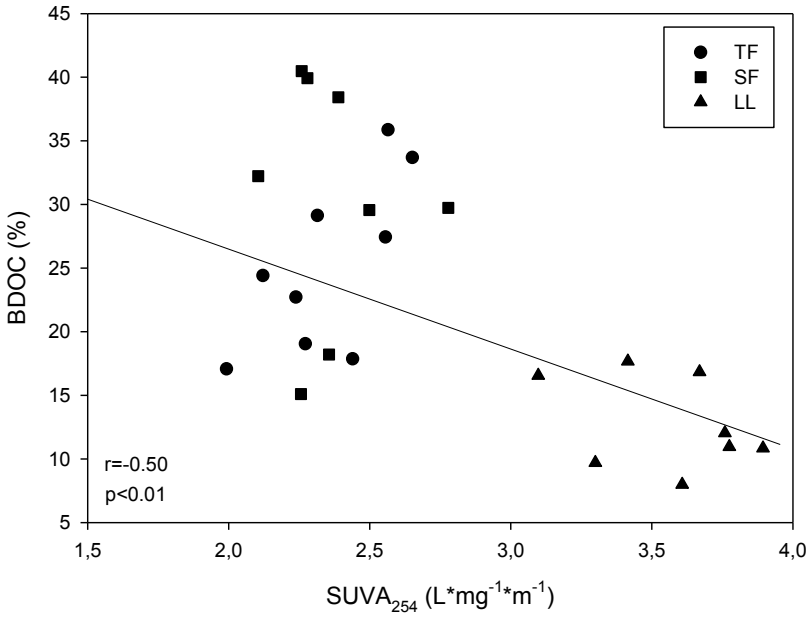

Figure 3.24: Linear regressions between %BDOC and initial $SUVA_{254}$

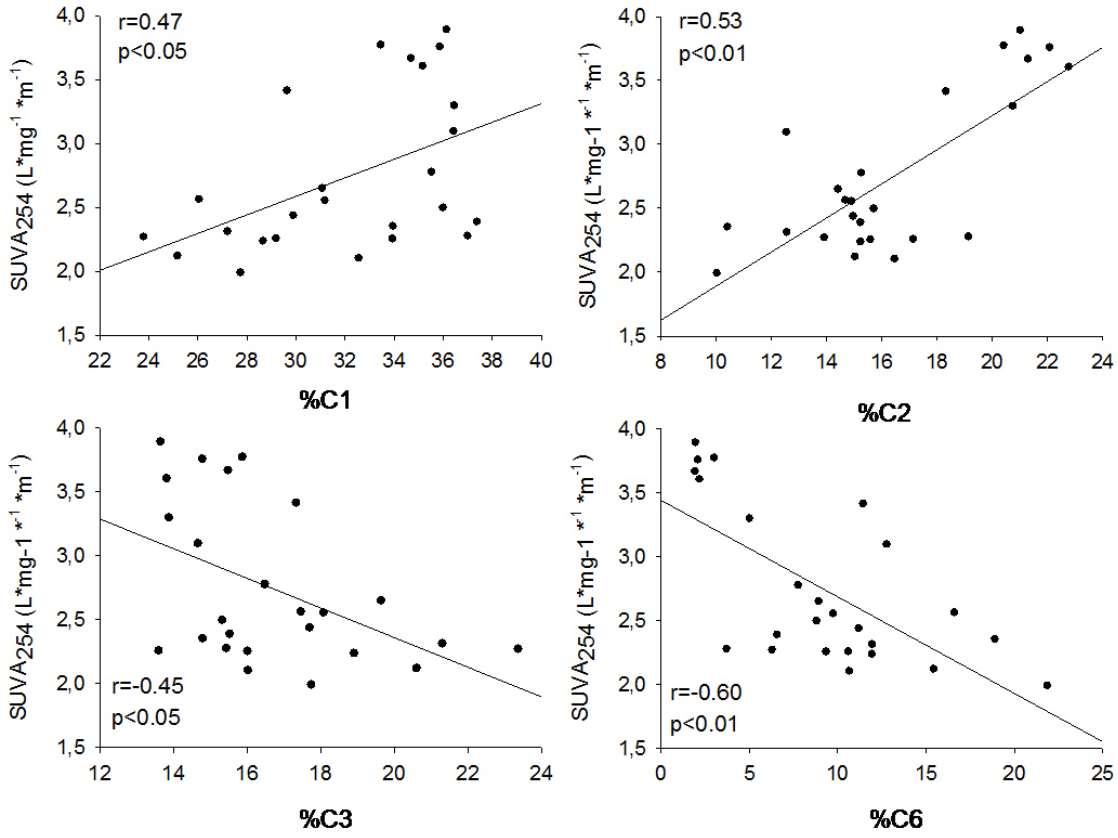

Figure 3.25: Linear regression between $SUVA_{254}$ and %PARAFAC components

3.3.2 Discussion

Generally the amount of biodegradable DOM was in the range of BDOC found by Qualls and Haines (1992) in deciduous forests throughfall samples (22–57%). The sample type was the main factor influencing the biodegradability of DOM in our solution samples. With BDOC amounts up to 40% of the initial DOC concentration as well as the highest degradations rates, stem flow samples showed to have the most bioavailable DOM. Throughfall samples with BDOC up to 36% contained DOM that seemed to be slightly less bioavailable. This could have been due to the high amount of condensed hydrocarbons in TF samples, which were identified by FT-ICR MS measurement (section 3.2). Also considering the sampling time of our samples (October), these were likely associated with combustion residues washed from canopy surfaces.

Lowest degradation rates and thus most stable DOM were found for litter samples (8–18% BDOC). This amount was comparable with results from Kalbitz et al. (2003), who found mean values of 8% BDOC when incubating extracts from spruce and beech forest fermentation layers (O_a). In litter layer (O_i) they found considerably higher BDOC values (65%). For I did not distinguish between Q_a and O_i layer and the sampling location was under the fermented layer, it is within reason, that the BDOC was closer to the O_i value.

Consistent with other studies (Kalbitz et al. 2003; Fellman et al. 2008a), I found a negative correlation between BDOC and aromaticity indicators ($SUVA_{254}$). This supported the assumption that especially aromatic structures are stable against rapid degradation. The significant positive correlation between $SUVA_{254}$ and %C1 in combination with the significant increase of component C1 after 28 days of incubation indicated either a transformation of former non-aromatic into aromatic compounds or a relative accumulation of the latter.

Previous studies described PARAFAC component C6, whose fluorescence resembles that of amino acid tryptophan, as protein-like (e.g. (Yamashita et al. 2010a; Murphy et al. 2013; Yu et al. 2015). Results from the structural DOM characterization (section 3.2) showed a positive correlation between component C6 and the protein-associated compounds of FT-ICR MS spectra supporting this assumption. Given the finding that carbohydrates and amino acids were typically utilized preferentially by microorganisms during degradation of different compounds in DOM solutions (Volk et al. 1997; Amon et al. 2001; Kalbitz et al. 2003), I expected a significant change of %C6 after 28 days of incubation. The fact that we found no significant change of %C6 during incubation might have indicated that amino acids were bound in and on humic substances and thus protected against degradation (Volk et al. 1997). Alternatively the compounds creating C6 fluorescence could have had no protein-based origin, but were phenolic compounds such as simple phenols and tannins which have been shown to contribute to this region of fluorescence (Maie et al. 2007; Hernes et al. 2009). I tend toward the first interpretation, because we found a significant negative correlation between $SUVA_{254}$ and %C6 (Figure 3.22), indicating a less aromatic composition of C6.

Considering the small but nonetheless significant changes in DOC concentration and absorption based $SUVA_{254}$ values for LL samples, I had expected to find changes in the other spectroscopic DOM characteristics as well. That I found different could be due to balanced changes of the relative shares of PARAFAC components used for comparison. Another explanation might be that only parts of the DOM able to absorb light are also able to emit light by fluorescence (Aiken 2014). In fact the combination of the low sensitivity of fluorescence and only small changes in LL DOM composition during the incubation might result in no visible changes of fluorescence.

Given the results of the chemical composition of DOM in section 3.2, showing distinct differences of tannin-related compounds between coniferous and beech forest samples, I expected a higher biodegradability for tannin-poor coniferous DOM. This would have been in agreement with degradation experiments with litter of different trees conducted by Don and Kalbitz (2005). In contrast, my result showed no significant differences between the biodegradability of solution samples from beech and coniferous forests. One possible explanation could be an optimal degradation of all samples, regardless of their origin and composition, based on the generation of inoculum using soils from all forests. Different forest management practice, including different tree species as well as different management intensities, developed different microorganism communities and thus enzymes activities (Purahong et al. 2014b; Hoppe et al. 2016). By including them all in one inoculum, it was ensured to have the optimum suitable organism and/or enzyme for all present DOM constituent.

4 Extended summary and synthesis

To test the effect of different freezing methods (standard freezing at −18 °C and fast-freezing with liquid nitrogen) on DOM concentrations and DOM properties of different forest ecosystem compartments, fresh and differently frozen throughfall, stemflow, litter leachate and soil solution samples were analyzed for DOC concentrations, UV-vis absorption and fluorescence as 3D EEMs. Subsequent PARAFAC modeling of EEMs resulted in four components (c1-c4) describing the fluorescence behavior of DOM.

As it shows, fast-freezing with liquid nitrogen was able to preserve bulk DOC concentrations, but not its composition. Fast-freezing with liquid nitrogen prevented a significant decrease of DOC concentrations observed after freezing at −18 °C. Nonetheless, the share of humic-like PARAFAC components c1 (EX_{max} < 250 nm (340 nm), EX_{max}: 480 nm) and c2 (EX_{max}: 335 nm, EX_{max}: 408 nm) to total fluorescence and the humification index (HIX) decreased after both freezing treatments, while the shares of component c3 (EX_{max}: < 250 nm (305 nm), EX_{max}: 438 nm) as well as $SUVA_{254}$ increased. The contribution of protein-like PARAFAC component c4 (EX_{max}: 280 nm, EX_{max}: 328 nm) to total fluorescence was not affected by freezing.

Freezing and thawing affected the DOC concentration, spectral absorption and fluorescence properties of terrestrial water samples (throughfall, litter leachate and soil solution). While I tested varying freezing procedures in this study, different thawing protocols for minimizing sample storage effects on DOM should be tested in future studies.

Given the results of the sample preservation investigation, I would recommend to measure samples immediately after the sampling to exclude all storage influenced changes of DOM quality and concentration. Due to logistical reasons, like three different sampling areas, several sampling days and different participating laboratories, frozen storage and transport was the only feasible solution for sample handling in this investigation. However, when quantifying the changes of DOM fluorescence due to freezing, slight but significant differences were found for C1, C2 and C3, leading to an overestimation of C1 and an underestimation of C2 and C3. No significant differences between differently stored samples were found for C4, C5 and C6. Also, considering that all samples were handed similarly and that all sample types were equally affected, I expect no significant influence when interpreting DOM quality trends in the following investigations.

In order to investigate the chemical composition and optical properties of throughfall, stemflow, litter leachate and soil solution DOM from forests with different management practice, I took samples selectively during the vegetation periods of 2011 to 2013. The conducted UV-vis absorption and fluorescence measurements as well as the subsequent modeling were the same as for the preservation tests. For more detailed chemical insights, FT ICR-MS spectra of selected samples, taken in October 2015, were measured.

FT-ICR MS measurement resulted in spectra with numerous peaks, which in parts, could be assigned to molecular formulas. To gain a broad qualitative overview, it was possible to

relate those compounds to major biochemical groups like lignins, tannins, proteins, condensed hydrocarbons and others, according to their H/C to O/C ratio.

Modeling the fluorescence EEMs of all samples with PARAFAC, a 6 component model (C1-C6), instead of only four components when modeling the smaller dataset of the freezing experiment, could be validated. These components could be described by comparison with previously published PARAFAC models as humic-like with terrestrial or microbial origin (C1-C5) and as protein-like (C6). Projecting the validated model on fluorescence measurements from the samples also measured with mass spectrometry, we could correlate the PARAFAC components with the biochemical groups and obtained additional chemical information. Additionally we calculated $SUVA_{254}$ values with absorption data and DOC concentrations.

Comparing all these parameters among different ecosystem compartments, I found distinct characteristics for each sample type. **Throughfall** showed the highest amount of protein related substances, visible in highest protein-like fluorescence and highest number of protein associated compounds. Additional, as indicated by low $SUVA_{254}$ values and a small number of lignin associated compounds, TF DOM contained the lowest amounts of humic, aromatic properties among all above ground sample types. An exception were condensed hydrocarbons, which are associated with combustion products, and showed to be highest in TF DOM, possible via deposition wash off from the canopy.

Following the second path precipitation takes through the canopy, we found a still high but slightly smaller amount of protein constituents in **stemflow** samples. Increasing $SUVA_{254}$ values and higher humic-like fluorescence indicating an increasing aromaticity caused either due to enhanced accumulation, or by increased conversion of former non-humic in aromatic DOM, induced by increased bark-water contact time compared with TF.

Reaching the ground and investigating **litter leachate** solutions, I found the most aromatic DOM composition compared to the other ecosystem compartments. This was indicated by the highest SUVA254 values, low protein-like additional to high humic-like fluorescence.

After percolating further downward and passing organic soil layers, the water reached mineral soil layers. DOC concentration as well as aromatic DOM components in **subsoil solution** decrease likely due to preferential adsorption of the latter. The remaining dissolved organic matter is exposed to ongoing degradation, resulting in an accumulation of microbial lignin degradation products and possible of refractory compounds.

I found significant differences of DOM composition between coniferous and beech forests for above ground samples (TF, SF, LL). These were mostly related to differences in the fractions associated with aromatic compounds like lignin and tannin. Following the water flow path below ground, DOM properties converged and vegetation related differences disappeared due to ongoing microbial processing and sorption processes. Remaining differences in DOM composition could be related to different abiotic factors like soil types and climatic variables in the exploratories.

I found no statistical influence of the forest management intensity index (ForMI), which is based on the proportion of harvested tree volume, the proportion of non-natural tree species, and the proportion of deadwood volume with saw-cuts, on optical DOM composition variables and DOC concentrations.

To investigate the influence of sample type and management practice on DOM biodegradability, a laboratory incubation experiments for 28 days with TF, SF and LL samples of managed and unmanaged beech forests as well as of coniferous stands were conducted. The same measurements than in the previous investigations (DOC concentrations, UV-vis absorption and fluorescence as 3D EEM scans) were applied to samples before and after the incubation.

I found a significant decrease of DOC concentrations for all samples, which could be adequately described using a single two parameter exponential model. The amount of assimilated and mineralized DOC (BDOC) after 28 days were highest for SF (max 40% CDOC) followed by TF (max 36% BDOC) samples, indicating the most bioavailable DOM in this sample types. LL showed two times lower values of degradation (max 18% BDOC) and thus the least bioavailable DOM.

A negative correlation between %BDOC and $SUVA_{254}$ as well as a significant increase of humic-like fluorescence component %C1 and $SUVA_{254}$ values along the incubation shows the stability of aromatic structures and their relative accumulation during microbial DOM degradation. I found no significant differences for BDOC and thus bioavailability between coniferous and beech forest stands. The same applies to differences between managed and unmanaged beech forests.

Considering the results of the chemical DOM characterization, the high amount of poor biological degradable condensed hydrocarbons in TF samples, could be the reason for its lesser bioavailability compared to SF samples.

The results of the incubation experiment in return, confirmed the assumption, that biological degradation processes are responsible for the increase in aromatic structures following the water from TF and SF to LL samples. The same could not be confirmed for the decrease of protein-like components.

5 General conclusions and prospects

DOC concentrations and optical DOM properties of different terrestrial ecosystem samples are influenced by freezing. Due to the fact, that all sample types are equally affected, it is possible to consider the under- or overestimations, caused by the storage protocol, when interpreting the results of DOM characterization. To asses, whether the thawing rather than the freezing process is responsible for changes in DOM properties, different thawing protocols should be tested in future investigations.

There are distinct chemical differences in DOM composition of throughfall, stemflow, litter leachate and soil solution along the water flow path in an European forested ecosystem. The incubation experiment revealed strong influence of sample type on biodegradability of DOM from above ground samples too. The higher amount of assimilated and mineralized DOC (BDOC) and the faster rate of degradation for stemflow and throughfall samples, indicating better bioavailable DOM compared to litter leachate.

I found, that above ground DOM composition but not its biodegradability is influenced by tree species. Following the water below ground, DOM properties converged and vegetation related differences disappeared, likely due to microbiological activity and sorption processes. This was best shown in the detailed high resolution FT-ICR MS spectra then in the sum parameter-like fluorescence measurements. Expected differences between coniferous and beech sample biodegradability might be masked by inoculum selection, which was chosen to supply the optimal microbial community. To test, whether different tree species related DOM needs different parts of the microbial community for degradation, measurement of enzyme activity might provide useful. Alternatively, cross testing with inoculums from the different tree type stands could reveal differences in DOM degradability. To further investigate the homogenization of chemical DOM properties when following the underground water flow path, sampling and high resolution measurements of deeper sample locations and aquifer sampling should be considered.

It was not possible to link DOM composition changes and biodegradability with indices of management intensity, which in my study were the ForMI and management categories like unmanaged forests or age-class forests. Generally the number of investigated plots per management category encompassed 6 plots for coniferous stands, 5 for managed and 6 for unmanaged beech forests, which is rather small for detecting potentially slight effects. So, additional plots might be necessary. The limitation on only one exploratory might be reasonable, to minimize the organizational effort. I can imagine two possible approaches to further reveal underlying effects of forest management practice. One might be to relate less broad and more specific proxies of management intensity to DOM quality variables of single sample types. For example, correlating understory vascular plant species richness or lichens diversity only to TF samples. While for SF DOM the richness of wood-inhabiting fungi, or for soil solution DOM the composition of soil algae might be relevant. To statistically remove all non-management related effects on DOM composition in order to investigate the residual information for management induced effects could be another manageable approach.

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