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Production and Biodegradability of Dissolved Organic Carbon from Different Litter Sources

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Abstract

The movement of carbon on Earth is based on exchange between pools that represent carbon in different physical forms, differing in chemical composition, structure and function. Dissolved organic carbon plays an important role in ecosystems because of its mobility, which can be relatively high in saturated soils, and because it is the most available fraction of organic matter for microorganisms in soil, being particularly active in microbial degradation processes through soil profile. The ability of moving through soil, that DOC has, makes it an essential part of the organic loading to the streams, forming a bridge between the carbon of terrestrial and aquatic systems. However, relatively little is known about the production and fate of DOC from its main source, which is plant organic matter. The correlation between the type of litter and the characteristics of the DOC produced from it represent the knowledge gap that this study aims to fill. The litter from six plant species was used to extract DOC over different extraction periods from one up to forty eight hours. Also, a degradation study on the DOC extracts was performed and the resulting degradation curves were analyzed in relation to the extraction time, percentage of aromaticity and to the nitrogen composition of the litter. The results showed that only in some of the species surveyed the DOC leaching from the wood litter is lower than the one from the leaf litter. Moreover, the DOC aromaticity did not increase over extraction time as was expected, but instead it tended to decrease. The degradation experiment showed an increase in lability until the 16 hours extraction, which was different to the expected pattern of decreasing lability as extraction time increased. Significant differences in DOC leaching rate and lability were also found between evergreen plant litter and summer green plant litter. The differences in production and degradability of the DOC are thus related to a wide range of factors, other than the chemical composition of the litter. Other factors such as physiological variations among species and plant structures appear to play a significant role in the DOC production. The results show that assumptions made in models about DOC production depending only on chemical structure of litter can possibly be improved by including physiological differences among species and morphological structures.

Content

Introduction

Anthropogenic activities have led to a changed balance of the Earth cycles, which has affected the climate system, with impacts on all the natural systems on the planet. Evidence of these changes has been shown in the IPCC report that summarize the variations in climate during the last hundred years (IPCC 2007)

The understanding of the biogeochemical cycles is crucial to assess the impacts of human activity in the local and global environment. Among these cycles, the carbon cycle is one of special interest, comprising two (CO_2 and CH_4) out of the main four gases (water vapor, CO_2, CH_4, and O_3) that cause the greenhouse effect are present in this cycle (Kiehl & Trenberth 1997).

The dissolved organic carbon (DOC) is produced from chemical leaching from organic material surfaces (Qualls et al. 1991; Zech et al. 1996), as a product of the biological decomposition of the organic matter (Guggenberger et al. 1994) and as a result of excretion of microbial metabolites and plant root exudates (Zsolnay & Steindl 1991; McDowell 2003). DOC plays an important role in the carbon cycle due to, among other things, its ability to move carbon hydrologically (laterally) between pools in the ecosystem (Hoover 2008).

However, the linkage between the terrestrial and aquatic carbon is still unclear. One of the biggest problems is to relate the DOC present in water bodies to its origins, since a labile part, originated from the partial degradation of the DOC, has high turnover rates and is usually consumed before it reaches water bodies (Blough & Green 1995).

Approximations have been made in several areas in order to understand the processes related to the DOC dynamic, i.e. studying the mechanistic ways that DOC moves through the hydrological system in the soil before reaching the surface water (Laudon et al. 2011), the lability of the DOC depending on the soil source (Bowen et al. 2009), and the influence of environmental conditions such as moisture in DOC generation and degradation (Guelland et al. 2013).

Moreover, even though DOC types derived from different plants have been studied (Don & Kalbitz 2005), most of these studies have been limited to comparisons

between plant species and ignore the morphological differences between parts of the same species. Specifically, a factor often neglected is the difference in production and lability of DOC between leaf and wood litter within a certain species.

According to the studies made by Parton (1994) wood material leaches considerably less DOC than the leaf litter. Moreover, Schreeg (2011) found that the DOC that leaches from the litter reaches an asymptote in the first days of extraction following the function of enzyme kinetics by Michaelis & Menten (1913). Marschner & Kalbitz (2003) stated that the most stable fraction of the DOC is formed by breakdown products of cellulose, hemicellulose and lignin. Lignin is the most thermally stable element of the cell wall and its degradation takes more time than any other structure in the plant cell (Hill 2006). Thus, it is expected that the leaching of lignin derived compounds would increase over time. To the best of my knowledge, there are only a few studies that identify the differences in the DOC degradation among different plant litter types (Lennon & Pfaff 2005). Nonetheless, approximations have been made in order to reach this goal (Bowen et al. 2009), but they have not managed to determine the potential degradability of DOC without an environmental influence. The extractions were made from soil and litter sources *in situ*, which can lead to difficulties when trying to understand the dynamics of the most labile part of the DOC.

In this study, an analysis of DOC differences between different litter sources, both among species and within species between their wood and leaf parts, was performed. The main aim was to find correlations between DOC source and characteristics of the DOC leached. In this sense and based in the theoretical background, it was hypothesized that 1) the wood litter would leach less amount as well as less degradable DOC than the leaf litter in all the species, 2) leaf litter will release most of its water extractable organic carbon during the first extraction times, while wood litter continue to leach significant amounts for longer times, and 3) the lability of the DOC will decrease with increasing extraction time while the aromaticity will increase since more lignin derived compounds would leach with time, leading to a coupling between the aromaticity and the lability of the DOC.

The plant species studied were *Betula, Picea abies, Pinus sylvestris, Vaccinium myrtillus, Vaccinium vitis-idaea and Deschampsia flexuosa.*

Background

Biogeochemical cycles

In order to understand the dynamics of climate change, it is important to undertake the study of biogeochemical cycles. The study of biogeochemical cycles refers to the cycling of elements or substances through biotic and abiotic pathways (Lyons 2001). They are called cycles because the elements involved move between Earth systems and their mass is conserved through the whole system. The influence of human activities in biogeochemical cycles leads to environmental issues such as global climate change and its consequences (Vitousek et al. 2015).

The most studied biogeochemical cycles are: oxygen, carbon, nitrogen, phosphorus, sulfur, water and rock cycle (Lyons 2001).

The carbon cycle

The carbon cycle is a key component to understand the effects of different human and non-human activities on climate change. In this cycle, carbon is distributed and interchanged among carbon pools. The carbon pools can be either sinks or sources of carbon (Bolin 1981; Siegenthaler & Sarmiento 1993). A distinction is made between organic carbon pools and inorganic carbon pools. The global inorganic carbon pool has a long term cycle where atmospheric carbon dioxide (CO_2) contributes to chemical weathering of rocks, eventually leading to burial of calcium carbonate at the ocean floors where the carbon is further cycled through the lithosphere. Organic carbon is fixed into organic matter across the biosphere via photosynthesis. Then, this carbon is decomposed in the soil or it is transported to aquatic systems ending up in coasts and oceans where it returns to the atmosphere as CO_2 form (Glok Galli et al. 2014).

The soil carbon pool is estimated to be in the range of 1500 - 2400 Pg C (1 Pg = 10^{15} g) and 1700 Pg C only in permafrost soils (IPCC 2013). Approximately 55 Pg C from the soil carbon pool belong to the carbon that resides in the fresh litter or detritus that lay in the surface of the soil (Hilbe 2000). The concentration of soil organic carbon decreases exponentially as the depth increases, showing that the main source of

organic carbon in soil comes from litter fall and root turnover, when they are not deep into mineral soil (Nakane 1976). The largest fraction of soil organic matter has been classified as humic material that as a product of microbial activity (Hilbe 2000).

The pool of organic carbon in soil consists of a small pool of fresh debris with a relatively short half-life near the surface, and a much larger pool composed of humic materials with a slow turnover time. Thus, the major part of the CO_2 emissions to the atmosphere can be attributed to the decomposition of the labile fraction of the carbon pool in the litter layer (Edwards & Sollins 1973; Bowden et al. 1993). The plant debris is decomposed in the soil, producing CO_2 that is released into the atmosphere. A significant part of it does not follow this process and is exported from the soil system by the ground water. This part, defined as lateral export, can represent annually 4 – 28% of annual net ecosystem exchange (NEE) (Oquist et al. 2014).

Lateral fluxes

Lateral fluxes are defined as the movement of carbon away from the places where the CO_2 is originally extracted from the atmosphere. This movement creates differences in the regional carbon stocks comparing to the initial uptake (Sarmiento & Sundquist 1992). The equation by which the lateral fluxes can be represented is shown as follows:

$$\boldsymbol{Ecosystem\ CO_2 sink} = \boldsymbol{Ecosystem\ carbon\ accumulation} + \boldsymbol{Lateral\ carbon\ flux} \quad \text{(Eq. 1)}$$

The understanding of the impact of the lateral fluxes in the global carbon budget is still not clear even though they have been estimated, as mentioned before, to be 4 – 28% of annual NEE (Öquist et al. 2014)

The dissolved organic matter and the dissolved organic carbon

Dissolved organic matter (DOM) has been defined as the total organic matter that passes through a certain pore size filter, typically 0.45 µm (range 0.2-1 µm). The size of the pore used to filter, as well as the filtration methodology, influence in a minor yet potentially significant way the concentration and properties of the DOM obtained

(Schnabel et al. 2002). Thus, in order to have the most accurate comparison of results, the same size of pores filtered should be used in the DOM estimation. The importance of DOM relies on its solubility, high mobility and because it often contains labile organic compounds that can serve as a nutrient source for microorganisms.

DOM is produced in different ways, for example, from chemical leaching of organic material on the surface of leaves, stems and plant litter (Qualls et al. 1991; Zech et al. 1996); as a product of the biological decomposition of the organic matter (Guggenberger et al. 1994); and as a result of excretion of microbial metabolites and plant root exudates (Zsolnay & Steindl 1991; McDowell 2003). Moreover, the amount of DOC that comes from the leaching of litter in forests is reported to be especially high compared to other sources (Mc Dowell et al. 1998). The DOM can leave the soil by several processes, such as utilization by microorganisms (Zsolnay & Steindl 1991; Qualls et al. 1991; Nelson et al. 1994), leaching by percolating water (Qualls et al. 1991) and the uptake of some organic molecules by plant roots, with the last process being small in magnitude compared to the other two (Kielland 1994; Näsholm et al. 1998).

The (DOC) represents approximately 50% of the total weight of all elements in the dissolved organic matter (Weishaar et al. 2003). The production, movement and fate of DOC is of special interest for understanding the overall carbon cycle (Yavitt & Fahey 1986). This interest is due to the significant importance (20-40%) of CO_2 emissions from litter mineralization to the entire soil carbon efflux (Joos et al. 2009; Kammer et al. 2012). Also, litter leaching of organic matter is essential for the supply of nutrients to the soil media (Mcdowell & Fisher 1976), and thus, it is important to understand the different characteristics of the leaching processes among the different plant species in the ecosystem. Moreover, the rate of mineralization from DOC to CO_2 can vary dramatically depending on the chemical structure of the compounds from a labile pool when it is highly biodegradable, to a stable pool when the biodegradability is low (Bowen et al. 2009). The more labile pool commonly has a large amount of monosaccharides, low molecular weight organic acids and aminosugars, while the stable pool is formed by recalcitrant and complex organic compounds, products of the microbial metabolism and the decomposition of complex polymers and lignocellulose (Koivula & Hänninen 2001),

Degradation experiments longer than 7 days can give a clear idea of the dynamism and size of the stable carbon pool. Nonetheless, shorter bioassays (highly labile DOC) can provide valuable information from the aquatic perspective. This is because of the fact that during episodes of high runoff the DOC can travel faster from soil to aquatic systems, where shot-term labile DOC can contribute to the substantial and large-scale CO_2 production rates recently found in streams (Hotchkiss et al. 2015; Berggren et al. 2010; Ågren et al. 2008; Berggren et al. 2010). However, for a broader understanding of labile DOC, experiments with periods of 7 days or longer should also be considered (McDowell et al. 2006).

Aromaticity of the DOC

The first time that the word aromatic was used as a chemical term was by Hofmann (1856) to characterize chemical compounds that had a phenyl radical. Aromaticity has not been defined as a function of chemical reactivity and stability, but as a function of the electronic structure of chemical compounds. Thus, it has been defined as aromatic compounds to any cyclic or polycyclic molecule or ion which has all the annular atoms participating in a group where all the π-electrons are disposed in a bonding molecular orbital between a closed shell (Badger 1969).

The aromaticity plays an important role for characterizing DOC (Weishaar et al. 2003). For instance, it has been stated that DOC rich in aromatic compounds and low in carbohydrates is a characteristic of the less biodegradable carbon pool of DOC (Kalbitz et al. 2003). Also, aromaticity in DOC has been proved to have correlations with its oxidation properties (Reckhow et al. 1990; Li et al. 2000; Westerhoff et al. 1999) and the ability to react with coagulants (Singer 1994).

Modeling dissolved organic carbon

Modeling of the behavior of DOC has been gaining special interest over the last 20 years. The dynamics of DOC in the environment have been described in models including soil and water carbon dynamics (Currie & Aber 1997; Kalbitz et al. 2000). In DOC modeling several aspects are taken into account, such as the relationship

between water fluxes and the DOC released from the soils (Boyer et al. 1996), geochemical interaction between DOC compounds and the soil structure (Cresser 1996), the decomposition of organic matter that becomes a potential source of DOC to the environment (Currie & Aber 1997) and the potential degradation of different kinds of DOC in the environment. Field results and laboratory experiments are used to parameterize models and validate their performance.

According to Parton (1994), leaves and wood litter that enter at the soil surface suffer a loss of water extractable organic carbon that is the amount of carbon that can be extracted with water and depends on the chemical structure of the litter. The potential amount of carbon that enters the ecosystem as DOC from the leaf and the wood litter is determined by the lignin: nitrogen ratio with the soluble fraction of the litter being between 5% and 25%.

In order to calculate the amount of carbon that passes to the soil system as DOC from the litter sources, the following equation stated by Parton et al (1994) has been used:

$$\boldsymbol{S_{fl,fr} = 0.25 - (0.018 * LN)} \quad \text{(Eq. 2)}$$

$$\boldsymbol{S_{fcwd} = 0.25 - (0.005 * LN)} \quad \text{(Eq. 3)}$$

Where S_{fl} and S_{fr} are the soluble litter and the soluble fraction of roots respectively, and S_{fcwd} is the soluble fraction of the woody debris. LN is the lignin: nitrogen ratio of every litter type.

In relation to the degradation rate of the extracted DOC, approximations have been done in order to simulate this process. One of these approximations is the one made by Tang (2015), which states that the mineralization of DOC pools follow first-order kinetics, being a function of the temperature response and the size of the DOC pool in the top and bottom layer.

$$\boldsymbol{M_D = \left(1 - \exp\left(-B_{DM} * B_{Ttop}\right)\right) * DOC_{top} + (1 - \exp(-B_{DM} * R_{Tbtm})) * DOC_{btm}}$$

(Eq. 4)

Where B_{DM} (day^{-1}) is the basal mineralization rate of the DOC pool at 20◦C, R_{Top} and R_{Tbtm} are the Q_{10} correlation of soil temperature at 0.25 m and 1.25 m which represent the middle point of the top and bottom layer respectively.

The formulas of Eq. 2, 3 and 4 are shown only to explain some assumptions regarding the production and lability of the DOC. Thus, they were not use in the present study for any type of calculation.

Methods

In order to investigate the amounts and characteristics of the extracted DOC from different litter sources, water extractions were performed on cut litter. Moreover, the extracted solutions were filtered and subjected to analysis of TOC and absorbance. The DOC extracts were, furthermore, subjected to a degradation experiment by adding a microbial inoculum and certain amounts of potentially limiting inorganic nutrients.

Litter collection and preparation

The samples were collected from the Vomb forest, that is situated at 55°40'03.9"N 13°35'13.0"E and presents 6 plant species. The Vomb forest is located in Scania, Sweden at approximately 20 km to the east of the city of Lund. The samples of *Betula* leaf litter and the *Deschampsia flexuosa* were collected in October 24th 2014 and were frozen until the day of the extraction. The twigs, needles, *Vaccinium myrtillus* leaf litter, *Vaccinium vitis-idaea* and the bark from the trees were collected in February 2015 from the same site. The species to study (Table 1) were: *Betula spp.* (birch)*,* which is a deciduous hardwood tree; *Picea abies* (Norway spruce) *and Pinus sylvestris (Scots Pine),* which are evergreen coniferous trees; *Vaccinium myrtillus* (European blueberry) that is a summer green shrub; *Vaccinium vitis-idaea* (lingonberry), which is a short evergreen shrubs; and *Deschampsia flexuosa* (wavy hair grass), which is a bunchgrass. Samples of leaf litter and wood litter were taken for each of the species, except for the *Vaccinium vitis-idaea* that presented difficulties when separating leaves from the rest of the plant, and the *Deschampsia flexuosa* that does not possess wood litter.

The leaf litter samples taken were the ones that showed to be about to fall from the trees. The leaves from *Picea abies* and *Pinus sylvestris* used in the experiment were the ones that showed brownish colors. The twigs were taken from trees that showed to be dead. In order to know if the tree was dead, several tree characteristics were evaluated. First, if the tree twigs showed a brown color instead of a green color when broken. Also, if the bark of the tree was lacking in some points of the trunk and there was no sign of recovering. Finally, if the tree showed branches on the ground around

it could suggest that the tree is dead. If these tree characteristics were present in the tree, branches and bark were taken from it.

The sampling was performed in an area of 100 square meters. The collection was done in two sampling spots for each species. Approximately 100 gr of each litter type were collected separately in previously marked self-adhesive bags. The number of plastic bags used for each litter type varied depending on the density of the litter. During the sampling, care was taken not to grind the litter in the bags.

Table 1. Abbreviations used for each sample. In order to identify leaf and wood litter, it has been placed an LL and a WL respectively at the end of each species. The *Vaccinium vitis-idaea* and the *Deschampsia flexuosa* had only one type of litter because of the difficulties of separating the wood and the leaf litter in the first case, and because of the absence of wood material in the *Deschampsia flexuosa.*

Sample	**Type**	**Assigned name (Abbreviation)**
***Betula spp.* leaf litter**	Summergreen tree	Betula LL
***Betula spp.* wood litter**	Summergreen tree	Betula WL
***Picea abies* leaf litter**	Evergreen tree	Picea abies LL
***Picea abies* wood litter**	Evergreen tree	Picea abies WL
***Pinus sylvestris* leaf litter**	Evergreen tree	Pinus sylvestris LL
***Pinus sylvestris* wood litter**	Evergreen tree	Pinus sylvestris WL
***Vaccinium myrtillus* leaf litter**	Summergreen shrub	Vaccinium myrtillus LL
***Vaccinium myrtillus* wood litter**	Summergreen shrub	Vaccinium myrtillus WL
Vaccinium vitis-idaea	Evergreen shrub	Vaccinium vitis-idaea
Deschampsia flexuosa	Grass	Deschampsia flexuosa

Sample cut and drying

The litter was cut and dried in order to have all the litter types in the same conditions that would lead to more accurate comparison of the extraction results (Schreeg 2011). Also, a litter drying was necessary for the carbon and nitrogen analysis. Each litter sample was cut with scissors to a size of 1 – 2 mm. The cut litter was placed separately by litter type in previously marked wet strength paper bags designed for drying. The bags were closed and placed inside a laboratory oven at 60 ºC for 48 hours.

Carbon and nitrogen analysis

An analysis of carbon and nitrogen composition of the litter was performed in order to find differences in the chemical composition of the litter and related to the DOC properties. The protocol for the sample preparation was designed based on the requirements of the Colorado Plateau Stable Isotope Laboratory of the Northern Arizona University.

The dried samples were pulverized using a food processing machine. The parts of the processing machine exposed to the litter were cleaned after every sample, so that the samples did not get contaminated. The pulverized litter samples were filtered using a metal sieve with a pore size of 250 µm. The metal sieve was washed with Mili-Q water and dried after being used in every litter type. The obtained fine powder was weighted using a micro analytical balance with 3 decimal places of a milligram. The amount of litter weighted per sample was between 4.00 to 6.00 µg. The samples were weighted inside 4x6-mm tin capsules. Three tin capsules were filled for each litter sample as replicates. Once the tin capsules contained the weighted sample, they were crushed into small balls and weighted again in order to confirm the final mass. All the samples were placed in a 96 well polyethylene plates. Each well in the plate had an alpha numeric position (rows A through H, columns 1 through 12). The

type of sample, an assigned name, weight and the respective alpha numeric position were written down in a paper form.

Once the plate was filled with the samples, it was closed and sealed before being sent to the Colorado Plateau Stable Isotope Laboratory of the Northern Arizona University. The samples were analyzed for carbon content and nitrogen content on an ECS4010 (Costech, Valencia, California, USA) or a NC2100 (Carlo Erba, Milan, Italy) Elemental Analyzer. The results of the analysis were given in percentage of carbon, percentage of nitrogen and carbon to nitrogen ratio.

Dissolved organic carbon extraction

The extractions were made based in the methodology suggested by Schreeg (2011) who evaluated several ways to perform DOC extractions in litter, referring to litter – solution ratio, oven dry temperature, size of the cut size of the litter and the speed at which the shaking process should be done. The procedure used in the present study is the one that Schreeg (2011) suggested as optimal for DOC extractions.

First, a portion of each sample was added to a 250 ml plastic bottle which contained Milli-*Q* water. The amount of sample added was 5 gr for every 100 ml of Milli-Q water in order to obtain a proportion of 1gr of litter for every 20 ml of Milli-Q water (Schreeg 2011). Two bottles were filled for every sample in every extraction time.

Milli-Q water is a type of purified water that has been defined as ‘ultrapure’ under the standards ISO 9001 v. 2000 and ISO 140001. It is produced by the Millipore corporation (Millipore 2015).

Moreover, the plastic bottles containing the samples and the Milli-Q water were set in a shaking table at a rate 180 oscillations per minute. The shaking intervals were 1, 4, 16, 24 and 48 hours. After every interval, the samples were subjected to vacuum filtration and through 1µm glass fiber filters. In the period of time between the shaking process and the beginning of the filtration, the samples were pre filtered using a sieve, so that the litter would stop leaching. After the filtration, the extracts were placed in 25 ml vials, marked and kept at 4 ºC for the further analysis.

Total organic carbon analysis

A TOC analysis was carried out in order to determine the amount of DOC present in each filtered extract. The TOC analysis was performed using a TOC-V CPN Shimadzu analyzer. This is a PC – controlled standard model (Shimadzu 2015).

The TOC analyzer uses combustion to determine the carbon concentrations. First, in order to eliminate the inorganic fraction of carbon, hydrochloric acid is added to the sample and then sample is sparged with sparge gas. Since some of the organic carbon might be lost with this procedure, the detected concentration is attributed to the non-purgeable organic carbon (NPOC).

Moreover, the sample is injected into the combustion tube where the NPOC of the sample is oxidized to CO_2. The CO_2 produced is detected by a non-dispersive infrared detector (NDIR) that is connected in series with a chemiluminescence detector.

The samples were diluted in order to be able to use the same calibration curve for all the measurements. Different dilution proportions were used for each extracted sample in order to obtain a concentration of carbon between 5 and 80 mg/l. A layer of Parafilm® was used to seal every vial. Two vials for every extract were placed in the TOC analyzer.

The DOC concentrations were evaluated as a function of time with a Michaelis-Menten and with a linear functions (Schreeg 2011).

Calculation of potential water extractable organic carbon

Schreeg (2011) did an approximation to simulate the extraction of the potential water extractable organic that can be obtained from litter sources using the Michaelis-Menten function.

The Michaelis-Menten model (Michaelis & Menten 1913) was originally an approach to enzyme kinetics. This function is an equation that relates the reaction velocity to the substrate concentration. The logic of the function is based on a system where an enzyme *E* binds reversibly to a substrate *S* to produce an enzyme-substrate complex

ES. Furthermore, a product *P* and the enzyme *E* are generated irreversibly from the complex *ES* (Eq. 5).

$$\boldsymbol{E + S \leftrightarrow ES \rightarrow E + P} \qquad \text{(Eq. 5)}$$

Under this logic, the equation that describes the system has been stated (Eq. 6).

$$\boldsymbol{v = \frac{V_{max}\,[S]}{K_M + [S]}} \qquad \text{(Eq. 6)}$$

The V_{max} represents the maximum velocity that the system can reach. K_m represents the the concentration of substrate at which the velocity of the system would be one half of the maximum velocity. [S] is the concentration of the substrate.

The plot of the Michaelis-Menten function (Figure 1) shows a curve with a slope that decreases overtime until the function reaches its maximum value at an asymptote defined as the maximum velocity of the system.

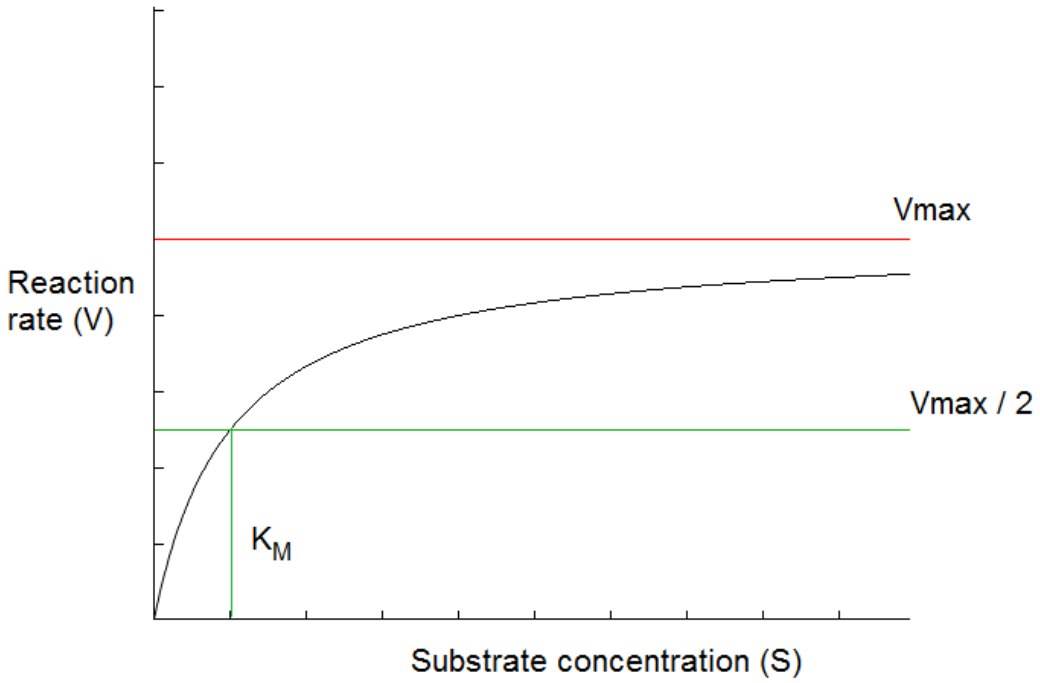

Figure 1. Michaelis - Menten saturation curve for enzyme reaction. The Vmax is the maximum rate that the system can achieve. The K_M is the Michaelis constant and represents the substrate concentration at which the reaction rate is half of the maximum Vmax. Adapted from "Die Kinetik der Invertinwirkung", by Michaelis, L. & Menten, M.L., 1913, *Biochem z*, pp. 333-369

The DOC extraction can be, thus, modeled using the Michaelis-Menten function (Schreeg 2011). In the extraction of DOC the concentration of TOC in the liquid phase will increase rapidly in the beginning of the experiment, but as time passes the concentration of TOC will not increase above a certain maximum value.

In the present study, the obtained DOC concentration data was simulated with the Michaelis-Menten model and with a linear model in order to determine if the DOC leaching can be adjusted to a Michaelis-Menten function.

Absorbance analysis

The absorbance was measured in Shimadzu UV-VIS 2600 Spectrophotometer equipment. The range of analysis was from 800 to 200 nanometers. This measurement was done in order to determine the different absorption values of the DOC extractions and furthermore calculate their respective percentage of

aromaticity. The utilization of the absorbance value as a tool to calculate the percentage of aromaticity was suggested by (Weishaar et al. 2003).

Degradation analysis

The degradation analysis was performed using a SensorDish reader (SDR) device, which is able to measure the concentration of dissolved oxygen in the samples over relatively short time steps (time step of 20 min was used in this study). This characteristic was of big importance for the purpose of the study because of the aim of characterizing relatively labile DOC degradation.

The DOC solutions were diluted to a concentration of 20 mg C /l in order to avoid excess in microbial production (Hongve et al. 2000), and furthermore were subjected to inoculation.

Inoculation

The inoculum used was a mix of an extraction from several order streams as well as from lakes around the region. The volume of inoculum added to the samples was 1ml for every 100 ml of sample (Kalbitz et al. 2003). It has been stated that the changes of DOC composition throughout the hydrological flow can make microbial communities adapt to different types of DOC (Myers et al. 2001; Judd & Kling 2002; Zak et al. 2003). However, Risse-Buhl et al. (2013) showed that when comparing microbial physiological capabilities, with inoculums obtained from soil, streams and ponds, leaded to a similar pattern in DOC consumption. Thus, it is expected that the results in the present study can be extrapolated to soil microbial DOC consumption.

Also, nutrients were added to the solutions. The nutrients used in the experiments were ammonium sulfate ($(NH_4)_2SO_4$) and monopotassium phosphate KH_2PO_4. The added amounts depended on the carbon concentrations in each solution. I aimed to obtain a final molar proportion of C:N:P:S:K ≤ 5:1:1:1:1 (Bowen et al. 2009).

The process of adding inoculum and nutrients started 8 days after the extraction period. In this period of time, the samples were kept in a refrigerator at 4ºC. This

delay was a necessary in order to analyze the DOC concentration of each sample. The DOC concentration was used to calculate how much the samples should be diluted in order to reach the targeted 20 mgC/l. The loss of DOC during this time was assumed to be small because the water had been filtered and then kept at a low temperature.

After the samples were taken out from the fridge, the samples remained at room temperature for approximately 5 hours, which is the amount of time that took to make the inoculum-nutrient-DOC solutions.

Dissolved oxygen measurements

The solutions and a duplicate for each sample were included in the experiment. The degradation experiments were performed in a SensorDish plate Reader (SDR). The duration of the experiment was for a period of 45 hours, with measurements of dissolved oxygen in each sample every 20 minutes. The vials used for the incubation had a volume of 5.2 ml. The amount added to each vial was enough to keep the sample without air bubbles that could interfere in the measurements.

Once the measurements of biodegradability were completed, they were analyzed with the software Matlab (Matlab, 2013). Since only degradability of the labile DOC pool is compared in this study, linear functions were attributed to each of the degradation curves in order to get a degradation rate.

It was found, during the incubation process, that concentrations of DOC in the samples (20 mg C/l) resulted to be too high for the amount of dissolved oxygen that the 5.2 ml SDR vials contained (~8 mgO_2/l). This fact was noted when, after approximately 45 hours of incubation, the vials ran out of dissolved oxygen. Furthermore, only the data corresponding to the first 45 hours of degradation were taken into account for this study.

Data treatment and statistics

DOC measurement

The measurement of DOC was performed in the TOC analyzer, which uses [mg/l] as a concentration unit. In order to have comparable values, the DOC concentration values were transformed to mg of DOC per grams of dry litter. Hence:

$$\boldsymbol{DOC}\ \left[\frac{\boldsymbol{mg}}{\boldsymbol{l}}\right] * \frac{\boldsymbol{0.02\ l}}{\boldsymbol{1000\ mg\ dry\ mass}} = \frac{\boldsymbol{mg\ DOC}}{\boldsymbol{g\ dry\ mass}} \qquad \text{(Eq. 7)}$$

Aromaticity

The absorbance was used to calculate the specific Ultra Violet absorbance (SUVA). The SUVA at 254 nm is strongly correlated to the percentage of aromaticity (Weishaar et al. 2003). Thus the $SUVA_{254}$ value was used to calculate the percentage of aromaticity of the DOC (Eq. 8).

$$\boldsymbol{SUVA_{254}} = \frac{\boldsymbol{UV_{254}}}{\boldsymbol{b{*}C}} \qquad \text{(Eq. 8)}$$

Where UV_{254} is ultraviolet absorption at 254 nm measured in the spectrophotometer, b is the optical path length in meters used by the equipment (0.01 m), and C is the DOC concentration of the samples in milligrams per liter measured in the TOC analysis.

Because of the correlation that exists between the percentage of aromaticity and the $SUVA_{254}$ in a solution (Eq. 2), an approach has been implemented in order to use the $SUVA_{254}$ to calculate the percentage of aromaticity (Weishaar et al. 2003):

$$\%\ \boldsymbol{Ar} = \boldsymbol{6.25} * (\boldsymbol{SUVA_{254}}) + \boldsymbol{3.63} \qquad \text{(Eq. 9)}$$

where %*Ar* is the percentage of aromaticity of the solution and $SUVA_{254}$ is the calculated value with Eq. 8 (L * mg C^{-1} * m^{-1}). The relationship between the percentage of aromaticity and the $SUVA_{254}$ were produced in a linear regression when comparing a known aromaticity of water samples with their respective value of $SUVA_{254}$. The linear regression resulted in a well fitted line with an r^2 value of 0.97 (Weishaar et al. 2003).

Units conversion of DOC degradation

In this study, the DOC degradation is estimated with the dissolved oxygen consumption. The respiratory quotient (RQ) is the ratio of metabolic gas exchange between the CO_2 and the O_2 consumed. Moreover, the RQ differs depending on the DOC type (Berggren et al. 2012). For instance, for carbohydrates, the RQ values is 1.000, for lipids is 0.696 and for proteins is 0.818 (Squires 1995). Moreover, since the most degradable part of the DOC is formed mainly by carbohydrates (Kalbitz et al. 2003), the RQ used in this study was 1.00. Thus,

$$((\boldsymbol{dO_2})_{\mathbf{0}} - \boldsymbol{dO_2}) \left[\frac{\boldsymbol{mg}}{\boldsymbol{l}}\right] * \frac{\mathbf{1}\,[\boldsymbol{mol\ O_2}]}{\mathbf{3200}\,[\boldsymbol{mg\ O_2}]} * \frac{\mathbf{1}\,[\boldsymbol{mol\ CO_2}]}{\mathbf{1}\,[\boldsymbol{mol\ O_2}]} * \frac{\mathbf{12000}\,[\boldsymbol{mg\ C}]}{\mathbf{1}\,[\boldsymbol{mol\ CO_2}]} *$$

$$* \frac{\mathbf{100}\,\%\ \boldsymbol{of\ initial\ TOC}}{\mathbf{20}\left[\frac{\boldsymbol{mg\ C}}{\boldsymbol{l}}\right]} = \boldsymbol{share\ of\ initial\ TOC}\ [\%] \qquad \text{(Eq. 10)}$$

Where $(dO_2)_0$ and dO_2 are the initial concentration of dissolved oxygen and the concentration of dissolved oxygen at the time (t) respectively.

Analysis of statistical significance

In order to compare the significance of differences between amounts of DOC leached by each litter type, a one way ANOVA was carried out. The ANOVA was conducted between the data of DOC concentrations of the extracts. Also, a one way

ANOVA was done in order to find significant differences between aromaticity values of the different extracts. In case of significance in the ANOVA with $p < 0.05$, a post hoc Tukey test was conducted in order to find significant differences between litter types.

For the evaluation of the significance of the positive correlation between percentage of nitrogen in dry litter and degradation rate of DOC a test for the significance of the correlation coefficient was done. Also, the relationship between the aromaticity of the DOC and its degradation rate was evaluated testing the significance of their correlation coefficient. The p value established as significant was $p < 0.05$. The ANOVA, the correlation coefficient significance test and the regression lines were performed in MATLAB, version 2013 (Mathworks Inc., Natick, MA, USA).

Results

Water Soluble Elements from Different Sources as a Function of Time – Hypothesis 1

The one way ANOVA test between DOC leaching data yielded significant variations among samples (p<0.05), F (9, 59) = 8.541, p = 1.33E-7. Furthermore, a Tukey HSD post hoc test was conducted finding significance (p<0.05) in amounts of DOC leached among litter types. Significant differences were found between *Betula* LL (mean = 117.58, standard deviation = 66.98) and *Betula* WL (mean = 36.90, standard deviation =18.02), and between *Vaccinium myrtillus* LL (mean = 131.63, standard deviation =62.2) and *Vaccinium myrtillus* WL (mean = 6.02, standard deviation =14.49). Moreover, no significant differences (p>0.05) were found between *Picea abies* LL (mean = 17.34, standard deviation =16.34) and *Picea abies* WL (mean = 6.05, standard deviation = 14.36) and between *Pinus sylvestris* LL (mean = 14.10, standard deviation =15.38) and *Pinus sylvestris* WL (mean = 7.44, standard deviation =14.33).

The leaf litter can be divided in two groups according to the obtained values of extracted DOC (Figure 2). The first group is *Picea abies* LL and *Pinus sylvestris* LL in one hand, which showed low amounts of water extractable organic carbon (they were not found to be significantly different in the Tukey HSD post hoc test, p>0.05). The other group is the *Vaccinium myrtillus* LL and the *Betula* LL which produced a higher amount of water extractable organic carbon (not significantly different to each other, p>0.05, when comparing with the Tukey HSD post hoc test). When comparing the values of extraction of any of the samples in the first group to the ones in the second group using the Tukey HSD post hoc test, the difference was statistically significant (p<0.05) for all comparisons.

The amount of water extractable organic carbon from the *Deschampsia flexuosa* was not significantly different to the values from needle leaves and from wood litter (p>0.05). However, they showed to be significantly lower than the values from the broad leaves (p<0.05).

Table 2. Parameterization of DOC extracted over time with Michaelis-Menten (MM) functions. Vm represents the maximum amount, or asymptote, of DOC that can be extracted per gram of dry litter [mg DOC / g dry litter]. K represents the time at which the DOC extracted is equal to Vm/2 [hours]. The "Maximum extraction value reached?" row shows if the extraction time of 48 hours was enough to reach the maximum extractable DOC of each sample. RMSE is the root mean square error of the measurements compared to the function.

	Parameter 1 (Vm) [mg DOC/ g dry litter]	**Parameter 2 (K) [hours]**	**Maximum extraction value reached?**	**R – square value**	**RMSE**
Betula LL	205	11.02	NO	0.918	4.82
Betula WL	48.71	1.982	NO	0.994	0.64
Picea abies LL	134.6	112.7	NO	0.727	1.08
Picea abies WL	10.78	8.332	YES	0.823	1.48
Pinus sylvestris LL	43.12	25.63	NO	0.982	4.31
Pinus sylvestris WL	26.15	37.41	NO	0.226	15.04
Vaccinium myrtillus LL	138.4	0.2055	YES	0.665	2.19
Vaccinium myrtillus WL	10.22	5.674	YES	0.982	3.31
Vaccinium vitis-idaea	142.2	25.89	NO	0.838	39.75
Deschampsia flexuosa	30.84	17.69	YES	0.918	3.25

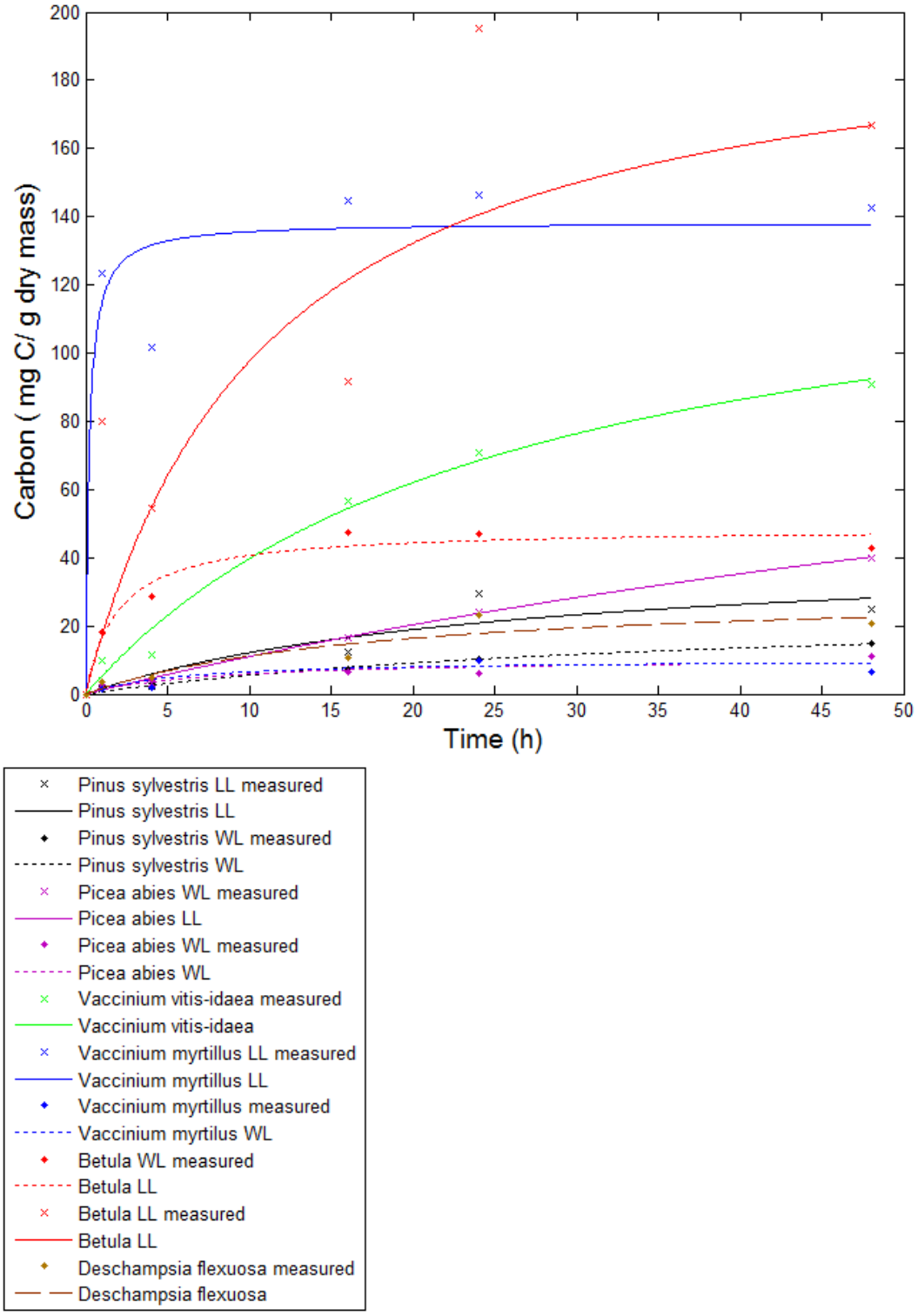

Figure 2. Amount of water extractable organic carbon obtained per gram of dry biomass plotted against the duration of the extraction time. The lines represent the Michaelis – Menten function adapted for the values of each sample. The data presented in dots represent the measured values of TOC for each sample at each extraction time. All the regression lines represent statistically significant relationships with the measured data ($p < 0.05$).

DOC leaching rate - Hypothesis 2

The obtained concentrations of DOC for each sample at different times were adequately predicted (Table 2) by the Michaelis-Menten function, excepting the *Vaccinium myrtillus* WL and LL litter, *Betula* LL and the *Picea abies* WL, where there were no signs of a plateau in DOC concentration, and thus a linear function had better fit (Figure 2).

The extraction period of 48 hours was enough for only 3 out of the 10 analyzed samples to reach the calculated maximum value of water extractable organic carbon (Vm). Only the *Picea abies* WL, the *Vaccinium myrtillus* LL and *Vaccinium myrtillus* WL reached the maximum value of water extractable organic carbon calculated (Table 2).

The sample that showed the highest amounts of DOC was the *Betula* LL even though the extraction time was not enough to reach the potential extractable concentration (Figure 2). The sample with the lowest amount of DOC extracted was the *Vaccinium myrtillus* WL.

Changes in Aromaticity as a Function of Time – Hypothesis 3

The values of aromaticity as a function of time were fitted to linear functions (Figure 3). Linear functions were used since, to the best of my knowledge, no function that describes the decreasing in DOC aromaticity as a function of extraction time has been established. All litter sources showed a decreasing trend in the aromaticity over time. The decreasing in aromaticity was more dramatic for the *Picea abies* Wood Litter, which decreased from 39.85% in the first hour to 9.51% after 48 hours. After the end of the study period, the aromaticity of almost all the samples decreased to a level of approximately 10% (Figure 3).

In the case of *Vaccinium myrtillus* Leaf Litter, the aromaticity remained almost constant throughout the study period, varying from 10.23% in the first hour to 9.49 after 48 hours. The sample with the lowest final aromaticity was the *Vaccinium vitis-idaea* with an aromaticity value of 5.7%, and the specie with the maximum final aromaticity was the *Deschampsia flexuosa* with 14.4% of aromaticity (Figure 3).

The one way ANOVA showed significant variations in aromaticity among litter type, $F(9,40) = 83.418$, $p < 0.05$. When comparing the leaf litter DOC aromaticity to the one from the wood litter using a post hoc Tukey test, none of the species showed significant differences ($p \geq 0.05$).

The leaf litter DOC aromaticity showed no differences among the species ($p \geq 0.05$). On the other hand, the only significant difference was found when comparing the aromaticity values obtained from *Deschampsia flexuosa* with *Betula* LL, *Betula* WL, and *Vaccinium vitis-idaea* ($p < 0.05$).

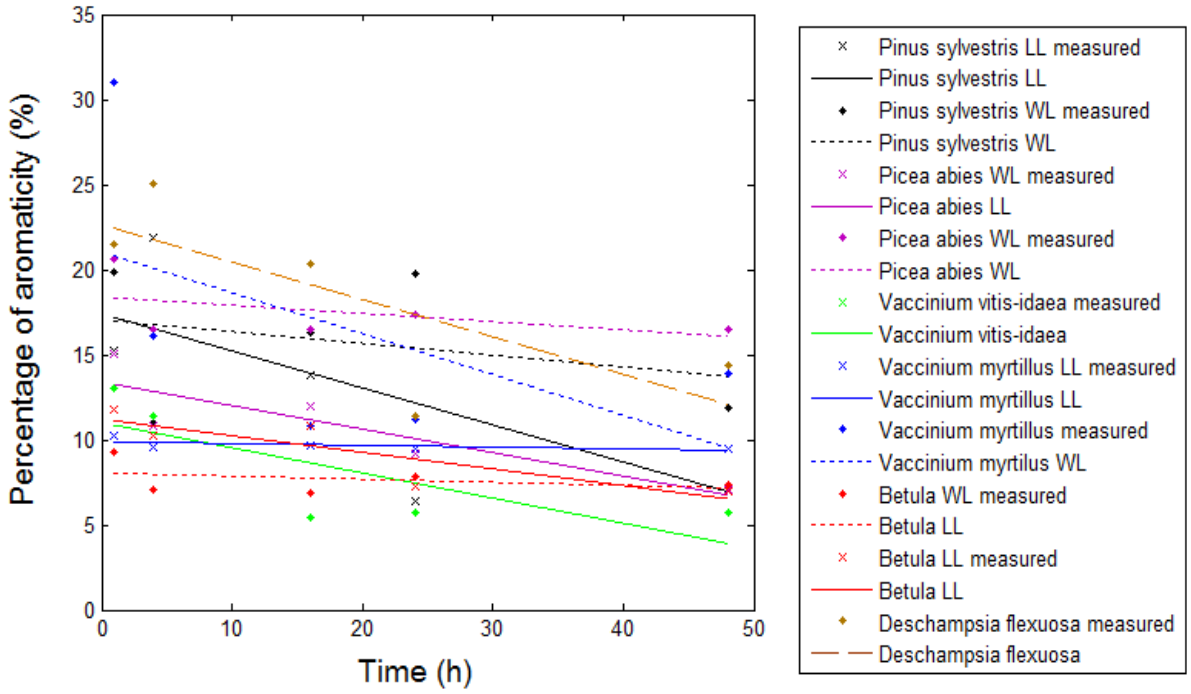

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Figure 3. Changes in DOC aromaticity as function of time. The percentage of aromaticity was calculated using the absorbance values at 254mm wavelength and the $SUVA_{254}$ – aromaticity relationship found by (Weishaar et al. 2003). The lines represent the simulated functions and the dots represent the calculated values.

Changes of biodegradability - Hypothesis 3

The results of the degradation experiment showed differences between extraction times (Figure 4) and between species (Figure 5). The samples that were extracted for 16 hours showed to be the most degradable in 8 out of the 10 cases (Figure 4). Only *Picea abies* LL and *Vaccinium myrtillus LL* showed a different pattern.

The sample that showed the higher total degradation rate was *Vaccinium vitis-idaea* and the lowest degradation rate was the one from *Pinus sylvestris* LL (Figure 4).

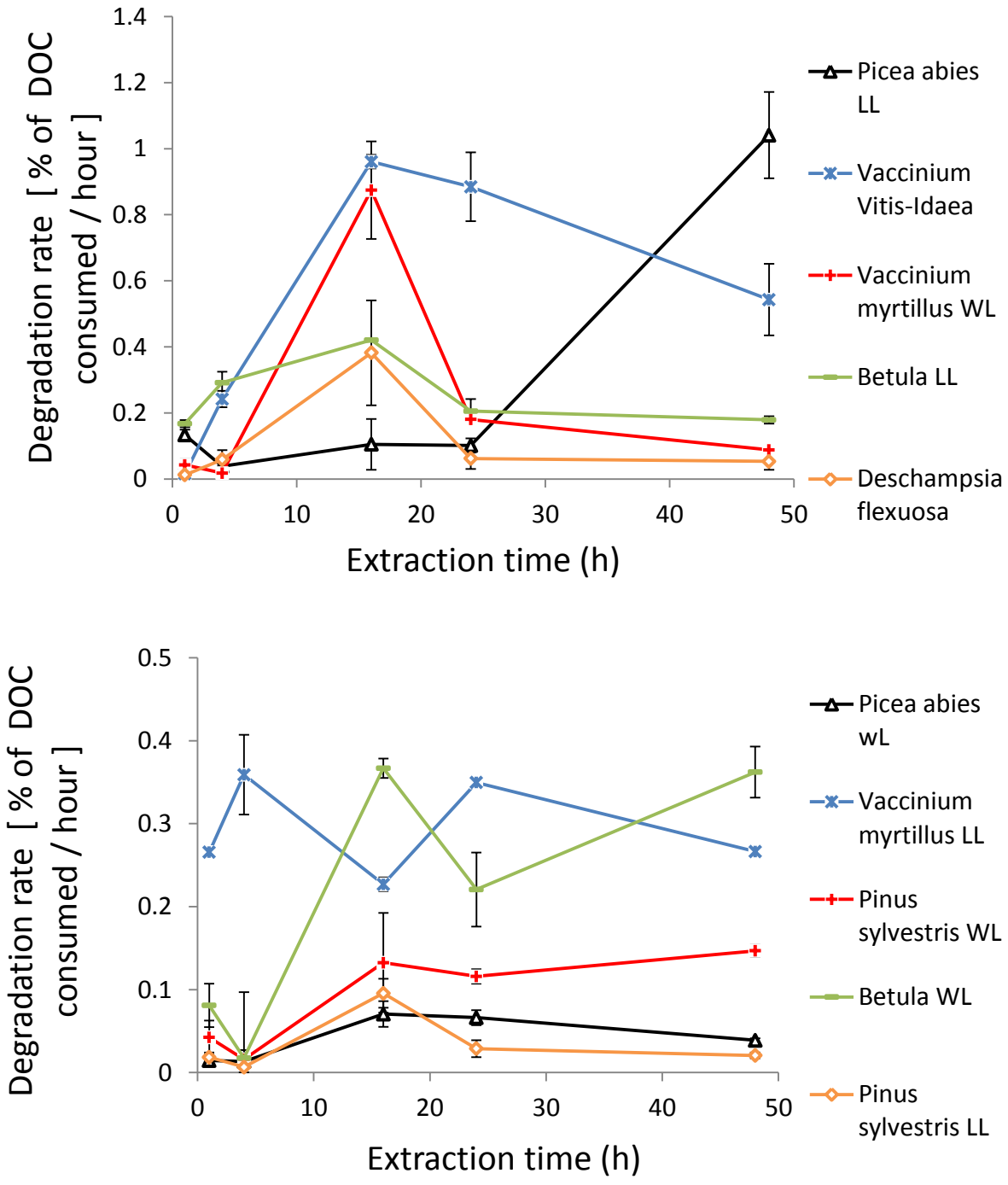

Figure 4 Degradation rate changes in time for all the samples studied. The degradation rates are shown with standard errors (n=2). The degradation rate shows how fast the DOC was consumed in the incubation experiment in the first 45 hours of experiment after the adding of nutrients and microbial inoculum.

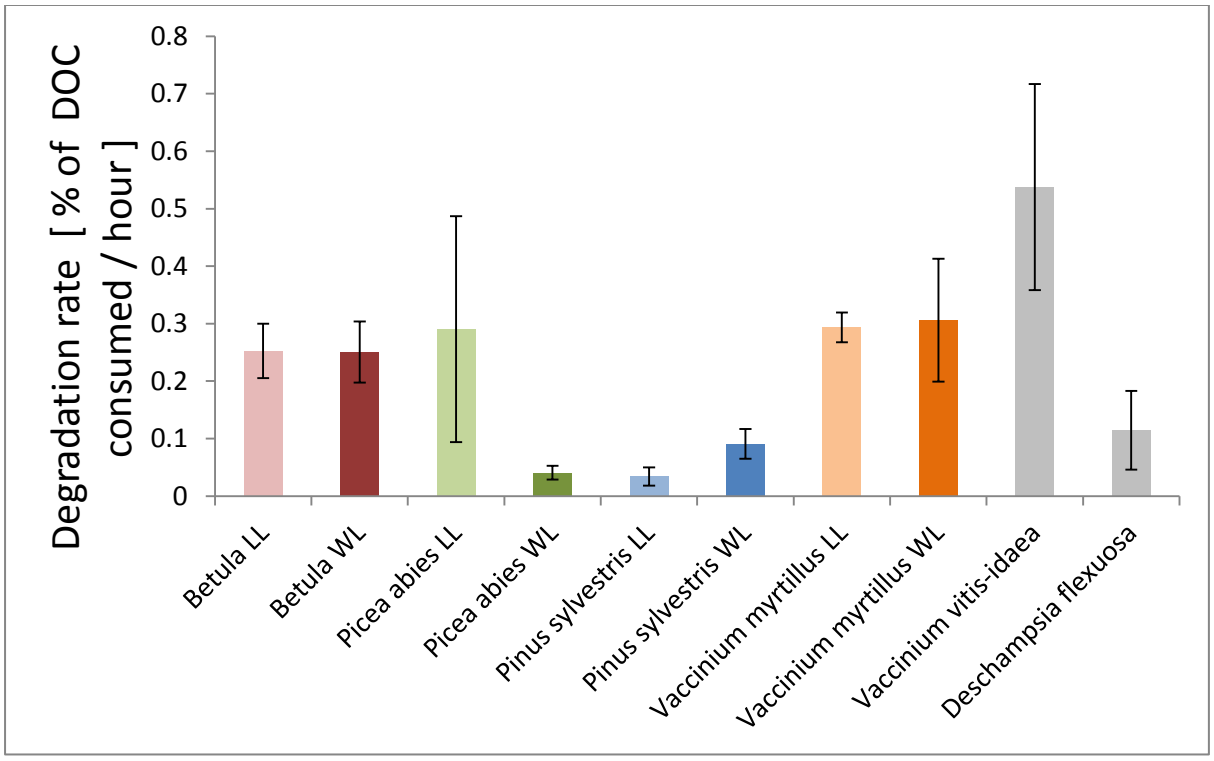

Figure 5 Mean degradation rates shown with standard errors (n=5). The mean degradation rate represents the mean of the slopes of the adapted DOC degradation line. The degradation rate shows how fast the DOC was consumed in the incubation experiment in the first 45 hours of experiment after the adding of nutrients and microbial inoculum. The graph shows an overview of the comparison in biodegradability of the DOC extracted from the studied species.

When comparing the rate of degradation of the samples to the measured aromaticity, a significant logarithmic correlation, like the one found by Kalbitz et al. (2003), was found in the test for analysis of the significance of the correlation coefficient ($p = 0.00024$, $n = 50$) (Figure 6).

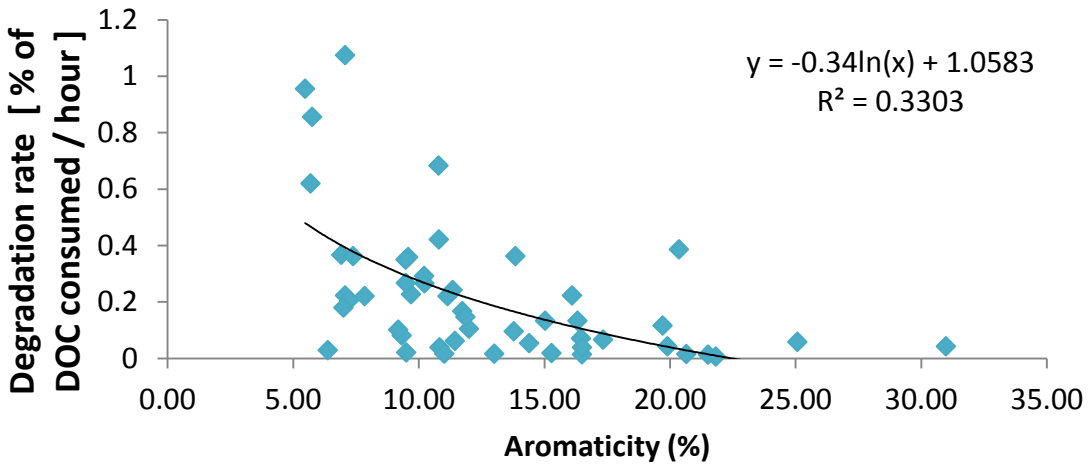

Figure 6. The relationship between the degradation rate of the DOC, which is the slope of the adapted degradation linear function, and the percentage of aromaticity calculated from the measure values of absorbance (p = 0.00024, n = 50). A logarithmic correlation was obtained.

The correlation between the nitrogen percentages in the litter source with the mean degradation rate (Figure 7) was significant with the test for analysis of the correlation coefficient (p = 0.0059, n = 10).

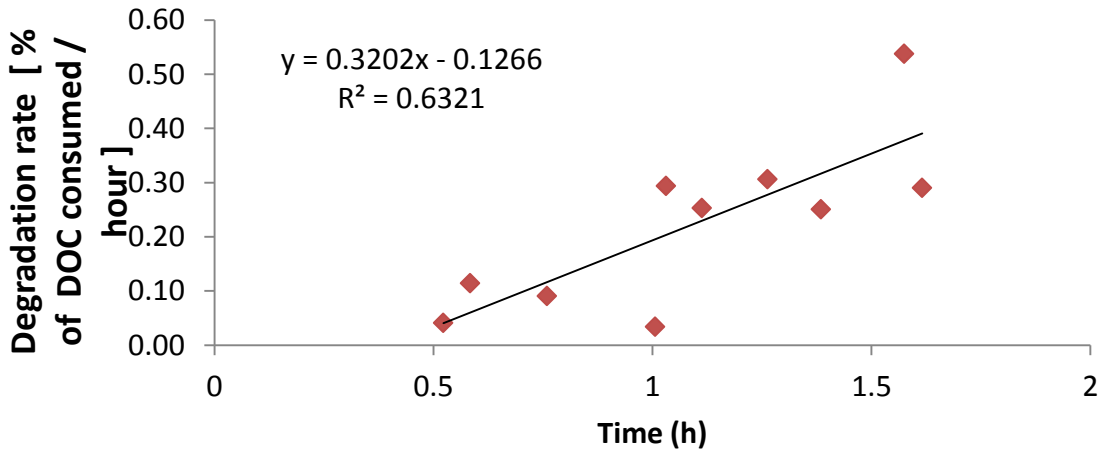

Figure 7. Relationship between the DOC degradation rate, which is the slope of the adapted degradation linear function, and the nitrogen percentage of the litter (p = 0.0059, n = 10).

Discussion

The results showed in general that the hypotheses could not be fully confirmed. The hypothesis that states that the amount of DOC extracted from leaf litter is bigger and more degradable than the one extracted from wood litter was not always the case. Also, some species, besides having similar chemical composition, leached significantly different amounts and types of DOC, suggesting that more factors, others than chemical composition, play role in the amount and lability of DOC leached. Moreover, since the aromaticity did not increase, it suggests that none, or few, lignin derived compounds were present in the extracted DOC. Furthermore, the degradability did not increase with time, suggesting changes in the composition of the DOC leached over time and also microbial activity during the extraction above the 16 hours.

DOC production

Hypothesis 1 referring to DOC leaching

The first part of the first hypothesis of this study was not confirmed. This happened because no significant differences were found between the leaf and the wood litter of the *Picea abies* and the *Pinus sylvestris* species.

The fact that the water extractable organic carbon from the leaf litter of the needle leaves did not differ significantly from their respective wood litter shows that the amount of DOC that will leach from these leaf litters will be on the same order than the ones from the wood litter. This statement represents a contradiction to the relation suggested by Parton (1994) which groups separately the leaf and the wood material when calculating the water extractable organic carbon.

Previous studies about lignin content on leaf litter (Johansson 1995) show low or no differences in lignin content between broad leaves and needle leaves. In this context, and taking into account the Parton et al. (1994) formula to calculate water extractable organic carbon, the concentration of DOC leached from needle leaves and broad leaves should be on the same order, which was shown to be inaccurate according to the results in this study (table 3). This fact can reveal that the amount of

water extractable organic carbon does not depend only on the amount of lignin of the species but also on the structure of the litter.

The differences in the amount of water extractable organic carbon between the needle leaves (*Picea abies* LL and *Pinus sylvestris* LL) and the broad leaves (*Betula* LL and *Vaccinium myrtillus* LL) could be attributed to the thickness of the cuticule in the type of leaves. The cuticule, which is a wax that prevents the water loosing through evaporation (Schreiber & Schonger 2009), is almost twice thicker in needle leaves compared to broad leaves (2,64 µm for *Vaccinium myrtillus,* 6.5 µm *Pinus sylvestris,* 4.9 µm *Picea abies*, 1.9 µm *Betula)*(Baig & Tranquillini 1975; Semerdjieva et al. 2003; Connor & Lanner 1991; Ashton et al. 1998). The presence of this tick wax may be preventing the needle leaves to loose inner substances when they are exposed to water in the same way that it prevents the needles to loose water to the environment. These results were also found by (Kalbitz et al. 2003) and were attributed to the difference in the thickness of the epidermic layers of the leaves.

The fact that the amount of water extractable organic carbon from the *Deschampsia flexuosa* was as low as the values obtained from wood litters and from needle leaves suggests that the grass is not a big source of water extractable organic carbon like the broad leaf litter is. This result was contrary to what was stated in hypothesis 1 when it was expected to obtain the same amounts of water extractable organic carbon from *Deschampsia flexuosa* than the rest of broad leaves litters. Moreover, it has to be taken into account that the samples of *Deschampsia flexuosa* used in this project were wilted by the time that the samples were taken from the forest. This fact might have leaded to an underestimation of the amount of DOC leached from *Deschampsia flexuosa* since it was exposed for an unknown amount of time to a natural leaching process in the environment before being extracted for this experiment.

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Hypothesis 2 referring to DOC leaching rate,

The second hypothesis was confirmed in this study for the broad leaves (*Betula* and *Vaccinium myrtillus*) but not for the needle leaves (*Picea abies* and *Pinus sylvestris*). The amount of DOC leached got close to the potential amount in the first hours for the broad leaves while their wood parts continued leaching. In the other hand, the

needle leaves did not follow the same pattern when leaching and their leaching rate did not decrease during the first extraction hours.

The part of the material that does not leach as DOC in the extractions might be the part that needs microbial activity for decomposition, mostly lignin, which would increase the amount of water extractable carbon transferred from the litter to the ecosystem over longer periods of time (Don & Kalbitz 2005). In order to estimate the rate at which the portion of litter, that is not the water extractable carbon, passes to the ecosystem as DOC, experiments using microbial degradation on the stable matter should be performed.

The fact that the water extractable organic carbon concentrations over time fitted with the Michaelis-Menten functions means that the leaching of water extractable organic carbon from a litter source in the first hours follows the logic explained by this function even though the extraction time was not enough to reach the maximum extractable DOC for 6 out of the 10 samples. The logic in the Michaelis-Menten function states that after a certain value of the on the independent variable, the dependent variable will not increase reaching an asymptote. This asymptote in the present study is represented by the maximum amount of water extractable organic carbon that can be extracted from a litter source before the microbial degradation of the lignin starts playing a significant role. These results corroborate the results by Schreeg (2011) who also managed to fit Michaelis-Menten functions to extracted DOC using different litter to solution ratios.

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Aromaticity of DOC

Hypothesis 3 referring to aromaticity

The third hypothesis stated in this study suggested that the aromaticity of the DOC extracted will increase over time. This fact could not be supported, and even a decrease of aromaticity was detected. The results were contrary to what was expected with the hypothesis.

Don & Kalbitz (2005) found that the amount of humic substances, measured with humification index, increased as degradation of the litter, measured in litter weight loss, increases. The humification index has been related to the percentage of aromaticity of the DOC (Kalbitz et al. 2003). The fact that aromaticity lightly decreased over time in the present study can suggest that little or no products of litter decomposition were present in the extracts. Also, the mass loss that Don & Kalbitz (2005) found represented up to 50% of the initial litter mass, which is considerably higher than the mass loss found in the present study (maximum ~20% of initial litter mass).

The small difference between the aromaticity of the water extractable organic carbon from the leaf and the wood litter of *Betula,* the *Pinus sylvestris* and the *Vaccinium myrtillus,* suggests that the separation of wood and leaf litter when trying to understand the DOC produced is not accurate. *Picea abies* was the only with significant differences in aromaticity between the wood and leaf litter. This, together with the results of the DOC production rate experiment, suggest that the estimation of water extractable organic carbon from the different sources should also take into account, besides the chemical composition, the plant structure which has been shown to play an important role.

The fact that the difference in aromaticity between the leaf litters was not significant might suggest that the compounds that are being obtained during the first hours of extractions come from the same cell structures and not from litter decay.

The similarity in the aromaticity of the water extractable organic carbon from the *Deschampsia flexuosa* with the ones obtained from the wood litters shows that when leaching DOC the *Deschampsia flexuosa* behaves more like a wood litter rather than a leaf litter. Moreover, the samples were collected from wilted grass in the field, which could suppose a significant loss of DOC with low aromaticity leaving only the lignin derived DOC which is more aromatic (Hernes et al. 2013).

The period of time used is not enough to determine more dramatic changes in aromaticity like the changes in humification index shown by Don & Kalbitz (2005). Since the products of the decay of the molecules of lignin are mostly aromatic and occur over longer periods of time, it can be speculated that the aromaticity should increase over time in periods up to one year as found by Don & Kalbitz (2005) .

Degradation rates

Hypothesis 3 referring the lability of DOC

The hypothesis 3 referring to the degradation of DOC could not be supported. This hypothesis stated that the lability of the DOC was expected to increase over time. Instead the degradation rate peaked at 16 hours. This fact suggests that the degradation of the components of the DOC changes over time and can be due to two factors. First, the extracted compounds change in the solution and become more degradable as time passes, reaching a peak around 16 hours. Second, the liberated DOC after 16 hours might start to get more stable than the one that is liberated in the first 16 hours. Also, Schreeg(2011) warned that with extraction periods above 24 hours, the autochthonous microorganisms might start interacting with the DOC, meaning that during the extraction process labile DOC is starting to get consumed inside the plastic bottles leaving only the more stable DOC. According to Marschner & Kalbitz (2003), a more stable pool can be derived from previous degradation steps.

The hypothesis that stated a correlation between aromaticity and degradation rate could be confirmed with the results (Figure 6).

Hypothesis 1 referring lability of DOC

The hypothesis 1 referring to lability stated that the DOC will be more labile when is originated from a wood litter source than from leaf litter sources. However, this hypothesis could not be proven since the results of the degradation of Pinus sylvestris LL water extractable organic carbon showed that this litter type had the lowest mean degradation rate. This fact suggests that the rate at which the water extractable organic carbon from leaf litters is consumed differs widely between species depending not only on the chemical composition of the litter but also on their internal structure. The influence of the chemical composition, and especially of the nitrogen percentage, could be confirmed with the results obtained, which showed a correlation between the nitrogen content of the litter with the degradation rate of the water extractable organic carbon.

Future directions

The results of the present study show that the difference in quantity and quality of the DOC produced between wood and leaf litter varies depending on plant species and on physiological characteristics of the plant parts. In this context, the estimation of the type and amount of DOC produced from plant litter is often subject to inaccurate assumptions. Thus, DOC production and lability in global carbon cycle models might be overestimated in some cases, and underestimated in some others. Because of the importance that DOC represents for the global carbon cycle, and thus in simulations of future scenarios under global warming conditions, more research in the production and fate of DOC in ecosystems is needed.

The collection process of leaves, twigs and tree bark can be decisive in the quantity and characteristics of the extracted DOC. Thus, it is desirable to have leaves, branches and tree bark in the exact state in which they naturally come in contact with the ground and begin their process of degradation.

The percentage of aromaticity can give an idea on the types of chemical compounds that are present in DOC solutions. However, by doing a fluorescence analysis and a further PARAFAC model it would be possible to identify in a more specific way the different components of extracted solutions.

Because of the unexpected DOC difference, in amounts and lability, between wood litter from twigs and wood litter from tree bark, it is recommended to perform future studies focused on distinguishing the variations in DOC characteristics among plant parts.

A degradation experiment with concentrations lower than 5 mg C/l can lead to a better understanding of the degradation of the most labile part of the extracts.

Conclusions

The research aim of the study was to test if the assumptions that are made in the determination of the amount of DOC leached from litter, the types of DOC and the lability of them, can be applied in a broader context. In this study, it was found that the assumptions can be used only up to a certain point when more factors start playing an important role in the DOC dynamics. These factors, such as plant physiology, have been shown in this study to play a major role when determining the DOC production and characteristics.

In this context, the results of this study are expected to stimulate further projects regarding more approximations of the characterization of amounts and types of DOC leached from litter based on chemical but also on physiological characteristics of the litter source.

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