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DYNAMICS OF DISSOLVED ORGANIC MATTER IN FOREST ECOSYSTEMS  
IN FLANDERS

Thesis submitted in fulfillment of the requirements for the degree of  
Doctor (PhD) in Applied Biological Sciences:  
Land and Forest Management

Dutch translation of the title:

DYNAMIEK VAN OPGELOST ORGANISCH MATERIAAL IN  
BOSECOSYSTEMEN IN VLAANDEREN

Illustrations on the cover:

The Overheide site located in forest reserve (front) and typical sequence of mineral soil horizons in a podzol profile at the Overheide site (back).

Citation: Vandenbruwane J. (2008). Dynamics of dissolved organic matter in forest ecosystems in Flanders. PhD thesis, Ghent University, p. 252.

ISBN-number: 978-90-5989-217-0

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## Woord vooraf

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*If all the year were playing holidays,  
to sport would be as tedious as to work.*

(W. Shakespeare)

Eindelijk! Het is er. Dat lang-verwachte en vaak-vervloekte “DOM”-boekje... Vier jaar lang hebben heel wat mensen met enthousiasme hieraan meegewerkt en zonder al deze helpende handen zou dit werk hier-en-nu niet zijn.

Stefaan en Georges; eerst en vooral dank om mij vier jaar geleden op dit project aan te werven. Bedankt ook voor de ruimte, het vertrouwen en de mogelijkheden die jullie mij geboden hebben om dit onderzoek te voeren. Daarnaast wil ik jullie bedanken voor jullie inzet bij het oneindig lezen, corrigeren en bijsturen van mijn schrijfselen.

Prof. Qualls, I’m very grateful for your hospitality, your well-founded advices and your motivating interest in this work. The stay at your department was an essential catalyst for the establishment of this manuscript. Thank you very much!

Daarnaast wil ik iedereen van de vakgroep bedanken voor de vier leuke jaren en de aangename, amuzante en familiale sfeer op “het eerste”. Tina en Sophie, bedankt voor de duizenden dringende en nog-dringendere analyses die jullie altijd met een glimlach hebben voltooid. Mathieu en Luc, bedankt voor de hulp bij het veld- en labowerk en voor de eerste-hulp-bij-droge-kelen. Mathieu, bedankt voor de afmattende, maar leuke fietstochten en ik hoop dat er nog veel zulke mogen volgen. Annemie, Ben, Bram, David en Sara; bedankt voor jullie aanmoedigingen. Steven, dank voor het handhaven van de orde, discipline en hiërarchie op

Eiland 134! Ook bedankt voor de buiten-professionele discussies. Daarnaast wil ik ook alle thesisstudenten bedanken die elk hun steentje hebben bijgedragen tot dit werk (Stijn, Koen, Monica, Veronica, Cesar en Winnok).

De goede samenwerking met het labo voor bosbouw was onontbeerlijk voor het succes van dit werk. In het bijzonder wil ik An en Guy bedanken om dit onderzoek op de rails te helpen zetten en de juiste richting aan te geven! An, ook bedankt voor de vele verhelderende gesprekken. Karen, Leen en Luc; bedankt voor de spannende tweewekelijkse ‘expedities’ naar Ravels en omstreken. Luc en Greet; heel erg bedankt voor de voorbereiding en analyses van de vele stalen en zoveel meer.

Kris, na 9 jaar ronddobberen in hetzelfde schuitje scheiden onze wegen... Maar ik ben ervan overtuigd dat onze wegen nog vaak zullen kruisen, zij het met één of ander schuimende-vloeistof-bevattend recipiënt in de hand...

Vader en moeder, Hendrik en Lisbeth; bedankt voor het warme nest waarin ik ben opgegroeid en waar ik nog altijd mag binnenfladderen! Vader en moeder, bedankt ook voor de vrijheid, het vertrouwen en de mogelijkheden die je ons hebt gegeven om ons te ontplooien!

Eveline, bedankt voor het verdragen van al mijn zottigheden, voor je goeie zorg, je luisterend oor en voor het afleiden van mijn gedachten! En weet: ik zie je graag!

Jeroen, januari 2008.

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## Symbols and Abbreviations

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$\alpha$	shape parameter in Van Genuchten equation
$\lambda$	exponent in the Mualem hydraulic conductivity function
$\theta_{\text{res}}$	residual water content
$\theta_{\text{sat}}$	saturated water content
$\ell_{\text{root}}$	relative root density
$a$	parameter accounting for presence of native adsorbed substances
$a$	y-intercept in the adapted Langmuir equation
$\text{Al}_{\text{ox}}$	oxalate extractable aluminum
$b$	parameter related to maximum adsorption capacity in the asymptotic negative exponential isotherm
$b$	intercept in the initial mass isotherm
$b$	parameter related to energy of adsorption in the Langmuir isotherm
BDOC	biodegradable dissolved organic carbon
C	amount of DOC or DON released during first extraction
CI	confidence interval
$C_i$	initial concentration
$C_e$	equilibrium concentration
CE	cellulose ester
CEC	cation exchange capacity
CL	critical load
CP	corsican pine stand
CPN	corsican pine stand under elevated N input
CRMa	coefficient of residual mass
CRM	certified reference material
$C_{\text{tot}}$	total carbon
DIC	dissolved inorganic carbon

DIN	dissolved inorganic nitrogen
DOC	dissolved organic carbon
DOC <sub>np</sub>	null-point DOC concentration
DOM	dissolved organic matter
DON	dissolved organic nitrogen
D <sub>root</sub>	root depth (SWAP)
DVS	crop development stage (SWAP)
DW	deionized water
ED	electrodialysis
EF	modeling efficiency
Fe <sub>ox</sub>	oxalate extractable iron
HTC	high temperature combustion
HTCO	high temperature catalytic oxidation
IEP	iso-electric point
IM	initial mass isotherm
IR	infrared
IRMS	isotope ratio mass spectrometer
K <sub>d</sub>	distribution coefficient
K <sub>sat</sub>	saturated hydraulic conductivity
k	parameter related to energy of adsorption (in asymptotic negative exponential isotherm)
k	release coefficient
k	freundlich parameter related to adsorption capacity
m	regression coefficient in the initial mass isotherm
MBC	microbial biomass carbon
MBN	microbial biomass nitrogen
MDL	method detection limit
ME	relative mean error
MWCO	molecular weight cut-off
N	number of extractions
n	Freundlich parameter related to adsorption intensity

n	shape parameter in Van Genuchten equation
N <sub>tot</sub>	total nitrogen
p	free throughfall coefficient (SWAP)
PES	poly-ethersulfone
PLFA	phospholipid fatty acid
PO	alkaline persulfate oxidation
POC	particulate organic carbon
PON	particulate organic nitrogen
PVC	polyvinyl chloride
Q <sub>o</sub>	maximum adsorption capacity in Langmuir isotherm
q <sub>e</sub>	amount of substance removed from or released to solution
R	cumulative amount of DOC or DON released
r <sub>crop</sub>	minimum crop resistance (SWAP)
RE	release or removal of DOM in initial mass isotherm
R <sub>max</sub> + C	total amount of DOC or DON that can be released from the soi
RMSE	root mean square error
RO	reverse osmosis
rpm	revolutions per minute
RSP	reactive soil pool
S	storage capacity of canopy (SWAP)
SB	silverbirch stand
SC	soil cover fraction
SOM	soil organic matter
TDN	total dissolved nitrogen
TSOC	total soluble organic carbon
TSOM	total soluble organic matter
TSON	total soluble organic nitrogen
WCO	wet chemical oxidation
X <sub>i</sub>	initial DOM concentration in solution
ZPC	zero point of charge



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# CHAPTER 1

## INTRODUCTION

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Illustration on p.1:

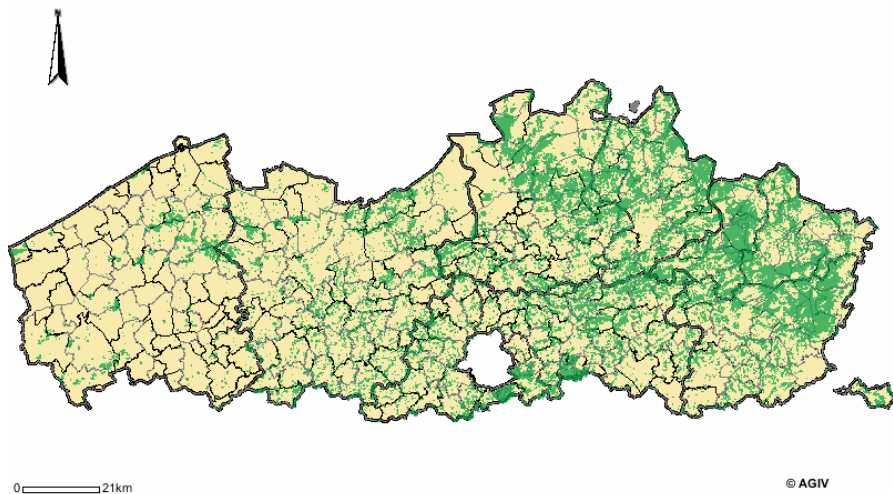
Open-field bulk precipitation collectors set up in a grassland.



## FOREST ECOSYSTEMS IN FLANDERS

### *Forest area, distribution and composition*

Flanders is one of the least forested (after Ireland and Denmark) and one of the most densely populated areas of Europe. The total forested area in Flanders amounts to 105 621 ha or only 8.3 % of the territory. The forest area in Flanders is very unevenly distributed, with particularly low percentages of forests in the provinces of West- and East-Flanders (Figure 1.1). The largest forest areas are mainly situated in the Campine area, covering parts of the provinces of Antwerp and Limburg. The forest area in Flanders is very scattered with more than half of the total area in units smaller than 100 ha (EFI, 2007).



**Figure 1.1. Forest (green) area and distribution in Flanders region resulting from forest mapping in 1990 (Source: Agiv, 2007)**

Although the wooded area stabilized from the post World War I era to present time, the public vision on the function of forests in Flanders has been changing constantly over the last century. Parallel to this vision, the forest policy and tree species composition has altered continuously. The industrial revolution and the two World Wars have largely impacted the natural character of forests in

Flanders. After 1945, the large demand for coniferous wood (esp. Scots pine) to provide props for the coal-mines led to large homogeneous coniferous plantations on sandy soils (like the Campine area). During the 70s and 80s mine pits were closed down and the intention grew to transform these former coniferous production plantation into multi-functional forests. In 1990, the Flemish Government Act on Forests was established which focussed on this multifunctionality of forests in Flanders. The different forest functions were grouped as: economic, social and educational, shelter, ecological and scientific functions. The gradual conversion from homogeneous coniferous forest stands towards heterogeneous mixed forests was one of the central points of an implementation order of 1991. As a result, the portion of coniferous forests to the total area decreased gradually from 53.3 % in 1970 to 39.1 % at present (EFI, 2007). Deciduous forests in Flanders are mainly composed of poplars, followed by oak and beech respectively.

The strategy of systematic conversion from coniferous to indigenous deciduous tree species will undoubtedly affect the overall biogeochemistry of these forest ecosystems (Menyailo et al., 2002). De Schrijver (2007) reported higher fluxes of anions (esp. nitrate and sulfate) and base cations under conifers compared to deciduous trees, resulting in a higher impoverishment and reduced buffering potential of the mineral soil. Differences in scavenging atmospheric pollution, root uptake and litter quality between both forest types will alter nitrogen cycling in the forest ecosystems in Flanders.

### *Nitrogen deposition*

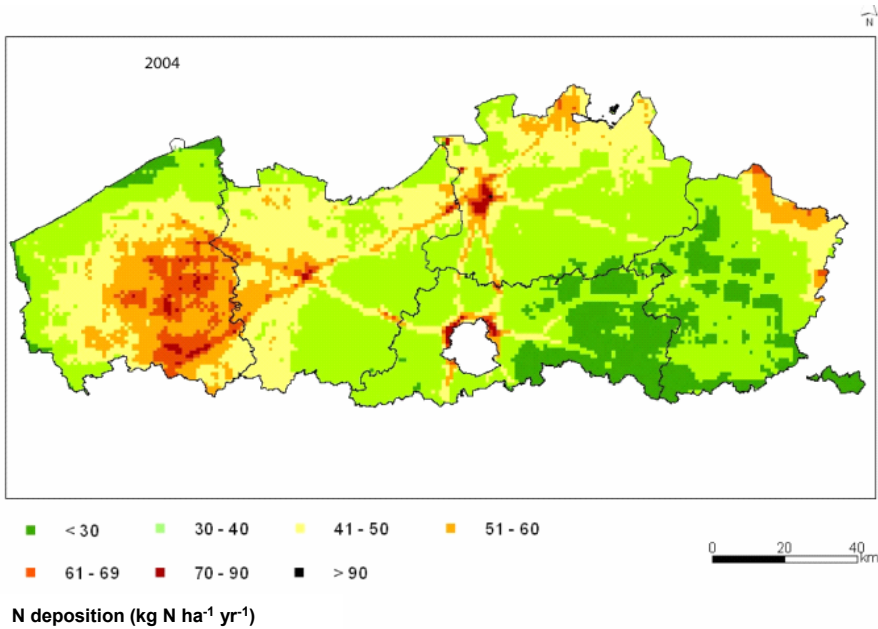
Nitrogen emissions to the atmosphere due to human activities remain elevated in industrialized regions of the world and are accelerating in many developing regions (Asman et al., 1988; Galloway et al., 2004). Approximately half of the total nitrogen deposited in Flanders region originates from abroad (Table 1.1). Total nitrogen emission is compounded of oxidized nitrogen forms ( $\text{NO}_y$ ) mainly originating from the combustion of fossil fuels and reduced nitrogen forms ( $\text{NH}_x$ ) principally emitted by agricultural activities.

**Table 1.1. Contribution of the different sectors to nitrogen deposition in Flanders region (Source: VMM, 2004)**

Sector	Contribution to NO <sub>y</sub> deposition (%)	Contribution to NH <sub>x</sub> deposition (%)	Contribution to total N deposition (%)
Housekeeping	3.6	0.0	1.6
Industry	3.8	0.7	2.1
Energy	2.6	0.0	1.2
Agriculture	6.1	48.7	29.5
Transport	32.0	1.9	15.5
Trade & Services	1.2	0.1	0.6
Import	50.7	48.5	49.5
<i>Total</i>	<i>100</i>	<i>100</i>	<i>100</i>

Since the agricultural and transport sectors are the main local emission sources in Flanders, the spatial distribution of the deposition of N from these low elevation sources are very uneven and located close to the source. Highest deposition of NO<sub>y</sub> is found along principal highways and around the big cities, whereas NH<sub>x</sub> deposition is mainly located in the regions with intensive livestock breeding (VMM, 2004). The spatial distribution of the total nitrogen deposition in Flanders is given in Figure 1.2. The average N deposition per community ranged from 22 to as much as 72 kg N ha<sup>-1</sup> in 2004 (MIRA, 2006). Although N deposition in Flanders shows a slightly, but significantly decreasing trend since 1990, depositions higher than 60 kg N ha<sup>-1</sup> still occur in the centre of West-Flanders, along the arterial roads and to a limited extent in the very north of the Campine Region and the northeast of the province of Limburg. Depositions are at present below 30 kg N ha<sup>-1</sup> in the eastern part of Flemish-Brabant and the south of the province of Limburg. This value is also the target set for average Flemish deposition in 2010.

Due to the roughness and the large contact surface with the atmosphere, forests are efficient air filters for capturing dry deposition in particular (e.g. dust particles, aerosols). Comparison of the deposition loads in forest stands and adjacent non-forested parcels (open field) indicated that the N deposition under forest is almost double than that in the open field (Genouw et al., 2006).



**Figure 1.2.** Spatial distribution of nitrogen deposition ( $\text{kg N ha}^{-1} \text{ yr}^{-1}$ ) in Flanders in 2004 (Source: VMM, 2004)

The large input of N containing compounds into natural ecosystems has serious consequences for the nutrient status (Magill et al., 1997) and overall ecosystem functioning (Aber et al., 2003; Magill et al., 2004). Direct results of excessive N input into (semi-) natural ecosystems are accelerated soil acidification (Vogt et al., 2006), lower biodiversity and nitrate leaching (MacDonald et al., 2002). The term *critical load* (CL) was introduced to express the tolerance of natural and semi-natural ecosystems to anthropogenic air pollution (Nillson and Grennfelt, 1988). It refers to the maximum input of one or more pollutants into the ecosystem concerned which does not lead to any significant harmful effect according to present knowledge. Critical loads can be determined based on the effects on e.g. biodiversity, forest vitality or nitrate leaching to groundwater. For Flemish forest ecosystems, critical load values were calculated for 1425 forest plots by Langouche et al. (2002) for two criteria: protection of herbal biodiversity and protection of undep groundwater for nitrate pollution (Table 1.2). The nitrogen deposition in the Flemish forests largely exceeds these critical loads.

**Table 1.2. Median critical load values for nitrogen and their exceedances in forest ecosystems (Adopted from Langouche et al., 2002)**

	Protection of Biodiversity		Protection of undep groundwater	
	Coniferous	Deciduous	Coniferous	Deciduous
Median critical load value (kg N ha <sup>-1</sup> yr <sup>-1</sup> )	10	15	26** (41)***	40 (69)
Median exceedance (kg N ha <sup>-1</sup> yr <sup>-1</sup> )*	36	26	22 (7)	0.2 (-27)
% of forest area where CL are exceeded	100	100	99 (69)	53 (12)

\* Median exceedance is the median value of the differences between the nitrogen deposition and the calculated critical load value for a total of 1425 forest plots;

\*\* Value calculated using the ecological value of 25 mg NO<sub>3</sub><sup>-</sup>-N l<sup>-1</sup> in percolating water;

\*\*\* Value calculated using the drinking water standard of 50 mg NO<sub>3</sub><sup>-</sup>-N l<sup>-1</sup> in percolating water.

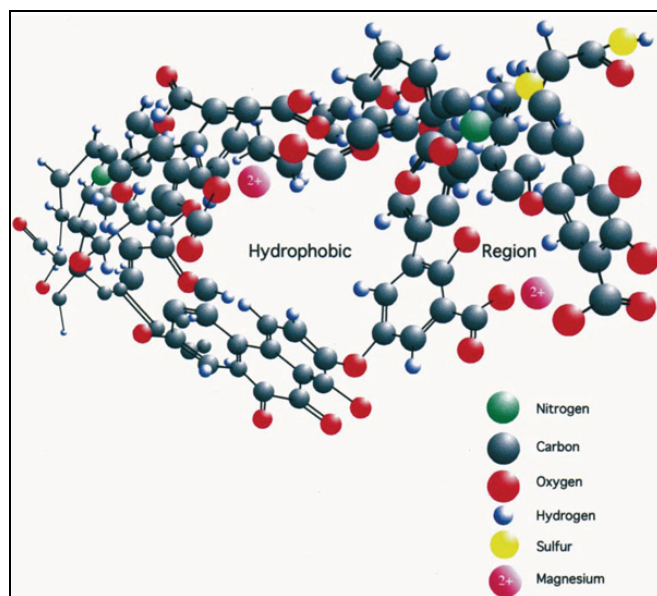
However, the high atmospheric nitrogen inputs in forest ecosystems in Flanders, and thus exceedance of the CL values (Table 1.2), has been going on for decades (Asman et al., 1988). Chronic nitrogen additions of this type have been reported to alter the N status of forest ecosystems drastically (Aber et al., 2003). De Schrijver (2007) observed considerable nitrate leaching throughout the entire year in a deciduous and coniferous forest stand in Flanders. This led to the conclusion that the availability of ammonium and nitrate was in excess of total combined plant and microbial nutritional demand, which is an indication for nitrogen saturation of ecosystems as defined by Aber et al. (1989).

## DISSOLVED ORGANIC MATTER

### *Terminology*

Organic matter (OM) in terrestrial and aquatic environments is a diverse mixture of organic compounds ranging from macromolecules (e.g. Figure 1.3) to low molecular weight compounds such as simple organic acids and short-chained hydrocarbons. Historically, organic matter in natural waters has been arbitrarily divided into dissolved (DOM) and particulate organic matter (POM), based on filtration through a filter with certain cut-off (Aiken, 2002). Dissolved material is typically operationally defined as the material passing a 0.45 µm filter (Zsolnay, 2003), although many ecology-based studies employ a 0.7 µm glass-fibre filter (Westerhoff and Mash, 2002). Since no natural cut-off exists between the

particulate and dissolved fractions, the distinction is arbitrary and the definition of the terms, therefore, is operational. The colloidal fraction, which consists of suspended solids that are operationally considered solutes, is distributed over both the dissolved and particulate fraction (Aiken, 2002).



**Figure 1.3. Chemical structure of a humic acid polymer (Source: von Wandruszka, 2000)**

DOM compounds are organic molecules that also may contain macronutrients such as N, P and S atoms (Figure 1.3) (Kaiser et al., 2001). Although the terms dissolved organic carbon (DOC), nitrogen (DON) and phosphorous (DOP) are sometimes used separately, the latter two merely refer to those DOM compounds which contain N and/or P.

### *Methodology*

Since the early 1950s, the level of awareness of dissolved organic matter as an active participant in the geochemistry, environmental chemistry, and ecology of freshwaters has grown enormously (Perdue and Ritchie, 2003). During the last decades, also increasingly more research has been done on DOM cycling in terrestrial ecosystems and particularly in the mineral soil (Zsolnay, 1996). The

qualification and quantification of DOM cycling generally involves three crucial steps: (i) sampling of the solution on site; (ii) sample preparation and (iii) effective chemical analysis (Kaplan, 1994).

The term *dissolved* in the definition of DOM refers to the *presence in the solution phase* at the time of sampling or “*the material which is truly dissolved in situ*” (Zsolnay, 2003). This has particular consequences for the sampling methods for DOM in mineral soil. The DOM indeed only exists, per definition, in the soil solution which is effectively under equilibrium with the surrounding mineral soil at the time of sampling. Suction cup and zero-tension lysimetry are the two methods that are used most frequently for sampling this equilibrium soil solution. Some researchers, however, have tried to quantify the DOM concentrations in soil solution by using a deionized water or salt solution extract of the bulk soil, viz. soluble organic matter (SOM) (e.g. Jones and Willet, 2006). These salt extractants release much larger OM pools from the soil than what is actually in solution *in situ* (Zsolnay, 2003). The SOM pool in mineral soil is less susceptible to temporal variations as a result of soil moisture content than is the DOM pool.

Besides, the term *dissolved* also refers to the (arbitrary) *size* of the DOM molecules. Since most sampling methods sample both POM and DOM, particulate OM has to be removed from the sampled solution prior to analytical determination of DOM (Kaplan, 1994). The high cost of the 0.45  $\mu\text{m}$  silver membrane filters has driven some researchers to use 0.7  $\mu\text{m}$  glass-fibre filters, since these filters are known to contain very few organic contaminants and adsorb very few DOM. The most widely used 0.45  $\mu\text{m}$  filters (eg. nitrocellulose) leach small amounts of DOM, though this can be overcome by rinsing with deionized water prior to filtration, or presents little problems in samples with high DOM concentrations.

The last step in the quantification of DOM concentrations is the effective chemical analysis. Sharp (2002) reviewed the available analytical methods for DOM determinations. Since carbon is the major element of organic matter, the concentration of DOM has generally been determined as dissolved organic carbon (DOC) (Ogawa and Tanoue, 2003). The oldest method used to determine DOC is by wet chemical oxidation (WCO) as described by Menzel and Vaccaro (1964).

Basically, in this analytical method organic compounds are converted to carbon dioxide in the presence of a chemical oxidant such as  $\text{KMnO}_4$ . Because of the presence of very stable organic molecules that are not chemically oxidisable, caution must be exercised in assessing DOM by wet oxidation methods (Perdue and Ritchie, 2003). Sugimura and Suzuki (1988) introduced a high temperature (catalytic) combustion (HTC) method that was reported to yield higher DOC concentrations in marine samples. Subsequently, this method was extensively explored and it was found to be a highly precise method for DOC determinations (Sharp, 1997). In addition, the international distribution of certified reference material (CRM) for DOC analyses made it possible to more correctly compare DOC values measured by different scientists. Consequently, both the accuracy and the precision of DOC measurements have been improved (Ogawa and Tanoue, 2003).

The quantification of dissolved organic nitrogen is somewhat more complicated. First, DON can not be quantified directly but can only be calculated as the difference between the total dissolved nitrogen concentration (TDN) and the inorganic nitrogen concentration (DIN) (Westerhoff and Mash, 2002). The subtraction of independently measured concentrations results in the accumulation of analytical variance in the DON determination (Lee and Westerhoff, 2005). Second, the quantification of TDN concentration requires a preparatory digestion step in which organic nitrogen forms are converted to a measurable inorganic nitrogen compound. Westerhoff and Mash (2002) reviewed the available digestion methods each with their limitations. Alkaline persulfate oxidation (PO) and high temperature catalytic oxidation (HTCO) are the two most common techniques used for the conversion of ON compounds into quantifiable N forms. The incomplete oxidation of some nitrogenous compounds undermines the DON determinations. Moreover, the lack of a CRM makes it impossible to determine the accuracy of DON measurements in complex field samples.



### *Ecological relevance*

Dissolved organic matter represents one of the most mobile and reactive organic matter fractions, thereby controlling a number of physical, chemical and biological processes in both terrestrial and aquatic environments. Zsolnay (1996; 2003) summarized the possible functions of DOM and their relationship to ecological problems (Figure 1.4).

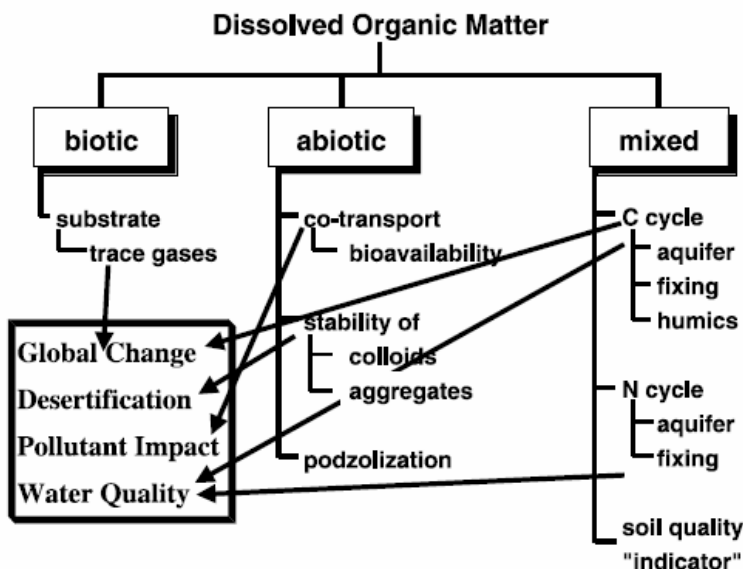


Figure 1.4. Schematic illustration of the possible functions of DOM and their relationship to ecological problems (Source: Zsolnay, 2003)

The downward movement and subsequent immobilization of DOM in mineral soils gives rise to soil formation, e.g. podzolisation (Lundström et al., 1995; Lundström et al., 2000; Zysset and Berggren, 2001). Besides, the presence of DOM in the soil solution can accelerate the weathering rates of some mineral soil constituents (Raulund-Rasmussen et al., 1998). The settling of DOM in deeper soil horizons significantly contributes to carbon sequestration in natural ecosystems (Kaiser and Guggenberger, 2000; Jimenez and Lal, 2006). Leaching of DOM to ground and surface waters results in loss of associated nutrients from the ecosystems (Kaiser et al., 2001) and eutrophication of ground and surface waters.

In aquatic environments, the easily oxidisable compounds in the DOM

can act as chemical- and biological-oxygen-demand compounds, thereby capable of depleting the oxygen concentrations in the aquifers and influencing aquatic biota (Lennon and Pfaff, 2005), methanogenesis (Lu et al., 2000) and denitrification rates (Siemens et al., 2003). Besides serving as electron source (McDowell et al., 2006), humic acids act as an electron mediator for the microbially catalyzed reduction of metals (Lovley et al., 1998) and organic pollutants. Besides, the presence of DOM in water can hamper its use for drinking water production. Membrane fouling and the formation of carcinogenic disinfection byproducts (DBP) during chlorination form the major restrictions (Westerhoff and Mash, 2002).

DOM can serve as a carrier of otherwise immobile and/or exogenous compounds within the environment. The complex-forming properties of DOM enhances the solubility of (heavy) metals (e.g. As, Cr, Cd, Pb,...) in both aquatic and terrestrial ecosystems (Ravichandran, 2004). This higher solubility results in a higher mobility and lower bio-availability and metal toxicity (Calace and Petronio, 2004). Sorption of (hydrophobic) organic pollutants (e.g. PCBs, PAHs, pharmaceuticals,...) to DOM may result in higher mobility and potential transfer of the pollutants between ecosystems (Jaffe, 1991).

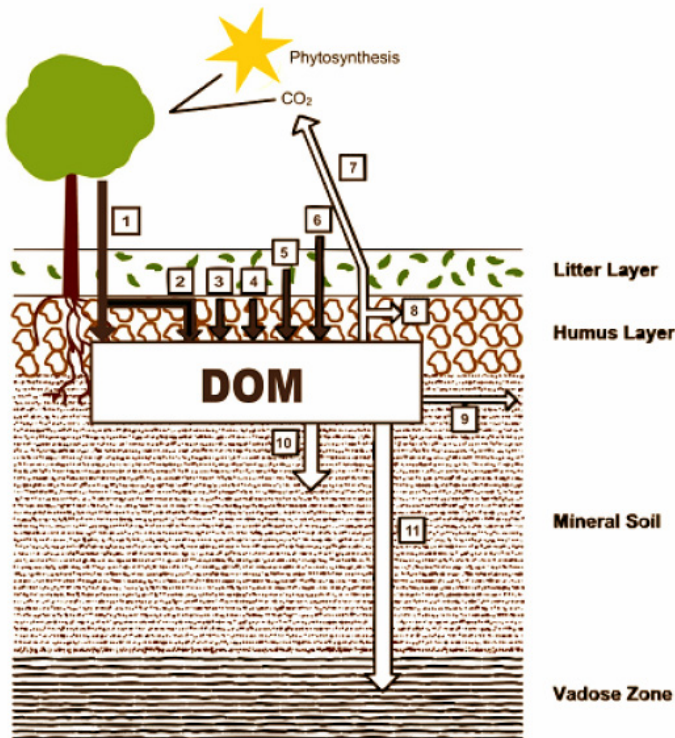
The acid properties of DOM have been noted and quantified from the earliest studies of DOM (Shapiro, 1957). The presence of carboxylic and phenolic functional groups in DOM molecules determine the water acidity. Therefore, the term *organic acids* has been used interchangeably with dissolved organic matter (Dai et al., 1996). The leaching of these organic anions lead to the existence of the anion deficit in solution inorganic cation-anion balances (Vance and David, 1991). The presence of humic substances has been shown to possess phytohormone-like activity in different forest ecosystems (Pizzeghello et al., 2002).

Perakis and Hedin (2002) reported that in unpolluted South American forests nitrogen is mainly lost via dissolved organic compounds. On average, 95 % of the TDN export from these pristine forest was in the form of DON (Hedin et al., 1995). Influences of human activities (esp. N deposition), however, have been

reported to result in a shift toward an abundance of inorganic nitrogen forms in soil leachates (van Breemen, 2002).

### *DOM dynamics in forest ecosystems*

Generally, dissolved organic matter originates from different sources in forest ecosystems and a large number of physical, chemical and biological processes determine its concentration in solution. The sources and sinks of DOM in forest ecosystems are schematically presented in Figure 1.5.



**Figure 1.5. Sources and sinks of dissolved organic matter (DOM) in soils (Sources: 1. Throughfall; 2. Root exudates; 3. Microbial lysis; 4. Humification; 5. Litter and root decomposition; 6. Organic amendments. Sinks: 7. Microbial degradation; 8. Microbial assimilation; 9. Lateral flow; 10. Sorption; 11. Leaching.) (Source: Bolan et al., 2004)**

The primal origin of all organic matter in natural ecosystems is photosynthesis. As precipitation water percolates through the forest canopy, DOM is released into the solution reaching the forest floor with throughfall [1]. Plant and

in particular tree roots exude organic substances in the soil solution and consequently act as a DOM source [2]. Microbial biomass is an important part of soil organic matter and acts as a source of DOM through death and subsequent lysis [3]. Humification of organic matter [4] and decomposition of litter and root biomass [5] feed the pool of DOM. Although normally not applicable in natural forest ecosystems, organic amendments [6] will also serve as an input for the DOM pool in soil.

The pool of DOM in the soil is subject to different chemical, physical and biological processes determining its size. Heterotrophic bacteria can utilize DOM as an energy source [7] or assimilate DOM in their biomass [8]. Lateral water flow [9] can export the available DOM from forest ecosystems situated on hill slopes. Although sorption is a reversible process, it has been shown to be a major sink for DOM in the mineral soil [10]. The DOM in the soil solution that is not adsorbed, degraded, taken up or exported via run-off is subject to leaching to deeper layers and groundwater with the downward water flux [11].

As a consequence of the multitude of sources and interactions, the overall DOM cycling is influenced by a wide range of sometimes correlated factors. Climatic factors (e.g. temperature, precipitation) determine organic matter production, degradation, hydrological fluxes. Besides the quantity and quality of the organic matter, the vegetation type also influences water fluxes. Soil type and properties, in turn, determine adsorption and degradation of DOM and favor DOM export through preferential flow via cracks. The overall nutrient status of the forest directly influences plant growth and indirectly the quantity and quality of the organic matter and water uptake. The dynamics of DOM are determined by these environmental conditions and thus are site-specific.

### *Research bottlenecks*

In the past decades, increasingly more studies focussed on the dynamics of dissolved organic matter in a variety of ecosystems. Concentrations and fluxes of dissolved organic compounds in forest ecosystems have been determined in

some regions of the world and the different processes involved with dissolved organic matter cycling have been studied extensively.

Despite this increasing knowledge on DOM cycling in terrestrial ecosystems, the extent and complexity of this matter creates many problems and leaves blank many questions. Zsolnay (2003) summarized the main problems and mis-understandings concerning dissolved organic matter. McDowell (2003) suggested three questions that would prove fruitful for future research on DOM in soils: (1) How large are various sources and sinks and how are they controlled?; (2) What is the ecological significance of dissolved organic nitrogen (DON) in comparison to dissolved organic carbon (DOC)?; (3) How are fluxes altered in human-dominated environments?.

## OBJECTIVES

The long-term high nitrogen inputs into semi-natural ecosystems via atmospheric deposition have been reported to alter biogeochemical cycles (Aber et al., 1989) and DOM cycling in particular (Pregitzer et al., 2004). This study focusses on the dynamics of dissolved organic carbon and nitrogen in temperate forest ecosystems in Flanders with historical high N deposition loads. Figure 1.6 gives a schematic overview of the structure of the thesis.

Dissolved organic nitrogen (DON) cannot be quantified directly in aqueous samples due to the presence of  $\text{NH}_4^+$ ,  $\text{NO}_2^-$  and/or  $\text{NO}_3^-$  in natural waters. Therefore, DON can only be calculated as the difference between total dissolved nitrogen (TDN) minus dissolved inorganic nitrogen (DIN). The current methodology of DON measurements shows several limitations and shortcomings. First, the selection of the most accurate and precise TDN analysis is of overriding importance for DON quantification. The subtraction of several independently measured concentrations accumulates analytical variance in the DON value. Under conditions with high mineral N contents, the contribution of DON to TDN can be masked by the high DIN content. The first objective of this study (**Objective 1**) was therefore to optimise the determination of dissolved organic nitrogen (DON) concentrations in aqueous samples with high DIN concentrations.

The potential impact of an elevated nitrogen deposition on DOM cycling in forest ecosystems is most often studied in laboratory and field manipulation experiments in which mineral nitrogen forms are artificially added to the ecosystem. However, these manipulation experiments all cover a limited time scale probably not reaching a new equilibrium situation in this relatively short time. Therefore, the second objective of this study (**Objective 2**) was to quantify concentrations and fluxes of DOC and DON in the different ecosystem strata of a deciduous and coniferous forest on sandy soil in Flanders that have been subjected to high nitrogen depositions for several decades. Temporal fluctuations in the different ecosystem strata (**a**) and the major sources and sinks (**b**) of DOM in these forest ecosystems were monitored during one year of field sampling. The potential influence of forest type (**c**) and of an additional gradient in atmospheric N deposition (**d**) on DOC and DON cycling was investigated. The export of DOC and DON to the groundwater (**e**) and the relative contribution of DON to total nitrogen leaching (**f**) was determined quantitatively under these specific circumstances.

Many studies have focussed on the release of DOM from forest floor material and the factors affecting this release. Although some potential interactions involved in the DOM release from forest floor material have been proposed, no clear mechanism has been put forward. Therefore, the third main objective of this study (**Objective 3**) was to elucidate the mechanism(s) regulating DOC and DON release from different types of forest floor material (**a**) and to determine if and to what extent the release mechanisms of DOC and DON are decoupled (**b**).

Microbial degradation and assimilation can affect the DOC and DON concentrations in the soil solution. Therefore, the fourth objective of this study (**Objective 4**) was to quantify the biodegradability of DOM released from the forest floor and to what extent biodegradability accounted for the removal of DOC and DON in the mineral soil.

Sorption to mineral soil particles has often been reported to be the major process regulating the DOM concentration in the soil solution and thus determining its leachability to groundwater. The fifth objective of this study

**(Objective 5)** was to find out if sorption was also the major DOM removal process in the sandy forest soils in Flanders **(a)** and what soil properties were of major importance for sorption in these podzolic soils **(b)**. Different isotherm models were compared for their capabilities to describe sorption to mineral soil **(c)** and the generation of field-representative parameter values **(d)**.

The sixth and last objective of this study **(Objective 6)** was to work out a new laboratory method to better describe DOM sorption in layered soil profiles. The possibilities to gain better insight in the specific sorption mechanisms **(a)** with this new technique were explored and the sorption-driven fractionation of DOM in mineral soils was investigated **(b)**.

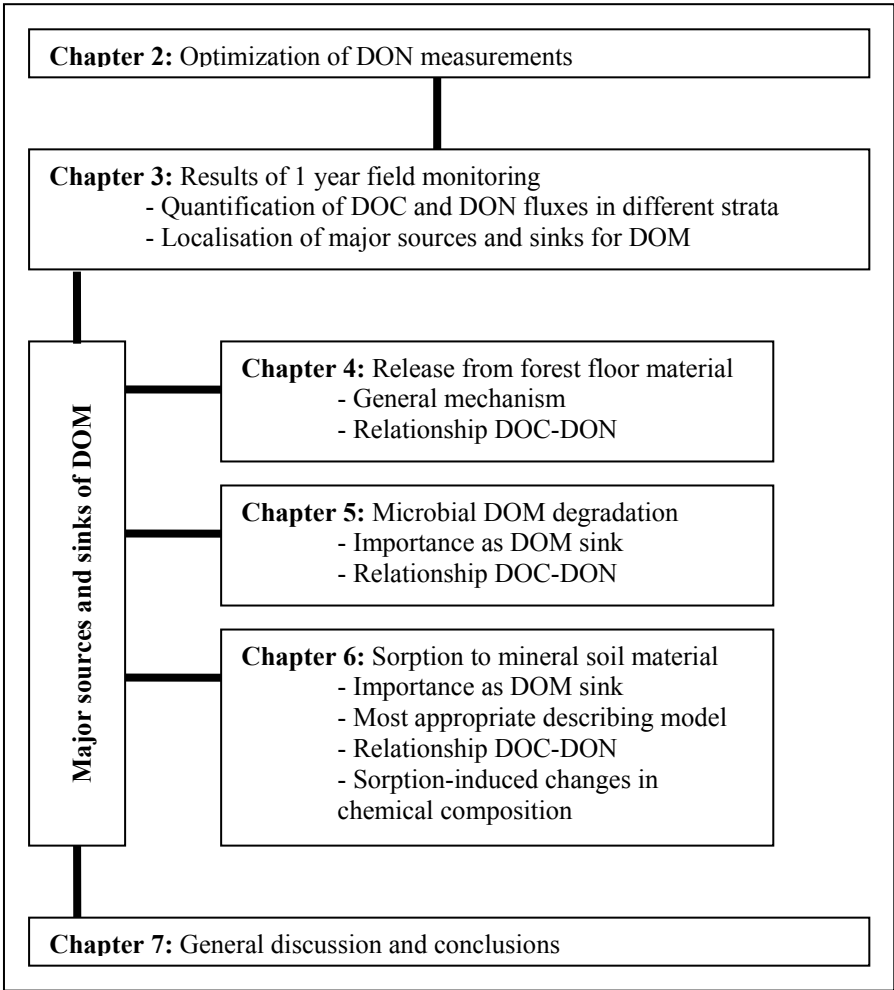


Figure 1.6. Schematic overview of the thesis



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## CHAPTER 2

# OPTIMIZATION OF DISSOLVED ORGANIC NITROGEN (DON) MEASUREMENTS IN AQUEOUS SAMPLES WITH HIGH INORGANIC NITROGEN CONCENTRATIONS

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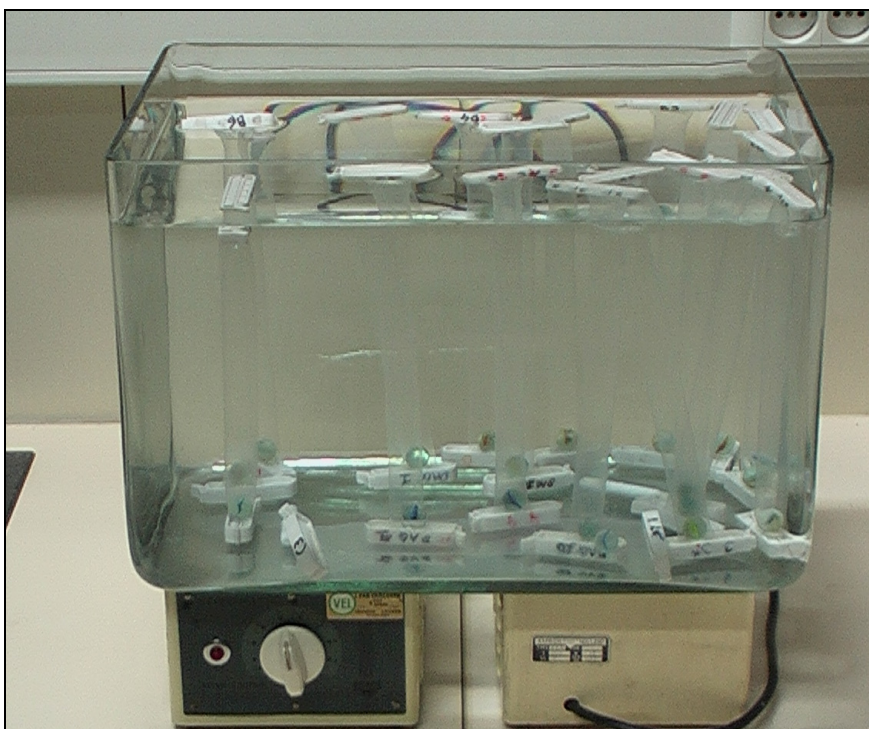


Illustration on p. 19:

Dialysis bags in container filled with phosphate-buffered deionized water and placed on two magnetic stirrers.

*This chapter has been published in:*

*Vandenbruwane J., De Neve S., Qualls R.G., Salomez J. and Hofman G. (2007). Optimization of dissolved organic nitrogen (DON) measurements in aqueous samples with high inorganic nitrogen concentrations. Science of the Total Environment 386: 103-113.*

## ABSTRACT

Since dissolved organic nitrogen (DON) concentrations in aqueous samples can only be determined by the subtraction of three independently measured concentrations (total dissolved nitrogen (TDN), nitrate and ammonium), analytical errors are compounded in the calculation of DON concentration. Several methods were tested to increase the recovery and precision of the DON determinations in aqueous samples with high inorganic nitrogen concentrations. The first step was the selection of the most accurate TDN analysis method. The persulfate oxidation (PO) method had a significantly higher recovery than the high temperature catalytic oxidation (HTCO) method. The second step involved the lowering of the DIN (dissolved inorganic N) / TDN ratio by applying three sample pretreatment procedures: conventional dialysis, dialysis against a buffered acceptor solution and concentration of the samples combined with the buffered dialysis. Despite the fact that DIN was only partially removed, conventional dialysis pretreatment resulted in higher precision and recovery of the DON determination compared to analysis of untreated samples. Dialysis of the samples against a buffered acceptor solution gave additional improvements in precision, recovery, and reproducibility. The concentration of aqueous samples by lyophilization, however, did not lead to higher recovery rates in the DON measurements.

## INTRODUCTION

During the last decade, dissolved organic matter (DOM) received increasingly greater attention in biogeochemical studies of natural ecosystems. Leaching of DOM gives rise to the loss of available nutrients (Qualls et al., 1991) and eutrophication of ground- and surface waters. Perakis and Hedin (2002) reported that nitrogen is lost from unpolluted South American forests mainly via dissolved organic compounds. Despite the growing interest in dissolved organic nitrogen (DON) cycling in natural ecosystems, the analytical methods still face serious limitations.

The DON concentration in water samples cannot be quantified directly, but must be calculated by subtracting dissolved inorganic nitrogen (DIN) concentrations from the total dissolved nitrogen (TDN) concentrations:

$$[DON] = [TDN] - [DIN]$$

where DIN is the sum of nitrite-, nitrate- and ammonium-nitrogen:

$$[DIN] = [NO_2^- - N] + [NO_3^- - N] + [(NH_3 / NH_4^+) - N]$$

Thus, the measurement of DON commonly involves three sequential steps: (1) measurement of inorganic nitrogen species; (2) measurement of TDN concentration; and (3) subtraction of both concentration values. Subtraction of several independently measured concentrations compounds analytical variance in the calculated DON concentration (Lee and Westerhoff, 2005):

$$\text{var}[DON] = \text{var}[TDN] + \text{var}[(NO_2^- + NO_3^-) - N] + \text{var}[(NH_3 / NH_4^+) - N]$$

where  $\text{var}[X]$  is the variance of each measurement  $[X]$ . In the case of high DIN/TDN ratios, the variance can be greater than the calculated DON concentration (Lee and Westerhoff, 2005). For example, in two studies negative DON concentrations were obtained in up to 15 % of the measurements (Solinger et al., 2001; Siemens and Kaupenjohann, 2002) because of high DIN concentrations.

Basically, two main principles can be applied to improve the DON measurements by lowering the variances on the separate analyses. The first is to optimize the digestion step. In contrast to the mineral nitrogen determination, the

TDN determination requires a preparatory digestion step, either chemical or by combustion. Westerhoff and Mash (2002) reviewed the available digestion methods for organic nitrogen. Kjeldahl digestion is the oldest method, but fails to analyze samples with low organic nitrogen concentration (Smart et al., 1981; Smart et al., 1983). A relatively recent widely used method to convert total nitrogen into quantifiable N-compounds is by high temperature catalytic oxidation (HTCO) (e.g. Merriam et al., 1996). Contradictory reports on the efficiency and accuracy of this last method have been published (Ammann et al., 2000; Sharp et al., 2004; Chen et al., 2005; Rogora et al., 2006). Huygens et al. (2005, 2007) modified this HTCO method and coupled the former to an isotope ratio mass spectrometer (IRMS) allowing on-line TD<sup>15</sup>N-measurements. Another very common and reliable digestion method for total nitrogen determination in aqueous samples is the alkaline persulfate oxidation (D'Elia et al., 1977). Several adaptations and expansions to this method have been proposed (Cabrera and Beare, 1993; Hagedorn and Schleppi, 2000; Doyle et al., 2004), and various factors and interferences affecting the digestion efficiency have been investigated (McKenna and Doering, 1995; Halstaed et al., 1999; Kowalenko and Babuin, 2003). Optimization of the digestion step can lead to more accurate TDN-measurement and thus improve DON determinations.

The second method that may improve the DON determination is based on increasing the DON/TDN-ratio in samples with high DIN/TDN ratios. Two pathways can be chosen to realize this goal: lowering the DIN concentration, or increasing the DON concentration in combination with partial DIN removal. Recently, two techniques have been proposed to lower the mineral nitrogen content of aqueous samples. The first procedure described by Lee and Westerhoff (2005) is based on a dialysis pretreatment. They experienced a slight loss (~10%) of DON possibly due to the adsorption of organics onto the dialysis membrane, permeation of low molecular weight fractions or biodegradation. However, they found that the dialysis pretreatment led to more accurate DON determinations than without dialysis when DIN/TDN ratios exceeded 0.6 mg N mg<sup>-1</sup> N. The second technique recently investigated to lower the DIN/TDN ratio is based on the

catalytic reduction of nitrate (Ambonguilat et al., 2006). Extensive testing in the presence of natural DOM, however, indicated that catalytic reduction of nitrate before DON determination cannot be used because of DON sorption onto the catalyst and low DIN removal efficiency. The second method based on increasing the DON concentration in combination with a partial DIN removal can be applied for samples with low absolute DON concentrations. Perdue and Ritchie (2003) reviewed the different methods to concentrate large quantities of natural organic matter (NOM) from many types of fresh waters. A recent study of Koprivnjak et al. (2006) reported the results of coupling reverse osmosis (RO) with electrodialysis to isolate natural organic matter from freshwaters. Since RO is designed for large water volumes (Serkiz and Perdue, 1990; Sun et al., 1995), it may be more practical to use either vacuum evaporation or lyophilization for small sample volumes. In these methods, solutes are concentrated by means of selective removal of water molecules by evaporation.

The aim of this study was to optimize the DON analysis in aqueous samples with high DIN/TDN ratio using various techniques. The first part of the study was intended to determine which of the two most common TDN analysis techniques is most suited for DON determinations in aqueous field samples. In a second part, we attempted to improve the recovery and precision of the TDN analysis by applying different sample pretreatment methods to lower the DIN/TDN ratio.

## **MATERIALS AND METHODS**

### *Samples and sample collection*

Samples of precipitation, throughfall, soil solution and groundwater were collected from three forested experimental plots in a homogeneous Silver birch (*Betula pendula* Roth.) stand at the National Forest Reserve of Ravels (51°25'N, 5°04'E), about 12 km northeast of Turnhout in Northern Belgium. The region is characterized by intensive livestock breeding which has caused high atmospheric nitrogen deposition rates. This resulted in throughfall and soil solutions with a

high ratio of DIN/TDN which, as described later, had an adverse effect on DON determinations and prompted this study. The soil under investigation was a podzol developed on sand deposits and is classified as a Typic Haplorthod according to USDA Soil Taxonomy. The average annual precipitation in the study area is 748 mm and the average annual temperature amounts to 9.5 °C.

Precipitation and throughfall water were collected using polyethylene funnels (15 cm diameter) supported by and draining into two-liter polyethylene bottles. The bottles were placed below ground level to keep the samples cool and to protect them from direct sunlight. Forest-floor leachate samples were collected using zero-tension lysimeters made of polyvinylchloride (PVC) installed beneath the organic layer. Soil solution samples under the mineral E, Bh and BC horizons were taken using acid washed ceramic suction cup lysimeters (Model 1900 Soil Water Sampler (with B02M2 cup), Soilmoisture Equipment Corp., Santa Barbara, USA). Perforated PVC tubes were installed to a depth of 2.5 m in order to sample the groundwater using a sampling bottle connected to a vacuum pump. All samples were filtered through 0.45 µm polyethersulfone (PES) membrane disc filters (Pall Supor®-450, Gelman Laboratory) and stored at -18 °C until used in the experiments.

To investigate the influence of the DIN/TDN-ratio on the performance of the DON determinations, a mix of soil solution samples was dialysed to lower the DIN-concentration. This dialysed solution was used to make up a series of 14 samples with equal DON concentration and increasing DIN concentration. These samples were prepared by mixing 90 ml of this dialysed solution with a certain volume of a DIN stock solution (750 mg NO<sub>3</sub><sup>-</sup>-N l<sup>-1</sup> and 250 mg NH<sub>4</sub><sup>+</sup>-N l<sup>-1</sup>) and added up to 100 ml with deionized water.

### *Sample pretreatment procedures*

The dialysis pretreatment procedure applied in this study was based on the system of Lee and Westerhoff (2005). Dialysis was conducted with a 20 mm diameter cellulose ester (CE) dialysis tube (Spectra Por, Spectrum Laboratories Inc., CA). The CE membrane has a nominal molecular weight cut-off of 100 Da.

Each dialysis tube was rinsed thoroughly with deionized water during several days until DOC concentration in the supernatant solution was less than  $0.1 \text{ mg C l}^{-1}$ . Samples were placed in cleaned dialysis tubes and were dialyzed against deionized water in a dark dialysis system. As a modification of the continuous flow-through system described by Lee and Westerhoff (2005), the acceptor solution (deionized water) in our experiments was discarded and refreshed completely at fixed time intervals (after 0, 24, 48, 72 and 96 hours). Sample volume changes were quantified by measuring the mass difference before and after dialysis. The TDN, DIN, DON and DOC concentrations were adjusted for these volume changes. Because of the amphoteric properties of the cellulose ester (CE) material, the dialysis membrane can have either a positive or negative charge, depending on the pH, ionic strength and ionic composition of the sample and acceptor solution (Lee and Westerhoff, 2005). Since the iso-electric point (IEP) of the CE membrane is 2.2 (Spectra Por, Spectrum Laboratories Inc., CA) and the pH of the deionized (DI) water was 6.3, the dialysis membrane will have a negative charge when using DI water as acceptor solution. As a consequence of this charge, the migration of positively charged compounds (like  $\text{NH}_4^+$ ) through the membrane is limited, if not blocked completely (Runge et al., 2005). The pH adjustment investigated in this study is a new modification of the conventional set-up of Lee and Westerhoff (2005) and its influence on DIN removal was therefore also investigated in this study.  $\text{H}_3\text{PO}_4$  and  $\text{KH}_2\text{PO}_4$  were added at a concentration of 4.7 mM and 5.3 mM respectively to buffer the acceptor solution at pH 2.2 and to maintain the ionic permeability (Runge et al., 2005).

For a selection of samples with low DON concentrations, we concentrated the samples using either lyophilization or vacuum rotary-evaporation, followed by the selective removal of DIN in order to decrease the DIN/TDN ratio. Preliminary experiments indicated that a significantly higher amount of organic nitrogen was lost using the rotary evaporation technique operating at  $\pm 49^\circ \text{C}$  compared to the freeze-drying procedure. Evaporation of volatile organics and adsorption onto the glassware were assumed to be the cause of these losses. A subset of aliquots of the test solutions was also concentrated using a lyophilization



device (Ankersmit, Heto FD3) operating at -53 °C and 442 mbar. The exact initial volume of sample solution was measured using a calibrated 250 ml flask. The solution was completely freeze-dried and re-dissolved in a known volume of deionized water. The degree of concentration was chosen not to exceed the persulfate digestion method's upper limit of 100 mg C l<sup>-1</sup> (Cabrera and Beare, 1993; Doyle et al., 2004).

### *Chemical analyses*

All samples were kept at -18 °C until analysis for DOC, TDN and DIN-content. The first step in the optimization of DON measurements involves the determination of the most accurate TDN analysis method. The TDN concentration of a selection of field samples was determined using the two most common analysis methods: high temperature catalytic oxidation (HTCO) and the alkaline persulfate oxidation (PO).

Total dissolved nitrogen concentration in a selection of aqueous field samples was measured using the HTCO method described by Merriam et al. (1996). In this method, all nitrogen forms are assumed to be converted to nitrogen oxides (NO<sub>x</sub>) at a fixed NO/NO<sub>x</sub> ratio by oxidizing the sample in a high-temperature furnace (680 °C) in the presence of a catalyst (Pt on alumina support). The NO<sub>x</sub> is reacted with O<sub>3</sub>, producing NO<sub>2</sub>, which is measured by chemiluminescence. The Shimadzu TOC-V system coupled with the TN-module (Shimadzu Corp., Japan) was used in this study.

Total dissolved nitrogen concentrations in the selected samples were also determined using the alkaline persulfate method described by Koroleff (1983). After autoclaving, the tubes were reweighed and these sample losses were used to correct the final NO<sub>3</sub><sup>-</sup>-concentration in the digested solutions. Nitrate in the digested samples was measured on a continuous-flow auto-analyzer (see below). The alkaline persulfate method was used for the determination of TDN concentration in all further experiments.

Total dissolved carbon (TDC) and dissolved inorganic carbon (DIC) were determined on a Shimadzu TOC-V analyzer (Shimadzu Corp., Tokyo, Japan) with

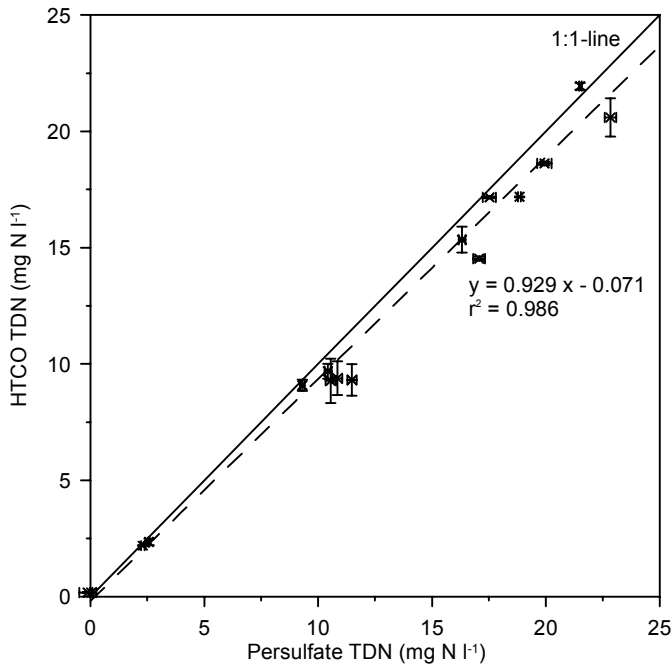
infrared (IR) detection following thermal oxidation. Dissolved organic carbon (DOC) was calculated as the difference between TDC and DIC. Samples were analyzed for DIN ( $\text{NO}_3^-$ -N +  $\text{NO}_2^-$ -N) and  $\text{NH}_4^+$ -N) on a continuous-flow auto-analyzer (ChemLab System 4). In this system, nitrate was converted to nitrite using enzymatic reduction and nitrite was measured colorimetrically at 520 nm after diazotation with sulfanilamide and N-(1-naphthyl)-ethylene diamine. Ammonium was determined colorimetrically at 650 nm after reaction with sodium salicylate and sodium dichloroisocyanurate. DON was then calculated as the difference between TDN and DIN.

Since highly colored natural DOM in water samples may add to the absorbance of the samples and thus interfere with the colorimetric DIN determination (Moliner-Martínez et al., 2006), a set of highly colored samples were run on the continuous-flow auto-analyzer without the addition of the respective color reagents. The resulting correction factors for nitrate and ammonium at 100 mg DOC  $\text{l}^{-1}$  were estimated to be 20.6  $\mu\text{g l}^{-1}$  and 0.8  $\mu\text{g l}^{-1}$  respectively.

## RESULTS AND DISCUSSION

### *HTCO versus persulfate oxidation*

Since accurate TDN analyses are the basis for reliable DON determinations, the two most common TDN methods were compared in this first experiment. A selection of various field solution samples were analyzed for TDN concentration using both the HTCO and PO method (Figure 2.1).



**Figure 2.1.** Comparison of total dissolved nitrogen (TDN) concentration measured by high-temperature catalytic oxidation (HTCO) and persulfate oxidation (PO) ( $n = 17$ ). Error bars represent 1 standard deviation (of replicate measurements of a single sample).

Although the measurements by the two methods were highly correlated ( $p < 0.001$ ), the slope of the regression line was 0.929 indicating that the persulfate digestion produces slightly higher (averaging 7.1 %) TDN values than does the HTCO method. A paired t-test of all the samples, comparing the difference between the two methods, indicated that this difference was highly significant ( $p < 0.001$ ). Recent work of Rogora et al. (2006) indicated a 4 % lower efficiency of the HTCO compared to PO on average for 793 freshwater samples. Ammann et al. (2000) also reported higher TDN recoveries in river water samples with the persulfate digestion compared to the HTCO method. Doyle et al. (2004) reported similar findings for K<sub>2</sub>SO<sub>4</sub>-soil extracts. Sharp et al. (2004) found significantly higher TDN-concentrations in 10 of the 13 seawater samples using persulfate oxidation compared to the HTCO method. They attributed this discrepancy to the possibility of incomplete conversion of some nitrogen compounds by the HTCO instruments and/or peak integration problems. Since mineral nitrogen recoveries

with HTCO were close to 100 % (99.3 % for nitrate and 100.5 % for ammonium using  $\text{KNO}_3$  as standard), the lower TDN efficiency is related to the organic nitrogen fraction in the samples. Incomplete combustion of high molecular N compounds and/or incomplete conversion of  $\text{NO}$  to  $\text{NO}_2$  are two possible pathways that may lead to lower TDN detection efficiencies.

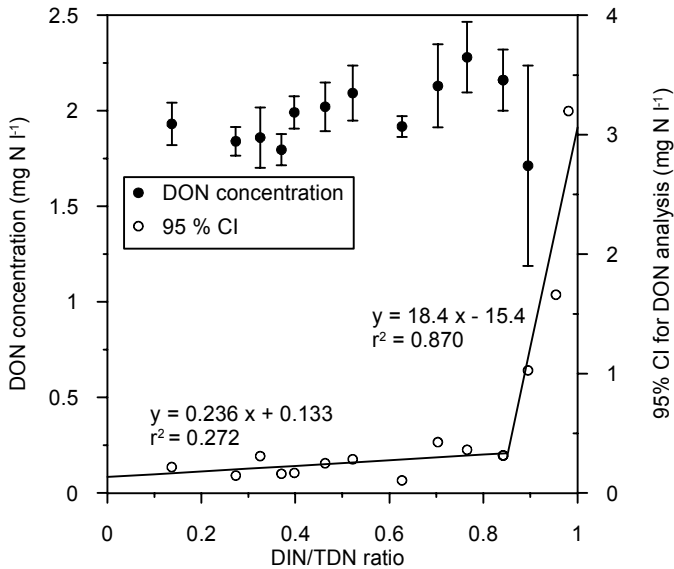
The observed 7.1 % lower TDN recovery of the HTCO method compared to the persulfate digestion had an important impact on the DON determination in aqueous samples with high DIN/TDN-ratio. The 7.1 % reduced TDN recovery resulted in a 26 % reduction in DON concentration in samples with a DIN/TDN-ratio of 0.73 i.e. the mean in this field study (see further). Since the persulfate oxidation method has proven to be the most reliable method for TDN measurements, this method was used for all further TDN analyses in this study.

### *Influence of DIN/TDN-ratio on DON measurement*

To investigate the influence of the DIN/TDN ratio on the recovery and precision of DON determinations, samples with equal DON concentration and increasing DIN concentrations were analyzed in this second experiment. The DON concentrations and 95 % confidence interval (95 % CI) on these values were plotted against the DIN/TDN ratio (Figure 2.2). As long as the DIN/TDN ratio was smaller than 0.85, the average measured DON concentration did not deviate significantly from its value at the smallest DIN/TDN ratio. The measured DON concentration was significantly different from the original concentration and became negative for DIN/TDN ratios of 0.95 and 0.98. The negative DON concentrations for these last two samples are related to the high  $\text{NH}_4^+$ -concentrations (10 and 25  $\text{mg N l}^{-1}$ ) and the lower TDN recovery at these high concentrations.

For DIN/TDN ratios  $\geq 0.85$ , the standard deviation and 95 % CI increased greatly. These findings are particularly important for samples with low DON and high DIN concentrations. They show that in such a situation there is a high probability for finding DON concentrations that are not significantly different from zero or that are even negative. These findings are in accordance with the

results of Lee and Westerhoff (2005), who found a higher analytical variance on the DON determination for DIN/TDN ratios greater than 0.6 in surface waters and finished drinking waters.



**Figure 2.2.** DON concentration ( $\pm$  st. dev.) ( $\bullet$ ) and 95 % confidence interval (95% CI =  $2.57 \cdot \sigma$ ) on the DON determination ( $\circ$ ) as a function of DIN/TDN ratio ( $n = 5$ ). The two solid lines represent the linear regressions for the 95 % C.I. on the DON determination as function of the DIN/TDN ratio (breakpoint at 0.85). The DON concentrations at DIN/TDN ratios of 0.95 and 0.98 were negative and thus omitted from this figure.

Since the standard deviation of the DON measurements is dependent on the DIN/TDN ratio, it is pointless to define one single value for the detection limit of the DON determination. Instead, it is more useful to define the 95 % CI as a function of the DIN/TDN ratio. Our results indicate that we can roughly divide the trend of the 95 % CI over the entire DIN/TDN range in two linear relationships; one for DIN/TDN ratios smaller than 0.85 and one for higher ratios (Figure 2.2). Clearly, lowering the DIN/TDN ratio can decrease the standard deviation and thus increase the precision of the DON measurements and selective DIN removal is expected to have a large impact on the precision and recovery of the DON determination for samples with DIN/TDN ratios  $\geq 0.85$ .

### *Results of the field monitoring*

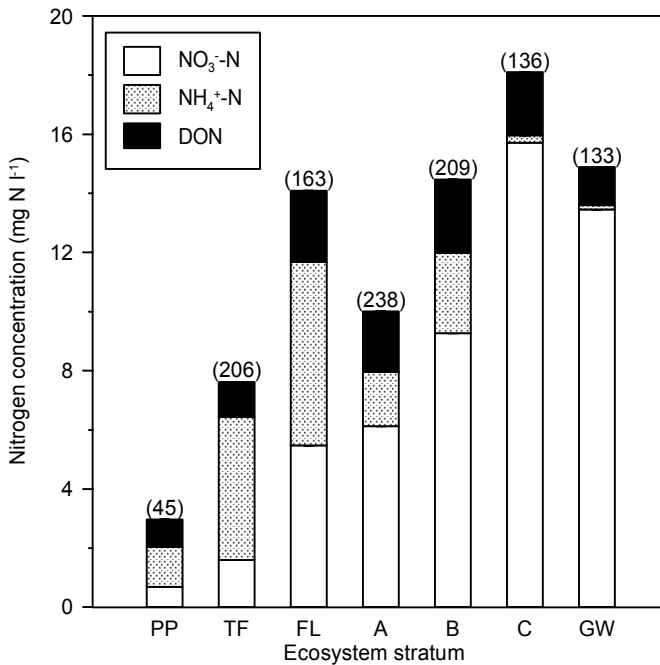
Table 2.1 summarizes the statistics of the chemical analyses of all aqueous samples during one year of field measurements. Due to the high nitrogen deposition originating from the intensive livestock agriculture in Flanders, most water samples in this ecosystem showed high DIN concentrations. On average, 73 % of TDN concentration consisted of mineral nitrogen species (nitrate + nitrite and ammonium). Nitrate was the most abundant nitrogen form in the field water samples, followed by ammonium and DON. The average DON concentration in the aqueous samples amounted to  $1.86 \text{ mg N l}^{-1}$ , but still 19 % of the analyzed samples had a DON concentration lower than the lowest method detection limit (MDL) at a DIN/TDN ratio of zero ( $0.41 \text{ mg N l}^{-1}$ ). Solinger et al. (2001) and Siemens and Kaupenjohann (2002) in whose studies soil solution samples also had high DIN/TDN ratios, found negative DON concentrations in 15 % of the cases.

**Table 2.1. Descriptive statistics of the chemical composition of water samples during the 1 year field monitoring program, based on 1130 observations**

	Mean	St. dev.	Median	Minimum	Maximum
$\text{NO}_3^- \text{-N} \text{ (mg l}^{-1}\text{)}$	6.99	10.23	3.33	0.01	91.35
$\text{NH}_4^+ \text{-N} \text{ (mg l}^{-1}\text{)}$	2.57	5.13	0.37	0.01	58.45
DIN (mg l <sup>-1</sup> )	9.51	12.23	5.17	0.02	102.98
TDN (mg l <sup>-1</sup> )	11.21	12.50	6.58	0.04	96.65
DIN / TDN	0.73	0.26	0.80	0.00	1.00
DON (mg l <sup>-1</sup> )	1.86	2.41	1.26	0.02	25.18
DOC (mg l <sup>-1</sup> )	32.39	31.28	25.72	0.05	528.1

The average nitrogen composition for the different strata of the forest ecosystem under investigation is represented in Figure 2.3. TDN concentration increased as the water percolates through the forest ecosystem. On average, DIN accounted for more than 70 % of TDN in all strata of the ecosystem. Ammonium seemed to be the major nitrogen form in the aboveground strata (precipitation, throughfall and forest floor leachate), whereas nitrate comprised the principal part in the belowground strata. The nitrate concentration increased from  $0.7 \text{ mg N l}^{-1}$  in precipitation water to  $15.7 \text{ mg N l}^{-1}$  in the soil solution of the C-horizon. The

average nitrate-N concentration measured in the groundwater ( $13.5 \text{ mg NO}_3^- \text{-N l}^{-1}$ ) during this monitoring program exceeded the limit of  $11.35 \text{ mg NO}_3^- \text{-N l}^{-1}$  of the European Nitrate Directive (91/676/EEC). The ammonium concentration in the water samples reached a maximum in the forest floor leachate ( $6.2 \text{ mg N l}^{-1}$ ) and was lowest in groundwater samples ( $0.1 \text{ mg N l}^{-1}$ ). DON concentrations ranged from  $0.9 \text{ mg N l}^{-1}$  in precipitation water to  $2.5 \text{ mg N l}^{-1}$  in the soil solution of the B-horizon.



**Figure 2.3.** Average nitrate-N, ammonium-N and DON concentrations in the different strata of the forest ecosystem in Flanders (PP = precipitation; TF = throughfall; FL = forest floor leachate; A = A-horizon; B = B-horizon; C = C-horizon; GW = groundwater). The number of samples between brackets.

As much as 38 % of the samples had a DIN/TDN ratio that was larger than the “breaking point” of 0.85. Seventy-two percent of these samples (i.e. 27 % of the total number of samples) with high DIN/TDN ratio had a 95 % C.I. larger than their intrinsic DON concentration. This means that there is a large probability of finding negative DON concentrations in this fraction of field samples (27 %).

These results show the importance of using a sample pretreatment method that decreases the DIN/TDN ratios under specific situations.

### *Potential of sample pretreatment procedures*

A selection of aqueous samples collected in the field was used to assess the potential of different pretreatment methods to optimize DON determinations. To investigate the absolute performance (precision) of each individual pretreatment procedure the nitrate, ammonia, TDN, DON and DOC concentrations were repeatedly measured in one single subsample of each of the selected field samples (replicated analyses) (Table 2.2). The recovery and reproducibility of the different pretreatment methods was estimated using the average concentrations and standard deviations resulting from the analysis of subsamples that were treated separately (method replicates) (Table 2.3).

The dialysis pretreatment described by Lee and Westerhoff (2005), in which aqueous samples are dialyzed against deionized water, resulted in a considerable decrease (on average 80 %) of the nitrate concentration in most samples in this experiment. Most nitrate was removed in samples with high initial nitrate concentration (E, Bs and BC-horizon samples), since concentration gradient is the driving force after dialysis. On the other hand, the removal efficiency of ammonium (67 %) was rather disappointing in contrast with the findings of Lee and Westerhoff (2005). They found an even faster permeation of ammonium than of nitrate. The poor migration of ammonium ions under these circumstances can be attributed to the presence of a net negative charge on the cellulose ester (CE) membrane. Since the iso-electric point (IEP) of the CE membrane is 2.2 and the pH of deionized water (DW) is situated around 6.3, the dialysis membrane is deprotonated and becomes negatively charged (Moran et al., 1999). This negative charge attracts cations (like  $\text{NH}_4^+$ ) and slows down or blocks the further permeation of cations through the dialysis membrane (Runge et al., 2005). The ammonium concentration in the groundwater sample increased significantly during dialysis treatment. This can be explained by the fact that all samples were treated simultaneously in one large container. The ammonium concentration in the



acceptor solution was probably higher than in the groundwater and blank samples, which resulted in reversed dialysis of ammonium. The inadequate removal of  $\text{NH}_4^+$ -ions in our experiments resulted in only partial decrease of the DIN concentration not leading to a substantial reduction of the standard deviation on the DON determinations (Table 2.2).

**Table 2.2. TDN, DIN, DON and DOC concentrations obtained without pretreatment (NP), with dialysis pretreatment (DP), with dialysis pretreatment against buffered acceptor solution (BDP) and with buffered dialysis after concentration pretreatment (CP). Average and standard deviation of replicated analytical measurements ( $n = 5$ ) on 1 single sample as a measure for method precision. Values between brackets indicate the percentage of the compound remaining in the sample compared to its initial concentration (NP).**

	Pretreat- ment	$\text{NO}_3^-$ ( $\text{mg N l}^{-1}$ )	$\text{NH}_4^+$ ( $\text{mg N l}^{-1}$ )	TDN ( $\text{mg N l}^{-1}$ )	DON ( $\text{mg N l}^{-1}$ )	DOC ( $\text{mg C l}^{-1}$ )
Precipitation	NP	$0.31 \pm 0.01$	$1.05 \pm 0.04$	$1.71 \pm 0.19$	$0.35 \pm 0.20$	$2.46 \pm 0.02$
	DP	$0.02 \pm 0.00$ (6)	$0.08 \pm 0.01$ (8)	$0.27 \pm 0.15$	$0.16 \pm 0.16$	$2.07 \pm 0.05$ (84)
	BDP	$0.00 \pm 0.00$ (0)	$0.04 \pm 0.00$ (4)	$0.34 \pm 0.07$	$0.30 \pm 0.08$	$2.39 \pm 0.13$ (97)
	CP	$0.00 \pm 0.00$ (0)	$0.13 \pm 0.00$ (12)	$0.76 \pm 0.01$	$0.64 \pm 0.01$	$1.02 \pm 0.01$ (41)
Throughfall	NP	$1.09 \pm 0.01$	$3.87 \pm 0.06$	$5.54 \pm 0.06$	$0.57 \pm 0.09$	$12.81 \pm 0.03$
	DP	$0.44 \pm 0.01$ (40)	$1.32 \pm 0.01$ (34)	$2.49 \pm 0.17$	$0.74 \pm 0.17$	$11.63 \pm 0.07$ (91)
	BDP	$0.00 \pm 0.00$ (0)	$0.65 \pm 0.01$ (17)	$1.25 \pm 0.10$	$0.60 \pm 0.10$	$10.83 \pm 0.16$ (85)
	CP	$0.00 \pm 0.00$ (0)	$0.54 \pm 0.01$ (14)	$1.38 \pm 0.01$	$0.83 \pm 0.01$	$9.94 \pm 0.20$ (78)
Forest-floor leachate	NP	$4.31 \pm 0.03$	$3.73 \pm 0.02$	$8.13 \pm 0.14$	$0.09 \pm 0.15$	$35.51 \pm 0.16$
	DP	$0.85 \pm 0.03$ (20)	$1.37 \pm 0.02$ (37)	$3.56 \pm 0.08$	$1.34 \pm 0.08$	$36.85 \pm 0.12$ (104)
	BDP	$0.00 \pm 0.01$ (0)	$0.55 \pm 0.01$ (15)	$1.96 \pm 0.05$	$1.41 \pm 0.05$	$28.89 \pm 0.22$ (81)
E-horizon	NP	$5.77 \pm 0.02$	$1.89 \pm 0.01$	$9.55 \pm 0.15$	$1.90 \pm 0.16$	$70.48 \pm 0.31$
	DP	$0.29 \pm 0.00$ (5)	$1.07 \pm 0.02$ (57)	$3.20 \pm 0.20$	$1.83 \pm 0.21$	$61.05 \pm 0.31$ (87)
	BDP	$0.00 \pm 0.00$ (0)	$0.15 \pm 0.01$ (8)	$2.14 \pm 0.13$	$1.99 \pm 0.13$	$59.35 \pm 0.32$ (84)
Bs-horizon	NP	$9.60 \pm 0.26$	$4.61 \pm 0.14$	$14.46 \pm 0.26$	$0.25 \pm 0.39$	$48.04 \pm 0.53$
	DP	$0.00 \pm 0.02$ (0)	$0.45 \pm 0.01$ (10)	$2.05 \pm 0.13$	$1.60 \pm 0.13$	$40.34 \pm 0.17$ (84)
	BDP	$0.00 \pm 0.01$ (0)	$0.29 \pm 0.01$ (6)	$1.63 \pm 0.06$	$1.34 \pm 0.06$	$43.09 \pm 0.38$ (90)
BC-horizon	NP	$25.48 \pm 0.23$	$0.37 \pm 0.01$	$28.31 \pm 0.54$	$2.39 \pm 0.58$	$37.57 \pm 0.07$
	DP	$0.69 \pm 0.03$ (3)	$0.12 \pm 0.01$ (32)	$2.06 \pm 0.13$	$1.25 \pm 0.13$	$20.42 \pm 0.07$ (54)
	BDP	$0.00 \pm 0.01$ (0)	$0.04 \pm 0.01$ (11)	$1.12 \pm 0.03$	$1.08 \pm 0.03$	$34.04 \pm 0.84$ (91)
Groundwater	NP	$11.56 \pm 0.13$	$0.06 \pm 0.01$	$11.69 \pm 0.24$	$0.07 \pm 0.27$	$10.21 \pm 0.03$
	DP	$4.26 \pm 0.06$ (37)	$0.13 \pm 0.01$ (217)	$5.11 \pm 0.06$	$0.72 \pm 0.09$	$8.44 \pm 0.02$ (83)
	CP	$0.00 \pm 0.00$ (0)	$0.01 \pm 0.00$ (17)	$0.27 \pm 0.01$	$0.26 \pm 0.01$	$5.80 \pm 0.02$ (57)

**Table 2.3. TDN, DIN, DON and DOC concentrations obtained without pretreatment (NP), with dialysis pretreatment (DP), with dialysis pretreatment against buffered acceptor solution (BDP) and with dialysis pretreatment after condensation (CP). Average and standard deviation of one single analytical measurement on replicated samples (n = 5) as a measure for method recovery and reproducibility. Values between brackets indicate the percentage of the compound remaining in the sample compared to its initial concentration (NP).**

Pretreat- ment	NO <sub>3</sub> <sup>-</sup> (mg N l <sup>-1</sup> )	NH <sub>4</sub> <sup>+</sup> (mg N l <sup>-1</sup> )	TDN (mg N l <sup>-1</sup> )	DON (mg N l <sup>-1</sup> )	DOC (mg C l <sup>-1</sup> )
Precipitation	NP	0.32 ± 0.01	1.00 ± 0.04	1.66 ± 0.03	0.34 ± 0.03
	DP	0.08 ± 0.04 (25)	0.20 ± 0.08 (20)	0.59 ± 0.25	0.31 ± 0.14
	BDP	0.00 ± 0.04 (0)	0.07 ± 0.02 (7)	0.38 ± 0.07	0.31 ± 0.07
	CP	0.00 ± 0.00 (0)	0.09 ± 0.03 (9)	0.34 ± 0.30	0.25 ± 0.26
Throughfall	NP	1.10 ± 0.02	3.85 ± 0.02	5.46 ± 0.07	0.52 ± 0.07
	DP	0.47 ± 0.04 (43)	1.36 ± 0.09 (35)	2.54 ± 0.16	0.71 ± 0.19
	BDP	0.00 ± 0.05 (0)	0.66 ± 0.15 (17)	1.31 ± 0.20	0.65 ± 0.07
	CP	0.00 ± 0.01 (0)	0.46 ± 0.06 (12)	0.93 ± 0.26	0.47 ± 0.21
Forest-floor leachate	NP	4.36 ± 0.08	4.27 ± 0.32	9.10 ± 0.56	0.48 ± 0.23
	DP	0.95 ± 0.08 (22)	1.48 ± 0.09 (35)	3.76 ± 0.18	1.33 ± 0.09
	BDP	0.00 ± 0.01 (0)	0.55 ± 0.10 (13)	1.78 ± 0.20	1.23 ± 0.13
E-horizon	NP	5.50 ± 0.16	1.88 ± 0.02	9.27 ± 0.19	1.89 ± 0.17
	DP	0.31 ± 0.07 (6)	1.11 ± 0.07 (59)	3.18 ± 0.17	1.76 ± 0.06
	BDP	0.00 ± 0.04 (0)	0.14 ± 0.04 (7)	1.93 ± 0.28	1.79 ± 0.22
Bs-horizon	NP	9.66 ± 0.13	4.66 ± 0.04	14.34 ± 0.30	0.03 ± 0.29
	DP	0.05 ± 0.07 (1)	0.53 ± 0.11 (11)	2.09 ± 0.17	1.51 ± 0.08
	BDP	0.00 ± 0.01 (0)	0.37 ± 0.12 (8)	1.73 ± 0.07	1.36 ± 0.07
BC-horizon	NP	24.76 ± 0.52	0.38 ± 0.01	27.74 ± 0.47	2.58 ± 0.34
	DP	0.85 ± 0.27 (3)	0.14 ± 0.02 (37)	2.17 ± 0.30	1.18 ± 0.30
	BDP	0.00 ± 0.01 (0)	0.07 ± 0.02 (18)	1.23 ± 0.03	1.16 ± 0.03
Groundwater	NP	10.84 ± 0.43	0.06 ± 0.00	11.52 ± 0.11	0.62 ± 0.44
	DP	4.60 ± 0.24 (42)	0.14 ± 0.01 (233)	5.45 ± 0.33	0.71 ± 0.15
	CP	0.00 ± 0.00 (0)	0.01 ± 0.00 (17)	0.27 ± 0.01	0.26 ± 0.01

Since the nominal molecular weight cut-off (MWCO) of the cellulose ester dialysis membrane used in these experiments was 100 Da, the size fraction of DOM smaller than this cut-off point (e.g. urea, free amino acids) can permeate through the membrane. Moreover, the negative charge on the membrane favors adsorption of DOM (esp. organic bases) by electrostatic attraction, which was clearly observed as a yellowish stain on the membrane. In addition, biodegradation of organics could occur resulting in a decreased DOM concentration in the sample. To obtain an indication of these DOM losses, DOC concentrations before and after dialysis were measured for the different samples (Table 2.3). On average,  $16 \pm 14$  % of the DOC (data not shown) was lost, either by biodegradation, adsorption to the membrane and/or migration to the acceptor solution. The loss of DOM ranged from 4 % (forest floor leachate) to 46 % (BC-horizon sample), but no clear trend could be found. Despite this considerable amount of DOM lost from the samples, the determined DON concentration after dialysis was even higher in 5

of the 7 samples (precipitation, throughfall, forest floor leachate, Bs horizon and groundwater samples) compared to the determination without pretreatment. This means that even though a considerable fraction of the DOM is lost from the samples during dialysis, the partial DIN removal still results in DON determinations with higher recovery and precision. Lee and Westerhoff (2005) reported an increased accuracy and precision of the DON determinations in spite of the 10-15 % DOC losses during dialysis of effluent samples of wastewater treatment plants.

Since water molecules can permeate through the dialysis membrane, the final sample volume will in most cases differ from the volume before dialysis. In our experiments, the sample volume increased with  $1.6 \pm 2.0$  % on average, ranging from a 5.6 % increase to a 0.4 % decrease (data not shown). The error on the weight measurements used for the generation of a correction factor introduced an additional source of variation on the DON determinations and thus a reduced reproducibility. Lee and Westerhoff (2005) also found an increasingly higher sample volume change with higher sample conductivity. In our experiment, a slight increase in variability on the DON determination as a result of this weighing error was found for samples with the lowest DON concentrations (precipitation and throughfall water) (Table 2.3). For the remaining samples, the decreased variability on the DON determinations due to the partial DIN removal exceeded the increased variability due to the sample volume change.

Despite the incomplete DIN removal, the partial DON removal and the increased variability due to the changes in sample volume, the use of the conventional dialysis pretreatment procedure resulted in more precise and reproducible DON determinations. Because of the lack of a certified reference sample for DON, increased accuracy as a result of pretreatment procedures can not be absolutely proven. However, the improved recovery observed in our results indicate that the dialysis pretreatment might increase the accuracy of the TDN analysis and thus of the DON determinations. Refreshing the acceptor solution on fixed occasions showed to give similar removal efficiency for nitrate, but lower

removal of ammonium compared to the flow-through system of Lee and Westerhoff (2005).

To maintain the ionic permeability of the cellulose ester membrane, Runge et al. (2005) suggested addition of one of a variety of different salts to the acceptor solution. In our experiments, we added a  $\text{H}_3\text{PO}_4/\text{KH}_2\text{PO}_4$ -buffer (pH 2.2) to the dialyzing solution in order to retain a high ionic permeation rate and to prevent a net charge development on the dialysis membrane. The addition of the phosphate buffer to the acceptor solution resulted in a complete removal of nitrate ions (Table 2.3) and a considerable higher removal efficiency of ammonium ions (88 %) compared to the unbuffered dialysis. These results confirm the hypothesis that buffering the dialysis solution at a pH of 2.2 keeps the membrane uncharged and maintains its permeability for (positively) charged ions. The higher overall DIN removal efficiency resulted in a lower standard deviation and thus more precise DON determinations for all samples (Table 2.2).

Since the addition of the phosphate buffer gives rise to an uncharged membrane and thus to a better ionic permeability, also charged DOM molecules passed the membrane more easily, resulting in a higher transfer to the dialyzing solution. In addition, we visually observed a decreased adsorption of DOC to the membrane (no yellow stain). With the buffered acceptor solution, only  $13 \pm 9 \%$  DOC was removed from the sample, compared to a total DOC removal of  $16 \pm 14 \%$  with the unbuffered dialysis. However, this slight difference in DOM removal had no significant influence on the absolute DON concentration in all cases compared to the conventional dialysis. The DOC concentration in the precipitation sample slightly increased during the buffered dialysis treatment. Again, this is a result of a higher concentration in the acceptor solution which gives rise to inverse dialysis: transfer from the acceptor solution to the sample. This could also be seen in the blank samples where the DOC concentration increased on average with  $1.2 \text{ mg DOC l}^{-1}$  (data not shown) during dialysis.

The presence of a higher ionic strength in the acceptor solution compared to the samples caused large sample volume changes during the buffered dialysis treatment. The volume of the samples decreased with  $30.8 \pm 9.8 \%$  on average,

ranging from 10.5 % to 37.6 % (data not shown) which was considerably more than during the unbuffered dialysis. The error on the weight measurements used for the generation of a correction factor introduced an additional source of variation on the DON determinations. Two samples (forest floor leachate and E-horizon solution) out of 6 showed a slightly lower reproducibility using the buffered dialysis compared to the unbuffered dialysis. The results of the other samples indicated a better reproducibility of the buffered dialysis pretreatment compared to the unbuffered system set-up. As a lower ionic concentration of phosphate suffices to sustain membrane permeability (Runge et al., 2005), the same buffer compounds can be added in lower absolute concentrations in order to decrease the difference in ionic strength between samples and acceptor solution. This modification might diminish the sample volume losses and thus increase the method reproducibility.

The introduction of the phosphate-buffer to the dialyzing solution can have several drawbacks. Since the pH of the buffered acceptor solution is 2.2 and  $H^+$ - and  $OH^-$ -ions can permeate through the dialysis membrane, the pH of the sample will inevitably drop. This pH-decrease can lead to precipitation of humic acids but this was not observed in our experiments. This potential for precipitation may limit the use of the buffered dialysis as a pretreatment step with some types of samples. Furthermore, phosphate-ions can permeate through the 100 Da CE membrane (Moran et al., 1999) which implies that this type of buffer can not be used to optimize dissolved organic phosphorus (DOP) determinations.

The combination of concentration by lyophilization and dialysis was also tested as a means of improving analysis of low DON concentrations. Three field samples (precipitation, throughfall and groundwater) with a low initial DON concentration were lyophilized followed by the buffered dialysis pretreatment (Table 2.2 and 2.3). Since the buffered dialysis procedure was used, most DIN was removed from the concentrated samples. This extensive DIN removal combined with the concentration procedure resulted in very low standard deviations on the DON determination for the three field samples under investigation (Table 2.2). These findings confirm the hypothesis based on the theoretical variance

calculations that the concentration pretreatment combined with partial DIN removal increases the precision of the DON determination.

On average,  $33 \pm 2$  % of the DOM was removed from the samples during this combined pretreatment procedure based on the DOC measurements (Table 2.3). This value is considerably higher than the  $13 \pm 9$  % observed for the buffered dialysis alone, which may indicate that DOM is lost during the concentration pretreatment. Koprivnjak et al. (2006) found average TOC recoveries of 84 % with the coupled application of reverse osmosis (RO) and electrodialysis (ED) for the concentration of river water samples. They found the smallest TOC losses during ED (4 %) which was preceded by the size exclusion based RO concentration method (12 % TOC removal). Possible loss pathways in our experiments are volatilization, since the lyophilisation is performed under a strong vacuum, and/or adsorption to the glassware. This larger part of DOM removed during the sample pretreatment will inevitably result in lower DON concentrations (Table 2.3). As a consequence, the concentration pretreatment of aqueous samples increased the precision of the DON determination, but failed to increase the recovery.

Another drawback of this pretreatment procedure is that the freeze-dried DOM may not be all redissolved in deionized water. The presence of these precipitates can decrease the reproducibility of the DOC and TDN analyses, since taking a representative subsample is of crucial importance for accurate chemical analyses. The lowered reproducibility of the TDN analysis resulted in less reproducible DON determinations (Table 2.3).

It should also be noted that the dialysis pretreatment, whether or not buffered, will inevitably change the chemical composition of DOM in the samples. The small molecules ( $< 100$  Da), which are believed to be the most labile, will permeate through the dialysis membrane, humic acids can precipitate and certain DOM fractions can be adsorbed to the dialysis membrane. Therefore, the use of dialysis pretreatment as a preparatory step for subsequent experiments involving chemical characterization should be considered with caution.

## CONCLUSIONS

The results of one year field monitoring in forest ecosystems in Flanders indicated that aqueous field samples had high inorganic nitrogen content. These conditions of high DIN/TDN ratios in samples taken for DON determination are not only specific for Flanders, but occur in all regions subjected to high atmospheric N deposition. This study focused particularly on the potential of several sample pretreatment methods to increase the performance of the DON determinations in samples with high DIN/TDN ratios. The experiments led to the following conclusions and recommendations:

1. Because of the slightly higher recoveries, the persulfate oxidation (PO) method is preferred over the HTCO method for TDN measurements for DON determinations.
2. Sample pretreatment methods that lower the DIN/TDN ratio are effective to increase the precision of the DON determinations under all DIN/TDN ratios. Selective removal of DIN is absolutely necessary for samples with DIN/TDN ratios  $> 0.85$ .
3. Batch refreshment of the acceptor solution results in similar dialysis performance as the continuous flow-through set-up of Lee and Westerhoff (2005).
4. Buffering the acceptor solution increases reproducibility of the dialysis pretreatment and increases the recovery and precision of the subsequent DON determinations.
5. Because of the low DOM recovery as indicated by DOC concentrations, lyophilization coupled with the buffered dialysis can not be used to optimize DON determinations in aqueous samples with low DON concentration and high DIN/TDN ratio.

Dialysis against a phosphate-buffered acceptor solution is a reliable, reproducible, though time-consuming, pretreatment method to increase the analytical recovery and precision of DON determinations in aqueous field samples with high DIN/TDN ratios. The only way to obtain an objective and absolute measure for the

accuracy of both the analysis and pretreatment methods is the use of samples with known initial dissolved organic and inorganic nitrogen concentration. Thus, there is a need for such certified reference sample for TDN and DON measurements that would be representative of naturally occurring DON.



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# **CHAPTER 3**

## **DISSOLVED ORGANIC CARBON (DOC) AND NITROGEN (DON) FLUXES IN DECIDUOUS AND CONIFEROUS FORESTS UNDER LONG-TERM NITROGEN DEPOSITION**

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Illustration on p. 43:

A monitoring plot in the Corsican pine stand at Overheide with four throughfall collectors and three lysimeters at different depths in the mineral soil.

*This chapter has been submitted as:*

*Vandenbruwane J., Wuyts K., De Schrijver A., Gielis L., De Neve S., Verheyen K. and Hofman G. Dissolved organic carbon (DOC) and nitrogen (DON) fluxes in deciduous and coniferous forests under long-term nitrogen deposition. Biogeochemistry (Submitted).*

## ABSTRACT

Recent studies have indicated that dissolved organic carbon (DOC) and nitrogen (DON) play an important role in C and N cycling in (semi-)natural ecosystems. Inorganic nitrogen deposition has been shown to alter the concentrations and fluxes of these dissolved organic substances and to increase dissolved organic matter (DOM) leaching losses from forests. In this study, concentrations and fluxes of DOC, DON and dissolved inorganic nitrogen (DIN) were quantified in deciduous and coniferous forests in Flanders under long-term nitrogen deposition. Furthermore, we aimed to investigate the impact of different levels of throughfall nitrogen deposition on DOM cycling in a coniferous stand. The input with precipitation was an important source of DON, though of minor importance for DOC. Passage of the aqueous solution through the forest floor largely increased the DOC fluxes. The release of DOM from the forest floor was minimally affected by forest type, but was strongly influenced by the proximity of the forest edge. Interferences resulting from the edge effect caused an 82 % increase of DOM release from the forest floor. Adsorption to mineral soil material rich in iron and aluminum oxyhydroxides was suggested to be the most important process removing DOM from the soil solution. This sorption mechanism largely determined DOM leaching losses and was responsible for substantial retention of DOM entering the mineral soil profile with forest floor leachate (67-84 %) and for further decrease of the DOC concentration (71-76 %) in the vadose zone below the rooting zone. Generally, DON was less reactive (i.e. less removal from the soil solution) than DOC, resulting in steadily decreasing DOC/DON ratios with soil depth. The relative contribution of DON (9-28 %) to total nitrogen leaching from these ecosystems was mainly determined by the extent of the DIN fluxes, which were in turn determined by the ecosystem DIN input with throughfall water.

## INTRODUCTION

Photosynthesis is the primal origin of all organic matter in forested ecosystems (Schulze, 2000) in which the living plants, the forest floor and the mineral soil comprise the largest pools (Janssens et al., 1999; Vande Walle et al., 2001). Soil organic matter is generally subdivided in two operationally defined fractions namely solid and dissolved organic matter (DOM). Dissolved organic matter (DOM) is defined as the continuum of organic compounds in the dissolved phase passing a filter with a certain pore size (mostly 0.45  $\mu\text{m}$ ). Consequently, dissolved organic carbon (DOC) and nitrogen (DON) are the carbon and nitrogen included in the DOM. The high mobility of DOM results in translocation of organic material and nutrients within and between ecosystems. The downward transport of DOM and associated elements with percolating water also plays a crucial role in soil formation (Zysset and Berggren, 2001), nutrient loss (Kaiser et al., 2001) and eutrophication of ground and surface waters.

Atmospheric deposition (Neff et al., 2002) and release from the canopy and the forest floor (Michalzik et al., 2001) are the principal sources of DOM in water passing through forest ecosystems. Adsorption to mineral soil, microbial degradation and leaching to groundwater are considered to be the major sinks or losses of DOM (Kalbitz et al., 2000). The concentrations, and thus fluxes, and chemical composition of DOM are related to a high number of external and internal chemical and physical factors (Kalbitz et al., 2000). Precipitation, average temperature (Christ and David, 1996) and the number of drying-rewetting cycles (Prechtel et al., 2000) can be considered to be more or less constant within certain climatic regions. External disturbing influences, mostly as a result of human activities, are much more variable and have been reported to change the chemical composition, concentrations and thus fluxes of DOM: sulfate concentration (Evans et al., 1988), ionic strength (Kalbitz et al., 2000; Münch et al., 2002), pH (Vance and David, 1992) and ecosystem nitrogen input (Pregitzer et al., 2004) with throughfall water were found to be the most important disruptions.

During the last century, the concentration of nitrogenous compounds in the atmosphere increased as a result of human activities (Asman et al., 1988). Combustion of fossil fuels and intensive livestock breeding are probably the two major sources of reactive N forms in the atmosphere (Galloway et al., 1995). This increased atmospheric nitrogen emission results in an elevated wet and dry deposition in aquatic and terrestrial ecosystems, causing changes in biogeochemical cycles (Aber et al., 1989), including cycling of DOM (McDowell et al., 2004).

Contradictory results have been found concerning the effect of elevated N deposition on DOM cycling within ecosystems and various mechanisms have been hypothesized and tested. Sjöberg et al. (2003) found no significant changes in DOC and DON production in mor humus after repeated N additions in a lab experiment. Also Raastad and Mulder (1999) found no significant effects on DOC and DON concentrations after 4 years of nitrogen addition to a forested catchment in Sweden. McDowell et al. (1998) reported small changes in DOC and large increases in DON concentrations leaching from the forest floor as a result of chronic nitrogen amendments in a long term field experiment. Currie et al. (1996) found fluxes of DON from the forest floor to be positively correlated with rates of N addition, but export of DON from the mineral soil appeared unaffected by 7 years of N amendments. Park et al. (2002) found a reduced DOC release, but increased release of DON and dissolved inorganic nitrogen (DIN) from a deciduous forest floor after  $\text{NH}_4\text{NO}_3$  amendments. Magill et al. (2000) found no effect of nitrogen additions on DOC concentrations, but reported increased DON concentrations in soil water at 60 cm depth. Pregitzer et al. (2004) reported dramatic increases in leaching losses of DOC and DON during a long-term N addition experiment in the field.

The potential impact of an elevated nitrogen deposition on DOM cycling in forest ecosystems is most often studied in laboratory and field manipulation experiments in which mineral nitrogen forms are artificially added to the ecosystem. However, these manipulation experiments all cover a limited time scale probably not reaching a new equilibrium situation in this relatively short

time. Therefore, the objective of this study was to quantify concentrations and fluxes of DOC and DON in the different ecosystem strata of a deciduous and coniferous forest on sandy soil in Flanders that have been subjected to high nitrogen depositions for several decades. Temporal fluctuations in the different ecosystem strata and the major sources and sinks of DOM in these forest ecosystems were monitored during one year of field sampling. The potential influence of forest type and of an additional gradient in atmospheric N deposition on DOC and DON cycling was investigated. The export of DOC and DON to the groundwater and the relative contribution of DON to total nitrogen leaching was determined quantitatively under these specific circumstances.

## MATERIALS AND METHODS

### *Site description*

The forest stands selected for this study are situated in the northern part of Belgium (province of Antwerp). The forests in this region are dominated by homogeneous plantations of Corsican pine (*Pinus nigra* ssp. *laricio* Maire) and Scots pine (*Pinus sylvestris* L.), a large part of them in conversion to mixed stands with silver birch (*Betula pendula* Roth.) and common oak (*Quercus robur* L.). The region is characterized by intensive livestock breeding and is therefore characterized by high atmospheric concentrations of reduced nitrogen. Three homogenous forest stands were selected (Figure 3.1). Two neighboring stands, namely one silver birch (SB) and one Corsican pine (CP) stand, were located in the middle of the forest complex ‘Overheide’, part of the state forest ‘Domeinbos Ravels’, both receiving background nitrogen deposition. A second Corsican pine stand (CPN) was selected in the forest complex ‘Tulderse Heide’, with an abrupt forest edge bordering undisturbed pasture and exposed to the prevailing westerly to south-westerly winds. The edge of this forest stand (CPN) was assumed to receive substantially higher N depositions compared to the interior parts of closed forest stands (SB and CP) (Spangenberg and Kölling, 2004). The major stand characteristics for the three forest stands are summarized in Table 3.1.

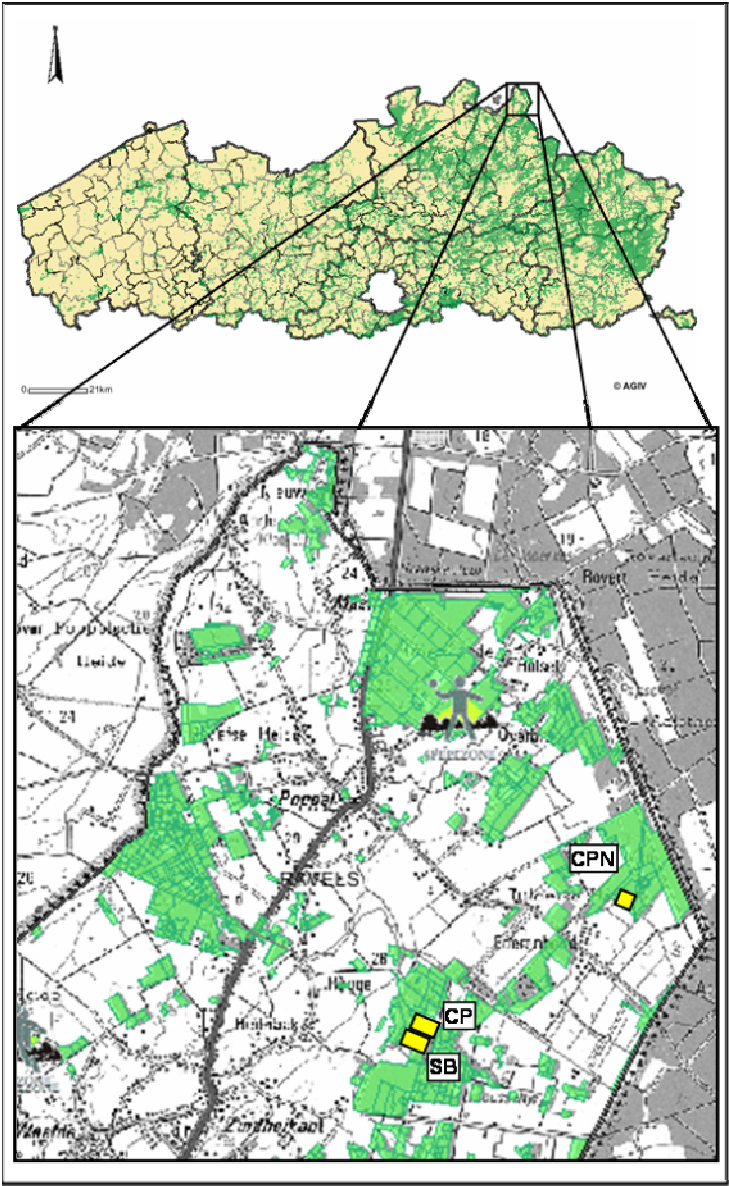


Figure 3.1. Location of the study plots in Flanders (Adapted from: Agiv, 2007)

**Table 3.1. Stand characteristics of the three selected forest stands**

Site code	SB	CP	CPN
Location name	Overheide	Overheide	Tulderse Heide
Longitude	51°25'27.0" N	51°25'28.6" N	51°26'11.7" N
Latitude	5°03'20.7" E	5°03'21.3" E	5°05'26.5" E
Elevation (m asl)	27.4	27.4	28.9
	<i>Betula pendula</i>	<i>Pinus nigra</i> ssp.	<i>Pinus nigra</i> ssp.
Tree species	Roth	<i>Laricio</i> Maire	<i>laricio</i> Maire
Age* (yr)	45	50	45
Stand density* (number ha <sup>-1</sup> )	891	722	nd <sup>†</sup>
Average tree height* (m)	17.4	17.2	15.4
Basal area* (m <sup>2</sup> ha <sup>-1</sup> )	21	41	nd
Average LAI* (m <sup>2</sup> m <sup>-2</sup> )	2.67	2.86	2.50
Current volume growth (m <sup>3</sup> ha <sup>-1</sup> yr <sup>-1</sup> ) <sup>‡</sup>	0.67	1.38	1.50
Distance to W-NW forest edge (m)	435	435	< 10

\* Results from complete stand survey in 2006;

<sup>†</sup>nd = not determined;

<sup>‡</sup>data deducted from Jansen et al. (1996).

The soils under the selected forest stands are podzols developed on sand deposits and classified as Typic Haplorthod according to USDA Soil Taxonomy. The main soil properties of the different mineral horizons at the three stands are presented in Table 3.2. Air-dried soil samples were homogenized, 2 mm sieved and analysed. The pH was measured potentiometrically in a 1 / 2.5 soil / 1 M KCl extract (pH-KCl) and in a 1 / 5 soil / water extract (pH-H<sub>2</sub>O). Total carbon (C<sub>tot</sub>) and total nitrogen (N<sub>tot</sub>) were measured by dry combustion at 850 °C using an elemental analyzer (Vario MAX CNS, Elementar, Germany). The NH<sub>4</sub><sup>+</sup>-acetate method was used to determine the cation-exchange capacity (CEC) of the soils (Kalra and Maynard, 1991). Particle-size distribution was determined by the pipette method (Gee and Bauder, 1986). Ammonium oxalate-extractable iron and aluminum (Fe<sub>ox</sub> and Al<sub>ox</sub>) was extracted using the method of Jackson et al. (1986) and then measured with an atomic absorption spectrophotometer (SpectrAA, Varian, Belgium).



**Table 3.2. Mineral soil properties to a depth of 90 cm at the three sites under investigation**

	Thickness (cm)	% sand (50- 200 µm)	% silt (2 - 50 µm)	% clay (0 - 2 µm)	CEC (cmol <sup>+</sup> kg <sup>-1</sup> )	pH-H <sub>2</sub> O (-)	pH-KCl (-)	C <sub>tot</sub> (g kg <sup>-1</sup> )	N <sub>tot</sub> (g kg <sup>-1</sup> )	Fe <sub>ox</sub> (g kg <sup>-1</sup> )	Al <sub>ox</sub> (g kg <sup>-1</sup> )
<i>SB</i>											
A	12 ± 1	92.5	6.9	0.5	9.00	4.18 ± 0.02	2.82 ± 0.01	37.7 ± 0.4	1.11 ± 0.03	0.38 ± 0.04	0.37 ± 0.01
E	5 ± 0	93.5	5.9	0.6	1.75	4.28 ± 0.05	3.14 ± 0.05	10.1 ± 0.1	0.30 ± 0.00	0.15 ± 0.02	0.18 ± 0.02
Bh	4 ± 1	92.2	6.4	1.4	23.40	4.01 ± 0.00	3.09 ± 0.06	63.2 ± 0.8	2.02 ± 0.05	1.43 ± 0.03	2.02 ± 0.02
Bs	7 ± 0	93.1	5.2	1.7	9.70	4.38 ± 0.01	4.08 ± 0.03	15.9 ± 0.3	0.56 ± 0.02	4.09 ± 0.04	1.72 ± 0.01
BC	30 ± 1	94.3	3.8	1.9	2.35	4.70 ± 0.01	4.63 ± 0.02	3.3 ± 0.0	0.15 ± 0.00	1.63 ± 0.05	1.51 ± 0.04
C	32 ± 2	96.2	2.6	1.2	1.55	4.78 ± 0.01	4.58 ± 0.08	1.4 ± 0.0	0.07 ± 0.03	0.74 ± 0.03	0.84 ± 0.03
<i>CP</i>											
A	17 ± 3	93.3	5.8	0.9	10.55	4.31 ± 0.07	2.99 ± 0.03	39.4 ± 1.4	1.42 ± 0.04	0.55 ± 0.05	0.86 ± 0.04
E	13 ± 1	95.1	4.7	0.2	1.30	4.53 ± 0.02	3.75 ± 0.02	4.4 ± 0.0	0.14 ± 0.02	0.11 ± 0.00	0.18 ± 0.01
Bh	7 ± 3	93.9	4.4	1.7	9.50	4.18 ± 0.03	3.61 ± 0.04	27.8 ± 0.2	1.10 ± 0.03	0.45 ± 0.02	1.44 ± 0.01
Bs	7 ± 2	93.5	4.7	1.8	11.50	4.18 ± 0.00	3.72 ± 0.06	27.9 ± 0.3	0.97 ± 0.03	0.55 ± 0.04	2.03 ± 0.09
BC	17 ± 2	94.4	4.5	1.1	3.20	4.53 ± 0.03	4.52 ± 0.10	5.4 ± 0.0	0.20 ± 0.01	0.80 ± 0.03	1.83 ± 0.05
C	29 ± 2	94.4	4.7	0.8	1.40	4.58 ± 0.02	4.59 ± 0.05	1.5 ± 0.1	0.09 ± 0.01	0.28 ± 0.01	0.82 ± 0.01
<i>CPN</i>											
A	17 ± 3	95.7	4.3	0.1	4.65	4.26 ± 0.04	2.83 ± 0.05	27.1 ± 0.1	0.74 ± 0.03	0.26 ± 0.00	0.20 ± 0.01
E	12 ± 5	95.7	4.2	0.1	1.25	4.31 ± 0.02	3.17 ± 0.01	7.4 ± 0.1	0.18 ± 0.01	0.10 ± 0.02	0.08 ± 0.01
Bh	13 ± 3	94.2	4.5	1.3	8.95	3.91 ± 0.02	3.09 ± 0.03	24.4 ± 0.5	0.80 ± 0.03	0.25 ± 0.03	1.12 ± 0.02
Bs	9 ± 5	94.9	3.6	1.5	7.05	4.08 ± 0.01	3.40 ± 0.01	14.6 ± 0.7	0.47 ± 0.05	0.26 ± 0.02	1.24 ± 0.02
BC	22 ± 2	98.6	0.9	0.5	3.10	4.51 ± 0.00	4.15 ± 0.05	4.6 ± 0.0	0.11 ± 0.02	0.37 ± 0.12	1.58 ± 0.06
C	17 ± 3	98.9	0.9	0.2	1.85	4.68 ± 0.01	4.54 ± 0.05	1.4 ± 0.0	0.06 ± 0.02	0.14 ± 0.01	0.06 ± 0.01

The forest floor of the three forest stands consisted of a succession of OL, OF and OH layers and were characterized by a sharp boundary between the OH and mineral A horizon. Consequently the forest floors were classified as mor humus types (Green et al., 1993). Forest floor material (Oi + Oe + Oa) from a 0.25 m<sup>2</sup> square was completely sampled in 9 replicates at each of the three forest stands and weighed field moist. Subsamples of these homogenized samples were taken for microbiological and chemical analyses. Portions of these subsamples were dried at 60 °C for 96 hours to determine moisture content and the amount of dry forest floor material per m<sup>2</sup> was calculated. The dried subsamples were ground using a ball-mill and stored in glass vials until used for further chemical analyses (Table 3.3).

**Table 3.3. Main properties of forest floor material and annual litterfall at the three selected forest stands**

	SB	CP	CPN
<i>Forest floor</i>			
Dry matter (DM) (kg m <sup>-2</sup> )	5.80 ± 1.50 <sup>a</sup>	5.30 ± 1.36 <sup>a</sup>	4.00 ± 0.50 <sup>b</sup>
Ash (%)	14.9 ± 14.7 <sup>a</sup>	19.8 ± 10.4 <sup>a</sup>	27.4 ± 12.9 <sup>a</sup>
C <sub>tot</sub> (%)	48.4 ± 4.1 <sup>a</sup>	45.8 ± 4.0 <sup>a</sup>	43.7 ± 1.4 <sup>a</sup>
N <sub>tot</sub> (%)	2.24 ± 0.23 <sup>a</sup>	1.70 ± 0.32 <sup>b</sup>	1.61 ± 0.33 <sup>b</sup>
C/N	21.6 ± 0.5 <sup>a</sup>	27.0 ± 3.1 <sup>b</sup>	27.2 ± 3.5 <sup>b</sup>
pH-H <sub>2</sub> O	3.79 ± 0.02 <sup>a</sup>	3.80 ± 0.03 <sup>a</sup>	4.02 ± 0.01 <sup>b</sup>
<i>Annual litterfall</i>			
Dry matter (kg m <sup>-2</sup> yr <sup>-1</sup> )	0.37 ± 0.02 <sup>a</sup>	0.82 ± 0.10 <sup>b</sup>	0.56 ± 0.13 <sup>a</sup>
C <sub>tot</sub> (%)	50.5 ± 0.1 <sup>a</sup>	49.4 ± 0.5 <sup>b</sup>	49.6 ± 0.6 <sup>b</sup>
N <sub>tot</sub> (%)	1.56 ± 0.05 <sup>a</sup>	0.82 ± 0.11 <sup>b</sup>	1.07 ± 0.10 <sup>a</sup>
C/N	32.4 ± 1.1 <sup>a</sup>	60.2 ± 12.2 <sup>b</sup>	46.4 ± 8.9 <sup>b</sup>

<sup>a,b,c</sup> Means with a different letter within the same line differ significantly at the  $p < 0.05$  level.

According to data obtained at the nearest meteorological station of the Royal Meteorological Institute of Belgium (KMI), i.e. at Retie (17-19 km south of the research sites), mean monthly temperatures vary from 5.5 °C in January to 19.2 °C in July. Average annual precipitation in the study area is 748 mm and average annual temperature amounts to 9.5 °C.

### *Experimental set-up and sample collection*

Within each of the three forest stands, three plots were established to monitor throughfall deposition, forest floor leachate, soil solution and groundwater concentrations. In SB and CP, the plots were installed in the middle of the stands to avoid edge influences, while in CPN, plots were installed within the first 10 m from the exposed forest edge. Water samples were collected and measured fortnightly between March 2005 and March 2006.

Open-field bulk deposition was collected in grassland (for SB and CP) or a recently afforested clear-cut (for CPN) in the immediate surroundings of the stands using four collectors. In each forested plot, four throughfall collectors were installed spaced 2 m apart. Stemflow was not collected because of its negligible contribution to the total ion flux reaching the forest floor in pine stands (Neirynek et al., 2004). For silver birch, its contribution to the total water flux and N flux to the forest floor is generally limited to 2 and 1 % respectively (Alcock and Morton, 1985). Open-field bulk and throughfall collectors consisted of a polyethylene (PE) funnel (177 cm<sup>2</sup>) supported by an opaque tube draining into a two-liter PE bottle. The bottles were placed below ground level to keep the samples cool and to protect them from direct sunlight. The use of a nylon wire mesh (1 mm mesh size) placed in the funnel's neck prevented contamination by large particles. At each sampling occasion, the sample volume in every collection bottle was measured on site and PE bottles and funnels were replaced by specimens that were rinsed with distilled water. Samples with visible contamination of animal excreta were discarded and the remaining samples of each plot were pooled volume-weighted to one bulk sample. A 300 ml subsample was taken for chemical analysis.

The forest floor leachate was collected using three zero-tension lysimeters per plot, installed directly underneath the forest floor. These lysimeters were made from 30 cm long rectilinear polyvinylchloride (PVC) guttering (338 cm<sup>2</sup>) covered with wire netting (1.5 mm mesh size) and connected with 20 cm flexible PVC tubing to a two-liter PE bottle installed beneath the lysimeter. At every sampling event, the three forest floor leachate samples of each plot were

pooled volume-weighted to one bulk sample and the PE sampling bottles were replaced by specimens that were rinsed with distilled water.

The soil solution in the different horizons was sampled using acid washed ceramic suction cup lysimeters (Model 1900 Soil Water Sampler with B02M2 cup, Soilmoisture Equipment Corp., Santa Barbara, USA) operating at -50 kPa. A preliminary experiment indicated that these lysimeters have least influence on the sampled DOC concentration compared to other types (Vandenbruwane et al., in press). One single lysimeter per plot was installed at each of three depths to capture the water percolating under the E, Bs and BC horizon. The lysimeters were installed 6 months before the start of the monitoring program and on average 3.2 l of soil solution was sampled by each lysimeter in order to bring them in equilibrium with the surrounding soil (solution) before effective sample collection.

For groundwater sampling, perforated PVC tubes (4 cm diameter) equipped with PE tubing to the bottom, were installed to a depth of 2.5 m in the mineral soil. At every sampling event, the depth of the groundwater table was measured using an electronic water level meter and the sampling tube was evacuated three times before effective sampling. The groundwater was pumped up through PE tubing draining into a Büchner flask which was connected to a vacuum pump.

The matric potential in the mineral soil of the SB and CP stand was monitored continuously using electronic tensiometers (T4, UMS GmbH, München, Germany) connected to a datalogger (DL2e, Delta-T Devices Ltd, Cambridge, UK), equipped with an external 12 V power supply (TV-Batt, UMS GmbH, München, Germany). Tensiometers were installed at three depths (below the E, Bh and BC horizons) in three plots in the SB and CP stands and the matric potentials were recorded at hourly intervals. The moisture content of the soil at these depths was determined gravimetrically at four occasions and converted to volumetric moisture content using the bulk densities measured on intact soil cores.

Soil temperature at different depths in the soil profile (15, 25, 65 and 90 cm) was recorded in one SB plot on an hourly base using electronic temperature probes with Fenwall-thermistor (Th2-f, UMS GmbH, München,

Germany) connected to a datalogger (DL2e, Delta-T Devices Ltd, Cambridge, UK) equipped with an external 12 V power supply (TV-Batt, UMS GmbH, München, Germany).

### *Chemical analyses*

Before analysis, all samples were filtered through 0.45 µm nylon syringe filters (Rotilab®, Carl Roth GmbH, Karlsruhe, Germany) and kept at -18 °C until analysis. All samples were analyzed for DOC, TDN,  $\text{NO}_3^-$  and  $\text{NH}_4^+$  concentration. Total dissolved carbon (TDC) and dissolved inorganic carbon (DIC) were determined on a Shimadzu TOC-V CPN-analyzer (Shimadzu Corp., Tokyo, Japan) with IR detection following thermal oxidation. Dissolved organic carbon (DOC) was calculated as the difference between TDC and DIC. Total dissolved nitrogen (TDN) was determined after alkaline persulfate oxidation (Koroleff, 1983) as  $\text{NO}_3^-$  with a continuous flow autoanalyser. Samples were analyzed for dissolved inorganic nitrogen (DIN) ( $(\text{NO}_3^- + \text{NO}_2^-)\text{-N}$  and  $\text{NH}_4^+\text{-N}$ ) using a continuous flow autoanalyser (ChemLab System 4) and dissolved organic nitrogen (DON) was calculated as the difference between TDN and DIN. A substantial subset of samples with various concentrations and origins, were analyzed for  $\text{NO}_2^-\text{-N}$ , but none were above the detection limit. Detection limits for nitrate-N and ammonium-N were 0.21 and 0.03 mg N l<sup>-1</sup> respectively, whereas the 95 % confidence interval (CI) and thus detection limit of the DON determinations was variable and depended on the DIN/TDN ratio (Vandenbruwane et al., 2007a). If nitrate, ammonium or DON concentrations were below the detection limit, concentrations were set to 50 % of the corresponding detection limit.

### *Calculation of solute fluxes*

Whereas the fortnightly water fluxes of precipitation and throughfall were measured on site, the water fluxes in the forest floor and mineral soil at different depths in the three forest stands were calculated using the water-balance model SWAP (Appendix 1). Fortnightly solute fluxes were calculated by multiplying the fortnightly water fluxes by the fortnightly solute concentration. The sum of these

fortnightly solute fluxes over the one year monitoring program yielded the annual solute flux for each stratum.

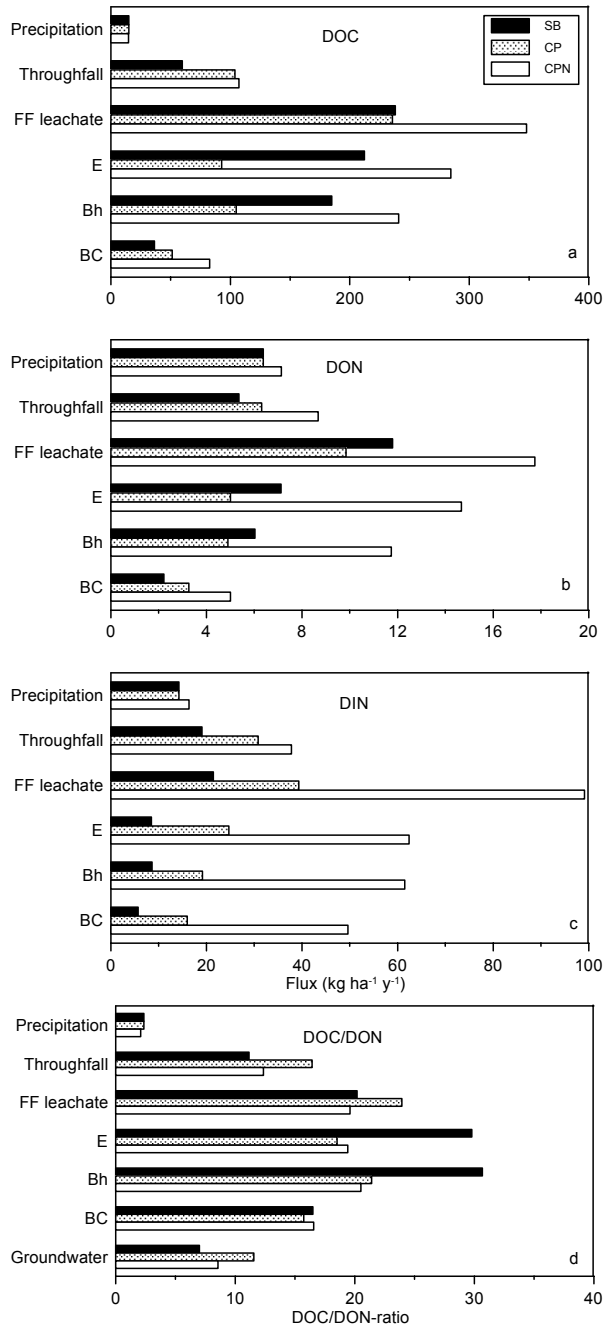
### *Statistical analyses*

Three sets of paired samples *t*-tests were used to detect significant differences in precipitation and throughfall DOC, DON and DIN concentrations between the three forest stands. Correlations between DOC and DON concentrations in forest floor leachates of each of the three stands and in the three mineral horizons of the three stands together were detected by linear regression analysis. The significance of the linear regressions was assessed using ANOVA. All statistical analyses were performed using SPSS 12.0. Since the solute fluxes with forest floor leachate and mineral soil solution result from calculations based on SWAP simulated water fluxes, no correct statistical analyses could be performed on the former values.

## **RESULTS**

### *Nitrogen deposition*

The bulk precipitation DIN deposition fluxes at the two sites were not significantly ( $p > 0.05$ ) different: 14.2 kg DIN ha<sup>-1</sup> yr<sup>-1</sup> at the Overheide site versus 16.4 kg DIN ha<sup>-1</sup> yr<sup>-1</sup> at the Tulderse Heide site (Figure 3.2). Also the bulk precipitation input of organic N did not differ between the two locations: 6.4 kg DON ha<sup>-1</sup> yr<sup>-1</sup> at the Overheide site and 7.1 kg DON ha<sup>-1</sup> yr<sup>-1</sup> at the Tulderse Heide site. As a result, the bulk TDN deposition with precipitation only differed about 3 kg N ha<sup>-1</sup> yr<sup>-1</sup> between the two sites. Thus, DIN contributed for about 70 % to the TDN deposition in this region and was mainly in the form of ammonium-N (65 % of DIN) (Table 3.4).



**Figure 3.2.** Annual fluxes of dissolved organic carbon (DOC) (a), nitrogen (DON) (b) and dissolved inorganic nitrogen (DIN) (c) and DOC/DON-ratio (d) in the different strata of the three forest stands in Flanders.

The DIN and DON throughfall fluxes differed significantly ( $p < 0.01$ ) between the two neighbouring stands; 19.1 and 5.4 kg N ha<sup>-1</sup> yr<sup>-1</sup> in the SB stand and 30.8 and 6.3 kg N ha<sup>-1</sup> yr<sup>-1</sup> in the adjacent CP stand. The location of the CPN plots at the west-southwesterly oriented forest edge resulted in an increased DIN and significantly higher ( $p < 0.01$ ) DON input in comparison to the CP plots: 37.8 and 8.7 kg N ha<sup>-1</sup> yr<sup>-1</sup> respectively. The difference in total nitrogen input with throughfall water between the two deposition loads was thus 9.4 kg N ha<sup>-1</sup> yr<sup>-1</sup> in total (7.0 kg DIN-N and 2.4 kg DON-N ha<sup>-1</sup> yr<sup>-1</sup>) or an increase of 25 %.

**Table 3.4. Average TDN composition (as % of TDN) in the different strata of the three forest ecosystems over the 1 year sampling period**

Stratum	SB			CP			CPN		
	NO <sub>3</sub> <sup>-</sup> -N	NH <sub>4</sub> <sup>+</sup> -N	DON	NO <sub>3</sub> <sup>-</sup> -N	NH <sub>4</sub> <sup>+</sup> -N	DON	NO <sub>3</sub> <sup>-</sup> -N	NH <sub>4</sub> <sup>+</sup> -N	DON
Precipitation	24	45	31	24	45	31	24	46	30
Throughfall	23	55	22	21	62	17	21	61	19
Forest floor	45	19	35	50	30	20	33	52	15
E-horizon	50	4	46	77	7	17	55	26	19
Bh-horizon	56	3	41	78	2	20	61	23	16
BC-horizon	67	5	28	81	2	17	90	1	9
Groundwater	83	3	14	88	1	11	93	1	6

### *Dissolved organic carbon*

#### *Temporal concentration patterns*

The temporal fluctuations in DOC concentrations in forest floor leachates and in soil solutions of the BC horizons are given in Figure 3.3a and 3.4a, respectively. The DOC concentration in the forest floor leachate is of particular interest since it reflects the amount and concentration of DOC entering the mineral soil. The BC horizon leachate is representative of the solution leaving the rooting zone and thus indicative of the leaching losses from the forest ecosystem.

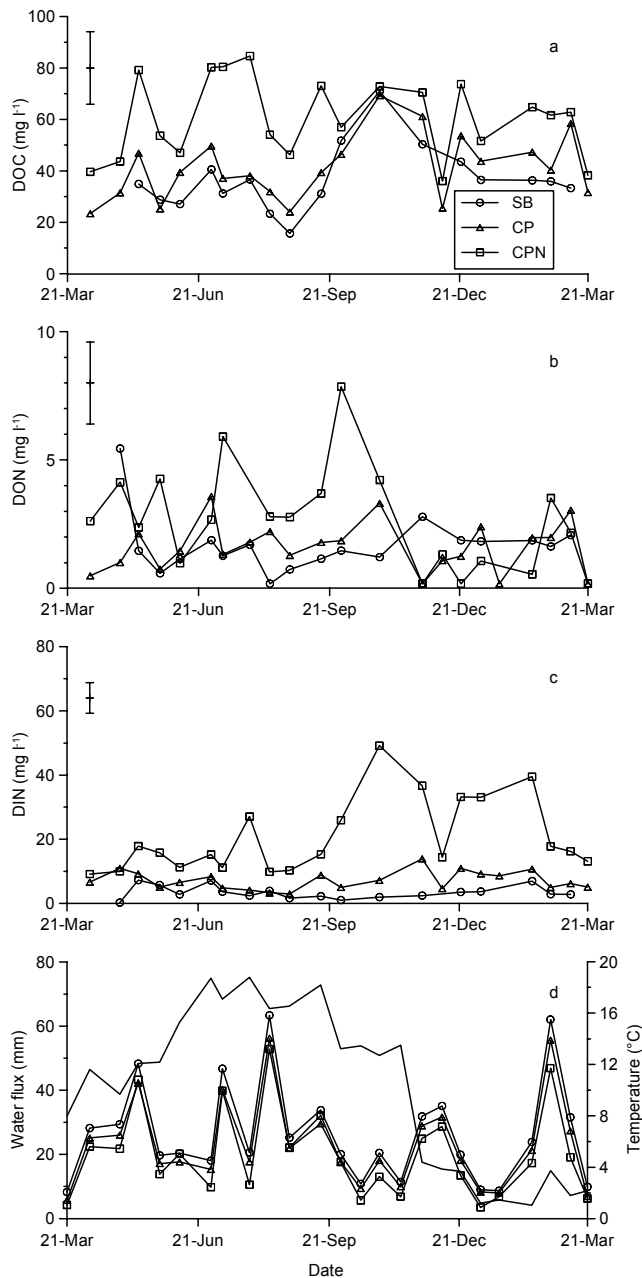
The DOC concentrations sampled below the forest floor at the SB and CP stands ran more or less parallel throughout the sampling period. The concentrations sampled under the SB forest floor were slightly lower than those



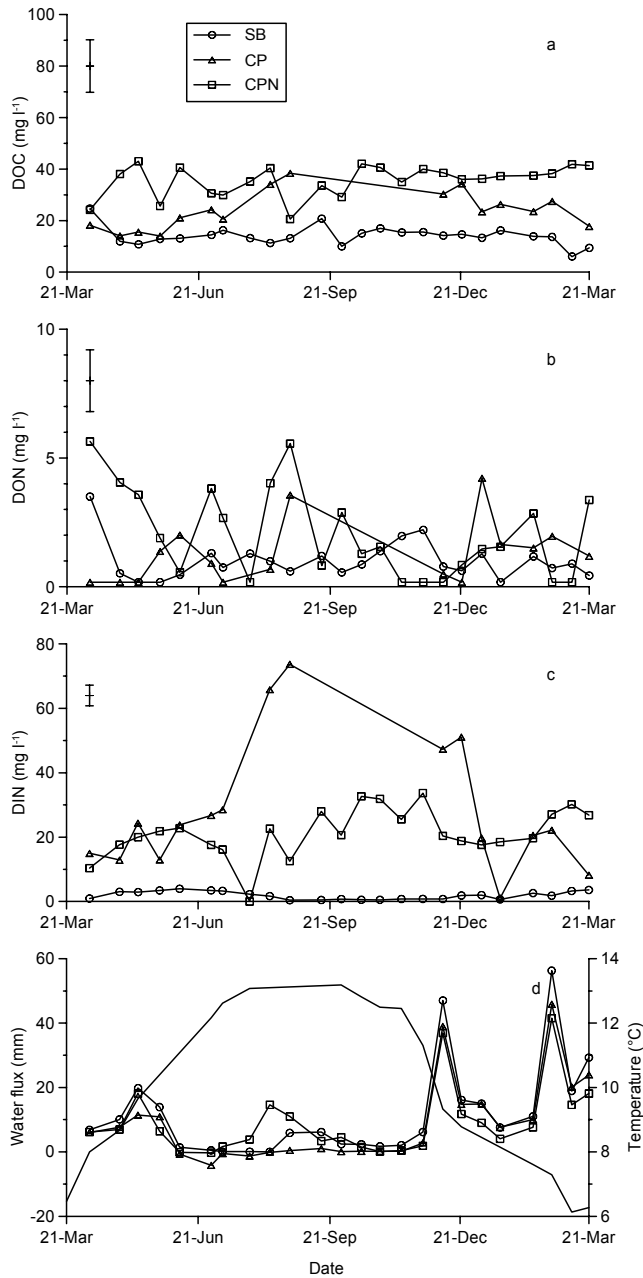
sampled under the CP forest floor, whereas the CPN forest floor released consistently higher concentrations compared to the CP forest floor. The course of the DOC concentrations in the forest floor leachate was obviously related to the water fluxes during the sampled fortnight and the preceding fortnight(s) (Figure 3.3d). For instance, considering the three dry fortnights in late May and June followed by a high water flux at the beginning of July, the DOC concentrations in the litter leachate increased steadily during the dryer weeks i.e. accumulation of leachable DOM occurred in the forest floor during dryer periods. This accumulation effect for DOC was also observed on July 27, October 26 and December 22. The inverse effect i.e. dilution as a result of a higher water flux occurred on August 10, December 9 and February 23.

In the SB and CP stands, slightly decreasing DOC concentrations were observed with increasing soil temperature. On the contrary, higher temperature rather increased DOC release from the CPN forest floor material.

The course of the DOC concentrations in the soil solution sampled under the BC-horizon showed similar patterns for the three forest stands; more or less constant concentrations were sampled throughout the year with limited seasonal fluctuations (Figure 3.4a). The restricted DOM fluctuations in the lower part of the soil profile compared to the larger variations in DOM concentrations entering the mineral soil suggest the presence of a mechanism controlling the DOM concentrations in the mineral soil.



**Figure 3.3.** Concentrations of dissolved organic carbon (DOC) (a), nitrogen (DON) (b) and dissolved inorganic nitrogen (DIN) (c) in the zero-tension lysimeter samples from the forest floor collected from 21 March 2005 to 21 March 2006. Water fluxes of litter leachate in the three forest stands and average air temperature during the measuring fortnights (d). Error bars indicate average standard deviations.



**Figure 3.4.** Concentrations of dissolved organic carbon (DOC) (a) and nitrogen (DON) (b) and dissolved inorganic nitrogen (DIN) (c) in the lysimeter samples from the BC horizon collected from 21 March 2005 to 21 March 2006. Water fluxes (+ = downward, - = upward) under the three forest stands and average soil temperature during the measuring fortnights (d). Error bars indicate average standard deviations.

*Annual fluxes in the ecosystem strata*

The annual DOC fluxes in the different strata of the three forest stands are given in Figure 2.2a whereas the net changes in DOC fluxes are summarized in Table 3.5.

**Table 3.5.** Net addition (indicated by a + sign) or removal (indicated by a – sign) ( $\text{kg ha}^{-1} \text{ yr}^{-1}$ ) of DOC, DON, and DIN from solution as it passed through each stratum or horizon. Calculated as: the flux through the stratum minus the flux through the stratum above.

Stratum	SB			CP			CPN		
	DOC	DON	DIN	DOC	DON	DIN	DOC	DON	DIN
Throughfall	45	-1.01	4.84	89	-0.06	16.6	92	1.54	21.4
Forest floor	178	6.43	2.37	132	3.52	8.48	240	9.07	61.3
E-horizon	-26	-4.67	-12.9	-143	-4.84	-14.6	-63	-3.08	-36.7
Bh-horizon	-27	-1.09	0.12	12	-0.10	-5.57	-44	-2.93	-0.87
BC-horizon	-148	-3.81	-2.94	-54	-1.63	-3.15	-158	-6.73	-11.87

The DOC flux in precipitation water measured in our study was  $15 \text{ kg ha}^{-1} \text{ yr}^{-1}$  at both sites. Passage of precipitation water through the SB canopy increased the DOC flux with  $45 \text{ kg DOC ha}^{-1} \text{ yr}^{-1}$  whereas the CP and CPN canopies contributed for 89 and  $92 \text{ kg ha}^{-1} \text{ yr}^{-1}$  in the throughfall water flux (Table 3.5). The observed differences in DOC release from the canopies can originate from differences in (i) interception of dry DOC deposition between needles and leaves and/or (ii) the release of plant derived DOC (e.g. waxes). The nature of the experimental set-up, however, did not allow a discrimination between these two DOC sources. We found DOC concentrations in throughfall water samples to be positively correlated with nitrate concentrations ( $r^2 = 0.21 - 0.50$ ) and to a lower extent with ammonium concentrations ( $r^2 = 0.01 - 0.32$ ) in the three forest stands.

Passage of throughfall water through the forest floor increased the DOC concentration substantially at the three stands, and thus the forest floor was by far the major source of DOC in these ecosystems (Table 3.5). The SB forest floor released  $36 \text{ kg DOC ha}^{-1} \text{ yr}^{-1}$  more than the CP forest floor. Despite the relatively small differences in inorganic N deposition between both Corsican pine stands, the

DOC released by the forest floor was  $132 \text{ kg ha}^{-1} \text{ yr}^{-1}$  in the CP stand and  $240 \text{ kg ha}^{-1} \text{ yr}^{-1}$  in the CPN stand. Average DOC fluxes under the forest floor of the three forest stands ranged from  $236 \text{ kg ha}^{-1} \text{ yr}^{-1}$  in the CP stand over  $238 \text{ kg ha}^{-1} \text{ yr}^{-1}$  in the SB stand to  $348 \text{ kg ha}^{-1} \text{ yr}^{-1}$  in the CPN stand.

Fluxes of DOC decreased steadily with depth in the mineral soil layers of the three forest ecosystems, though the major DOC retention took place in different horizons in the different stands. In the SB and CPN stand, relatively small amounts of DOC were retained in the A and E horizons and the Bh horizons (Table 3.5). Most DOC ( $148 \text{ kg ha}^{-1} \text{ yr}^{-1}$ ) was removed between the lower boundary of the Bh horizon and the lower boundary of the BC horizon. In the CP stand in contrast, DOC was mainly removed from the soil solution in the upper horizons.

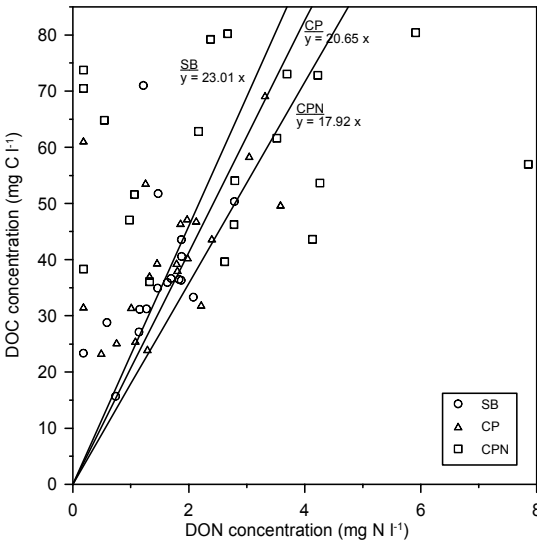
The DOC fluxes leaching under the rooting zone ranged from  $37 \text{ kg ha}^{-1} \text{ yr}^{-1}$  in the SB stand over  $51 \text{ kg ha}^{-1} \text{ yr}^{-1}$  in the CP stand to  $83 \text{ kg ha}^{-1} \text{ yr}^{-1}$  in the CPN stand (Figure 3.2a). There was obviously an influence of tree species on the DOC leaching from the soil profile; the DOC flux in the BC soil solution of the CP stand was markedly higher compared to the SB stand. Furthermore, both Corsican pine stands substantially differed in their annual DOC efflux pointing towards the influence of the forest edge proximity. The entire mineral soil profile of the SB, CP and CPN stand retained 201, 185 and  $265 \text{ kg DOC ha}^{-1} \text{ yr}^{-1}$  respectively or 84, 79 and 76 % of the DOC entering the mineral soil with forest floor leachate respectively.

Average groundwater DOC concentrations were low and more or less constant throughout the year at the three forested sites and showed a similar pattern as the DOC concentrations under the BC-horizons (data not shown). Since the DOC concentrations in the groundwater amounted to only 24-29 % of the DOC concentration at 65 cm, further DOC removal processes must be active in the deeper mineral soil (parent material).

# *Dissolved organic nitrogen*

## *Temporal concentration patterns*

The courses of the DON concentrations in the forest floor leachate of the SB and CP stands showed similar temporal patterns as the course of the DOC concentrations (Figure 3.3), apart from the larger scatter on the DON measurements. As a result, strong positive correlations ( $p = 0.063$  resp.  $0.013$ ) were found between DOC and DON concentrations in these forest floor leachates of the SB and CP stands (Figure 3.5). On the contrary, the course of the DON concentration in the CPN forest floor leachate did not exhibit a similar temporal pattern as DOC and there was no significant correlation between DOC and DON concentrations in the CPN forest floor leachate ( $p = 0.481$ ) (Figure 3.5).



**Figure 3.5. Correlations between DOC and DON concentrations in the forest floor leachate from the three forest stands.**

The DON concentration in the humus percolate of the SB stand was lower during summer and early autumn, but then increased steadily and exceeded the concentration under the CP stand during winter. There was apparently a clear influence of the forest edge proximity on the DON release from the Corsican pine

forest floor, especially during summer and early autumn. Similar as for DOC, the DON concentration in the forest floor leachate is related to the water fluxes during the sampled fortnight and the preceding fortnight(s) in the SB and CP stands (Figure 3.3). Decreasing DON concentrations were observed with increasing soil temperature in the SB and CP forest floor leachates. On the contrary, higher temperature increased the DON release from the CPN forest floor material.

The DON concentration in the soil solution of the BC horizon at the SB stand showed limited temporal variations. On the contrary, fluctuations in the DON concentrations sampled below the BC horizon of the CP and CPN stands displayed larger variability than the corresponding DOC concentrations.

#### *Annual fluxes in the different strata*

The DON fluxes measured in the bulk precipitation flux amounted to 6.4 kg DON ha<sup>-1</sup> yr<sup>-1</sup> at the proximity of the SB and CP stands and 7.1 kg DON ha<sup>-1</sup> yr<sup>-1</sup> at the CPN stand, whereas throughfall water DON deposition fluxes ranged from 5.4 kg ha<sup>-1</sup> yr<sup>-1</sup> in the SB stand over 6.3 kg ha<sup>-1</sup> yr<sup>-1</sup> in the CP stand to 8.7 kg ha<sup>-1</sup> yr<sup>-1</sup> in the CPN stand. Dissolved organic nitrogen was thus retained in the forest canopies of the SB and CP stands and released at the CPN site (Table 3.5).

Similar as for DOC, the forest floor was the largest source of DON in the three forest stands releasing 6.4, 3.5 and 9.07 kg ha<sup>-1</sup> yr<sup>-1</sup> in the SB, CP and CPN stand respectively (Table 3.5). The location of the CPN stand close to the forest edge apparently had a positive effect on the release of DON from the forest floor of the CPN stand.

In the SB stand, most DON was removed between the forest floor and the lower boundary of the E horizon (4.7 kg ha<sup>-1</sup> yr<sup>-1</sup>) and in the Bs and BC horizons (3.8 kg ha<sup>-1</sup> yr<sup>-1</sup>). Similar as for DOC, most DON removal in the CP stand occurred in the upper horizons (A and E), whereas the Bs and BC horizons retained most DON in the CPN stand. The total amount of DON retained by the mineral soil of the SB, CP and CPN stand was 9.6, 6.6 and 12.7 kg ha<sup>-1</sup> yr<sup>-1</sup> or 81, 67 and 72 % of the DON entering the mineral soil respectively. Hence, DON

contributed for a substantial amount to nitrogen retention in mineral soils: 38, 22 and 20 % of the N retained in mineral soils of the SB, CP and CPN stand was in the form of DON. The annual DON fluxes leaving the rooting zone of these forest ecosystems were 2.2, 3.3 and 5.0 kg ha<sup>-1</sup> yr<sup>-1</sup> in the SB, CP and CPN stand respectively (Figure 3.2b).

The DON concentrations further decreased by an additional 23, 14 and 32 % between the lower boundary of the BC horizon and the groundwater in the SB, CP and CPN stand respectively. Consequently, further DON removal processes are active in the deeper mineral soil.

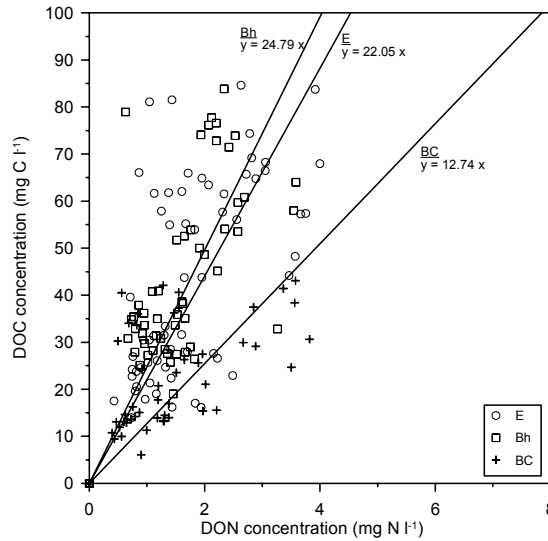
#### *Relationship between DON and DOC*

The DON concentrations in the forest floor of SB and CP stands and in the different mineral soil compartments of the three forest stands were positively correlated with the measured DOC concentrations (Figures 3.5 and 3.6). We found an average DOC/DON ratio in the mineral soil solutions of these ecosystems of about 24.

Generally, passage of rainwater through the forest canopy increased the DOC/DON ratio substantially, from 2.1-2.4 to 11-16 (Figure 3.2). The forest floor leachate had DOC/DON values of 20, 24 and 20 in the SB, CP and CPN stand respectively which were lower than the C/N ratios of the respective forest floor materials (22, 27 and 27 respectively).

In the lower part of the mineral soil, the DOC/DON ratio steadily decreased from 21-31 under the Bh horizon to 7-12 in the groundwater, indicating a strong preferential removal of DOC compared to DON. Generally, DON showed smaller changes within the forest ecosystem compared to DOC, pointing towards lower overall reactivity of N-containing DOM compounds.





**Figure 3.6.** Average concentrations of DON versus DOC in the soil solutions sampled below E, Bh and BC horizons at the three forest stands.

#### *Contribution of DON to TDN*

On average, about 30 % of the TDN flux with precipitation water consisted of organic nitrogen compounds (Table 3.4). The increased DIN fluxes, as a result of the passage of precipitation water through the forest canopies at the three stands, in combination with the limited DON release (or even retention in the SB and CP stands) from the canopies, resulted in a substantially reduced contribution of DON to TDN fluxes (Table 3.4). DON represented 22 % of TDN in throughfall water in the SB stand, but only 17 and 19 % in the CP and CPN stands respectively. The percentage-wise larger release of DON from the SB and CP forest floors resulted in an increased contribution of DON to TDN in the forest floor leachates. On the contrary, extremely high DIN release from the CPN forest floor lowered the DON contribution to TDN in forest floor percolates to only 15 %. The contribution of DON to TDN decreased with depth in the mineral soil in the SB and CPN stand, whereas it remained rather constant in the CP stand.

## DISCUSSION

### *Major sources and sinks of DOC and DON*

Although atmospheric deposition was of minor importance as a source of DOC in these ecosystems, input with precipitation was the major net source of DON. Neff et al. (2002) indicated that the major sources of atmospheric organic nitrogen (AON) include byproducts of reactions between  $\text{NO}_x$  and hydrocarbons, marine and terrestrial sources of reduced (amino acid) N and the long-range transport of organic matter (dust, pollen etc.) and bacteria.

The net throughfall deposition of DON is the result of a combination of wet and dry deposition and interaction processes in the forest canopy. DON can be retained and/or converted in the canopy by: (i) (physico-chemical) retention; (ii) (microbial) conversion to DIN and/or (iii) active uptake by canopy lichens and microorganisms during passage through the canopy. It can also be released in forest canopies by (i) leaf washing of dry deposited DON; (ii) leaf leaching of DON and (iii) microbial DON formation from DIN (Lovett and Lindberg, 1993). However, our dataset does not allow to indicate the mechanisms responsible for the differences in throughfall DON fluxes observed between the three forest stands.

The highest DOC, DON and DIN concentrations and fluxes were found in the forest floor leachate at the three stands. The lower C/N ratio of the DOM released from the forest floor material compared to the solid phase C/N is somewhat in contradiction to the general assumption that DON release follows that of DOC (the passive carbon vehicle). However, several studies (e.g. McDowell et al., 1998; Michalzik and Matzner, 1999; Michel and Matzner, 1999; Solinger et al., 2001) found higher C/N ratios of DOM in forest floor leachate compared to the solid phase organic matter. Brookshire et al. (2007) presented two hypothetical mechanisms for the stoichiometric N enrichment of DOM: (i) abiotic reaction between DOM and inorganic nitrogen and (ii) the microbial (mycorrhizal) incorporation of DIN with subsequent release of DON. Davidson et al. (2003) described an abiotic mechanism producing DON through reaction of nitrite with

DOM. Colman et al. (2007), however, found that this apparent abiotic N incorporation was likely the result of iron interference with nitrate measurements.

Our results show that the mineral soil appeared to be the major sink for DOC and DON. Since most DOM retention was found in the Bs and BC horizons, both rich in iron and aluminum oxyhydroxides, recognized as strong sorbents for DOM (Gu et al., 1994; Kaiser et al., 1996), sorption is believed to be the main removal mechanism. Also Qualls and Haines (1992) indicated that adsorption, rather than biodegradation, is more likely responsible for maintaining low DOC concentrations in the mineral soil. Fritze et al. (2000) found up to three times higher microbial biomass in the upper mineral horizons compared to Bs and BC horizons of podzol profiles (where most DOC removal took place).

We found decreasing DOC/DON ratios with increasing soil depth in the three forest stands, which is in accordance to the findings of Michalzik et al. (2001) and Lajtha et al. (2005). The latter attributed this difference to the preferential sorption of high C/N hydrophobic DOM to mineral soil constituents over hydrophilic DOM with a much lower and less variable C/N ratio. Several studies found higher DOC/DON ratios in the hydrophilic and hydrophobic acid fractions (which were found to be preferentially sorbed to iron and aluminum oxyhydroxides) and lower ratios in the hydrophilic neutrals fraction (e.g. Qualls and Haines, 1991).

Substantial amounts of DOC (71-76 %) and DON (14-32 %) were retained between the lower boundary of the mineral soil profile (< 65 cm) and the groundwater. Schwesig et al. (2003) found that DOC leaching from the rooting zone is highly resistant to mineralization and concluded that the further decrease of DOC concentrations in the subsoil could not be attributed to mineralization. Siemens et al. (2003) showed that substantial amounts of DOM were removed by sorption in deeper mineral subsoil, and Jardine et al. (2006) indicated the importance of DOC accumulation in lower horizons for carbon sequestration.

*Influence of the forest type*

Although the adjacent SB and CP stands are subject to identical bulk precipitation deposition, the DIN (and other ions) throughfall water fluxes significantly differed between both stands. It is generally known that, under identical site and meteorological conditions, coniferous forests receive more atmospheric deposition via throughfall water than deciduous forest types (for a review, see De Schrijver et al., 2007b). This difference can be explained by (i) the summer-green character of the deciduous forest (Rothe et al. 2002), (ii) differences in vegetation structure such as leaf area index (LAI), canopy height and canopy closure (Erisman and Draaijers, 2003) and (iii) differences in form or shape of leaves or needles (Smith, 1981). As a result, observed differences in DOM dynamics between forest types are not solely due to inherent differences in DOM quality and quantity, but may result from indirect differences in biogeochemical and hydrological cycles which are related to the forest type. The coniferous forest had higher DOC and DON throughfall water fluxes compared to the deciduous forest which can result from different canopy interactions between the studied forest types.

In contrast to the observed throughfall water fluxes, the forest type only slightly affected the DOC and DON fluxes via forest floor leachate. This is more or less what could be expected, since a similar amount of forest floor material was present in both stands (Table 3.3). Although both forest types did not differ substantially in the percentage DOC retention within the mineral soil profile (84 % in SB and 78 % in CP), there was obviously a difference in the location where most DOM was retained. The high DOM retention in the upper mineral horizons in the CP stand are most likely due to the presence of Bs and BC horizon material overlying the podzol as a result of the excavation of canals before reforestation in 1871-1887. A higher percentage of the DON input with forest floor leachate was retained in the mineral soil of the SB stand (81 %) compared to the CP stand (67 %), suggesting a difference in the distribution of N over the different DOM fractions. Since the CP stand intercepted more DIN than the SB stand, this finding is in accordance with the findings of McDowell et al. (2004) that proportionally

more DON is in the hydrophilic fractions as a result of chronic nitrogen amendments. Although the DOC/DON ratio in most strata differed substantially between both stands, the DOM leaching from the BC horizon showed a similar DOC/DON ratio under both forest types.

The major differences between the two forest stands were found in the relative contribution of DON to TDN fluxes in the different strata. Whereas DON was a substantial part of total nitrogen fluxes in the deciduous forest (28 - 46 %), its contribution to TDN was only 20 % at most in the coniferous forest. Since absolute DON fluxes differed only slightly between both forest types, the differences in the DON/TDN ratio were mainly due to large differences in DIN fluxes. The higher DIN leaching under the coniferous compared to deciduous forest is in agreement with several other studies (for a review see De Schrijver et al., 2007b).

#### *Influence of forest edge proximity*

One of the main objectives of this study was furthermore to investigate the effect of different levels of N throughfall deposition on the dynamics of DOC and DON. Therefore, a forest stand was selected with the forest edge oriented to the prevailing wind direction. Forest edges are more effective at filtering atmospheric deposition than forest interiors; they are subject to a so-called *edge effect* on throughfall deposition (Draaijers, 1993). This edge effect causes an up to fourfold increase of throughfall N deposition compared to the forest interior and decreases exponentially with increasing distance from the forest edge (for an overview, see De Schrijver et al., 2007a). As a result, the CPN stand received higher TDN inputs with throughfall water compared to the CP stand although the bulk nitrogen deposition with precipitation did not significantly differ between the two sites under investigation (Overheide and Tulderse Heide).

Overall, larger fluxes of DOC, DON and DIN were found in the CPN stand compared to the CP stand. These differences are mainly introduced during passage of the water through the forest floor material, which points towards a major impact of N deposition and forest edge proximity on DOM release from the

forest floor. Although a lower amount of forest floor material was present in the CPN stand, more DOM was released compared to the CP stand pointing towards a higher turnover of organic material. Various hypothetical mechanisms have been formulated and tested which may be responsible for the enhanced DOM leaching as a result of increased nitrogen deposition (Carreiro et al., 2000; Burton et al., 2004; Frey et al., 2004; DeForest et al., 2005; Blackwood et al., 2007), but results were often not consistent. It is questionable whether the relatively small increase in measured N input (25 %) can result in the dramatically increased DOM release from the forest floor (82 %). However, two other factors could have induced the elevated DOM release:

- (i) The N throughfall deposition measured between March 2005 and March 2006 is relatively low compared to earlier studies in the same region under similar conditions (Neiryneck et al., 2002). Although the N deposition more than doubled between 1870 and 1980 (Asman et al., 1988), recent data indicated that N deposition in Flanders decreased with 18 % between 1990 and 2004 (MIRA, 2006). Thus, although the N deposition and the difference in N input between both stands is relatively small to date, the biogeochemical cycles of all observed stands, but especially of the CPN stand because of its proximity to the forest edge, are probably still influenced by the historical very high nitrogen inputs with precipitation which lasted for several decades. Wuyts et al. (in press) reported increased depositions of DIN but also of other ions (e.g.  $\text{SO}_4^{2-}$ ,  $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$ ,  $\text{Cl}^-$  and  $\text{Na}^+$ ) in forest edges compared to forest interiors in two forest types comparable to our study. The physico-chemical release from the forest floor is, due to the colloidal properties of DOM, to a considerable extent influenced by the ionic strength and ionic composition of throughfall water (Kalbitz et al., 2000).
- (ii) Besides the elevated ion throughfall deposition flux, the proximity of a forest edge furthermore influences the microclimatic conditions (like light intensity, air temperature, relative humidity, soil moisture and litter moisture) (Davies-Colley et al., 2000; Marchand and Houle, 2006) which

in turn also might affect DOM dynamics. Therefore, observed differences in DOM dynamics between both coniferous stands (CP and CPN) were probably due to a combination of factors influenced by an edge effect of which increased N input is one.

The combination of higher DOC fluxes entering the mineral soil with forest floor leachate, together with an almost equal percentagewise retention (by sorption) in the mineral soil (78 % in CP and 76 % in CPN) results in larger amounts of C retained in the mineral soil of the CPN stand. This is in agreement with the findings of Hyvönen et al. (2007) concerning the impact of long-term N addition on carbon stocks in soils in northern Europe. Högberg (2007) even suggested the possibility of using N fertilization in forests to increase C sequestration. Besides, the DOC and DON fluxes leaving the rooting zone were substantially increased as a result of the forest edge effect. Similarly, Pregitzer et al. (2004) reported DOC and DON exports to be elevated by more than 3 and 6 times respectively after 8 years of artificial nitrate additions as  $\text{NaNO}_3$  ( $30 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ ).

The overall higher DIN fluxes in the CPN stand resulted in a smaller contribution of DON to TDN. Park and Matzner (2006) also found that the contribution of nitrate increased whereas that of DON decreased with increasing throughfall nitrogen input. Beside differences in total DIN fluxes between the CP and CPN stands, our results indicated that there is obviously a difference in the location within the ecosystem strata where maximum nitrification occurs. At the CP stand, most nitrification occurred in the forest floor, whereas in the CPN stand, most nitrification occurred in the upper mineral horizons (A and E).

### *DON and N leaching*

Although several studies pointed out that nitrogen leaching in oligotrophic ecosystems mainly occurs in the organic form (Currie et al., 1996; Perakis and Hedin, 2002; Qualls et al., 2002), we found a limited contribution of DON to total nitrogen leaching (9-28 %) in our study. Contrary to the order of absolute DON leaching fluxes ( $\text{CPN} > \text{CP} > \text{SB}$ ), the contribution of DON to total

nitrogen leaching followed the order SB>CP>CPN. This indicates that the relative contribution of DON to TDN leaching was mainly determined by the extent of the DIN fluxes. These DIN fluxes were, in turn, controlled by the overall throughfall N input and by the ecosystem N status. This is in accordance with the findings of Park and Matzner (2006) that the contribution of nitrate increased whereas that of DON decreased with increasing throughfall nitrogen input. The contribution of DON to the total dissolved nitrogen concentration in groundwater was only 6-14 %, probably as a result of additional DON retention in the deeper mineral horizons. Although DON is sometimes overlooked in studies dealing with N leaching and input-output balances, our results indicate that its contribution in N cycling is not negligible and should be taken into account.

While the number of measurements was limited spatially, these data indicated that the total nitrogen input (DON + DIN) with throughfall water was higher than the total N output as leaching to groundwater in the three replicated plots of the SB and CP stand. This means that N still accumulates in these forest ecosystems. The opposite was found in the three plots of the CPN stand, namely the export of nitrogen was larger than the input with throughfall water. These findings show that this ecosystem released nitrogen instead of taking it up, which may be an indication for nitrogen (over)-saturation as a result of the long-term high nitrogen deposition.

## CONCLUSIONS

Input with precipitation was the major source of DON, whereas the forest floor was the major source of DOC in these forest ecosystems. Most DOC and DON in forest floor leachate was removed during passage through the mineral soil, resulting in constant low DOM concentrations leaching from the rooting zone. Several indications were found that sorption to mineral soil material is the main mechanism responsible for this DOM retention. Our data indicated that substantial amounts of DOM were further removed from the soil solution between the lower part of the rooting zone and the groundwater, most likely by sorption.



The lower reactivity of N containing DOM compounds resulted in substantial differences in DOC/DON ratios between ecosystem strata.

Although the limited number of comparative measurements, our data indicated small differences between the studied forest types. Whereas the forest type apparently influenced the DOC and DON fluxes within the ecosystem, both forest types showed only minor differences in percentagewise retention (by sorption) in the mineral soil and in DOM leaching. The major effect of forest type was apparently found in the relative contribution of DON to TDN; due to the higher DIN canopy interception, lower DON/TDN ratios occurred in the coniferous forest.

The differences in DOM release from the forest floor between both coniferous stands are probably not only due to the relatively small additional DIN input, but rather to long-term N deposition and a combination of factors related to the proximity of the forest edge. The higher DOM release from the forest floor as a result of edge effect, in combination with an equal percentagewise DOM retention in the mineral soil, resulted in (i) larger amounts of DOM being retained in the mineral soil and (ii) larger absolute DOM leaching losses from the coniferous ecosystem. Since the effect of the forest edge proximity on DOM cycling is introduced in the forest floor, the mechanisms and factors influencing the DOM release from forest floor material deserve further research.

The contribution of DON to TDN leaching was mainly determined by the DIN fluxes. Since these DIN fluxes are in turn determined by canopy interception of N, the DON/TDN ratio is influenced by forest type and forest edge proximity. The contribution of DON to ecosystem N inputs and outputs is apparently not negligible although it has frequently been omitted from ecosystem nutrient balances.



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## CHAPTER 4

# FACTORS AFFECTING DISSOLVED ORGANIC MATTER (DOM) RELEASE FROM FOREST FLOOR MATERIAL

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Illustration on p. 77:

Forest floor material sampled in the silver birch (left) and Corsican pine stand (right) at Overheide.

*This chapter has been submitted as:*

*Vandenbruwane J., Leroy B., Van den Bossche A., De Neve S. and Hofman G.  
Factors affecting dissolved organic matter (DOM) release from forest floor  
material receiving chronic atmospheric nitrogen deposition in Flanders. European  
Journal of Soil Science (Submitted).*

## ABSTRACT

Although the forest floor has frequently been identified as the major source of dissolved organic matter (DOM) in forest ecosystems, an overall picture of the interactions and the relationships between the major processes involved in the DOM release from the forest floor is lacking. Based on the general knowledge of individual processes, a conceptual model was designed combining these separate mechanisms. The central point within this conceptual model is the existence of a pool of potential DOM in the forest floor, which is replenished by the decomposition of solid organic matter. Physico-chemical mechanisms determine the solubilization of DOM from this pool, whereas microbial degradation is a sink for solubilized DOM. The amount of DOM remaining in solution is subject to export via convective transport with the percolating water. Based on this conceptual model, explanations have been formulated for differences in DOM release between deciduous and coniferous forest floors and for the increased DOM release as a result of increased N deposition observed during field experiments. Laboratory release experiments showed that microbial activity is responsible for the decreased C/N ratio of DOM compared to solid organic matter probably as a result of the preferential degradation of carbohydrates and the incorporation of inorganic nitrogen into the microbial biomass followed by lysis after cell death.

## INTRODUCTION

During the last decades, extensive research has been done on cycling of dissolved organic matter (DOM) in both aquatic and terrestrial ecosystems. This mobile fraction of the total organic matter pool is highly susceptible to transfer within and between ecosystems. Downward movement of DOM in mineral soil is essential to soil formation, export of carbon and nitrogen from the ecosystem and may contribute to eutrophication of ground- and surface waters. The presence of DOM in polluted soils can increase the mobility and thus transport of heavy metals and organic pollutants.

The dynamics of dissolved organic carbon (DOC) and nitrogen (DON) in temperate forest ecosystems have extensively been described and a synthesis can be found in Michalzik et al. (2001). Atmospheric deposition and release from the forest canopy and from the forest floor have been found to be the major sources of DOM in the aboveground strata of these ecosystems. Dissolved organic matter entering the mineral soil is subject to various processes controlling the concentration in soil solution. Plant-uptake, microbial degradation and adsorption to mineral soil material are the primal sinks for DOM in the soil profile. The fraction of DOM that is not taken up, degraded or adsorbed is susceptible to leaching from the system.

Although the forest floor material has been widely recognized as the most important source of DOM in forest ecosystems and extensive research on specific release mechanisms has been done, the mechanisms and factors affecting the DOM release have as yet not been identified unambiguously. Kalbitz et al. (2000) reviewed the controls on the dynamics of dissolved organic matter in soils. Among other things, they tried to find correlations between solid phase properties, solution phase properties and environmental conditions and DOM concentrations and fluxes from the forest floor. Fröberg et al. (2007) stated that the DOC concentration is controlled not only by its production, but also by its consumption through microbes, hydrologically driven transport and physico-chemical retention. They suggested that the net release is thus a result of a complex interaction

between abiotic and biotic factors, with the relative importance of each process not yet known.

In their review, Kalbitz et al. (2000) did not find a clear trend in the influence of increased nitrogen deposition on DOM release from the forest floor and to date research results are often contradictory. McDowell et al. (1998) found little changes in DOC concentrations (10-30 % increase) associated with elevated N inputs, but did observe significant increases in DON concentrations. Also Pilkington et al. (2005) found increased leaching of DON from the Oh horizon by the addition of  $40 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ . Currie et al. (1996) found no significant responses to N treatment of both DOC and DON concentrations, but DOC/DON ratios did show significant responses. Pregitzer et al. (2004) reported that the export of both carbon and nitrogen from northern hardwood ecosystems was dramatically increased by chronic nitrate additions. On the contrary, Sjöberg et al. (2003) found no effect on DOC and DON production in mor humus under Spruce but measured lower C mineralization as a result of long-term N addition. Michel et al. (2006) pointed out that the chemical composition of DOM is altered as a result of higher mineral N inputs.

Extensive research has been done on the potential mechanisms involved in ecosystem and particularly forest floor layer responses on elevated N deposition. Although Magill and Aber (2000) found increased respiration in forest floor material as a result of increased mineral N input, most studies point towards a suppressed microbial respiration (e.g. Burton et al., 2004). A decrease in overall microbial biomass (Andersson et al., 2004; Burton et al., 2004; DeForest et al., 2004) and microbial activity (Carreiro et al., 2000; DeForest et al., 2004) have been put forward as potential effects of elevated N deposition. Increased N deposition has been shown to influence extracellular enzyme activities (Saiya-Cork et al., 2002; Sinsabaugh et al., 2002; Burton et al., 2004; DeForest et al., 2004; Kang and Lee, 2005) in general and oxidative enzyme activities (Gallo et al., 2004; Waldrop and Zak, 2006) in particular. Although Blackwood et al. (2007) and DeForest et al. (2004) did not observe changes in the structure and function of soil microbial communities as a result of increased nitrogen deposition, several

studies (Frey et al., 2004; Gallo et al., 2004; Chung et al., 2007) indicated shifts in microbial communities. The relative abundance of fungi was found to decrease and that of bacteria to increase with increasing ecosystem N status and fertility (Pennanen et al., 1999; Frey et al., 2004).

The main objective of this study was to design a conceptual model of the major processes involved in the DOM release from forest floor material. Based on this model, possible explanations were investigated for various observations concerning the DOM release from forest floors of different origins.

## **MATERIALS AND METHODS**

### *Field solution sampling*

During the one year sampling campaign (Chapter 3), samples of the throughfall water and forest floor leachate were analyzed for pH, cation ( $\text{Na}^+$ ,  $\text{K}^+$ ,  $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$ ,  $\text{Al}^{3+}$ ) and anion ( $\text{Cl}^-$ ,  $\text{SO}_4^{2-}$ ) concentrations, DOC, TDN,  $\text{NO}_3^-$  and  $\text{NH}_4^+$ -content. The pH was measured potentiometrically (Thermo Orion model 420 A+),  $\text{K}^+$ ,  $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$ ,  $\text{Na}^+$  and  $\text{Al}^{3+}$  concentrations were determined using flame atomic absorption spectrophotometry (SpectrAA 220, VARIAN) and  $\text{Cl}^-$  and  $\text{SO}_4^{2-}$  were measured with ion chromatography (ICS-90, DIONEX). Concentrations of DOC, TDN and DIN were determined using the methods described in Chapter 3.

Fortnightly water fluxes entering and leaving the forest floor were calculated using the SWAP model (Van Dam et al., 1997) as described in Appendix 1, whereas fortnightly solute fluxes were calculated by multiplying the fortnightly water fluxes by the fortnightly solute concentration. The summation of these fortnightly solute fluxes over the entire sampling year yielded the annual solute flux.

### *Litterfall and forest floor sampling*

Litterfall in the three forest stands was collected in 9 replicates using round collection baskets ( $0.25 \text{ m}^2$ ) equipped with nylon wire netting at 60 cm above the soil surface for 1 year. An inert PVC weight was used to prevent the



samples to be blown over the edge of the basket. Samples were collected monthly and dried at 60 °C for moisture content determination. All dried samples from each collection basket were bulked, ground roughly and subsamples were ground using a ball-mill for further chemical analyses.

The forest floor of the three forest stands consisted of a succession of OL, OF and OH layers and were characterized by a sharp boundary between the OH and mineral A horizon. Consequently the forest floors were classified as mor humus types (Green et al., 1993). Forest floor material (Oi + Oe + Oa) from a 0.25 m<sup>2</sup> square was completely sampled in 9 replicates at each of the three forest stands and weighed field moist. Subsamples of these homogenized samples were taken for microbiological and chemical analyses. Portions of these subsamples were dried at 60 °C for 96 hours to determine moisture content and the amount of dry forest floor material per m<sup>2</sup> was calculated. The dried subsamples were ground using a ball-mill and stored in glass vials until used for further chemical analyses. Nitrogen mineralization and nitrification rates, microbial biomass carbon (MBC) and nitrogen (MBN), pH, mineral nitrogen (NO<sub>3</sub><sup>-</sup>-N and NH<sub>4</sub><sup>+</sup>-N) and phospholipids fatty acids (PLFAs) were determined on field moist subsamples. The field moist forest floor material required for the laboratory release experiment was stored at 4 °C until use.

The pH of the field moist forest floor material was determined potentiometrically (Thermo Orion model 420 A+) in 1:10-water extracts (w / w). Microbial biomass C and N were determined by the fumigation-extraction method according to Voroney et al. (1993) using a 24 h chloroform fumigation time, a 0.5 M K<sub>2</sub>SO<sub>4</sub> extractant, a soil-to-extractant ratio of 1:5 and a conversion factor  $k_{EC}$  of 0.25. The DOC and TDN of the extracts were determined using the method for aqueous samples (see above). Total soluble organic carbon (TSOC) and nitrogen (TSON) were determined as the DOC and DON in the extracts from unfumigated forest floor samples. The chemical composition of litterfall and forest floor material was determined on 105 °C dried and ball-mill ground subsamples. Total C (C<sub>tot</sub>) and N (N<sub>tot</sub>) were measured by dry combustion at 850 °C using an elemental analyser (Vario MAX CNS, Elementar). K<sup>+</sup>, Na<sup>+</sup>, Ca<sup>2+</sup>, Mg<sup>2+</sup> and P<sub>tot</sub>

contents were extracted with 1 M  $\text{HNO}_3$  from the organic materials after incineration for 4 h at 450 °C. The P concentration was measured colorimetrically after complexation with nitrovanadomolybdate at 425 nm with a photometer (Universal Photometer, Vitatron). The concentration of  $\text{K}^+$  and  $\text{Na}^+$  was measured with a flame photometer (Elex 6361, Eppendorf) in an air-propane flame at 768 nm and 589 nm respectively, while for the  $\text{Ca}^{2+}$  concentration an air-acetylene flame was used at 623 nm. The concentration of  $\text{Mg}^{2+}$  was determined by atomic absorption spectrometry (SpectrAA Atomic Absorption Spectrometer, Varian) at 285 nm.

Nitrogen mineralization and nitrification rates of the forest floor material were measured during laboratory incubations. After removal of living plant material (mosses, tree roots and herbal vegetation), field moist forest floor material of each stand was thoroughly mixed and put into PVC tubes (height = 18 cm; inner diameter = 4.6 cm). The organic material in the tubes was slightly compacted in order to reach a bulk density similar as under field conditions. After filling the tubes distilled water was added to obtain a gravimetric moisture content of 200 % (Vervaeke et al., 2003) and the tubes were covered with a single layer of pin-holed gas permeable parafilm to minimize water loss. The tubes were incubated at 15 °C for 122 days. Samples were taken in three replicates at increasing time intervals destructively by removing and emptying three tubes per treatment which were stored at -18 °C until analysis. The moisture content during the incubation was checked and adjusted by adding deionized water as needed every two weeks. Samples were extracted with 1 M KCl at a 1:5 (w/w) ratio and mineral nitrogen ( $(\text{NO}_3^- + \text{NO}_2^-)\text{-N} + \text{NH}_4^+\text{-N}$ ) concentrations were measured with a continuous-flow auto-analyzer. Net N mineralization rate was identified as the slope calculated by linear regression of the temporal DIN ( $(\text{NO}_3^- + \text{NO}_2^-)\text{-N} + \text{NH}_4^+\text{-N}$ ) patterns. Nitrification rate was determined as the slope resulting from the linear regression of the temporal nitrate patterns.

Lipid extraction and fatty acid analysis were performed using a procedure based on a modified Blight and Dyer (1959) technique. In short, lipids were extracted from 1 g lyophilized forest floor material with a single-phase mixture of

chloroform, methanol and phosphate buffer (1:2:0.8, v/v/v). The resulting lipid material was fractionated into neutral, glyco- and phospholipids by silica-bonded phase columns. The phospholipids were trans-esterified to fatty acid methyl esters through a mild alkaline methanolysis and then analyzed by gas chromatograph/mass spectrometer using a Thermo Focus GC with a Varian Capillary column CP Sil 88 (100 m x 0.25 mm i.d., 0.2  $\mu$ m film thickness) combined with a Thermo DSQ-MS EI with quadrupole. The identification and quantification of the fatty acids was conducted using nonadecanoic acid methyl ester (C19:0) as internal standard. The weights of individual PLFAs were measured as ng g<sup>-1</sup> dry material. All samples were analyzed using a set of 33 fatty acids. Fatty acids not included in this set were discarded since they were unreliably quantified in many plots due to their low abundances. Fatty acids were designated as X:Y $\omega$ Z, where X is the number of C atoms, Y the number of double bonds and Z indicates the position of the first double bond from the aliphatic ( $\omega$ ) end of the molecule. Cis and trans isomers are indicated by c or t. Branched-chain fatty acids are indicated by the prefixes i or a for iso and anteiso-branchings respectively; the prefix br refers to a branched chain at unknown position and cy designates cyclopropyl fatty acids. The 10Me-fatty acids have a methyl group attached to the 10th carbon atom from the carboxyl end of the molecule. The dataset of all fatty acids per sampling date was simplified by using certain marker fatty acids of selected microbial groups, following Kozdroj and van Elsas (2001). For Gram-positive bacteria the sum of iC15:0, aC15:0, iC16:0, iC17:0 and aC17:0 was used. The fatty acid cyC17:0 was considered to be typical for Gram-negative bacteria, while for the actinomycetes, the sum of the 10Me fatty acids (10MeC16:0 and 10MeC18:0) was regarded as a reliable indicator. The C18:2 $\omega$ 6,9c was used as a signature fatty acid for fungi, and 16:1 $\omega$ 5c as a signature fatty acid for arbuscular mycorrhizal fungi. For the ratio of bacteria to fungi the fatty acids iC15:0, aC15:0, C15:0, iC16:0, iC17:0, aC17:0, C17:0, cyC17:0 and cyC19:011,12 $\omega$ c were chosen to represent bacterial PLFAs, while C18:2 $\omega$ 6,9c was used as indicator of fungal biomass (Fröstegård et al., 1993; Pennanen et al., 1996; Bossio and Scow, 1998; Marschner et al., 2003). The data

of the marker fatty acids of selected microbial groups and of the microbial biomass C were subjected to analyses of variance (ANOVA) tests and significant differences between means were determined by Tukey's test.

#### *Laboratory release experiment*

The release of DOM from forest floor material was investigated under controlled laboratory conditions. Polyvinylchloride (PVC) tubes (height = 15 cm; inner diameter = 7.7 cm diameter) equipped with a PVC netting and glass-fiber filter (Whatman GF/F) at the bottom, were filled with an amount of field moist forest floor material corresponding to the amount of material on 32 cm<sup>2</sup> in the field and covered with the same glass-fiber filters. The amounts of forest floor material used corresponded with 18.6 g, 17.0 g and 12.8 g dry SB, CP and CPN material respectively. For each location (SB, CP, CPN), six replicated tubes were filled with forest floor material of which three were sterilized with mercury chloride (HgCl<sub>2</sub>) (SB<sub>sterile</sub>, CP<sub>sterile</sub>, CPN<sub>sterile</sub>). Mercury chloride has been proposed as a sterilant that does not alter the overall structure of the organic matter as drastically as other sterilization methods (Wolf et al., 1989; Wolf and Skipper, 1994). To test the possible effect of a microbial inoculum on DOM release, three tubes were filled with 99 % (w/w) CP forest floor material and inoculated with 1 % (w/w) CPN forest floor material (CP<sub>inoc</sub>). Before the start of the incubation, entire samples were submerged for 12 hours in an artificial DOM-free throughfall solution. This throughfall solution was made by dissolving MgO, AlCl<sub>3</sub>.6H<sub>2</sub>O, CaCl<sub>2</sub>.2H<sub>2</sub>O, Na<sub>2</sub>CO<sub>3</sub>, K<sub>2</sub>SO<sub>4</sub> and KCl to obtain a similar ionic concentration and composition as the average of the annual concentrations at the three sites. In order to sterilize the samples, HgCl<sub>2</sub> was amended to the artificial throughfall solution at a concentration of 20 mg HgCl<sub>2</sub> l<sup>-1</sup>, whereas in the control treatments an equal molar amount of CaCl<sub>2</sub> was added to the solution to achieve the same ionic strength. After the submersion, samples were allowed to drain by gravity for 6 hours followed by percolation at a constant flow rate maintained by a peristaltic pump of 100 ml h<sup>-1</sup> to achieve a final litter to solution ratio of 1:25. This

percolation prior to the start of the experiment was performed to flush out most soluble organic matter released after sterilization.

During the 40 day incubation at 20 °C, samples were repeatedly leached with the artificial throughfall solution at a soil to solution ratio of 1:10 and a flow rate of 100 ml h<sup>-1</sup>. Mercury chloride was added to the artificial throughfall solution of the sterile samples at a concentration of 5 mg l<sup>-1</sup> and the ionic strength of the solution used for the non-sterile samples was adjusted with CaCl<sub>2</sub>. The PVC tubes were capped between consecutive leaching occasions in order to maintain optimal moisture contents. All aqueous samples were filtered through 0.45 µm polyethersulfone (PES) membrane disc filters (Pall Supor®-450, Gelman Laboratory) and stored at -18 °C until analysis. Samples were analyzed for NO<sub>3</sub><sup>-</sup>-N, NH<sub>4</sub><sup>+</sup>-N, DON and DOC (see before) and the cumulative DOC and DON release was calculated per mass unit of C<sub>tot</sub> or N<sub>tot</sub> respectively.

## RESULTS

### *Field observations*

The results of the one year field monitoring campaign revealed large differences in the patterns of DOC and DON release from the forest floor of the three stands. Two remarkable trends were observed in these data:

(i) The net annual release of DOC expressed per surface area was obviously higher in the SB stand compared to the CP stand (Table 4.1). However, this difference is relatively smaller when the DOC release is expressed per mass unit of total carbon (C<sub>tot</sub>) in the forest floor (Table 4.2): 6.34 and 5.43 g DOC kg<sup>-1</sup> C<sub>tot</sub> yr<sup>-1</sup> in the SB and CP stand respectively. Also the amount of DON released from the forest floor material differed between both forest types; 4.95 and 3.91 g DON kg<sup>-1</sup> N<sub>tot</sub> yr<sup>-1</sup> in the SB and CP stand respectively. The nitrogen deposition or forest edge proximity in general apparently increased the absolute DOC and DON release from the forest floor in the coniferous stands substantially (by 83 and 157 % respectively). The difference in DOC and DON release between CP and CPN was even larger when it was expressed per mass unit

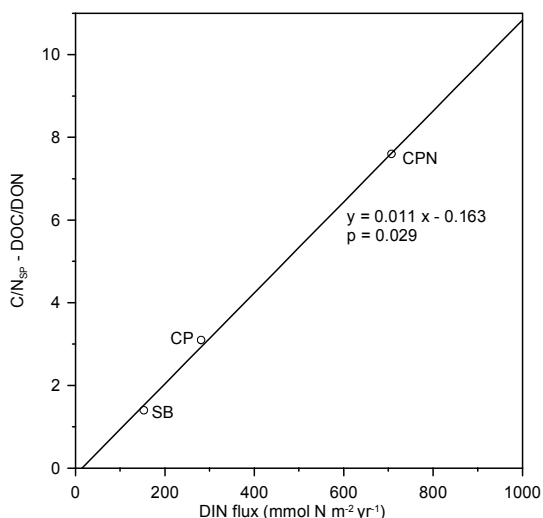
Table 4.1. Fluxes of DOM, cations and anions in throughfall and forest floor leachate samples in the forest stands under investigation

Properties	SB			CP			CPN		
	Throughfall	FF leachate	Release*	Throughfall	FF leachate	Release*	Throughfall	FF leachate	Release*
Water flux (mm yr <sup>-1</sup> )	654	649	-5	569	564	-5	504	497	-7
DOC (mol C m <sup>-2</sup> yr <sup>-1</sup> )	0.498	1.982	1.484	0.864	1.961	1.097	0.893	2.897	2.004
DON (mmol N m <sup>-2</sup> yr <sup>-1</sup> )	38.3	84.2	45.9	45.1	70.2	25.1	62.0	126.7	64.7
DOC / DON	11.2	20.2		16.4	23.9		12.4	19.6	
NO <sub>3</sub> <sup>-</sup> -N (mmol m <sup>-2</sup> yr <sup>-1</sup> )	40.2	107.7	67.4	55.7	175.5	119.8	69.1	274.7	205.5
NH <sub>4</sub> <sup>+</sup> -N (mmol m <sup>-2</sup> yr <sup>-1</sup> )	96.0	45.4	-50.6	164.5	105.3	-59.2	200.8	432.8	232.0
DIN (mmol m <sup>-2</sup> yr <sup>-1</sup> )	136.2	153.1	16.9	220.2	280.8	60.6	269.9	707.5	437.5
H <sup>+</sup> (mmol m <sup>-2</sup> yr <sup>-1</sup> )	1.1	71.1	70.0	0.6	145.5	144.9	0.4	31.4	31.0
Na <sup>+</sup> (mmol m <sup>-2</sup> yr <sup>-1</sup> )	36.9	54.8	17.9	54.1	81.1	27.0	79.0	134.3	55.3
K <sup>+</sup> (mmol m <sup>-2</sup> yr <sup>-1</sup> )	55.5	86.4	30.9	48.5	67.1	18.6	50.6	118.0	57.4
Mg <sup>2+</sup> (mmol m <sup>-2</sup> yr <sup>-1</sup> )	9.5	12.3	2.8	6.7	13.9	7.2	10.6	26.9	16.3
Ca <sup>2+</sup> (mmol m <sup>-2</sup> yr <sup>-1</sup> )	8.6	28.8	20.2	8.3	25.9	17.6	12.3	46.3	44.0
Al <sup>3+</sup> (mmol m <sup>-2</sup> yr <sup>-1</sup> )	0.70	4.42	3.72	0.90	2.73	1.83	1.21	6.13	4.92
Cl <sup>-</sup> (mmol m <sup>-2</sup> yr <sup>-1</sup> )	58.0	70.0	12.0	66.9	96.5	29.6	106.2	156.9	50.7
SO <sub>4</sub> <sup>2-</sup> (mmol m <sup>-2</sup> yr <sup>-1</sup> )	35.8	39.2	3.4	55.0	66.3	11.3	81.8	115.8	34.0
Cations (mmol(+) m <sup>-2</sup> yr <sup>-1</sup> )	227.8	353.2	125.4	300.4	486.8	186.4	380.2	881.3	501.1
Anions (mmol(-) m <sup>-2</sup> yr <sup>-1</sup> )	169.8	256.1	86.3	232.6	404.6	172.0	338.9	663.2	324.3
Anion deficit (mmol(-) m <sup>-2</sup> yr <sup>-1</sup> )	58.0	97.1	39.1	67.8	82.2	14.4	41.3	218.1	176.8

\* Calculated as: (Forest floor leachate flux – Throughfall flux). Negative values indicate uptake by the forest floor

$C_{\text{tot}}$  and  $N_{\text{tot}}$  (154 % and 260 % increase respectively). These findings point towards a relatively limited influence of forest type, but a very large impact of the forest edge proximity on DOM release. Leaching of C and N with DOM constituted 9.5, 3.3 and 8.7 % of annual C input and 11.1, 5.2 and 15.2 % of annual N input with litterfall to the SB, CP and CPN forest floor respectively (Table 4.3).

(ii) Different relationships were found between DOC and DON concentrations in the forest floor leachates (Figure 3.5). Fairly strong correlations were found between DOC and DON concentrations in the forest floor leachates of the SB and CP stands. On the contrary, the release of DON was apparently largely independent of that of DOC in the CPN forest floor. Furthermore, the DOC/DON ratio of the DOM in forest floor leachate was smaller than the solid phase C/N ratio of the forest floor material in the three stands whereas this difference tended to increase with increasing DIN flux (Figure 4.1). These findings point towards different mechanisms involved in the DOC and DON release and/or a stoichiometric N enrichment of the DOM in the forest floor in presence of substantial amounts of DIN.



**Figure 4.1.** Course of the difference in solid phase C/N ( $C/N_{\text{sp}}$ ) ratio and the DOC/DON ratio as a function of annual DIN flux at the three forest stand

**Table 4.2. Chemical and microbiological properties of forest floor material at the three stands under investigation**

	SB	CP	CPN
<i>Chemical properties</i>			
Dry matter (DM) (kg m <sup>-2</sup> )	5.80 ± 1.50 <sup>a</sup>	5.30 ± 1.36 <sup>a</sup>	4.00 ± 0.50 <sup>b</sup>
Ash (%)	14.9 ± 14.7 <sup>a</sup>	19.8 ± 10.4 <sup>a</sup>	27.4 ± 12.9 <sup>a</sup>
C <sub>tot</sub> (%)	48.4 ± 4.1 <sup>a</sup>	45.8 ± 4.0 <sup>a</sup>	43.7 ± 1.4 <sup>a</sup>
N <sub>tot</sub> (%)	2.24 ± 0.23 <sup>a</sup>	1.70 ± 0.32 <sup>b</sup>	1.61 ± 0.33 <sup>b</sup>
C/N	21.6 ± 0.5 <sup>a</sup>	27.0 ± 3.1 <sup>b</sup>	27.2 ± 3.5 <sup>b</sup>
TSOC (g kg <sup>-1</sup> DM)	0.40 ± 0.06 <sup>a</sup>	0.50 ± 0.04 <sup>b</sup>	0.34 ± 0.06 <sup>a</sup>
TSO <sub>N</sub> (g kg <sup>-1</sup> DM)	0.09 ± 0.02 <sup>ab</sup>	0.10 ± 0.01 <sup>a</sup>	0.07 ± 0.01 <sup>b</sup>
NO <sub>3</sub> <sup>-</sup> -N (mg kg <sup>-1</sup> DM)	3.06 ± 0.35 <sup>a</sup>	1.71 ± 0.22 <sup>b</sup>	9.56 ± 2.42 <sup>c</sup>
NH <sub>4</sub> <sup>+</sup> -N (mg kg <sup>-1</sup> DM)	42.1 ± 22.1 <sup>ab</sup>	35.5 ± 3.9 <sup>a</sup>	51.2 ± 10.2 <sup>b</sup>
DIN (mg kg <sup>-1</sup> DM)	45.1 ± 21.8 <sup>ab</sup>	37.2 ± 4.0 <sup>a</sup>	60.7 ± 12.2 <sup>b</sup>
pH-H <sub>2</sub> O	3.79 ± 0.02 <sup>a</sup>	3.80 ± 0.03 <sup>a</sup>	4.02 ± 0.01 <sup>b</sup>
P <sub>tot</sub> (mg kg <sup>-1</sup> DM)	75.1 ± 10.8 <sup>a</sup>	62.0 ± 9.0 <sup>ab</sup>	59.9 ± 9.0 <sup>b</sup>
Na (g kg <sup>-1</sup> DM)	0.19 ± 0.03 <sup>a</sup>	0.20 ± 0.04 <sup>a</sup>	0.27 ± 0.10 <sup>a</sup>
K (g kg <sup>-1</sup> DM)	1.02 ± 0.12 <sup>a</sup>	0.84 ± 0.21 <sup>a</sup>	0.89 ± 0.17 <sup>a</sup>
Ca (g kg <sup>-1</sup> DM)	0.88 ± 0.50 <sup>a</sup>	0.13 ± 0.13 <sup>b</sup>	0.49 ± 0.27 <sup>a</sup>
Mg (g kg <sup>-1</sup> DM)	0.59 ± 0.11 <sup>a</sup>	0.35 ± 0.07 <sup>b</sup>	0.54 ± 0.12 <sup>a</sup>
<i>Microbiological properties</i>			
MBC (g kg <sup>-1</sup> DM)	5.13 ± 1.31 <sup>a</sup>	4.86 ± 1.73 <sup>a</sup>	6.18 ± 2.20 <sup>a</sup>
MBN (g kg <sup>-1</sup> DM)	0.96 ± 0.30 <sup>a</sup>	0.95 ± 0.33 <sup>a</sup>	1.13 ± 0.32 <sup>a</sup>
PLFA <sub>total</sub> (μg kg <sup>-1</sup> DM)	65.9 ± 11.2 <sup>a</sup>	54.5 ± 6.0 <sup>a</sup>	49.9 ± 9.9 <sup>a</sup>
Gram+ (ng kg <sup>-1</sup> DM)	11959 <sup>a</sup> (18.3 <sup>a</sup> )	10343 <sup>ab</sup> (19.0 <sup>a</sup> )	8088 <sup>b</sup> (16.3 <sup>a</sup> )
Gram- (ng kg <sup>-1</sup> DM)	782 <sup>ab</sup> (1.2 <sup>a</sup> )	1101 <sup>a</sup> (2.0 <sup>b</sup> )	810 <sup>b</sup> (1.7 <sup>b</sup> )
Fungi (ng kg <sup>-1</sup> DM)	16862 <sup>a</sup> (25.0 <sup>a</sup> )	11404 <sup>a</sup> (21.1 <sup>a</sup> )	10270 <sup>a</sup> (20.4 <sup>a</sup> )
Actinomycetes (ng kg <sup>-1</sup> DM)	4480 <sup>ab</sup> (7.0 <sup>ab</sup> )	5090 <sup>a</sup> (9.4 <sup>a</sup> )	2845 <sup>b</sup> (5.8 <sup>b</sup> )
Mycorrhizae (ng kg <sup>-1</sup> DM)	1635 <sup>a</sup> (2.5 <sup>a</sup> )	698 <sup>b</sup> (1.3 <sup>b</sup> )	1033 <sup>c</sup> (2.1 <sup>a</sup> )
Protozoa (ng kg <sup>-1</sup> DM)	2953 <sup>a</sup> (4.5 <sup>a</sup> )	2399 <sup>a</sup> (4.3 <sup>a</sup> )	2165 <sup>a</sup> (4.2 <sup>a</sup> )
Bacteria/Fungi	1.46 <sup>a</sup>	1.67 <sup>a</sup>	1.53 <sup>a</sup>
Gram+/Gram-	16.29 <sup>a</sup>	9.42 <sup>b</sup>	10.28 <sup>b</sup>
N-mineralization*** (mg N kg <sup>-1</sup> N <sub>tot</sub> d <sup>-1</sup> )	103.0 ± 4.7	151.5 ± 19.9	483.2 ± 57.2

<sup>a, b, c</sup> Means with a different letter within the same line differ significantly at the  $p < 0.05$  level;

\* values for the different microbial groups indicate absolute PLFA amounts. Numbers between brackets indicate the relative contribution (%) of that group to total PLFAs;

\*\* estimated as difference between field DIN fluxes in throughfall and forest floor percolate;

\*\*\* quantified in laboratory N-mineralization experiment.



**Table 4.3. Amount and chemical composition of litterfall material at the three sites under investigation**

Properties	SB	CP		CPN	
		Needles	Cones	Needles	Cones
Dry matter ( $\text{kg m}^{-2} \text{yr}^{-1}$ )	$0.37 \pm 0.02^a$	$0.57 \pm 0.05^b$	$0.25 \pm 0.05$	$0.48 \pm 0.07^b$	$0.08 \pm 0.06$
Ash (%)	$2.76 \pm 0.05^a$	$1.78 \pm 0.16^b$	$1.01 \pm 0.17$	$1.69 \pm 0.04^b$	$0.96 \pm 0.31$
$C_{\text{tot}}$ (%)	$50.5 \pm 0.1^a$	$49.9 \pm 0.2^b$	$48.1 \pm 0.9$	$49.8 \pm 0.4^b$	$48.6 \pm 1.0$
$N_{\text{tot}}$ (%)	$1.56 \pm 0.05^a$	$0.95 \pm 0.13^b$	$0.54 \pm 0.08$	$1.13 \pm 0.06^b$	$0.68 \pm 0.13$
C/N	$32.4 \pm 1.1^a$	$53.3 \pm 7.4^b$	$89.9 \pm 13.3$	$44.3 \pm 2.2^b$	$73.0 \pm 12.9$
$P_{\text{tot}}$ ( $\text{mg kg}^{-1} \text{DM}$ )	$79.4 \pm 3.0^a$	$41.2 \pm 8.1^b$	$42.9 \pm 6.2$	$39.5 \pm 12.3^b$	$47.1 \pm 0.9$
Na ( $\text{g kg}^{-1} \text{DM}$ )	$0.33 \pm 0.02^a$	$0.37 \pm 0.03^{ab}$	$0.26 \pm 0.01$	$0.53 \pm 0.08^b$	$0.26 \pm 0.06$
K ( $\text{g kg}^{-1} \text{DM}$ )	$4.81 \pm 0.42^a$	$2.17 \pm 0.34^b$	$4.03 \pm 0.66$	$2.84 \pm 0.10^c$	$3.25 \pm 2.34$
Ca ( $\text{g kg}^{-1} \text{DM}$ )	$2.68 \pm 0.09^a$	$2.18 \pm 0.30^{ab}$	$0.00 \pm 0.00$	$1.38 \pm 0.24^b$	$0.07 \pm 0.10$
Mg ( $\text{g kg}^{-1} \text{DM}$ )	$1.95 \pm 0.04^a$	$0.62 \pm 0.07^b$	$0.38 \pm 0.04$	$0.67 \pm 0.03^b$	$0.42 \pm 0.01$

<sup>a, b, c</sup> Means with a different letter within the same line differ significantly at the  $p < 0.05$  level.

### *Laboratory release experiment*

To exclude possible influences of external factors on the DOM release from forest floor material, a release experiment was performed under controlled laboratory conditions. Under similar (micro-)climatic conditions (temperature, moisture content and drying-rewetting cycles) and with an identical artificial throughfall solution, observed differences in DOM release can be attributed to inherent differences in the forest floor material. This laboratory experiment exhibited the following striking results:

(i). In general, the DOC release from sterilized samples largely exceeded the release from control samples (Figure 4.2). This is in accordance to the findings of Stutter et al. (2007) who attributed the initial increased DOM release after sterilization to death and lysis of microbial cells, which is rapidly flushed out, whereas they ascribed the continued elevated DOM release to suppressed microbial immobilization or decomposition of DOM. They also found that active biological functioning is not required to mobilize significant quantities of DOM over extended periods, although biological processes may have been responsible for DOM production prior to sterilization. This is in accordance to the findings of Qualls (2000) which could not find evidence for microbial production of DOC

from forest floor material in a 21 day  $\text{HgCl}_2$  sterilization experiment. Lensi et al. (1991) found that residual enzyme-activities after sterilization might be responsible for several degradation processes.

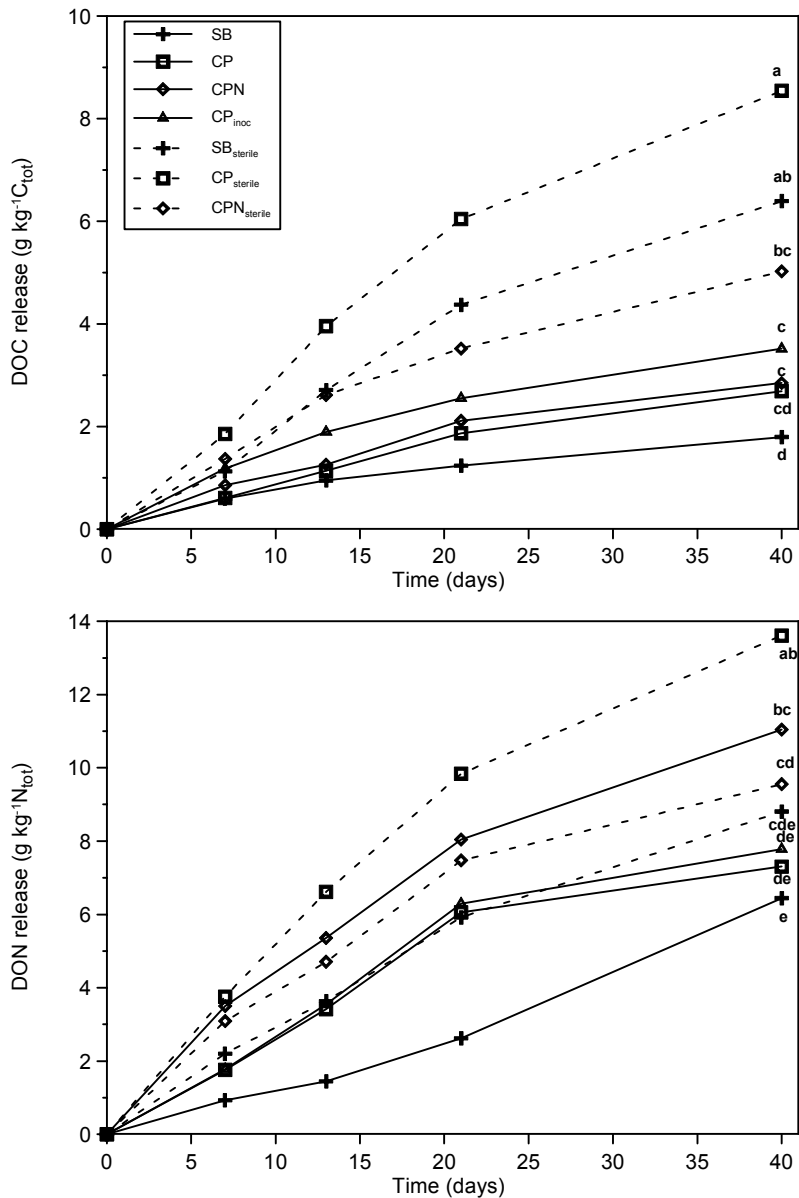


Figure 4.2. Cumulative release of dissolved organic carbon (DOC) and nitrogen (DON) from forest floor material of different origins under laboratory conditions. Letters indicate significant differences.

Since in our study, most DOM resulting from the sterilization should have been leached before the experiment and since the cumulative amount of DOC released from the sterilized samples is rather inversely correlated to the amount of microbial biomass carbon (MBC), we suggest that the increased DOC release after sterilization was mainly due to the suppressed microbial degradation of DOC. Kristensen and McCarty (1999) also found that the addition of  $\text{HgCl}_2$  led to complete inhibition of  $\text{CO}_2$  production in soils. The relative differences between the cumulative DOC released from the sterile and control samples differs between the three forest floor materials: on average, 72, 69 and 43 % of the total amount of DOM released is apparently degraded in the SB, CP and CPN control samples respectively.

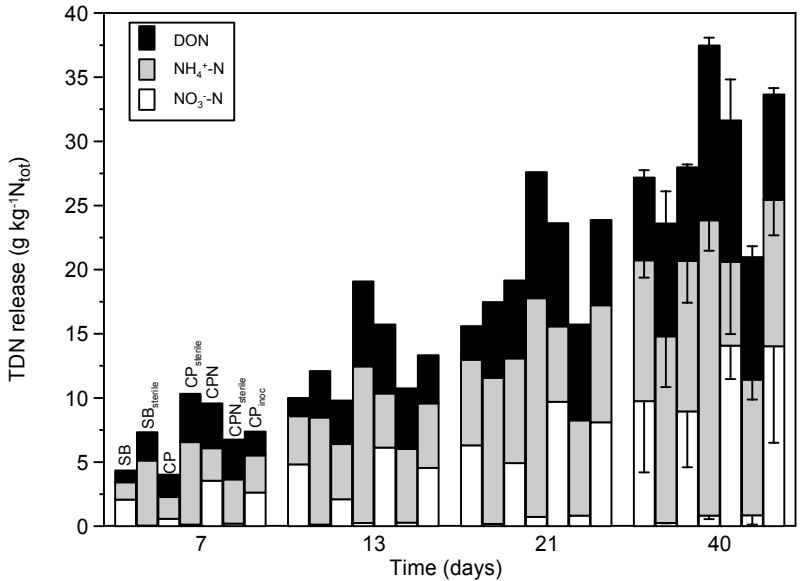
(ii). Contrary to its influence on DOC release, the sterilization of the forest floor materials did not show the same impact on DON release (Figure 4.2). The DON release from the SB and CP material was minimally, though positively, affected by sterilization, whereas cumulatively less DON was released from the CPN material after sterilization. As a result of the different impact of sterilization on DOC and DON release, the DOC/DON ratio of the DOM released from the sterile samples was significantly larger compared to the controls (Table 4.4). If the increase in DOM released after sterilization would originate from the lysis of dead microbial biomass, with a typical C/N ratio of 7.4 (Cleveland and Liptzin, 2007), the DOC/DON ratio would rather tend to decrease. The lower DOC/DON ratio in the control samples compared to the sterile samples is thus due to the presence of microbial activity by (i) the preferential degradation of low C/N DOM fractions and/or (ii) the stoichiometric N enrichment of the DOM.

**Table 4.4. C/N ratios of forest floor material and DOM in forest floor leachates under field conditions and different laboratory treatments**

	C/N			DOC/DON	
	Solid phase	Field	Control	$\text{HgCl}_2$ -sterilized	Inoculated (CPN)
SB	$21.6 \pm 0.5^a$	20.2	$5.99 \pm 0.49^b$	$16.5 \pm 4.5^a$	n.d.
CP	$27.5 \pm 1.1^a$	23.9	$6.48 \pm 1.95^b$	$17.0 \pm 3.3^c$	$12.2 \pm 1.8^c$
CPN	$27.2 \pm 1.4^a$	19.6	$7.13 \pm 0.75^b$	$17.5 \pm 3.2^c$	n.d.

<sup>a, b, c</sup> Means with a different letter within the same line differ significantly at the  $p < 0.05$  level.

(iii). The  $\text{HgCl}_2$  sterilization also impacted on the amount and composition of the total dissolved nitrogen release (TDN) (Figure 4.3). Without sterilization, the TDN release followed the order  $\text{SB} < \text{CP} < \text{CPN}$ , with most nitrate formation in the CPN material. Since no nitrate was found in the sterile samples, sterilization apparently blocked nitrification completely. Similar to the findings of Stutter et al. (2007), continued release of ammonium has been observed from the sterilized samples, which can be attributed to (i) the deamination of nitrogenous organic compounds and the proteolysis of damaged microbial cells as a result of residual enzyme-activities (Lensi et al., 1991) and/or (ii) the gradual release of  $\text{NH}_4^+$  retained on the cation exchange sites (Stutter et al., 2007).



**Figure 4.3. Cumulative nitrate, ammonium and DON release from forest floor material of different origins. Error bars indicate single standard deviations on cumulative concentrations of individual N compounds.**

(iv). The differences in DOC release from the different forest floors as observed under field conditions could not be found under laboratory conditions. Whereas the DOC release in the field followed the order  $\text{CPN} \gg \text{SB} > \text{CP}$ , the cumulative DOC release from the control samples in the laboratory followed the order  $\text{CP} \approx \text{CPN} > \text{SB}$ . These findings indicate that the differences in DOC release

from the forest floor of the three stands are a result of differences in either climatic conditions or throughfall ionic composition. The DOC release from sterilized samples showed even an inverse order ( $CP > SB > CPN$ ) compared to the field observations, which follows the order of the total soluble organic carbon (TSOC) pools measured before the experiment (Table 4.2). Besides, the size of this pool at the start of the experiment was inversely correlated with the annual DOC release in the field. This indicates that the DOC released from sterile samples originates from the gradual solubilization of DOC from a large potential DOC pool. Consequently, the ionic concentration and/or composition of the throughfall solution are believed to play a key role in this solubilization process, pointing towards a physico-chemical release mechanism.

(v). Inoculation of the CP forest floor material with CPN material had a slightly positive effect on the initial DOC release (first 13 days), whereas the release of DON was rather unaffected. As a result, the average DOC/DON ratio of the DOM released from the  $CP_{inoc}$  samples was significantly higher than that of the CP control samples, though not significantly different from that of the sterilized CP samples. These findings would suggest that the inoculation had a similar, though smaller effect on the DOM release as the  $HgCl_2$  sterilization.

## DISCUSSION

### *A conceptual model*

The combination of the field observations together with the findings of the laboratory release experiment allowed the design of a conceptual model of the processes involved in the DOM release from forest floor material (Figure 4.4). The microbially mediated decomposition of soil organic matter (SOM) produces DOM, leading to the replenishment of the potential DOM pool. The DOM present in this pool is subject to microbial degradation and physico-chemical solubilization. The size of the potential DOM pool is thus determined by the balance between the rate of formation and the rate of removal (degradation and leaching). The solubilized DOM in the forest floor material is liable to leaching to

the mineral soil with the percolating water. Thus, the net DOM release from the forest floor is generally determined by 4 distinct processes: DOM formation, physico-chemical mobilization, microbial degradation and the hydrological export.

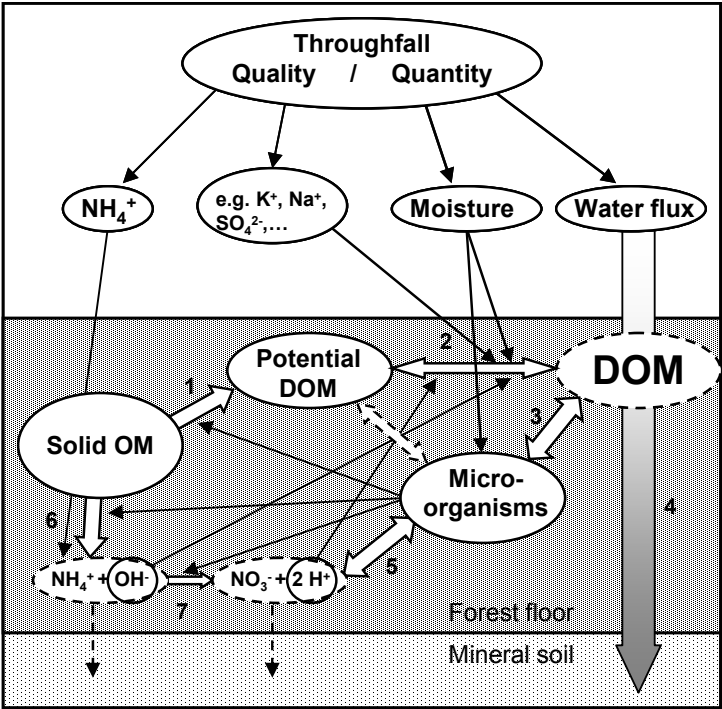


Figure 4.4. Conceptual model of the main processes involved in the formation and release of DOM from forest floor material (1: Enzymatic DOM decomposition; 2: Physico-chemical solubilization; 3: Microbial DOM degradation; 4: Convective DOM transport; 5: Microbial DIN release/ incorporation; 6: Ammonification; 7: Nitrification).

(i) DOM formation

Solid organic matter decomposition is ultimately a result of the catabolic activities of saprotrophic communities associated with organic matter resources. At the molecular level, these catabolic activities are directly mediated by extracellular enzymes (Cotrufo et al., 2000). The first action in enzyme-mediated decomposition of complex organic substrates is their depolymerisation by cleavage of intermonomeric bonds, leading to the production of low molecular weight molecules (amino acids, disaccharides, dipeptides etc.) which can

subsequently be assimilated and mineralized by the saprotrophic communities (Mayer, 1993). Fungi, rather than bacteria, have been shown to play a pivotal role in the degradation of recalcitrant litter C sources (Suberkropp and Weyers, 1996; Møller et al., 1999). Guggenberger et al. (1994) suggested that fungi are the most important agents in the process of DOM production, probably because of incomplete degradation of organic matter by fungi.

Because of the complex chemical nature of plant tissue, total degradation requires the interaction between many classes of enzymes (e.g. Klyosov, 1990). As no single microorganism is able to produce all the enzymes necessary for the complete degradation of plant residues, the decomposition process requires the succession and interaction of many different species of microorganisms. The production of enzymes by the soil microbial community is dependent on site-specific factors (i.e. temperature, moisture and nutrient availability), which control microbial growth. Once enzymes are released into the environment, litter chemistry becomes the primary determinant of enzyme activity.

Guggenberger et al. (1994) found that the DOM release into forest floor solution is related to microbial activity by oxidative degradation of plant-derived organic matter (e.g. water-soluble lignin and lignocellulose fragments) and by production of microbial metabolites (e.g. polysaccharides). Also Kalbitz et al. (2006) found that lignin degradation controls the production of dissolved organic matter in decomposing foliar litter. Besides, the microbial biomass itself serves as an important pool of potential DOM through cell death and subsequent lysis. However, Qualls (2000) did not find evidence for microbial production of DOC in HgCl<sub>2</sub> poisoned versus non-poisoned forest floor material over a 21 day incubation and extraction period.

#### *(ii) Physico-chemical DOM solubilization*

The gradual release of DOM from sterile forest floor material under laboratory conditions indicated the presence of a large pool of soluble organic matter in the forest floor material of the three stands. This is in accordance to the findings of Tipping (1998) who postulated that a pool of potential DOM exists as

part of solid organic matter “*that is not in solution but is part of the soil solids and able to pass into solution under realistic soil conditions*”. Consequently, the actual DOM concentration in the aqueous solution is mainly controlled by abiotic processes such as desorption and dissolution from this potential DOM pool (Christ and David, 1996a; Kalbitz et al., 2000; Qualls, 2000; Yano et al., 2000).

Recent studies have emphasized the physico-chemical factors controlling the release of DOM from peaty material (Stutter et al., 2007) and forest subsoils (Münch et al., 2002). As a result of its colloidal and amphoteric properties, the net charge of DOM molecules and thus their solubility is determined by the ionic concentration and composition of the surrounding solution. Kalbitz et al. (2000) reviewed the influences of pH, ionic strength, cation and anion concentrations and composition on DOM dynamics. At low pH, DOM solubility is generally low (David et al., 1989) and molecules are more condensed than at higher pH. Monovalent cations (like  $\text{Na}^+$  and  $\text{K}^+$ ) can replace protons causing a conformational expansion of DOM molecules giving rise to a higher DOM solubility. Skjellberg and Magnusson (1995) found that  $\text{Na}^+$  ions showed highest extractability of DOC from soil. On the contrary, chemical reactions between anionic functional groups of organic molecules and solution polyvalent cations can reduce the surface charge density, alter the structural conformation and consequently reduce solubility. Furthermore, anions can affect the DOM release by displacing DOM from the available sorption sites (Kalbitz et al., 2000). The strength of displacement seems to be related to the valence, with  $\text{PO}_4^{3-} > \text{SO}_4^{2-} > \text{Cl}^-$  (Gu et al., 1994). Sulfate is the dominant anion in precipitation, throughfall and soil solutions in forest soils of many regions, and competition between sulfate and DOC for sorption sites is a well established phenomenon (e.g. Vance and David, 1992). Reemtsma et al. (1999) found sodium exchange, calcium decomplexation and displacement of sorbed organic anions to be the major release mechanisms for DOM from soil by esp. monovalent salt solutions. Calcium decomplexation is the process in which dissolved organic matter is solubilized from organic matter in calcium complexes and includes the disaggregation of immobilized aggregates into mobile colloids.



It is obvious that the ionic concentration and composition of the aqueous solution, and thus the physico-chemical solubilization of potential DOM from the forest floor, are influenced by various other processes (Figure 4.4). Throughfall water and the release during SOM mineralization are the major sources of cations and anions in the forest floor. Different deposition and interception rates of atmospheric ions result in differences in the composition and amount of ions entering the forest floor with throughfall water. The  $H^+$  concentration in forest floor material is mainly determined by atmospheric acidifying deposition (Vogt et al., 2006), ammonification (proton consuming) and nitrification (proton producing) rates and the ratio of the latter two.

*(iii) Microbial DOM degradation*

In soils, DOM may be the most important carbon source since soil microorganisms are basically aquatic and all microbial uptake mechanisms require a water environment (Metting, 1993). Furthermore, the soluble state is presumably a prerequisite for the diffusion of substrates through microbial cell membranes (Marschner and Kalbitz, 2003).

Marschner and Kalbitz (2003) reviewed the factors controlling the DOM biodegradability in soils. Beside the intrinsic quality of DOM, soil and solution properties and some external factors determine the DOM biodegradability. They pointed out the pH dependence of DOM biodegradability: at low pH, DOM molecules are more condensed than at higher pH, while  $Na^+$  or  $K^+$  can cause an expansion of DOM molecules giving rise to a higher degradability of this DOM. On the contrary, the presence of  $Al^{3+}$  has been shown to have an inhibitory effect on DOM mineralization (Schwesig et al., 2003). Moreover, van Hees et al. (2002) found that the mineralization rate of DOC is concentration dependent. Cleveland et al. (2004) suggested that a substantial fraction of the DOM produced in the litter layer is rapidly decomposed following production.

*(iv) Hydrological DOM export*

The water input with throughfall can also play an important role in both production and mobilization of DOM in the forest floor (Park and Matzner, 2003). The formation of water-soluble organic materials can be increased through the positive effect of moisture on microbial activity (Falkengren-Grerup and Tyler, 1993) or through the rewetting effect after dry periods (Christ and David, 1999b). Since substantial amounts of potentially soluble organic materials exist in an adsorbed phase (Christ and David, 1996a), the amount of percolating water can play a crucial role in mobilizing DOM from different sources (Tipping et al., 1999). Increased water flow and high leaching frequency were found to increase DOM release in some laboratory leaching experiments (Christ and David, 1996a; Göttsche et al., 1996). Water fluxes have also been suggested to be a key driver of DOM export either from the forest floor (Michalzik et al., 2001) or the whole soil profile (Tipping et al., 1999). Park and Matzner (2003) concluded that the magnitude of potentially soluble organic matter and water flux seems to play a more important role in regulating the actual DOM release from the forest floor than a straightforward relationship between microbial activity and DOM production.

*Effect of tree species*

The field observations showed a higher annual release of DOC and DON from the SB than from the CP forest floor (Table 4.1). Higher amounts of  $H^+$  ions were released from the CP forest floor mainly as a result of the higher nitrification. This higher proton concentration may have reduced DOM solubilization in the field. Together with the lower water flux (Table 4.1), this lower physico-chemical DOM solubilization has resulted in a lower overall DOM release. Furthermore, the higher pool of total soluble organic carbon (TSOC) remaining in the CP forest floor (Table 4.2) points towards the accumulation of DOM in the potential DOM pool. This is in accordance to the higher concentration of total water-extractable DOC found in the humus layer under birch than under pine as reported in other

studies (Smolander and Kitunen, 2002; Suominen et al., 2003; Kiikkilä et al., 2005). The equal percentagewise difference in cumulative DOC release between control and sterilized samples of both forest floors revealed that more or less similar fractions of DOM were degraded in the SB (72 %) and CP (68 %) forest floor. No significant differences between SB and CP could be found in DOC/DON ratios of the DOM released from control and sterile forest floor samples (Table 4.4), pointing towards an independence of the DOC/DON ratio on forest type and solid phase C/N under similar conditions. Since there were no differences in MBC, MBN and PLFA<sub>total</sub> (Table 4.2), the slightly higher DOM release from the SB forest floor compared to the CP forest floor seems thus mainly a result of the higher physico-chemical DOM solubilization as a result of lower H<sup>+</sup> formation in the SB forest floor.

#### *Effect of nitrogen deposition and forest edge proximity*

Despite the slightly lower water flux, the DOC and DON release was much higher from the forest floor in the CPN stand compared to the CP stand (by 154 % and 260 % respectively). This higher DOM release concurred with a 7 times higher DIN release (Table 4.1), pointing towards a higher net N mineralization rate (219 %). This significantly elevated N mineralization was also found in the laboratory N mineralization experiment (Table 4.2). Vestgarden et al. (2003) found that N additions lead to increased net N mineralization rates in the forest floor of a Scots pine stand. Falkengren-Grerup et al. (1998) also found that high nitrogen deposition in recent decades has favored ammonification. They attributed this enhanced mineralization to the increased N pool in the soil and the altered quality of the organic matter, the microbial community adapting to the acidified soils and the increased nitrification. We could only find an increased mineral N pool in the CPN stand, a significantly higher pH and Ca and Mg content of the forest floor material, less Gram-negative bacteria and actinomycetes and more mycorrhizae (Table 4.2). Michel and Matzner (1999) found a significant positive correlation between N-mineralization and the pH of the forest floor material, and they also found a positive correlation between N mineralization and

DOC and DON release in Oe samples. Furthermore, the field observations showed a lower contribution of nitrate to DIN release in the CPN forest floor (39 %) compared to the CP forest floor (62 %), resulting in a substantially (4.6 times) lower  $H^+$  release. This lower proton release together with the higher cation release, points towards an enhanced physico-chemical DOM solubilization. Furthermore, the close relationship between  $NH_4^+$  and DOM release could also be attributed to the formation of ‘soluble salts’ of  $NH_4^+$  and humic and fulvic acids (Norman et al., 1987).

The results of the laboratory release experiment showed a lower DOM degradation in the CPN forest floor (43 %) compared to CP material (68 %). Sinsabaugh et al. (2004) found a reduction in oxidative enzyme activities, especially the lignin-degrading phenol oxidase and peroxidase activity, as a result of inorganic nitrogen additions to different forest floors, resulting in higher DOC concentrations. Smemo et al. (2007) found increased polyphenolic and total aromatic contents of DOC leached from forest floor material receiving chronic experimental  $NO_3^-$  deposition. Mercer et al. (1996) found that extracellular peroxidase production is common, though quantitatively variable, for actinomycetes. We found significantly lower actinomycetal PLFA contents and lower contribution of actinomycetes to total PLFAs in the CPN stand compared to the CP stand (Table 4.2). Trigo and Ball (1994) suggested that actinomycetes play a role in the formation of humic substances in soils and composts. Sinsabaugh et al. (2004) suggested that the increased concentration of phenolic compounds might have inhibitory effects on extracellular enzyme activity (EEA) and microbial metabolism, and thus increase DOM export by reducing consumption (Freeman et al., 2001).

Inoculation of the CP forest floor with CPN forest floor material showed a similar, though smaller, effect as the  $HgCl_2$  sterilization during the first 13 days of incubation especially on the DOC release. The increased release of DOC and DIN might be a result of suppressed DOM degradation perhaps as the result of addition of (poly)phenolic compounds with the inoculum. This is in accordance to the findings of Kanerva et al. (2006) that the addition of the heavy tannins

(polyphenolic) fraction, common in pine humus (Suominen et al., 2003), to soil reduced respiration and slightly increased net N mineralization as a result of lower N immobilization in the microbial biomass. They suggested that these polymerized condensed tannins have a toxic or protein-precipitating effect. As a result of the higher peroxidase and phenol oxidase activity in the CP forest floor, the inhibitory effect of the inoculum (polyphenols) was only temporary and gradually decreased during the first 13 days. Furthermore, some studies (e.g. Baldwin et al., 1983) have found that phenolic compounds also show an inhibitory effect on nitrification. This is in accordance to our field observations; a higher abundance of  $\text{NH}_4^+$  was found in the forest floor leachate of the CPN stand compared to the CP stand. This inhibition of nitrification results in a lowered  $\text{H}^+$  production or even a net  $\text{H}^+$  consumption (as a result of ammonification) resulting in an increased physico-chemical DOM solubilization. The lower proton production is evidenced by the lower proton flux (Table 4.1) and the significantly higher pH (Table 4.2) of the CPN forest floor compared to the CP. Further research is required to confirm this hypothesis.

#### *Relation between DOC and DON*

The field observations showed that the C/N ratio of the DOM leaching from the three forest floors was lower than the C/N ratio of the solid phase. This is in agreement with the findings of several experimental N addition studies (e.g. McDowell et al., 2004; Pregitzer et al., 2004). Brookshire et al. (2007) suggested two major pathways for this stoichiometric enrichment of DOM: (i) stoichiometric alteration of the SOM pool and (ii) direct N enrichment of DOM. Since the C/N ratio of the forest floor material was apparently not affected by the higher N inputs in our study, our results point towards direct N enrichment of DOM.

Furthermore, the difference between C/N ratio of the forest floor material and the DOM released from it tended to increase with increasing DIN fluxes. An increase of DON release with increasing N availability was observed in several experimental field studies with manipulation of mineral N inputs (Currie et al., 1996; McDowell et al., 1998). Three mechanisms might be responsible for the N

enrichment of the DOM released from the forest floor: (i) preferential degradation of dissolved organic substances with high C/N ratios (e.g. carbohydrates) (Kalbitz et al., 2003); (ii) the abiotic DON formation from the chemical reaction of DIN with DOM (Davidson et al., 2003) and/or (iii) microbial assimilation or incorporation of inorganic nitrogen which is later released as DON (Aber et al., 1998).

Sterilization of the forest floor material in the laboratory experiment resulted in a significant increase of the DOC/DON ratio of the DOM released from the three forest floors, indicating that microbial activity is responsible for the decreased DOC/DON ratio compared to the solid phase C/N ratio, either by a preferential degradation of high C/N DOM or active microbial N incorporation. Moreover, the absolute DON release from the CPN forest floor decreased as a result of the sterilization suggesting that N is microbially incorporated in the DOM in this forest floor. This is in accordance with the hypothesis of Aber et al. (1998) that microbial assimilation of DIN and subsequent exudation of amine-rich DON is an important pathway lowering the DOC/DON ratio in soil solution under conditions of N saturation. Furthermore, McDowell et al. (2004) found that an increased mineral N input increased the contribution of hydrophilic fractions to DON, suggesting that compounds with microbial origin such as amino acids and amino sugars have increased in concentration due to N saturation. Microbial biomass C/N ratios typically average around 7.4 (Cleveland and Liptzin, 2007), which is considerably lower than the DOC/DON ratios in most aqueous solutions. Release of bacterial cell components through several biological processes (including direct release, viral lysis and grazing) results in a decrease in the bulk DOC/DON ratio. Furthermore, Ogawa et al. (2001) showed that marine bacteria produce highly refractory DON from glucose and ammonium with a bulk DOC/DON ratio of 8.9 after a 365-days incubation.

## CONCLUSIONS

The annual DOM flux with forest floor leachate accounted for 3.2 to 9.5 % of carbon input and 5.2 to 15.2 % of nitrogen input to the forest floors in this study with aboveground litterfall.

From the combination of field data and observations during the laboratory release experiment, we proposed a conceptual model of the major processes involved in the release of DOM from forest floor material. The leaching of DOC from the forest floor is controlled not only by its (enzymatic) production, but also by its consumption through microbes, its physico-chemical retention and by the hydrologically driven transport. The net release is thus a result of complex interactions between abiotic and biotic processes, with the relative importance of each process, in turn, depending on different other external or internal factors.

The results of the one year field measurements showed that more DOC and DON were released from SB forest floor material compared to the CP material. Because of the limited differences in chemical and microbiological properties between both forest floors, our data suggest that this difference in DOM release from both forest floors mainly resulted from the difference in  $H^+$  budget. A higher atmospheric input of acidifying deposition, together with higher N mineralization and nitrification rates resulted in a higher proton production in the coniferous forest floor. The lower pH suppressed physico-chemical solubilization of DOM, which was confirmed by the presence of a higher extractable organic matter pool.

The strong increase in DOM release from a coniferous forest floor under elevated nitrogen deposition in comparison with one under background N deposition is probably a result of a complex interaction of different mechanisms. First, the high DIN content of the CPN forest floor probably resulted in a suppressed peroxidase activity, leading to the accumulation of soluble (poly)phenolic compounds. These organic compounds probably inhibited nitrification resulting in a lower net  $H^+$  formation inducing in turn physico-chemical DOM solubilization. Furthermore, the less acidic conditions in the forest

floor favored mineralization and thus DOM production. Second, the coniferous stand at the forest edge also intercepted more anions and cations, promoting the physico-chemical solubilization of DOM. The foregoing chain-reaction examples the complex nature of the mechanisms and the fragile equilibria involved in the DOM release from forest floors.

The results of the field measurements revealed a (partial) decoupling of DOC and DON release from the forest floor. The laboratory release experiment indicated that microbial activity is responsible for the lower DOC/DON ratio compared to the C/N ratio of the forest floor material. Two simultaneous processes are probably involved: the preferential degradation of high C/N DOM (e.g. carbohydrates) together with the assimilation of DIN in the microbial biomass followed by release of low C/N DOM after death and lysis of the microbial cells.



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# CHAPTER 5

## BIODEGRADABILITY OF DISSOLVED ORGANIC MATTER

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Illustration on p. 107:

Biodegradability test: soil solution samples incubated in the lab for one year.

*This chapter has been submitted as:*

*Vandenbruwane J., Sleutel S., De Neve S., Qualls R.G. and Hofman G.*

*Biodegradability of dissolved organic carbon (DOC) and nitrogen (DON) from different origins and soil depths. Soil Biology & Biochemistry (Submitted).*

## ABSTRACT

Large amounts of dissolved organic carbon (DOC) and nitrogen (DON) enter the mineral soil with forest floor leachate, but the concentrations of dissolved organic matter (DOM) that percolate through the mineral soil to deeper horizons or to the ground water are fairly low due to mineralization, sorption and/or precipitation processes. The relative contribution of each of these processes to DOM retention is still largely unknown. The objective of this study was to quantify the biodegradability of DOC and DON from different sources and from different depths in the mineral soil. The degradation rates of DOC in forest floor leachate from three different forest stands and in mineral soil solution from a coniferous stand, were determined in a 365 day batch incubation experiment. Initially, all samples were found to be largely depleted in labile DOC, with mean half-life values of the remaining DOC ranging from 1.9 to 6.8 years. No significant differences in DOC degradation could be found between the forest floor leachates of three forest stands. The DOC biodegradability tended to increase with increasing soil depth, probably as a result of the adsorption of the more refractory hydrophobic and hydrophilic acid fraction to mineral soil material. A consistent, though not significant, decrease in DOC/DON ratio over incubation time was found in all samples pointing towards the microbial incorporation of inorganic nitrogen followed by release of organic N compounds into the solution. The limited degradability of DOC and DON found in this study suggests that mineralization was not the major process responsible for the low DOM concentrations in the subsoil.

## INTRODUCTION

During the last decades, dissolved organic matter (DOM) dynamics in both aquatic and terrestrial ecosystems have received increasingly more attention. Beside its contribution to soil formation (weathering and podsolization), DOM largely determines the solubility and mobility of (heavy) metals and organic pollutants in soils. In addition, DOM contains organically bound nutrients such as N, P and S, and DOM dynamics will therefore affect their mobility and availability (Kalbitz et al., 2000).

Although sorption has been determined to be the fastest and largest sink for DOM in mineral soils (Guggenberger et al., 1998), McCracken et al. (2002) found that only about 50 % of the DOC retained by the mineral soil was found as soil organic carbon (SOC), suggesting that microbial decomposition is a significant factor regulating the removal of (adsorbed) DOC. In soils, DOM may be the most important carbon source since soil microorganisms are in a sense aquatic and all microbial uptake mechanisms require a water environment (Metting, 1993). Furthermore, the soluble state is presumably a prerequisite for the diffusion of substrates through microbial cell membranes so that the degradation of solid phase organic matter or large molecules can only occur after dissolution or hydrolysis by exoenzymes (Marschner and Kalbitz, 2003). Besides, the importance of DOM biodegradation in mineral soils lies in the fact that it yields both energy and limiting nutrients and that it can alter the DOM delivery to aquatic ecosystems (McDowell et al., 2006).

The distinction between DOM *bioavailability* and *biodegradability* was put forward by Marschner and Kalbitz (2003). They stated that, in the context of DOM, the term *bioavailability* describes *the potential of microorganisms to interact with these substances*. The term *biodegradability* refers to *the actual utilization of organic compounds by soil microorganisms*, which still encompasses two alternative or sequential processes. Biodegradation can denote (i) the microbial uptake or breakdown of the original compounds which are then used for the biosynthesis of microbial cell materials and (ii) the complete mineralization to

obtain energy and inorganic nutrients. Marschner and Kalbitz (2003) reviewed the factors controlling DOM bioavailability and degradability in the mineral soil and found that the size of the pores, soil aggregation, sorption and drought to be the main controls on DOM bioavailability. Dissolved organic matter biodegradability is largely controlled by the intrinsic DOM quality parameters (e.g. molecular size, chemical structure), by soil and solution properties (e.g. nutrients, salts, pH, O<sub>2</sub>, metal concentration, organic compounds, composition of microbial community) and by external factors (e.g. temperature, moisture, wet-dry cycles).

Batch incubations in solution culture, flow-through reactors (Yano et al., 1998) and addition of <sup>14</sup>C-labelled DOM to soil (Qualls and Bridgman, 2005) are the three common experimental methods used for the determination of DOM biodegradability under laboratory conditions (Marschner and Kalbitz, 2003). Trulleyová and Rulík (2004) compared different batch methods in their ability for accurate biodegradable DOC (BDOC) quantification. McDowell et al. (2006) compared batch, kinetic and bioreactor methods in their comparability and reproducibility to determine BDOC from different terrestrial sources. Beside the choice of the experimental method to determine BDOC, there are different strategies to quantify effective DOM degradation. The two most common quantification methods are measurement of the CO<sub>2</sub> efflux from the samples and/or measurement of the decline in DOC concentration. However, the last method requires the simultaneous quantification of microbial biomass and particulate matter formation. Marschner and Kalbitz (2003) pointed out that knowledge about the size of the biodegradable DOM fraction is not sufficient, since samples can contain similar BDOM concentrations that can differ substantially in their degradation rates depending on intrinsic properties.

Although the biodegradability of DOC and different DOC fractions from various sources has been studied extensively, very little is known about the microbial degradation of dissolved organic nitrogen (DON). Few attempts have been made to indirectly quantify DON degradability relative to DOC based on changes in DOC/DON ratios. Qualls and Haines (1992) measured the initial and final DOC/DON ratio in a 134-day batch incubation and did not find a faster decay

of DON compounds compared to DOC. Cook and Allan (1992) found a gradually decreasing DOC/DON ratio in the hydrophobic acids fraction during a 210-day in vitro incubation of soils. Kiikkilä et al. (2005) tried to determine the short-term biodegradability of DON originating from different tree species during a 15-day batch experiment by measuring the DON removal from the solution, but did not find a significant reduction of the DON concentrations. Cleveland et al. (2004) reported the occurrence of both N immobilization in MBN and N mineralization of DON during a 100-day batch incubation experiment.

The objective of this study was to find out if biodegradation was potentially responsible for the substantial reduction in DOC and DON concentrations during percolation through the mineral soil. In a companion study, concentrations of forest floor water percolating through the soil were substantially reduced even though the soil was a poor sandy podzol. In addition, the differences in biodegradability of DOM originating from different forest floors and different soil horizons were investigated.

## **MATERIALS AND METHODS**

### *Sample collection*

Samples of forest floor leachate (H) in the 3 forested experimental stands (SB, CP and CPN) and mineral soil solution at different depths in the CPN stand (Chapter 3) were collected on a fortnightly basis between September and November 2005 for this biodegradability test. All samples were filtered through 0.45 µm polyethersulfone (PES) membrane disc filters (Pall Supor®-450, Gelman Laboratory) and stored at -18 °C until used in the experiments.

### *Incubation*

Samples taken at different times and locations differed substantially in DOC and DON concentration. In order to obtain measurable DOC and DON concentrations in all cases and to facilitate comparison between soil horizons (Qualls and Haines, 1992), the soil solution samples originating from the mineral

soil were concentrated using a centrifugal vacuum evaporator (Laborota 4000 efficient, Heidolph, Schwabach, Germany) (Cook and Allan, 1992) to obtain a final DOC concentration in the range of 40-60 mg DOC l<sup>-1</sup>.

Since DON biodegradability during incubations was assessed by determining the removal rate of DON from the solution, accurate and precise DON measurements are indispensable. Selective removal of mineral nitrogen from aqueous samples by means of a buffered dialysis pretreatment has been found to increase both the recovery and precision of DON measurements (Vandenbruwane et al., 2007). Therefore, all aqueous samples were dialyzed for 2 days using this method before the start of the incubation. Since both protons and hydroxyl ions can permeate through the dialysis membrane (Vandenbruwane et al., 2007), the pH of the samples had to be readjusted to the original values prior to incubation.

For the preparation of the inoculum (Qualls and Haines, 1992), a mixture of the forest floor material and A horizon mineral soil originating from the three sites was blended in deionized water. This suspension was sequentially filtered through a 37 µm glass-fiber filter and a 0.45 µm polyethersulfone (PES) membrane disc filters (Pall Supor®-450, Gelman Laboratory). The 0.45 - 37 µm fraction was washed with groundwater and resuspended in a mixture of the DOM solutions used in the experiment (H<sub>SB</sub>, H<sub>CP</sub>, H<sub>CPN</sub>, E<sub>CPN</sub>, Bh<sub>CPN</sub> and BC<sub>CPN</sub>). This suspension was pre-incubated for 16 hrs to initiate the microbial growth, and thus to prevent a lag phase during the actual incubations. Subsequently, this solution was again filtered through 37 µm and 0.45 µm filters and the 0.45 - 37 µm fraction was resuspended in 20 mL groundwater.

For the incubation experiment, 900 ml of each DOM solution was put in an acid-washed 1 L Erlenmeyer flask in three replicates. Each flask was inoculated with 1 ml of the prepared inoculum and covered with gas-permeable parafilm (Pechiney Plastic Packaging Co., Menasha, WI, USA) to prevent excessive evaporation. To compare the mineralization of the natural substrates with a very easily mineralizable substrate (Qualls, 2004), three glucose controls (Glc) were treated in a similar fashion as the DOM samples. To this end, glucose was added to deionized water to obtain a final concentration of 50 mg glucose-C l<sup>-1</sup>. To

provide the necessary nutrients for microbial activity,  $\text{NH}_4\text{Cl}$  and  $\text{K}_2\text{HPO}_4$  were added to these glucose-control samples. All samples were incubated in a climate chamber at 15 °C in the dark for 365 days. The flasks were regularly opened and gently shaken to aerate the solutions. Representative subsamples (60 ml) were taken at increasing time intervals on days 0, 5, 8, 13, 21, 42, 63, 84, 211 and 365 immediately after vigorous shaking. Twenty ml of each sample was not filtered and directly stored at -18 °C before analysis. The remaining 40 ml of each sample was filtered through 0.45  $\mu\text{m}$  polyethersulfone (PES) membrane disc filters (Pall Supor®-450, Gelman Laboratory) and stored at -18 °C before analysis.

### *Chemical analyses*

The unfiltered samples were analyzed for total organic carbon (TOC) and total nitrogen (TN) concentrations, whereas TOC, TN and dissolved inorganic nitrogen (DIN) concentrations were determined in the filtered samples. Total carbon (TC) and inorganic carbon (IC) were determined on a Shimadzu TOC-V analyzer (Shimadzu Corp., Kyoto, Japan) with IR detection following thermal oxidation. Total organic carbon (TOC) was calculated as the difference between TC and IC. Total nitrogen (TN) concentrations were measured after alkaline persulfate oxidation (Koroleff, 1983) as  $\text{NO}_3^-$  with a continuous-flow auto-analyzer. Samples were analyzed for DIN ( $(\text{NO}_3^- - \text{N} + \text{NO}_2^- - \text{N})$  and  $\text{NH}_4^+ - \text{N}$ ) on a continuous-flow auto-analyzer (ChemLab System 4).

### *Calculations*

The concentrations of dissolved organic carbon (DOC) and nitrogen (DON) and particulate organic carbon (POC) and nitrogen (PON) in the solutions were calculated as:

$$\text{DOC} = \text{TOC}_{\text{filtered}} \quad [5.1]$$

$$\text{POC} = \text{TOC}_{\text{unfiltered}} - \text{TOC}_{\text{filtered}} \quad [5.2]$$

$$\text{DON} = \text{TN}_{\text{filtered}} - \text{DIN}_{\text{filtered}} \quad [5.3]$$

$$\text{PON} = \text{TN}_{\text{unfiltered}} - \text{TN}_{\text{filtered}} \quad [5.4]$$



The particulate fractions thus consisted of both the precipitates formed by coagulation of DOM molecules and the microbial biomass larger than 0.45  $\mu\text{m}$ . The degradation of dissolved organic carbon (DOC) was quantified as its removal from the solutions. The course of the DOC concentrations followed first order kinetics as was also found by Merckx et al. (2001) and Cleveland et al. (2004). Equation 5.5 was fit to the percentagewise removal of DOC with time:

$$DOC_{\text{degraded}} (\%) = 100 \cdot (1 - e^{-k \cdot t}) \quad [5.5]$$

with  $DOC_{\text{degraded}}$  the percentage of initial DOC concentration degraded after  $t$  days and with  $k$  the degradation rate ( $\text{days}^{-1}$ ). This model was fit to the data points by non-linear curve fitting using the Levenberg-Marquardt algorithm (SPSS 12.0). The half-lives ( $t_{1/2}$ ) (days), representing the time needed to degrade 50 % of the initial DOC concentration, was then calculated as:

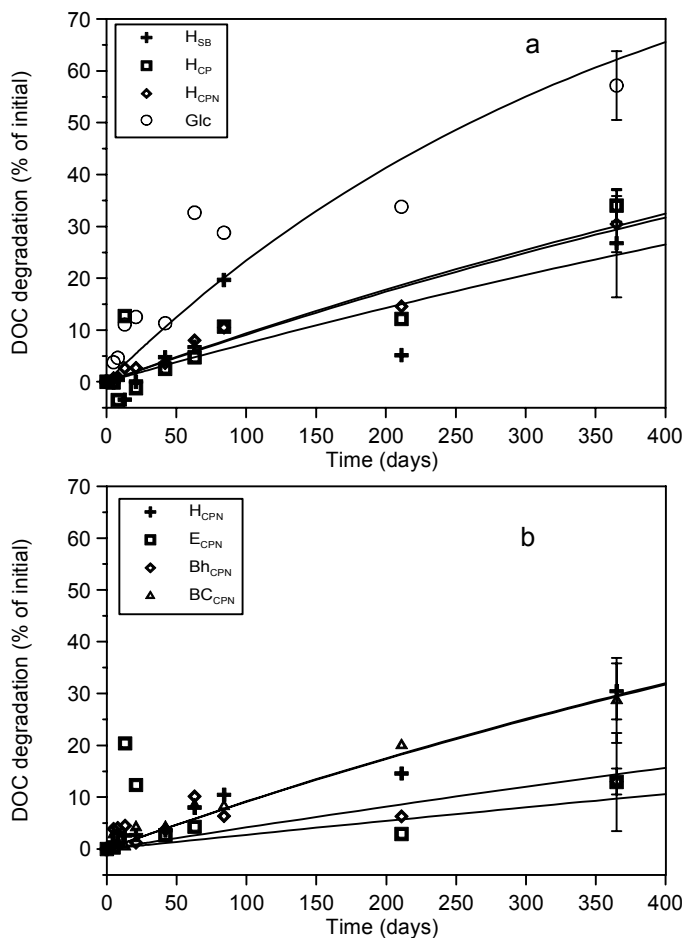
$$t_{1/2} = \frac{0.693}{k} \quad [5.6]$$

## RESULTS AND DISCUSSION

Contrary to the findings of several other DOM biodegradability studies (e.g. Kalbitz et al., 2003), our data did not allow a discrimination between an easily degradable and a more stable dissolved organic matter pool (Figure 5.1).

Since the half-life of the labile DOC pool is typically in the range of 4.8 h – 8 days (Qualls and Haines, 1992; Kalbitz et al., 2003; Schwesig et al., 2003 a,b; Don & Kalbitz, 2005; Qualls & Bridgham, 2005; McDowell et al., 2006), our findings suggest that the DOM sampled in the field is depleted in labile compounds resulting in overall slower decay rates. Also Schwesig et al. (2003b) found that more than 95 % of the DOC in Oa and mineral soil solutions sampled in the field were in the form of slowly degradable compounds with half-lives ranging from years to decades. McDowell et al. (2006) found a much larger amount of DOM in the labile fraction and higher degradation rates in an aqueous litter extract compared to forest floor leachate samples collected in the field using lysimeters. Cleveland et al. (2004) suggested that field DOM collection may underestimate

the initial biodegradability of DOM generated in the litter layer due to rapid decomposition following production. Besides, the substantial DOM degradation during the last 154 days indicates the absence of an extremely recalcitrant residual DOM fraction as is commonly found. The absence of a clear rapidly degradable and of a recalcitrant DOM pool disabled the use of the commonly used double exponential model to describe DOM degradation (Kalbitz et al., 2003). The DOC decomposition in this experiment followed a negative exponential relationship as found in other studies (Merckx et al., 2001; Cleveland et al., 2004).



**Figure 5.1.** Course of the cumulative percentage of DOC degraded during the 365 day incubation for the different origins (a) and different soil depths (b)

The biodegradation rates of natural DOM ( $2.8 - 9.8 \times 10^{-4} \text{ day}^{-1}$ ) found in our study (Table 5.1) are in the range ( $1.4 - 21.0 \times 10^{-4} \text{ day}^{-1}$ ) of the slowly degradable DOM fraction reported by other studies (e.g. Qualls and Haines, 1992; Schwesig et al., 2003b; McDowell et al., 2006). Glucose was mineralized faster and to a larger extent (57 %) than any other substance investigated (Figure 5.1). DOM can be used (i) as an energy substrate, where organic carbon is largely mineralized to  $\text{CO}_2$ , and (ii) it can be used for microbial growth if essential nutrients like N and P are present that are needed for the synthesis of new biomass. While glucose can be taken up directly and is easily metabolized by cells, it has been shown in many studies that the complete mineralization of the C is delayed for periods approaching 1 yr. Qualls (2004) listed three explanations for the persistence of C originating from glucose: (i) a proportion of the glucose C is used to form cellular components that are inherently difficult to decompose after death of the cell; (ii) these components are otherwise stabilized by humification; or (iii) a fraction can remain present in living cells. Qualls (2004) concluded that the apparent incomplete mineralization of glucose is attributable to the transformation of more refractory cellular components. Also Ogawa et al. (2001) found that bacteria produce refractory DOM from labile substrates.

**Table 5.1. Degradation rate ( $k$ ), half-lives ( $t_{1/2}$ ) based on DOC degradation and initial and final DOC/DON ratios of the dissolved organic matter in the forest floor leachate samples from different tree species and at different depths in the mineral soil at the CPN site resulting from the 365-day incubation**

Source	$k$ ( $\times 10^{-4} \text{ days}^{-1}$ )	$t_{1/2}$ (years)	Initial DOC/DON	Final DOC/DON
H <sub>SB</sub>	7.70 (1.69) <sup>†</sup>	2.5	34.2 (0.9) <sup>‡</sup>	29.2 (5.7)
H <sub>CP</sub>	9.80 (1.55)	1.9	36.9 (3.0)	27.0 (4.0)
H <sub>CPN</sub>	9.54 (0.55)	2.0	33.3 (4.7)	27.7 (2.6)
E <sub>CPN</sub>	2.80 (2.35)	6.8	31.2 (2.6)	28.0 (0.0)
Bh <sub>CPN</sub>	4.27 (0.95)	4.4	31.0 (1.9)	28.1 (2.7)
BC <sub>CPN</sub>	9.63 (0.55)	2.0	30.5 (4.3)	29.7 (4.1)
Glucose	26.64 (3.96)	0.7	2741 (215)	23.9 (8.5)

<sup>†</sup> asymptotic standard error;

<sup>‡</sup> standard deviation.

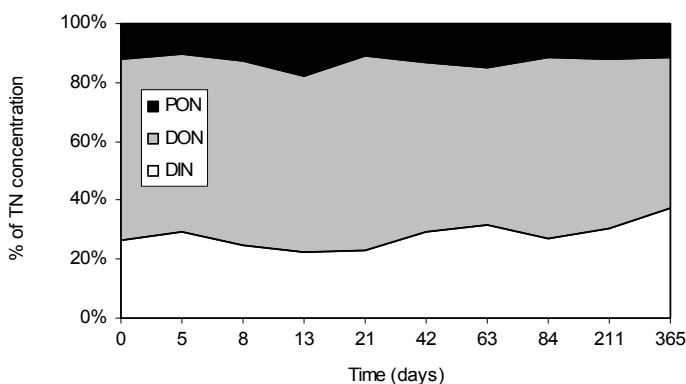
The nature of the batch experimental set-up could lead to some bias in estimates of DOM biodegradability by various interferences compared to field conditions. First, the dialysis pretreatment applied to reduce the DIN concentration in the samples is known to remove substances smaller than the molecular weight cut-off (MWCO) of the membrane. The cellulose-ester membrane used had a MWCO of 100 Da, which implies that small organic compounds (e.g. urea, some amino acids, carbohydrates (Schulten and Schnitzer, 1998)) can permeate through the membrane. Vandenbruwane et al. (2007) have reported that on average 13 % of DOC was lost during buffered dialysis pretreatment. These low molecular weight (LMW) organic acids have been shown to be mineralized very fast (van Hees et al., 2002). Marschner and Kalbitz (2003) also found a three- to four-fold higher biodegradability of the size class  $< 1$  kDa compared to the size class  $< 10$  kDa or in the bulk DOM solution. However, Amon and Benner (1996) found opposite results for aquatic DOM with bacterial DOC utilization 1.4 - 4-fold faster in the high molecular weight (HMW) ( $> 1$  kDa) fractions compared to the LMW ( $< 1$  kDa) fractions. Because of these contradictory results in literature, it is impossible to predict whether the dialysis pretreatment results in an over- or underestimation of DOM biodegradability and degradation rate.

Second, Trulleyová and Rulík (2004) also found that the use of free, suspended bacteria in a batch incubation like in this experiment lead to BDOC values 5-25 % lower compared to the use of an attached bacterial inoculum.

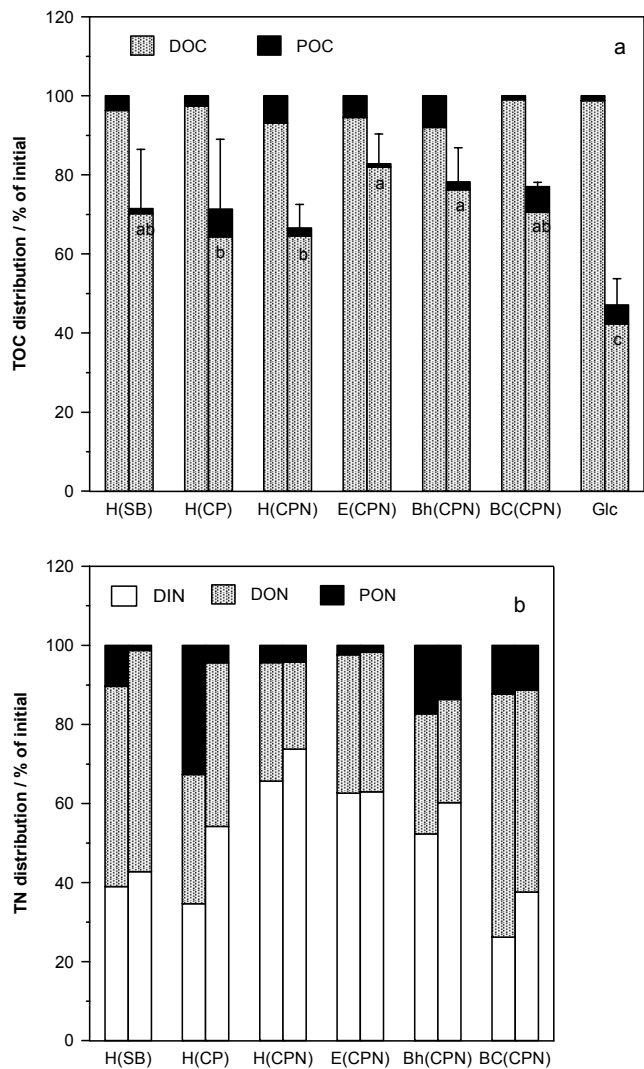
And third, the biodegradation rates determined in laboratory experiments can substantially deviate from these occurring in the mineral soil environment. The presence of the mineral soil medium can have contrasting effects on DOM biodegradability. On the one hand, sorption to mineral surfaces is considered to be one of the most important stabilization processes resulting in reduced biodegradability of DOM (Kaiser and Guggenberger, 2000). However, Qualls and Bridgham (2005) could not find support for this idea from the addition of  $^{14}\text{C}$ -labelled DOM to soils of a weathering chronosequence with various sorption capacities. On the other hand, Guggenberger and Kaiser (2003) hypothesized that natural soil surfaces are covered by biofilms with a high affinity for DOM.

Furthermore, these biofilms show relatively higher microbial densities compared to batch cultures and thus allow more intensive microbial interactions with DOM and its degradation products within the biofilm (Marschner and Kalbitz, 2003).

In contrast to the determination of DOC biodegradability, DON degradation is more complicated. The nature of the batch incubation experiments gives rise to a continuous removal of C from the system, whereas N is continuously (re)-cycled between the DIN, DON and PON pools in the same environment (Figure 5.2). Under dark conditions (in absence of photosynthesis), the dissolved inorganic carbon (DIC) pool is steadily depleted by volatilization of  $\text{CO}_2$  to the atmosphere. Under acidic (absence of considerable  $\text{NH}_3$ -volatilization) and oxic conditions (in absence of considerable denitrification), no inorganic nitrogen (DIN) will leave this closed system resulting in an overall decrease in TC/TN ratio of the incubated solutions. As an inherent result of this continuous cycling of N between the different pools in the closed system, it is impossible to calculate biodegradation rates for DON as is done for DOC. The DON concentration at the end of the experiment was larger than the initial concentration in 4 of the 7 samples (Figure 5.3b).



**Figure 5.2.** Course of the three TN fractions (DIN, DON and PON) in the  $\text{BC}_{\text{CPN}}$  sample during the 365 day incubation



**Figure 5.3.** Initial and final total organic carbon (TOC) (a) and total nitrogen (TN) (b) composition in aqueous forest floor leachate (*H*) samples of the silverbirch (*SB*), corsican pine (*CP*) and corsican pine stand under elevated N deposition (*CPN*), in mineral soil solution at three depths (*E*-, *Bh*- and *BC*-horizons) in the *CPN* site and in a glucose (*Glc*) control sample. (a, b and c indicate significant differences between treatments at  $p < 0.05$ -level).

*Effect of tree species and N load*

During the 365-day incubation experiment, substantial amounts of DOC were removed from the forest floor solutions originating from the different sites;

on average 27, 34 and 31 % of the initial DOC was degraded in the  $H_{SB}$ ,  $H_{CP}$  and  $H_{CPN}$  samples after 1 yr respectively (Figure 5.3a). There was no significant difference in DOC removal from the three forest floor leachate samples. The major part of this DOC removal can be attributed to mineralization, while the contribution of particulate organic carbon (POC) slightly increased only in the  $H_{CP}$  sample. The formation of this POC can be ascribed to assimilation of carbon in the microbial biomass and/or coagulation of organic compounds (Marschner and Kalbitz, 2003). The DOC in the  $H_{SB}$  sample showed a slightly lower decay rate than that in  $H_{CP}$  and  $H_{CPN}$  samples (Figure 5.1 and Table 5.1). Kiikkilä et al. (2005) compared the degradability of DOM extracted from a silver birch forest floor with that of Scots pine and Norway spruce and also found a lower degradation of DOM from the silver birch forest floor. They found that most DOC removal was from the hydrophilic fraction in forest floor extracts for all tree species. But they found a slightly lower abundance of hydrophilic compounds in the extract from silver birch humus. Suominen et al. (2003) characterized the chemical composition of dissolved organic matter in water extracts from humus layers taken from silver birch and Scots pine stands. They found a higher abundance of smaller DOC compounds ( $< 10$  kDa) in the birch forest floor extract compared to that of the pine stand. Amon and Benner (1996) found that the LMW DOC compounds ( $< 1$  kDa) are less bioreactive and more diagenetically altered than the bulk HMW DOC in marine and freshwater samples. Don and Kalbitz (2005) also found a considerably faster degradation rate of DOC extracted from decomposed coniferous litter compared to deciduous litter. There was obviously no influence of N deposition load ( $H_{CP}$  versus  $H_{CPN}$ ) on the biodegradation rate of DOC in forest floor leachate samples (Figure 5.1a). Also Yano et al. (2000) could not find a significant effect of N-treatment on the percentage biodegradable DOC neither in pine nor hardwood stands.

### *Effect of soil depth*

On average, only 13 % of initial DOC was removed from the  $E_{CPN}$  sample, which is significantly less than the 31 % found for the forest floor leachate

( $H_{CPN}$ ) (Figure 5.3), indicating a lower biodegradability. Moreover, the DOC degradation rate was lower by 3.4-fold between the forest floor leachate and the *E* horizon leachate (Table 5.1) as a result of the passage through the upper mineral horizons (*A* and *E*). These findings suggest a relative depletion of easily degradable DOC fractions since these upper mineral horizons have also been shown to contain a large and active microbial biomass (Fritze et al., 2000). Bacteria have been shown to rapidly utilize labile compounds and produce refractory DOM (Ogawa et al., 2001). Also Qualls and Haines (1992) found that DOM originating from the *A* horizon showed the lowest biodegradability with a 13.9 % loss on average after a 134-day incubation in comparison to other ecosystem strata.

The DOM sampled below the *Bh* horizon showed a slightly higher biodegradability (15 %) and degradation rate than that below the *E* horizon (Figure 5.1 and Table 5.1). The biodegradability of DOM further increased to 29 % below the *BC* horizon. The DOC removal rate found in the *BC* leachate approached that of the solution entering the mineral soil ( $H_{CPN}$ ) (Figure 5.1). This is in accordance with the findings of Qualls and Haines (1992) who reported a similar DOC degradation rate and biodegradability in the soil solutions sampled below the *B* horizon and the *Oa* horizon.

Qualls and Haines (1992) attributed the increased biodegradability with mineral soil depth to the selective removal of the more refractory fractions by adsorption. As found by Dai et al. (1996) and Kaiser and Guggenberger (2000), the hydrophobic and to some minor extent the hydrophilic acid fractions are preferentially sorbed to mineral surfaces, especially to Fe and Al oxyhydroxides. Consequently, the hydrophilic (neutrals) fraction becomes more dominant with increasing depth and decreasing DOC concentrations. Furthermore, since hydrophilic neutrals have been shown to be highly degradable (Qualls, 2005), the biodegradability of DOM may increase with increasing soil depth (Marschner and Kalbitz, 2003).



*Relation DOC versus DON*

The DOC/DON ratio in all DOM samples decreased gradually during the 365-day incubation (Table 5.1). These findings indicate that there is apparently no preferential degradation of N-containing compounds, since a preferential hydrolysis of organic N fractions would tend to increase the DOC/DON ratio of the remaining organic substrate (Qualls and Haines, 1992). McGill and Cole (1981) presumed that nitrogen mineralization depended on carbon mineralization instead of the selective hydrolysis of nitrogenous organic compounds. Qualls and Haines (1992) found in general that DON was as refractory as the DOC in forest throughfall, soil solution and stream water.

Generally, two mechanisms can be put forward that may be responsible for the lowered DOC/DON ratio: (i) preferential degradation of organic substances with high DOC/DON ratios and/or: (ii) microbial assimilation or incorporation of inorganic nitrogen with subsequent release as DON. Mechanism (i) is probably not the main process leading to a decreased DOC/DON ratio during incubations. Kalbitz et al. (2003) found that carbohydrates (included in the hydrophilic neutrals fraction (Qualls and Haines, 1992)) are preferentially utilized by microorganisms during degradation of the different DOM compounds. The results of Qualls and Haines (1991) showed that the DOC/DON ratio of this hydrophilic neutrals fraction was not significantly different from that of the bulk DOM solution sampled in different ecosystem strata. Thus, degradation of this fairly labile DOM fraction would not lead to a substantial change in the DOC/DON ratio. In addition, the DOM fraction with the highest DOC/DON ratio (hydrophobic acids and neutrals) was found to be least degradable (Kalbitz et al., 2003). It is thus not very plausible that this first mechanism is the driving force behind the decreased DOC/DON ratio. The second mechanism (ii) assumes microbial immobilization of nitrogen forms followed by the release of bacterial cell components into the solution. This is in accordance with the hypothesis of Aber et al. (1998) that microbial assimilation of DIN and subsequent exudation of amine-rich DON is an important pathway lowering the DOC/DON ratio in soil solution under conditions of N saturation. Microbial biomass C/N ratios typically average around 7.4

(Cleveland and Liptzin, 2007), which is considerably lower than the DOC/DON ratios in most aqueous solutions. Release of bacterial cell components through several biological processes (including direct release, viral lysis and grazing) may result in a decrease in the bulk DOC/DON ratio. Ogawa et al. (2001) showed that marine bacteria produce DON from glucose and ammonium with a bulk DOC/DON ratio of 8.9 after a 365-days incubation. They also found that this microbially produced dissolved organic matter is highly refractory. Our findings are support this last hypothesis. In the glucose control sample, a C/N ratio of 11.3 was found for the particulate organic fraction which mainly consisted of recently formed microbial biomass. Furthermore, we found a significant decrease in the DOC/DON ratio in this glucose control after 1 yr, indicating microbial incorporation of inorganic nitrogen and subsequent release as DON into the solution. The presence of sufficient nutrients (e.g. N and P) in all samples allowed substantial microbial growth and thus incorporation of inorganic nitrogen into biomass. Subsequent release of refractory DOM from the low C/N microbial biomass results in a decreasing DOC/DON ratio and lowering of the bulk DOM biodegradation rate.

## CONCLUSIONS

The bulk DOM pool in the forest floor leachate of the three forest stands was largely depleted in labile compounds. The microbial biomass present in the forest floor probably consumed this fraction very rapidly, resulting in relatively stable DOM released to the mineral soil over a long period of time. No significant effects of tree species or N load could be detected in the biodegradability of DOC in the forest floor leachates. Passage of the soil solution through the upper mineral horizons (*A* and *E*) with a large and active microbial population, leads to a reduction in decay rate and biodegradability of DOC. The biodegradability of DOC in the soil solution, however, increases with depth probably as a result of the preferential sorption of highly refractory hydrophobic acids to Fe and Al oxyhydroxides in the *Bs* horizon.

We found no evidence for preferential degradation of nitrogen-containing organic compounds. Instead, DON was most likely formed by assimilation of DIN in the microbial biomass and subsequent solubilization of these compounds resulting in a decreased DOC/DON ratio after incubation. The nature of the batch incubation experiments makes it very difficult to measure the effective biodegradability of native DON, since N is continuously cycled between the DON, DIN and PON pools. The incorporation of mineral N in DOM through the microbial assimilation and excretion pathway has ecological consequences since the mobility of N may be reduced by the conversion from inorganic to organic N forms. Besides, adsorption of N-containing DOM compounds to mineral soil material may determine N retention in and N leaching from terrestrial ecosystems. This mechanism of microbial DIN incorporation and subsequent DON formation might partly explain the decoupling of DOC and DON dynamics under conditions of N saturation as stated by McDowell (2003).

The observed yearly degradation of DOM in forest floor leachates of 27 - 34 % was consistently higher than at lower depths in the mineral subsoil. Indeed, the mineral soil has been shown to retain from 76 to 84 % of the DOM in the forest floor leachate during percolation. This indicates that other processes (e.g. sorption and/or precipitation) are more important mechanisms controlling DOM leaching.



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## CHAPTER 6

### **SORPTION OF DISSOLVED ORGANIC MATTER (DOM) TO MINERAL SOIL**

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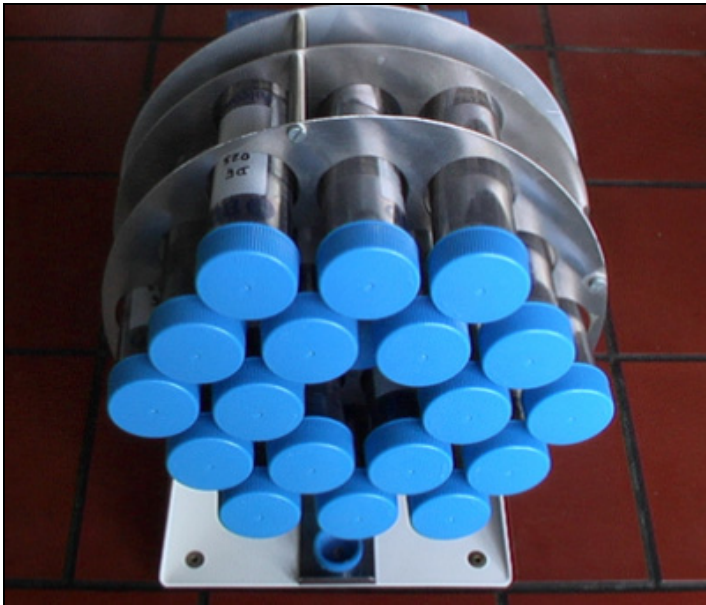


Illustration on p. 127:

Batch adsorption experiment: centrifuge tubes shaking for 24 h on a rotary shaker.

*Part 1 of this chapter has been published in:*

*Vandenbruwane J., De Neve S., Qualls R.G., Sleutel S. and Hofman G. 2007.  
Comparison of different isotherm models for dissolved organic carbon (DOC) and  
nitrogen (DON) sorption to mineral soil. Geoderma 139: 144-153.*

*Part 2 of this chapter has been submitted as:*

*Vandenbruwane J., Sleutel S., Qualls R.G., De Neve S. and Hofman G. Sequential  
sorption experiments for describing dissolved organic matter (DOM) sorption in  
layered soil profiles. Soil Science Society of America Journal (Submitted).*

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## **Part 1: Comparison of different isotherm models for dissolved organic carbon (DOC) and nitrogen (DON) sorption to mineral soil**

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### **ABSTRACT**

The concentration of dissolved organic matter (DOM) in the soil solution is predominantly determined by sorption processes to mineral surfaces. We compared four models describing DOM sorption isotherms to mineral soil material using the results of batch experiments for dissolved organic carbon (DOC) and nitrogen (DON) sorption in different horizons of a podzol. The Initial Mass and modified Langmuir isotherms could be fit to the data points in all cases. The three curvilinear models (Langmuir, Freundlich and exponential model) were found to result in similar goodness-of-fit and parameter estimates. We found that the most widely used, linear model (the Initial Mass isotherm) could be used for horizons with very small sorption capacity and horizons almost saturated with DOM, but failed to describe sorption in the deeper, DOM-unsaturated horizons characterized by a curvilinear shape of the sorption isotherm. The modified Langmuir isotherm was found to describe DOM sorption to mineral soil best and gave realistic parameter estimates, including a measure for maximum sorption capacity of the soil. Comparing the results of the sorption experiments with DOM concentrations in soil solution in the field indicated that the sorption isotherms can serve as a basis for larger DOM leaching models.

## INTRODUCTION

The reactivity and mobility of most chemical elements in soil is determined by the fraction in the dissolved phase, namely its concentration in the soil solution. A number of microbial, chemical and physical processes control the DOM concentration in the soil solution and thus the leaching to ground and surface waters. Sorption onto the mineral soil material has been reported to be the predominant factor in DOM removal from the percolating soil solution (Qualls, 2000; Guggenberger and Kaiser, 2003).

Jardine et al. (1989) elucidated the mechanisms operating during dissolved organic carbon adsorption on mineral soil. A portion of the DOC seemed to be electrostatically bound to the soil via anion exchange, but physical adsorption driven by favourable entropy changes was found to be the predominant mechanism of DOC retention by the soil. Gu et al. (1994) investigated the interactions involved in sorption of natural organic matter on iron oxide. Their results indicated that ligand exchange between carboxyl / hydroxyl functional groups of DOM and iron oxide surfaces was the dominant interaction mechanism, especially under acidic or slightly acidic conditions.

The general method used to obtain sorption characteristics of a certain chemical involves the equilibration of different solute concentrations with the sorbent under investigation and measuring the amount of adsorbed solute. Basically, there are two main methods to generate adsorption isotherms, batch experiments and flow (miscible displacement) techniques. The flow systems seem to more closely approximate field conditions by maintaining narrow solid / solution ratios and by allowing the solid phase to be at rest relative to a mobile fluid phase containing a constant, low concentration of sorbate. Miller et al. (1989) compared batch- and flow-generated anion adsorption isotherms and found that the shapes of the isotherms generated by the two methods were very similar although the flow-generated isotherms were displaced slightly upward relative to the batch experiments. Qualls and Haines (1992) measured DOC adsorption isotherms using continuous, unsaturated flow-through intact soil cores and



compared these results with those of the batch experiments. They found a lower sorption affinity of the soil for DOC in the batch experiments compared to the flow-through technique. Guggenberger and Zech (1992) found higher affinities of the soil for DOC with batch experiments compared to the miscible displacement method. Because the differences between the sorption isotherm parameters obtained by the flow system and batch experiments seem not to be consistent, we used the more simple and rapid batch experiments to produce sorption isotherms for this study.

### THEORY: ADSORPTION ISOTHERM MODELS

Travis and Etnier (1981) reviewed the existing isotherm models describing sorption of reactive solutes to soil in general. Different linear and non-linear models are currently used to describe the adsorption isotherms for natural dissolved organic matter sorption to mineral soil. The most common model based on simple partitioning is called the *initial mass* (IM) isotherm (Equation 6.1) and was first used for DOC adsorption to soil by Nodvin et al. (1986). This model is based on the linear adsorption isotherm (Travis and Etnier, 1981), but additionally accounts for substances initially present within the soil:

$$RE = m \cdot X_i - b \quad [6.1]$$

with  $RE$  the release (negative value) or removal (positive value) of DOM ( $\text{mg kg}^{-1}$  soil),  $m$  the regression coefficient similar to the partition coefficient,  $X_i$  the initial concentration of DOC in solution ( $\text{mg kg}^{-1}$  soil) and  $b$  the intercept ( $\text{mg kg}^{-1}$  soil). The regression coefficient ( $m$ ) can be used to calculate the distribution coefficient ( $K_d$ ) (Nodvin et al., 1986):

$$K_d = \frac{m}{1-m} \cdot \frac{V}{M} \quad [6.2]$$

with  $V$  the volume of solution and  $M$  the mass of soil. The distribution coefficient,  $K_d$ , like the partition coefficient,  $m$ , is a measure for the affinity of the substance under investigation for the soil. The intercept and slope values of the regression

can be used to calculate the reactive soil pool (*RSP*) of DOM (Nodvin et al., 1986):

$$RSP = \frac{b}{1-m} \quad [6.3]$$

Nodvin et al. (1986) defined this *RSP* as “the amount of reactive substance present in the soil with respect to the mass of soil”. Because for adsorption isotherms, the mass of DOM adsorbed per mass of dry soil ( $q_e$ ) is generally plotted against the equilibrium DOM concentration in solution ( $C_e$ ), equation 1 can be written using the above defined parameters  $K_d$  (Equation 6.2) and *RSP* (Equation 6.3) and the general relationship between  $q_e$  and  $C_e$  (Equation 6.4):

$$q_e = (C_i - C_e) \cdot \frac{V}{M} \quad [6.4]$$

with  $q_e$  representing the amount of a substance removed from or released to the solution with respect to the mass of soil ( $\text{mg kg}^{-1}$ ),  $C_i$  the initial DOM concentration ( $\text{mg l}^{-1}$ ),  $C_e$  the equilibrium DOM concentration ( $\text{mg l}^{-1}$ ). This results in the following form of Equation 6.1:

$$q_e = K_d \cdot C_e - RSP \quad [6.5]$$

The major advantage of this linear model is its ease to use (linear regression) and also the ability to fit this model in all circumstances. The major disadvantage is the lack of an adsorption maximum and thus its applicability is limited to lower adsorbate concentrations (Nodvin et al., 1986).

A second model used to describe DOM sorption to mineral soil is the Langmuir equation:

$$q_e = \frac{Q_o \cdot b \cdot C_e}{1 + b \cdot C_e} \quad [6.6]$$

with  $Q_o$  the Langmuir parameter related to maximum adsorption capacity ( $\text{mg kg}^{-1}$ ) and  $b$  the Langmuir parameter related to energy of adsorption (Langmuir, 1918). Harter and Baker (1977) reviewed the applicability of this equation to soil sorption phenomena. Gu et al. (1994) implemented the Langmuir model to describe DOC sorption on iron-oxide and adapted the model to describe desorption reactions. The major advantage of the Langmuir equation is the fact

that it provides an estimate for the adsorption maximum of the adsorbent and a coefficient that is a measure of the adsorbate-adsorbent bonding energy.

Because the original form of this equation does not take into account the presence of native adsorbed organic matter, Lilienfein et al. (2004a) proposed to add a parameter  $a$  to the Langmuir equation:

$$q_e = \frac{Q_0 \cdot b \cdot C_e}{1 + b \cdot C_e} - a \quad [6.7]$$

where  $a$  represents the y intercept which describes the DOM released at low initial DOM concentrations. The principal drawback of the above modification (Equation 6.7) is the fact that it is no longer possible to transform the equation into the linear form as is commonly done to solve the Langmuir equation and that iterative non-linear methods must be used. Kumar and Sivanesan (2005) found, however, that the non-linear methods may be a statistically better way to obtain the adsorption parameters from adsorption data because of the effect of transformation on the distribution of error.

The third model used to describe dissolved organic matter sorption to soil is based on the empirical Freundlich isotherm:

$$q_e = k \cdot C_e^{1/n} \quad [6.8]$$

with  $k$  the Freundlich parameter related to adsorption capacity and  $n$  the Freundlich parameter related to adsorption intensity. The advantage of this model is the ease of solving the equation (the logarithmic form is a linear function), but the lack of an adsorption maximum is its major drawback. As for the Langmuir equation, the original form of the Freundlich equation does not take into account the presence of native adsorbed organic matter. Therefore, we propose to add a parameter  $a$  to the Freundlich equation:

$$q_e = k \cdot C_e^{1/n} - a \quad [6.9]$$

Another possible empirical model that we propose for the adsorption of dissolved organic matter to mineral soil is the asymptotic negative exponential curve:

$$q_e = b \cdot (1 - e^{-k \cdot C_e}) - a \quad [6.10]$$

with  $b$  the parameter related to maximum adsorption capacity ( $\text{mg kg}^{-1}$ ),  $k$  the parameter related to energy of adsorption and  $a$  the parameter ( $\text{mg kg}^{-1}$ ) accounting for the presence of native adsorbed substances ( $\text{mg kg}^{-1}$ ).

In most adsorption studies, the IM model has been used to describe DOM sorption to mineral soil even for curvilinear isotherms. This has resulted from the apparent inability to fit the conventional Freundlich or Langmuir isotherms to data with a negative intercept caused by the presence of indigenous DOM (Nodvin et al., 1986; Vance and David, 1992; Kaiser and Zech, 1997; Ussiri and Johnson, 2004). In models describing DOC transport in terrestrial ecosystems, the simple Initial Mass isotherm has been used to describe DOC sorption (Neff and Asner, 2001; Michalzik et al., 2003).

This comparative study was performed to determine which of the proposed models best describes DOM sorption reactions in mineral soil. The specific objective of this study was to evaluate which of the four models (1) is most suitable to describe DOC and DON sorption to mineral soil and (2) yields relevant parameters with a physical meaning. The structure of this chapter is given in the schematic overview (Figure 6.1).

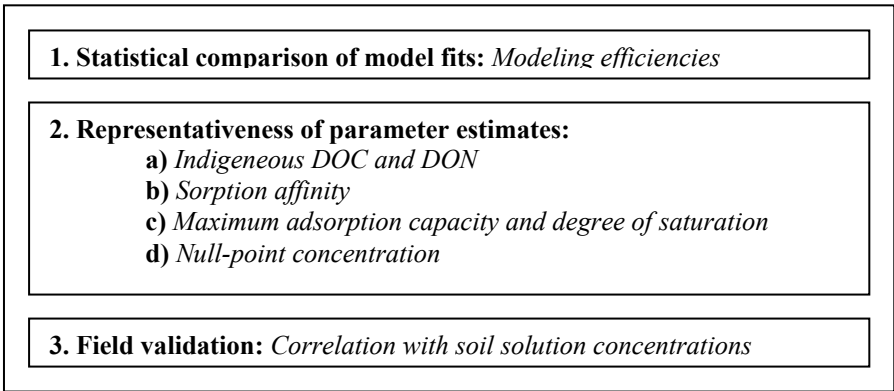


Figure 6.1. Schematic overview of part 1 of Chapter 6

## MATERIALS AND METHODS

### *Laboratory experiments*

To mimic the field situation where the humus percolate enters the mineral soil, an artificial forest floor leachate was used as DOM stock solution for the adsorption experiments (Lilienfein et al., 2004a, b). The entire organic layer (Oi (1 cm) + Oe (3 cm) + Oa (1 cm)) originating from a homogeneous Silver Birch (*Betula pendula* Roth.) stand from the study site, was placed on netting and was percolated every 2 hours with 1 l deionized water till a final solid:solution-ratio of 1/10 was reached. This percolate was filtered through 0.45  $\mu\text{m}$  polyethersulfone (PES) membrane disc filters (Pall Supor®-450, Gelman Laboratory) and was stored at -18 °C until used in the adsorption experiments. The chemical composition of this stock solution is given in Table 6.1. The stock DOM solution was diluted to six DOM concentrations between 0 and 100 mg DOC l<sup>-1</sup> in order to cover the complete range of sorption reactions (from desorption to saturation) (Table 6.2). To exclude a possible influence of different ion concentrations between dilutions, the original stock DOM solution was diluted with a solution having an identical inorganic ion composition and pH as the stock solution (Table 6.1).

Field moist soil samples were taken from the different mineral horizons of the podzol soil. Samples were homogenized, sieved over a 2 mm mesh to remove most fine roots and kept field moist at 4 °C until used in the experiment. Subsamples were air dried for chemical analysis (Table 3.2).

To generate the sorption isotherm data, an amount of field moist soil corresponding with 4 g dry material was weighed in a centrifuge tube in three replicates and 40 ml of the solutions with different concentrations was added (Table 6.2). The centrifuge tubes were shaken for 24 h at room temperature (20 - 22°C) on a rotary shaker (1 rpm) and afterwards centrifuged for 15 min at 3000 rpm (1000 g). The supernatant was filtered through 0.45  $\mu\text{m}$  polyethersulfone (PES) membrane disc filters (Pall Supor®-450, Gelman Laboratory) and the samples were stored at -18 °C until analysis. All aqueous

samples were analyzed for DOC, TDN,  $\text{NO}_3^-$  and  $\text{NH}_4^+$ -content using the methods described in Chapter 3.

**Table 6.1. Chemical composition of the litter leachate used as stock solution for the adsorption experiments (n = 3)**

pH	$4.37 \pm 0.02^\dagger$
EC ( $\mu\text{S cm}^{-1}$ )	$96.67 \pm 1.53$
DOC ( $\text{mg C l}^{-1}$ )	$134.1 \pm 3.2$
DON ( $\text{mg N l}^{-1}$ )	$5.47 \pm 0.08$
$\text{NO}_3^-$ -N ( $\text{mg N l}^{-1}$ )	$1.95 \pm 0.03$
$\text{NH}_4^+$ -N ( $\text{mg N l}^{-1}$ )	$2.89 \pm 0.03$
$\text{K}^+$ ( $\text{mg l}^{-1}$ )	$5.77 \pm 0.06$
$\text{Na}^+$ ( $\text{mg l}^{-1}$ )	$5.07 \pm 0.08$
$\text{Ca}^{2+}$ ( $\text{mg l}^{-1}$ )	$4.80 \pm 0.10$
$\text{Mg}^{2+}$ ( $\text{mg l}^{-1}$ )	$1.28 \pm 0.11$
$\text{Fe}^{3+}$ ( $\text{mg l}^{-1}$ )	$0.19 \pm 0.02$
$\text{Al}^{3+}$ ( $\text{mg l}^{-1}$ )	$0.52 \pm 0.03$
$\text{Cl}^-$ ( $\text{mg l}^{-1}$ )	$2.02 \pm 0.05$
$\text{SO}_4^{2-}$ ( $\text{mg l}^{-1}$ )	$3.72 \pm 0.05$
$\text{PO}_4^{3-}$ ( $\text{mg l}^{-1}$ )	$3.77 \pm 0.06$

<sup>†</sup> Standard deviation

**Table 6.2. The six DOM concentrations used to measure the adsorption isotherms and their anticipated sorption reactions**

	DOC concentration $\text{mg C l}^{-1}$	DON concentration $\text{mg N l}^{-1}$	Expected reaction
1	$0.91 \pm 0.21$	$0.10 \pm 0.06$	DOM-release
2	$2.04 \pm 0.14$	$0.15 \pm 0.07$	Little reaction
3	$4.92 \pm 0.31$	$0.39 \pm 0.35$	Little reaction
4	$22.05 \pm 0.61$	$0.86 \pm 0.09$	DOM-sorption
5	$44.17 \pm 1.20$	$1.55 \pm 0.06$	DOM-sorption
6	$88.93 \pm 3.63$	$3.27 \pm 0.11$	Sorption maximum

<sup>†</sup> Standard deviation

To evaluate the model parameter accounting for the initially adsorbed DOC and DON present in the soil, a sequential extraction procedure (Qualls, 2000) was performed. Therefore, an amount of field moist soil corresponding with 4 g dry material was weighed in a centrifuge tube in triplicate and 50 ml of

deionized water was added. The centrifuge tubes were shaken for 24 h at room temperature (20 – 22°C) on a rotary shaker (1 rpm) and afterwards centrifuged for 15 min at 7000 rpm (5500 g). Forty milliliters of the supernatant was aspirated using a glass pipette and filtered through 0.45 µm polyethersulfone (PES) membrane disc filters (Pall Supor®-450, Gelman Laboratory). The samples were stored at -18 °C until analysis for DOC, TDN, NO<sub>3</sub><sup>-</sup> and NH<sub>4</sub><sup>+</sup>-content as described in Chapter 3. Again 40 ml of deionized water was added to the centrifuge tubes and the procedure described above was repeated 15 times. An exponential curve was fitted to the cumulative amounts of DOC and DON released from the soil as a function of the number of extractions by omitting the first extraction to obtain a better fit (Qualls, 2000).

$$R = C + R_{\max} \cdot (1 - e^{-k(N-1)}) \quad [6.11]$$

With  $R$  the cumulative amount of DOC resp. DON released after  $N$  extractions (mg kg<sup>-1</sup>),  $C$  the amount of DOC/DON released during the first extraction (mg kg<sup>-1</sup>),  $(R_{\max} + C)$  the total amount of DOC resp. DON that can be released from the soil (mg kg<sup>-1</sup>),  $k$  the release coefficient and  $N$  the number of extractions.

### Statistical analysis

The four models were fit to the data points by non-linear curve fitting using the Levenberg-Marquardt algorithm (SPSS 12.0). The model performance was evaluated using following criteria: the relative mean error (ME),

$$ME = 100 \cdot \frac{\left( \frac{1}{n} \cdot \sum_{i=1}^n (P_i - O_i) \right)}{\bar{O}} \quad [6.12]$$

the root mean square error (RMSE) (Bernoux et al., 1998),

$$RMSE = \left( \frac{1}{n} \sum_{i=1}^n (P_i - O_i)^2 \right)^{\frac{1}{2}} \quad [6.13]$$

the coefficient of residual mass (CRM<sub>a</sub>) (Loague and Green, 1991),

$$CRM_a = \left( \sum_{i=1}^n O_i - \sum_{i=1}^n P_i \right) / \sum_{i=1}^n O_i \quad [6.14]$$

and the modeling efficiency (EF) (Loague and Green, 1991),

$$EF = \left( \sum_{i=1}^n (O_i - \bar{O})^2 - \sum_{i=1}^n (P_i - O_i)^2 \right) / \sum_{i=1}^n (O_i - \bar{O})^2 \quad [6.15]$$

where  $P_i$  is the amount of DOC / DON released or adsorbed predicted by the model;  $O_i$  is the average DOC / DON released or adsorbed as found in the experiment,  $n$  is the number of samples and  $\bar{O}$  is the mean of all  $O_i$  values.

### *Field validation*

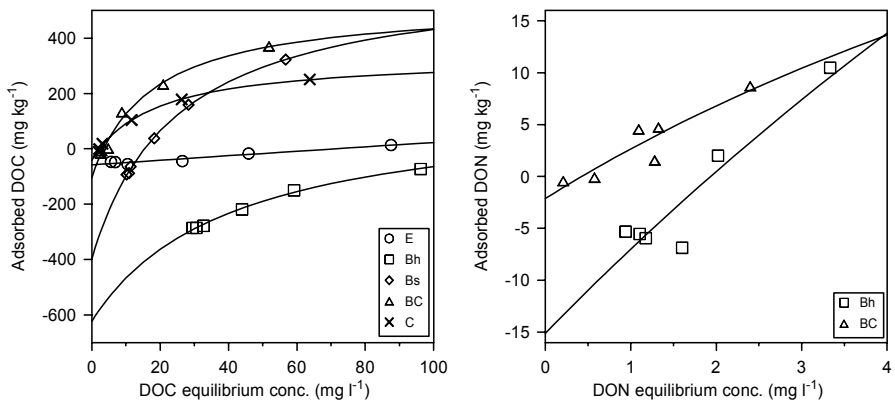
To compare the results of the laboratory sorption experiments with the field soil solution concentrations, acid washed ceramic suction cup lysimeters (Model 1900 Soil Water Sampler (with B02M2 cup), Soilmoisture Equipment Corp., Santa Barbara, USA) were installed at three depths in three replicates at the study site. The three depths were chosen so that the seepage water directly under the E, Bh and BC horizon was collected. Zero-tension lysimeters made of polyvinylchloride (PVC) were placed in nine replicates under the litter layer to collect the seepage water entering the mineral soil. Soil solution samples were collected fortnightly for 1 year and stored at -18 °C until analysis as described in Chapter 3.

## **RESULTS AND DISCUSSION**

The linear and horizontal shape of the sorption curve near the  $X$ -axis for the upper horizon ( $E$ ) implies an almost unaltered DOC concentration during this sorption experiment, meaning that there is very little DOC initially adsorbed and that this horizon has a very low adsorption capacity (Figure 6.2). These findings correspond with the general properties of the albic horizon: a white sandy subsurface horizon without iron oxides and clay. The sorption curve of the Bh-horizon is a curved line that remains below the  $X$ -axis, indicating that DOC is desorbed under all circumstances in this experiment and that this horizon has a high concentration of initially adsorbed DOC. These findings are in agreement with the general characteristics of the spodic  $Bh$  (humus accumulation) horizon. The sorption isotherms of the deeper horizons ( $Bs$ ,  $BC$  and  $C$ ) all show a similar



curvilinear shape. The *Bs*-horizon has a higher concentration of initially adsorbed DOC than the two deeper horizons, and the parent material (*C*) represents a lower adsorption maximum than the above lying *BC*-horizon. Similar results were obtained for distinct horizons of a Typic Haplorthod in New Hampshire, USA (Ussiri and Johnson, 2004) and a Haplic podzol in the Hohe Matzen, Germany (Guggenberger and Kaiser, 2003). The greater scatter of data for the DON sorption experiment were influenced by a low DON concentration and high average standard deviation, which could mainly be attributed to the high DIN/TDN ratio (Lee and Westerhoff, 2005) ranging from 0.47 to 0.98 at a more or less constant DIN concentration of  $4.84 \text{ mg N l}^{-1}$ . Because all four models could only be fit in 2 (*Bh* and *BC*) of the 5 horizons, only these were considered in the further analyses.



**Figure 6.2.** DOC (left) and DON (right) sorption to different horizons of a podzol (Solid lines represent Langmuir model fit)

### 1. Statistical comparison of model fits: Modeling efficiencies

The relative mean error (ME) for DOC was of the same order of magnitude for all four models in the upper horizons (*E* and *Bh*) (Table 6.3).

For the deeper horizons, the linear model (IM model) resulted in a systematic higher mean error than the curvilinear alternatives. The RMSE indicated a similar efficiency of the four models only for the upper horizon (*E*), but better fits of the curvilinear models for the other horizons. For DOC, none of the models showed a systematic bias as indicated by the CRMa values close to

zero (data not shown). The modeling efficiency (EF) yields a measure for the correspondence between the observations and the model predictions, with EF = 1 implying a perfect fit of the model to the data points (Loague and Green, 1991). The modeling efficiencies of all four models were similar for the upper two horizons (*E* and *Bh*), but the curvilinear models had higher EF values in the deeper horizons.

**Table 6.3. Results of the measures for modeling efficiencies of the four models in the different soil horizons**

		Initial Mass		Langmuir		Freundlich		Exponential	
		DOC	DON	DOC	DON	DOC	DON	DOC	DON
E	ME (%) <sup>‡</sup>	3.1		3.3		2.3		3.2	
	RMSE <sup>§</sup>	5.39		5.45		4.96		5.48	
	EF <sup>#</sup>	0.950		0.949		0.958		0.948	
	R <sup>2</sup>	0.95		0.95		0.96		0.95	
Bh	ME (%)	-0.5	18.2	0.5	19.4	0.0	10.5	0.5	19.0
	RMSE	16.02	2.07	5.01	2.14	11.03	1.89	4.46	2.12
	EF	0.994	0.890	0.999	0.883	0.997	0.909	0.999	0.886
	R <sup>2</sup>	0.96	0.89	1.00	0.88	0.98	0.91	1.00	0.89
Bs	ME (%)	9.9		-0.8		1.6		-0.4	
	RMSE	35.06		5.02		10.95		4.57	
	EF	0.958		0.999		0.996		0.999	
	R <sup>2</sup>	0.95		1.00		0.99		1.00	
BC	ME (%)	5.2	-2.3	-0.8	-3.3	-1.3	-1.3	-0.3	-3.1
	RMSE	47.71	1.21	16.74	1.24	18.46	1.21	17.60	1.23
	EF	0.948	0.955	0.994	0.953	0.992	0.955	0.993	0.954
	R <sup>2</sup>	0.89	0.86	0.99	0.85	0.98	0.86	0.99	0.85
C	ME (%)	6.3		1.1		0.5		1.5	
	RMSE	34.95		3.58		3.30		5.37	
	EF	0.951		0.999		1.000		0.999	
	R <sup>2</sup>	0.87		1.00		1.00		1.00	

<sup>‡</sup>ME = relative mean error;

<sup>§</sup>RMSE = root mean square error;

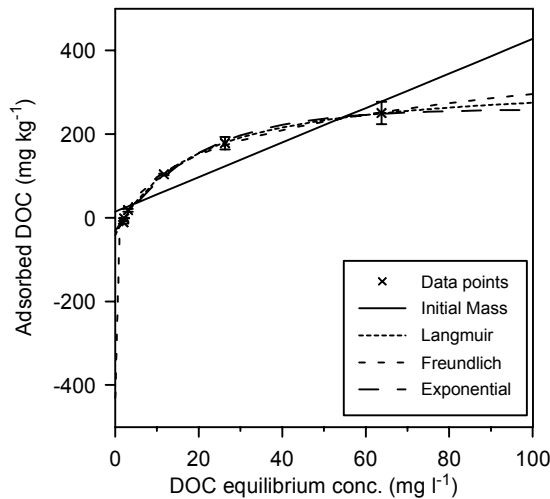
<sup>#</sup>EF = modelling efficiency.

In general there was no marked difference in modeling efficiency between the three curvilinear models for DOC sorption to mineral soil

(Figure 6.3). From Figure 6.3 it is apparent that a careful choice of the initial DOC concentrations is of principal importance to distinguish between the linear and curvilinear shape of the sorption isotherm. Although used in many studies for its simplicity, the use of the Initial Mass isotherm should be limited to mineral soil horizons with high initial DOC concentrations (near saturation) or to sorption isotherms in a narrow DOC concentration range for deeper, unsaturated horizons.

For DON, there were no remarkable differences in modeling efficiency between the models for the two horizons. We conclude that DOM sorption isotherms are generally best described by the modified Langmuir model because this model can be fit in all cases (both DOC and DON) and because this model can describe both linear and curvilinear isotherms.

Since the 5 horizons of the podzol used in this study cover a fairly wide range of soil properties, ranging from low to high sorption capacity (Fe- and Al-oxyhydroxides) and from low to high saturation (C-content), these findings are likely applicable to other soil types.



**Figure 6.3.** DOC sorption isotherm of the C horizon: data points and the four describing models

## 2.Representativeness of parameter estimates

### a) Indigenous DOC and DON

The parameters RSP of the IM-isotherm and the a-term of the curvilinear models are a measure of the initially adsorbed DOC or DON present in the soil (Tables 6.4 and 6.5).

For DOC, this term is of the same order of magnitude for the four models in the E-horizon, but differs substantially in the other horizons. The negative RSP values for the BC and C horizon are physically meaningless, which supports the finding that it is better not to use the IM approach for deeper (unsaturated) soil horizons in a wide DOM concentration range. For DON concentrations, this term is of the same order of magnitude for all models.

**Table 6.4. Sorption isotherm parameters of the four models for DOC sorption to the different horizons of the podzol**

	Initial Mass		Langmuir		Freundlich		Exponential	
E	K <sub>d</sub>	0.81	Q <sub>o</sub>	2600.92	k	0.23	b	807.90
	RSP	57.49	a	57.93	a	53.4	a	57.90
			b	0.32 x 10 <sup>-3</sup>	n	0.79	k	1.04 x 10 <sup>-3</sup>
	DOC <sub>np</sub>	71.33	DOC <sub>np</sub>	71.54	DOC <sub>np</sub>	72.04	DOC <sub>np</sub>	71.40
Bh	K <sub>d</sub>	3.32	Q <sub>o</sub>	783.40	k	33.38	b	545.48
	RSP	377.14	a	623.12	a	516.27	a	547.67
			b	24.82 x 10 <sup>-3</sup>	n	1.75	k	21.44 x 10 <sup>-3</sup>
	DOC <sub>np</sub>	113.49	DOC <sub>np</sub>	156.61	DOC <sub>np</sub>	120.62	DOC <sub>np</sub>	/
Bs	K <sub>d</sub>	8.96	Q <sub>o</sub>	1032.95	k	427.66	b	739.71
	RSP	156.92	a	398.08	a	856.14	a	335.82
			b	40.79 x 10 <sup>-3</sup>	n	3.97	k	38.91 x 10 <sup>-3</sup>
	DOC <sub>np</sub>	17.51	DOC <sub>np</sub>	15.37	DOC <sub>np</sub>	15.69	DOC <sub>np</sub>	15.55
BC	K <sub>d</sub>	7.77 n <sup>†</sup>	Q <sub>o</sub>	630.72	k	492.12	b	476.39
	RSP	-0.05 n	a	104.15	a	606.42	a	83.72
			b	57.88 x 10 <sup>-3</sup>	n	5.73	k	56.55 x 10 <sup>-3</sup>
	DOC <sub>np</sub>	-0.01 n	DOC <sub>np</sub>	3.42	DOC <sub>np</sub>	3.31	DOC <sub>np</sub>	3.42
C	K <sub>d</sub>	4.13 n	Q <sub>o</sub>	374.99	k	384.16	b	291.20
	RSP	-15.01 n	a	41.77	a	431.03	a	31.10
			b	55.04 x 10 <sup>-3</sup>	n	7.21	k	51.16 x 10 <sup>-3</sup>
	DOC <sub>np</sub>	-3.63 n	DOC <sub>np</sub>	2.28	DOC <sub>np</sub>	2.29	DOC <sub>np</sub>	2.21

<sup>†</sup>n = no physical meaning

**Table 6.5. Sorption isotherm parameters of the four models for DON sorption to the different horizons of the podzol**

		Initial Mass		Langmuir		Freundlich		Exponential
Bh	K <sub>d</sub>	7.23	Q <sub>o</sub>	201.54	k	2.09	b	159.35
	RSP	14.13	a	15.11	a	8.34	a	14.76
			b	41.89 x 10 <sup>-3</sup>	n	0.54	k	50.16 x 10 <sup>-3</sup>
	DON <sub>np</sub>	1.95	DON <sub>np</sub>	1.93	DON <sub>np</sub>	2.12	DON <sub>np</sub>	1.94
BC	K <sub>d</sub>	4.31	Q <sub>o</sub>	67.11	k	3.79	b	50.66
	RSP	1.77	a	2.12	a	1.35	a	2.03
			b	76.63 x 10 <sup>-3</sup>	n	0.90	k	95.58 x 10 <sup>-3</sup>
	DON <sub>np</sub>	0.41	DON <sub>np</sub>	0.42	DON <sub>np</sub>	0.40	DON <sub>np</sub>	0.43

The results of the linear regressions between the amount of initially adsorbed DOC and DON measured by the sequential extraction method ( $C + R_{max}$ ) (Equation 6.8 and Table 6.6) and the corresponding values estimated by the four isotherm models (Table 6.4) are given in Table 6.7. The linear regressions are significant ( $p < 0.05$ ) for the values of DOC resulting from the fit by the Initial Mass, Langmuir and the exponential isotherm models. In the case of DON, only the Initial Mass and Langmuir isotherms yielded enough data points to be able to perform the linear regression. The slope was similar for both models, but the regressions were not significant.

**Table 6.6. Parameters resulting from the fit of the exponential function (Equation 6.11) to the 15 data points of the sequential extraction procedure.**

		C* <sup>§</sup>	R <sub>max</sub>	C+R <sub>max</sub> <sup>§</sup>	k <sup>#</sup>	r <sup>2</sup>
DOC	E	65.3 ± 2.9	141.5 ± 3.7	206.8 ± 4.7	0.187 ± 0.014	0.994
	Bh	391.9 ± 16.1	2313.0 ± 44.6	2704.9 ± 47.4	0.118 ± 0.005	0.999
	Bs	82.3 ± 2.9	455.3 ± 10.8	537.6 ± 11.2	0.102 ± 0.005	0.999
	BC	12.1 ± 1.0	210.9 ± 303.7	223.0 ± 303.7	0.012 ± 0.188	0.985
	C	7.7 ± 0.7	173.1 ± 369.7	180.8 ± 369.7	0.009 ± 0.021	0.981
DON	E	7.31 ± 0.36	17.52 ± 8.18	24.83 ± 8.19	0.043 ± 0.028	0.967
	Bh	18.89 ± 1.07	138.89 ± 5.42	157.78 ± 5.52	0.088 ± 0.007	0.998
	Bs	7.65 ± 0.87	117.35 ± 20.39	125.00 ± 20.41	0.043 ± 0.010	0.996
	BC	5.33 ± 0.69	336.15 ± 831.24	341.48 ± 831.24	0.006 ± 0.016	0.989
	C	1.82 ± 0.33	29.40 ± 10.75	31.22 ± 10.76	0.037 ± 0.018	0.987

\*C = the amount of DOC/DON released during the first extraction (mg kg soil<sup>-1</sup>);

§C+R<sub>max</sub> = the total amount of DOC/DON that can be released from the soil (mg kg soil<sup>-1</sup>);

#k = release coefficient.

Although the model estimates for the amount of initially adsorbed DOC and DON are simply extrapolations of the data points ( $y$ -intercept), the above findings indicate that these estimates are correlated with the analytical measures, especially for the Initial Mass, Langmuir and exponential models, and hence have practical use and importance. Despite the correlation between the estimated values of the amount of indigenous DOC and DON and the analytically measured values ( $C + R_{max}$ ), the absolute values differ substantially. This difference is a result of the contrasting methods of determination. The method used to determine the amount of indigenous DOC and DON analytically is based on the sequential extraction till the soil is exhausted. On the other hand, the estimated value of the amount of indigenous DOC and DON is based on the results of one single extraction.

**Table 6.7. Results of the linear regression between measured ( $C+R_{max}$  of sequential extraction) and estimated values ( $y$ -intercepts of the four isotherm models) for the amount of indigenous DOC based on the data points of the 5 horizons.**

	Slope	Intercept	$r^2$	sign. (F)
Initial Mass	$0.141 \pm 0.026$	$6.96 \pm 32.59$	0.906	0.013*
Langmuir	$0.210 \pm 0.061$	$83.46 \pm 75.94$	0.797	0.041*
Freundlich	$0.038 \pm 0.153$	$463.08 \pm 190.52$	0.020	0.818
Exponential	$0.186 \pm 0.051$	$68.13 \pm 63.04$	0.817	0.035*

*b) Sorption affinity*

The distribution coefficient ( $K_d$ ) of the Initial Mass, the Langmuir parameter ( $b$ ) related to the energy of adsorption, the Freundlich parameter ( $n$ ) related to adsorption intensity and the exponent ( $k$ ) of the exponential function all represent a measure for the bonding energy between DOC or DON and the mineral soil material (Table 6.4 and 6.5). A comparison of the absolute values of the different models has little meaning since they are obtained in a different way and have other units, though the estimates for the different horizons can be compared with each other.

In the case of quasi-linear isotherms, the use of the curvilinear models gives rise to non-realistic estimates for the sorption parameters, despite overall

good fit of the models. For example in the *E* horizon data, the maximum adsorption capacity is overestimated and the bonding energy underestimated.

*c) Maximum adsorption capacity and degree of saturation*

Only the Langmuir and exponential isotherms yield an estimate for the maximum adsorption capacity of the soil, namely  $Q_0$  and  $b$  respectively. The DOC adsorption capacities of these sandy soils seemed to be highly correlated with the amounts of oxalate extractable iron and aluminum present in the soil (data not shown). This corresponds with the general knowledge of DOM sorption to soils with low clay content and with iron- and aluminum oxy-hydroxides (Kaiser et al., 1996). The slope of the linear regression provides a measure for the amount of DOC and DON that can be adsorbed per unit of oxalate-extractable iron and aluminum present in the soil: 266 and 176 mmol DOC per mol oxalate-extractable iron plus aluminum for the Langmuir and exponential isotherm model respectively. These results lie in the range of the findings of other studies. Gu et al. (1994) found a maximum DOC sorption onto hematite at pH 4.1 of 22.2 mmol C per mol Fe. Kaiser and Zech (1997) reported a maximum adsorption of 756 mmol C per mol Fe onto goethite at pH 3.95. The DON adsorption capacities of these sandy soils seemed not to be correlated with the amounts of oxalate extractable iron and aluminum present in the soil (data not shown).

As the fit of the Langmuir and exponential isotherm models results in estimates for the amount of initially adsorbed DOC and for the maximum adsorption capacity, the ratio of both values could be used to calculate a measure for the degree of saturation of the potential binding sites. The degree of saturation was 80 and 100 % for the Bh-horizon, 39 and 45 % for the Bs-horizon, 16 and 18 % for the BC-horizon and 11 % for the C-horizon for the Langmuir and exponential models, respectively. The high degree of saturation of the Bh-horizon corresponds with desorption of DOC under all circumstances in this sorption experiment.

*d) Null-point concentration*

The null-point concentration of DOC ( $DOC_{np}$ ) or DON ( $DON_{np}$ ) is the equilibrium DOC or DON concentration at which there is no net removal (adsorption) or release (desorption) of DOC or DON from the solution, i.e. the  $x$ -intercept of the fitted model curves (Mendoza and Barrow, 1987). This means that the equilibrium DOM concentration ( $C_e$ ) equals the initial DOM concentration ( $C_i$ ) at this point. If the initial concentration is lower than the null-point concentration, desorption of DOM will take place; if the initial concentration is higher than the null-point concentration, adsorption will take place:

$$DOC_{np} = C_e = C_i \Leftrightarrow q_e = 0$$

The fit of the four models to the DOC and DON values results in similar null-point concentrations obtained by the different isotherm models for each horizon (Table 6.4 and 6.5). The Initial Mass isotherm yielded realistic results for the upper horizons (*E*, *Bh* and *Bs*), but gave inconsistent outcomes for DOC sorption to deeper soil layers (negative null-point concentrations) as a result of the negative RSP values. The three curvilinear models (Langmuir, Freundlich and the exponential isotherm) yielded almost identical null-point concentrations for the *E*, *Bs*, *BC* and *C* horizons.

*3. Field validation: correlation with soil solution concentrations*

As a final analysis, we tested whether the results of the batch adsorption experiments could be related to soil solution concentrations in the field. The soil solutions sampled in the field are considered to be in equilibrium with the surrounding mineral soil.

To predict the equilibrium DOC and DON concentrations in the soil solution at different depths, the mineral soil is considered to be a succession of different layers (horizons). The annual average DOC and DON concentration of the humus percolate as collected using the zero-tension lysimeters ( $C_{HP, measured}$ ) is the only given boundary condition of this layered system. This DOC and DON concentration was assumed to be the initial concentration ( $C_i$ ) of the *E*-horizon (Equation 6.16), and the calculated equilibrium concentration of this horizon was,



in turn, used as the initial concentration of the underlying horizon. This procedure was repeated for all lower horizons (Equations 6.17-6.20).

$$C_{i,E} = C_{HP,measured} \quad [6.16]$$

$$C_{i,Bh} = C_{e,E} \quad [6.17]$$

$$C_{i,Bs} = C_{e,Bh} \quad [6.18]$$

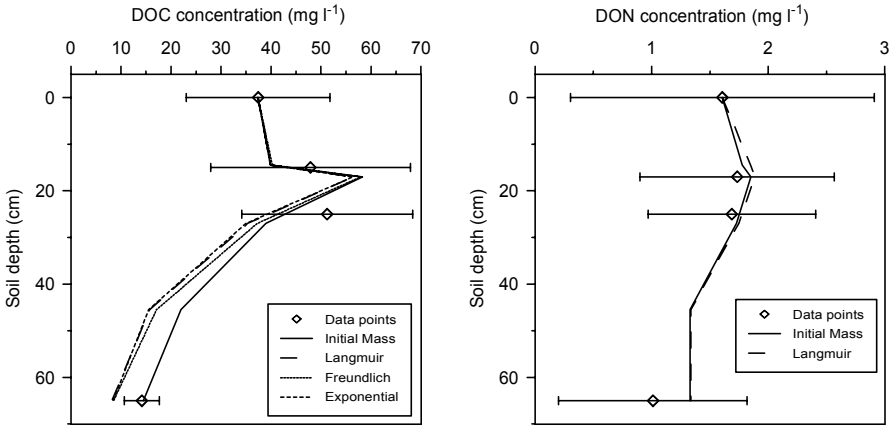
$$C_{i,BC} = C_{e,Bs} \quad [6.19]$$

$$C_{i,C} = C_{e,BC} \quad [6.20]$$

To solve these equations, the equilibrium concentrations ( $C_e$ ) should be expressed as a function of initial concentrations ( $C_i$ ). Therefore, Equation 6.4 was substituted into either Equation 6.5, 6.7, 6.9, or 6.10 and the resulting equation was solved for  $C_e$  for the given  $C_i$  using the calculated adsorption parameters for each model, derived from the adsorption experiments (Table 6.4), for each horizon. The  $V/M$  term (Equation 6.4) was kept the same as in the batch experiments. The method of trial and error was used to solve the equation for  $C_e$  in the case of the non-linear equations.

The predicted soil solution DOC and DON concentrations at different depths were then compared to the observed soil solution concentrations (Figure 6.4). The Initial Mass isotherm seemed to give the best results under these circumstances. However, these findings should be interpreted with caution; the sorption isotherms of all horizons were obtained using an initial DOC solution with identical chemical composition. This is probably not the case under field conditions, given the fact that the chemical composition of the soil solution changes as a result of the sorption reactions. Kaiser and Zech (1997 & 2000) recorded a slight preferential adsorption of the hydrophobic DOC fraction over the hydrophilic fraction. This would imply that the deeper soil horizons adsorb less DOC under field conditions than what is calculated by the sorption isotherms and thus the model applications should overestimate DOC sorption and underestimate the DOC concentrations especially in the deeper soil horizons. This is true for the curvilinear models which show significantly lower concentrations than the soil

solution at 65 cm depth. The DOC concentrations in the soil solution confirm the findings of the batch sorption experiments. They showed that DOC in the soil solution was neither sorbed nor desorbed in the *E*-horizon, that DOC is apparently desorbed in the *Bh*-horizon, and that DOC was adsorbed in the deeper horizons.



**Figure 6.4.** Measured soil solution DOC (left) and DON (right) concentrations and DOC and DON concentrations calculated with the different models for the different depths in a podzol

The Initial Mass and the Langmuir models were the only ones yielding significant fits for DON sorption in all horizons. Both isotherm models give rise to similar DON concentrations in the different horizons and both overestimate the DON concentrations in the soil solution in the different horizons. The DON concentration pattern in the soil profile is similar to that for DOC: little net change in the *E*-horizon, desorption in the *Bh*-horizon and adsorption in the deeper horizons. Nevertheless, as the sorption experiments indicated, the results of the field measurements showed that DON is less susceptible to sorption than DOC. Also Kaiser and Zech (2000) found a smaller overall retention of organic nitrogen by the mineral phases and subsoils than that of organic carbon. They attributed this difference to the fact that the hydrophilic fraction contained more nitrogen than the hydrophobic fraction and that the hydrophilic fraction has a lower sorption affinity to soil.

Despite a number of assumptions and simplifications, the fits of the isotherm models based on the results of batch experiments seem to describe the field DOC and DON concentrations in the different horizons well.

## CONCLUSIONS

Despite its simplicity, the most widely used linear sorption isotherm model (Initial Mass) seemed to successfully describe DOM sorption phenomena in soil horizons with low sorption capacity or cases within a narrow concentration range. Nevertheless, this model can not be used over a wide DOM concentration range where the sorption maximum is likely to be reached. The modified Langmuir isotherm, with the additional parameter accounting for indigenous DOM, proved to be the best model to describe DOM sorption to mineral soil horizons in all cases. The fit of this model gives rise to realistic parameter estimates in most cases and produces a measure for the adsorption maximum of the soil.

On the other hand, simulated DOC and DON concentrations based on sorption isotherms from simple batch-experiments were very similar to concentrations measured in the field indicating that the sorption process is one of the major control mechanisms for DOC and DON concentration in the soil solution. An understanding of the form of the adsorption isotherm models can provide a basis for modeling DOM leaching in mineral soil profiles.



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## **Part 2: Sequential sorption experiments for describing dissolved organic matter (DOM) sorption in layered soil profiles**

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### **ABSTRACT**

The transport of dissolved organic matter (DOM) is responsible for the translocation of pollutants, (heavy) metals and nutrients within and between ecosystems. Since sorption was reported to control DOM concentrations in the soil solution, sorption isotherms are believed to form the basis for DOM transport models in mineral soils. In this study, a sequential batch experiment was developed to describe DOM sorption and transport in layered soil profiles. Despite the more restricted range and the larger scatter of the isotherm data, this method has several advantages. Since the soil solution sequentially runs through the succession of the different horizons, it gives insight in the sorption-driven chemical fractionation of DOM in these profiles. Preferential sorption of both hydrophobic and hydrophilic acids fractions in the horizons rich in iron and aluminum oxyhydroxides was evidenced in this study. The equilibrium concentrations and chemical compositions in the sequential batch adsorption experiments matched well with the DOM concentrations obtained in a field study. We concluded that the results of sequential sorption experiments can be used as a solid base for modeling DOM sorption and transport through layered soil profiles.

## INTRODUCTION

Soil organic matter generally can be viewed as consisting of two operationally defined fractions viz. solid and dissolved organic matter (DOM). The last one is by definition dissolved in the soil solution ( $< 0.45 \mu\text{m}$ ) and as a result of its inherent mobility it contributes to the translocation of organic matter within and from the soil profile. The downward movement of DOM together with its associated elements can result in a variety of (soil) processes ranging from the migration of (heavy) metals and organic pollutants to soil formation and eutrophication of ground and surface waters. A number of microbial, chemical and physical processes determine the DOM concentration in the soil solution and thus the potential leaching to ground and surface water. Physical sorption onto mineral soil surfaces has been found to be the fastest and predominant factor controlling the DOM concentration in percolating soil solution (Qualls, 2000; Guggenberger and Kaiser, 2003).

In the past, different techniques have been used to describe DOM sorption to mineral soil. The most widespread method is conventional *batch adsorption experiments*, in which an amount of soil is equilibrated with an excess of solution at different initial DOM concentrations. Besides its major advantages (speed and simplicity), this method is known to have some serious shortcomings. These include breakdown of soil aggregates and solubilization of otherwise insoluble soil components as a result of vigorous sample agitation and also soil/solution ratios that are much smaller than under natural conditions (Bond and Phillips, 1990). The second technique commonly used to generate sorption isotherms is the *miscible displacement method*. The principle of this technique is that a solution with certain concentration and composition is pumped through an intact or repacked soil column at constant saturated or unsaturated flow. This method has the advantage to closely approach field conditions with respect to soil/solution ratio and soil structure. Different studies have compared these two techniques for the adsorption of a wide range of chemicals (Miller et al., 1989; Bond and Phillips, 1990; Guggenberger and Zech, 1992; Qualls and Haines, 1992;

Johnson and Farmer, 1993) and found fairly similar isotherms resulting from the two methods. This implies that batch sorption experiments are a valuable technique to quantify adsorption in homogeneous soils or individual mineral horizons.

However, natural soil profiles are rarely homogeneous, but frequently show horizontal stratification in the mineral soils. In addition, DOM is a poly-disperse mixture of organic molecules ranging from simple amino acids to complex humic substances with different sorption affinities for mineral soil material. As a consequence of these two heterogeneities, the passage of DOM through mineral soil material results in shifts in chemical composition (Kaiser and Zech, 1997), the so-called *chromatographic effect* (Siemens et al., 2003). Banaitis et al. (2006) investigated sorption-driven fractionation of DOM and found increasing sorption with increasing DOM molecular weight and humification. Kaiser and Zech (1997) indicated that the change in chemical composition is not only due to adsorption, but is a combined effect from both adsorption and desorption as a result of the competitive sorption of the different DOM fractions. Kawahigashi et al. (2006) found the DOM fractionation due to sorption to be dependent not only on DOM composition but also influenced by soil horizon and soil type. Consequently, certain DOM fractions are likely to have a higher sorption affinity for specific horizons in a layered soil profile. These fractionation phenomena obviously may undermine the quality of the DOM transport models in layered soil profiles based on conventional batch experiments (Li and Shuman, 1997). In this conventional technique, the same initial DOM solution is used for all horizons of a layered soil profile resulting in an overestimation of sorption reactions in the mineral soil. In this paper, a new technique is put forward that may account for the shift in both DOM concentration and chemical DOM composition throughout the mineral soil profile. In this technique an initial DOM solution is used for horizon  $n$  that has been equilibrated with all  $n-1$  above-lying horizons. In this way, we might get better insight in the sorption processes taking place throughout an entire mineral soil profile.

The objective of this study was to investigate whether *sequential batch adsorption experiments* yield more realistic isotherms than the conventional batch adsorption experiments for describing dissolved organic carbon (DOC) and nitrogen (DON) transport through soil profiles. The new technique was also analyzed for its ability to account for the shifts in chemical composition of DOM as a result of the preferential sorption of certain DOM fractions. The performance of the sequential batch experiments to predict DOC and DON concentrations and compositions was quantified by comparison with representative field data.

## MATERIALS AND METHODS

### *Soil and experimental solution*

The soils used in this study were identical to those of the previous sorption experiments (Chapter 6, Part 1). The artificial forest floor stock solution was obtained in an identical way as described in Part 1 of this chapter. The chemical composition of this stock solution is given in Table 6.8. This DOM stock solution was diluted to yield six DOM solutions with concentrations between 0 and 62 mg DOC l<sup>-1</sup> in order to cover the complete range of sorption reactions (from desorption to saturation) (Table 6.9). To exclude a possible influence of different ion concentrations between dilutions, the original stock DOM solution was diluted with a solution having an identical inorganic ion composition and pH as the stock solution (Table 6.9).



**Table 6.8. Chemical composition (mean  $\pm$  st.dev.) of the litter leachate used as stock solution for the adsorption experiments (n = 3)**

pH	4.00 $\pm$ 0.01 <sup>†</sup>
EC ( $\mu$ S cm <sup>-1</sup> )	124.85 $\pm$ 2.98
DOC (mg C l <sup>-1</sup> )	61.92 $\pm$ 3.82
DON (mg N l <sup>-1</sup> )	3.63 $\pm$ 0.35
NO <sub>3</sub> <sup>-</sup> -N (mg N l <sup>-1</sup> )	2.80 $\pm$ 0.30
NH <sub>4</sub> <sup>+</sup> -N (mg N l <sup>-1</sup> )	2.90 $\pm$ 0.26
K <sup>+</sup> ( $\mu$ mol l <sup>-1</sup> )	74.87 $\pm$ 1.09
Na <sup>+</sup> ( $\mu$ mol l <sup>-1</sup> )	164.15 $\pm$ 0.75
Ca <sup>2+</sup> ( $\mu$ mol l <sup>-1</sup> )	31.80 $\pm$ 0.12
Mg <sup>2+</sup> ( $\mu$ mol l <sup>-1</sup> )	22.45 $\pm$ 2.38
Fe <sup>3+</sup> ( $\mu$ mol l <sup>-1</sup> )	1.565 $\pm$ 0.27
Al <sup>3+</sup> ( $\mu$ mol l <sup>-1</sup> )	38.95 $\pm$ 3.83
Cl <sup>-</sup> ( $\mu$ mol l <sup>-1</sup> )	349.2 $\pm$ 0.85
SO <sub>4</sub> <sup>2-</sup> ( $\mu$ mol l <sup>-1</sup> )	554.15 $\pm$ 1.77
PO <sub>4</sub> <sup>3-</sup> ( $\mu$ mol l <sup>-1</sup> )	40.80 $\pm$ 5.09

<sup>†</sup> Standard deviation**Table 6.9. The six DOM concentrations (mean  $\pm$  st.dev.) used to measure the adsorption isotherms and their anticipated sorption reactions**

	DOC concentration (mg C l <sup>-1</sup> )	Expected reaction
1	2.04 $\pm$ 1.21	DOM-release
2	6.89 $\pm$ 0.39	Little reaction
3	15.36 $\pm$ 1.02	Little reaction
4	28.33 $\pm$ 3.25	DOM-sorption
5	47.89 $\pm$ 0.17	DOM-sorption
6	61.92 $\pm$ 3.82	Sorption maximum

<sup>†</sup> Standard deviation

## *Experimental set-ups*

### *Standard batch adsorption experiment*

To generate the batch adsorption isotherm data, an amount of field moist soil corresponding with 5 g dry material was weighed in a centrifuge tube in three replicates and 50 ml of the solutions with different concentrations was added (Table 6.9). The centrifuge tubes were shaken for 24 h at room temperature (20 – 22°C) on a rotary shaker (1 rpm) and afterwards centrifuged for 15 min at 3000 rpm (1000 g). The supernatant was filtered through 0.45 µm PES membrane disc filters and the samples were stored at -18 °C until analysis.

### *Sequential batch adsorption experiment*

The main drawback of the above described batch adsorption experiments is that it uses a solution with identical DOM composition for all horizons of an entire soil profile. In order to more closely simulate the real DOM-soil interactions in layered soil profiles, we designed a sequential adsorption experiment in which we first equilibrated a DOM solution with the upper mineral horizons and then used this equilibrated solution as the initial solution for the next horizon. This procedure was continued for all horizons. To do this, an amount of field moist soil (A-horizon) corresponding with 5 g dry material was weighed in centrifuge tubes in eighteen replicates and 50 ml of the initial DOM solution was added. The centrifuge tubes were shaken for 24 h at room temperature (20 – 22°C) on a rotary shaker (1 rpm) and afterwards centrifuged for 15 min at 3000 rpm (1000 g). The supernatant of all samples was filtered through 0.45 µm PES membrane disc filters and three samples were stored at -18 °C until analysis. The remaining 15 aqueous samples were bulked and used as initial solution for the underlying E-horizon. Then, an amount of field moist soil from the E-horizon corresponding with 4.5 g dry material was weighed in centrifuge tubes in fifteen replicates and 45 ml equilibrated solution of the A-horizon was added. This procedure was continued till the lowest (C) horizon with gradually decreasing masses of soil and volumes of

solution since not all the liquid was recovered in each step. The entire sequential adsorption procedure was repeated for a total of 5 initial DOM concentrations (nos 1, 3, 4, 5 and 6 in Table 6.9). Since the chemical fractionation procedure that was applied requires larger solution volumes, the sequential experiment was repeated on a larger scale only with one initial DOM concentration similar to that of the average annual humus percolate concentration found in the field (no. 5 in Table 6.9).

### *Isotherm model*

A modified Langmuir model was previously found to be the best in describing DOM sorption to mineral soil material (Vandenbruwane et al., 2007b), but the higher variability on sorption isotherm data of the sequential batch experiments did not yield realistic parameter estimates for this model and were therefore omitted from further analysis. There may be several reasons for the unsuccessful fit of this isotherm model. First, the low sorption capacity and relatively high degree of saturation of the upper horizons (*A*, *E* and *Bh* horizons) gives rise to quasi-linear sorption isotherms and thus non-realistic estimates for the adapted-Langmuir sorption parameters. Second, due to the sorption reactions in the upper horizons, the initial concentrations for the lower horizons lay within a relatively small range, not approaching the adsorption maximum resulting in quasi-linear relationships in relatively narrow equilibrium concentration ranges. And third, as a consequence of the succession of the different horizons, the chemical composition of the initial solutions is different at different concentrations and for the different subsurface horizons as a result of chemical fractionation during both adsorption and desorption reactions.

For this reason, the generally applicable Initial Mass (IM) isotherm (Equation 6.21) was fit to the data points using linear regression analysis (SPSS 12.0):

$$q_e = K_d \cdot C_e - RSP \quad [6.21]$$

where  $K_d$ , the distribution coefficient, is a measure for the affinity of the substance under investigation for the soil and  $RSP$ , the *reactive soil pool*, is defined as “the

*amount of reactive substance present in the soil with respect to the mass of soil'* (Nodvin et al., 1986).

### *Field validation*

The field validation of the sorption isotherm results obtained in these experiments was similar as the validation in the previous part (Chapter 6, Part 1). Composite solution samples of the litter leachate and C-horizon were collected in March and September 2005 for the chemical fractionation procedure.

### *Chemical analyses*

All aqueous samples were analyzed for DOC, TDN,  $\text{NO}_3^-$  and  $\text{NH}_4^+$ -content as described in Chapter 3.

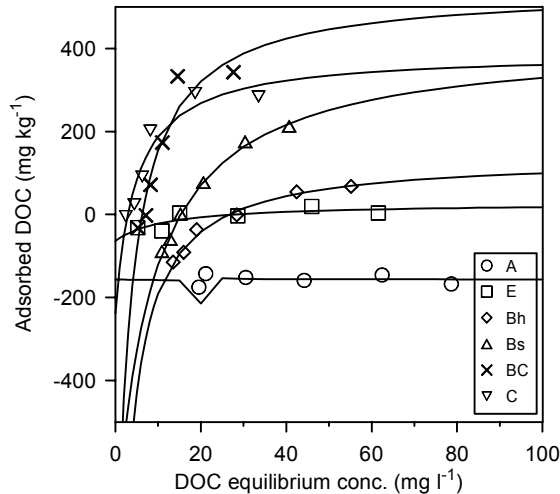
Dissolved organic carbon and nitrogen were fractionated according to their chemical properties by the procedure of Qualls and Haines (1991). This method is based on adsorption of DOM onto 3 different resins resulting in 6 defined fractions: total bases, phenols, hydrophobic acids and neutrals and hydrophilic acids and neutrals (Leenheer, 1981; Qualls and Haines, 1991). We used the nonionic XAD-8 resin (Supelite DAX-8, SUPELCO, Bellefonte, USA), the  $\text{H}^+$ -saturated AG-MP-50 cation-exchange resin (Bio-Rad Laboratories, Inc., Hercules, USA) and the Duolite A-7 anion-exchange resin (Sigma-Aldrich, Inc., St. Louis, USA).

The pH of a selection of aqueous samples was measured potentiometrically (Thermo Orion model 420 A+, Orion Europe, Cambridge, England),  $\text{Na}^+$ ,  $\text{K}^+$  and  $\text{Ca}^{2+}$  concentrations were quantified using a flame photometer (Elex 6361, Eppendorf AG, Hamburg, Germany),  $\text{Mg}^{2+}$ ,  $\text{Fe}^{2+}$  and  $\text{Al}^{3+}$  concentrations were determined using flame atomic absorption spectrophotometry (SpectrAA 220, Varian, Sint-Katelijne-Waver, Belgium) and  $\text{Cl}^-$  and  $\text{SO}_4^{2-}$  were measured with ion chromatography (ICS-90, Dionex Corp., Sunnyvale, USA).

## RESULTS AND DISCUSSION

### *Comparison of batch experiments*

The DOC sorption isotherms resulting from the conventional batch experiments for the six mineral horizons of the podzol are graphically represented in Figure 6.5.



**Figure 6.5.** DOC sorption to different horizons of a podzol as determined by the conventional batch experiments

The linear and horizontal shape of the DOC sorption curve for the *A*-horizon indicates no sorption but constant release of DOC under all circumstances. This implies that the *A*-horizon is even oversaturated with respect to DOM. The quasi-linear shape of the sorption isotherm of the *E*-horizon close to the *x*-axis suggests that DOC passes this horizon without changes in absolute concentrations. The saturation of the available binding sites in both the *A* and *E* horizons could be expected since both horizons have fairly high carbon contents and are relatively poor in iron and aluminum oxyhydroxides (Table 3.2). The sorption isotherm of the *Bh*-horizon shows a curvilinear shape reaching an asymptote only slightly above the *x*-axis which indicates a very high amount of initially adsorbed DOC and a sorption capacity that is almost saturated. These findings are in agreement with the general characteristics of the spodic *Bh* (humus accumulation) horizon.

The three deeper horizons were characterized by a similar curvilinear sorption isotherm. The isotherm of the *Bs*-horizon reflects a relatively high amount of initially adsorbed DOM and a high sorption capacity. The *BC* and *C* horizons have lower contents of initially adsorbed DOM and more available binding sites.

The parameters resulting from the fit of the IM isotherm to the data of the conventional and sequential batch adsorption experiments are listed in Table 6.10. As a consequence of the succession of the different horizons, the chemical composition of the initial solutions is different for the different subsurface horizons as a result of chemical fractionation during both adsorption and desorption reactions and gives rise to lower  $r^2$ -values for the IM isotherm as an inherent consequence of the sequential experimental procedure. On the other hand, due to the sorption reactions in the upper horizons, the initial concentrations for the lower horizons lay within a relatively small range, not approaching the adsorption maximum resulting in quasi-linear relationships in relatively narrow equilibrium concentration ranges. These concentration ranges correspond very well with the concentrations found in the field and are thus of practical interest for modeling DOM sorption and transport.

**Table 6.10. Parameter estimates for the Initial Mass (IM) isotherm model for the six horizons of the podzol resulting from the conventional and sequential batch sorption experiments**

	Conventional batch experiment			Sequential batch experiment		
	$K_d^\dagger$ (l kg <sup>-1</sup> )	RSP <sup>‡</sup> (mg kg <sup>-1</sup> )	$r^2$ -	$K_d$ (l kg <sup>-1</sup> )	RSP (mg kg <sup>-1</sup> )	$r^2$ -
A	0.388	178	0.047	0.388	178	0.047
E	0.752	29.0	0.526	0.031	-54.1	0.000
Bh	4.25	144	0.890	3.55	134	0.376
Bs	10.3	171	0.932	9.74	123	0.382
BC	17.1	63.0	0.734	2.34	-61.3	0.086
C	9.10	-36.8	0.693	2.71	4.54	0.500

<sup>†</sup> distribution coefficient

<sup>‡</sup> reactive soil pool.

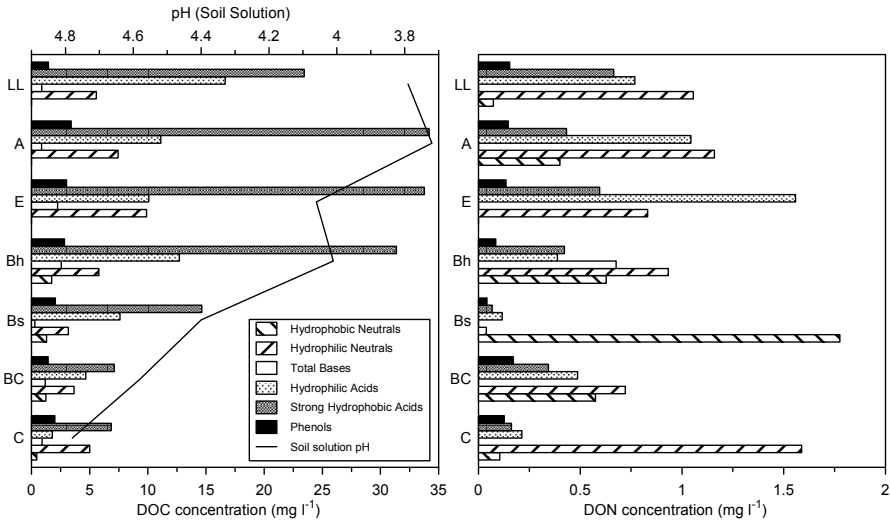
Based on the IM parameter estimates, overall lower DOC sorption affinities were obtained with the sequential experiment compared to the

conventional batch experiment (Table 6.10). The differences were small in the *A*, *E*, *Bh* and *Bs* horizons, but were substantial in the two deepest mineral layers. The highest sorption affinity of DOC was found in the *BC*-horizon in the conventional batch experiment and in the *Bs*-horizon in the sequential experiments. These findings are in agreement with the general assumption that the more sorption-reactive substances are selectively more retained in the upper horizons and thus are not available for sorption in the lower horizons. In this way, the solution is depleted in more reactive substances as it enters deeper in the profile resulting in lower sorption affinities in these deeper horizons (see further). As a result, sorption in the deeper horizons (*BC* and *C*) is overestimated by the isotherms based on the results of the conventional batch experiments. The highest estimates of the RSP, as a measure for the release of initially adsorbed DOC, based on both methods are found in the *A*, *Bh* and *Bs* horizons. This is in accordance with the high total carbon content of these horizons.

### *Sorption-driven fractionation*

The chemical composition of the artificial litter leachate used in the sequential batch experiments indicates an abundance of acidic DOM fractions (Figure 6.6). Hydrophobic and hydrophilic acids comprised 49 % and 35 %, respectively, of total DOC and 24 % and 28 % of total DON. Thus, about 84 % of total DOC and 52 % of total DON belonged to substances with strong acidic properties. The hydrophilic neutrals fraction comprises up 12 % of total DOC and 39 % of total DON, whereas phenols, hydrophobic neutrals and total bases were found only in very low concentrations. These results are in agreement with the findings of Moore and Matos (1999) who determined the chemical composition of DOC in extracts of organic material from different sources. They found the hydrophobic acid fraction to be the largest proportion (ranging from 18 to 56 %) of total DOC and hydrophilic acids the second largest fraction (ranging from 10 to 47 %). Kaiser et al. (1996) found a similar chemical composition of DOC extracted from a mor Oa horizon. Vance and David (1991) found that the sum of organic acids totaled 92 % of the DOC in litter leachate samples of a northern

hardwood forest during a two-year period, with hydrophobic acids being the larger fraction at 60 %. Several studies (Kaiser et al., 1996; Kaiser, 2003; Ussiri and Johnson, 2004) support our findings that nitrogen is more abundant in the hydrophilic fractions, especially the hydrophilic neutral fraction.



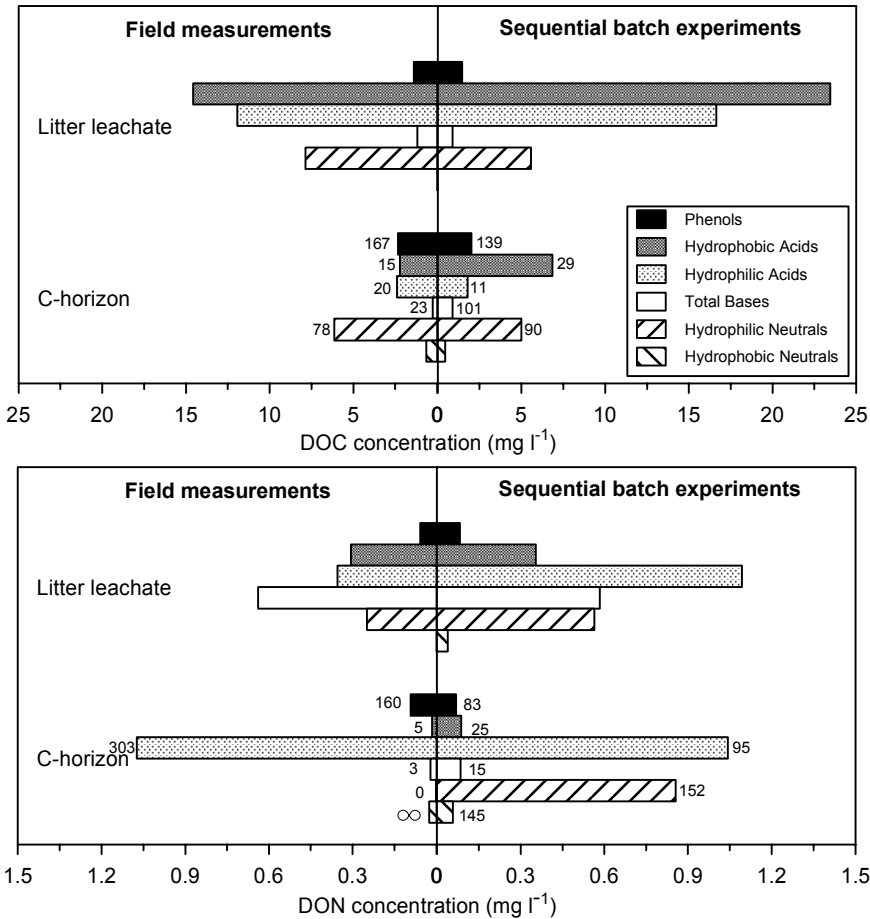
**Figure 6.6.** Course of the DOC and DON composition and pH in the soil solution under the different layers of the podzol as obtained with the sequential batch adsorption experiments (LL = litter leachate).

As the initial DOC concentration of 47.9 mg l<sup>-1</sup> was equilibrated with the A-horizon soil material in the sequential sorption experiment, a net release of DOC in the form of hydrophobic acids, phenols, and hydrophilic neutrals was observed while concentrations of the total base and hydrophobic neutral fractions remained the same (Figure 6.6). But, hydrophilic acids were adsorbed to the mineral soil material of the A-horizon. For DON, hydrophilic acids and hydrophobic and hydrophilic neutrals were released whereas hydrophobic acids were adsorbed in the A-horizon. As reported in numerous studies (Dunnivant et al., 1992; Kaiser and Zech, 1997), competitive sorption of hydrophobic and hydrophilic acids could be observed in this horizon, with hydrophilic acids displacing the indigenous hydrophobic organic substances. The passage of water with this particular DOC concentration and composition through the E-horizon is characterized by slight net release of indigenous DOC (1.8 mg l<sup>-1</sup>), although the discrete fractions behave



differently. The *Bh*-horizon material released on average  $2.1 \text{ mg l}^{-1}$  total DOC during the sequential batch experiment, mainly hydrophobic neutrals and hydrophilic acids. Hydrophilic neutrals and hydrophobic acids, on the contrary, were slightly retained in this horizon. As for the *A*-horizon, we noticed competitive sorption of the different DOC fractions in this horizon. Probably, hydrophilic acids and hydrophobic neutrals were displaced by hydrophobic acids and hydrophilic neutrals. All acidic DON fractions (phenols, hydrophobic and hydrophilic acids) tended to be adsorbed, while bases and both neutral DON fractions were released in the *Bh*-horizon. The *Bs*-horizon characterized by a high content of iron and aluminum oxyhydroxides, adsorbed 50 % of the DOC leaching from the *Bh*-horizon. All chemical DOC fractions were adsorbed, but both the hydrophobic and hydrophilic acid fractions were adsorbed most strongly. This is in agreement with the findings of Kaiser (2003) who reported that the presence of acidic moieties (total acidity and content of carboxylic C) is the major determinant of the extent of DOM sorption to goethite. All DON fractions were strongly adsorbed in the *Bs*-horizon except the hydrophobic neutrals fraction which was released to a relatively large extent. A net adsorption of DOC ( $7.6 \text{ mg l}^{-1}$ ) was found in the *BC*-horizon, with a slight release of hydrophilic neutrals and total bases. The hydrophobic neutrals fraction of DON was adsorbed to the *BC*-horizon material whereas all other fractions were released. However hydrophilic neutrals and phenols were slightly released in the *C*-horizon, there was a net adsorption of DOC of  $4.5 \text{ mg l}^{-1}$ . The *C*-horizon material released DON in the hydrophilic neutrals fraction, but all other DON fractions showed net adsorption.

Comparison of the chemical composition of the litter leachate and the *C*-horizon leachate indicates that the hydrophobic and hydrophilic acid fractions play the major role in the adsorption of both DOC and DON in this podzol profile (Figure 6.7). On average, 71 % and 89 % of the hydrophobic acids and hydrophilic acids present in the litter leachate respectively, were removed during equilibration with the different horizons. This finding differs from several other studies (Vance and David, 1989; Dunnivant et al., 1992; Gu et al., 1994; Kaiser and Zech, 1997; Ussiri et al., 2004) which reported preferential adsorption of hydrophobic DOC



**Figure 6.7. Chemical DOC and DON composition in humuspercolate and C-horizon samples from field experiment and from the sequential batch experiment. Numbers indicate the percentages of each fraction remaining in the C-horizon leachate relative to the amounts in the humuspercolate.**

constituents. Phenols and hydrophobic neutrals were slightly released from the different horizons of this podzol whereas the other fractions (hydrophilic neutrals and total bases) showed minor changes after passage through the entire profile. Qualls and Haines (1991) also found that the contribution of hydrophilic neutrals in total DOM increased dramatically (2.5-fold) and consistently with depth. For DON, phenols, hydrophobic and hydrophilic acids were adsorbed during passage through the podzol profile, whereas hydrophilic and hydrophobic neutrals were released. These findings are an indication that the shift in chemical composition of

DOM as a result of passage through the profile is a result of competitive exchange reactions of DOM subcomponents with the solid phase (Jardine et al., 1989; Dunnivant et al., 1992; Kaiser and Zech, 1997). The higher abundance of DON in the hydrophilic neutrals fraction combined with the lower reactivity of this fraction results in a smaller DOC/DON ratio after passage through this mineral soil profile. This implies that DON shows a higher susceptibility for leaching to the ground water as could be expected from the DOC/DON ratio entering the mineral soil. Lajtha et al. (2005) found that DOC concentrations decreased through the soil profile to a greater degree than did DON. They attributed this difference to the preferential sorption of high C/N hydrophobic DOM in upper horizons over hydrophilic DOM with a much lower and less variable C/N ratio.

#### *Factors controlling removal of DOM in the profile*

Data in Figure 6.6 and Table 6.9 indicate that concentration and removal of both hydrophilic and hydrophobic acids in the soil solution through the soil profile is related to the content of Fe and Al oxyhydroxides and the soil carbon content. It is the general assumption of many studies that ligand exchange on iron and/or aluminum oxyhydroxides is the major adsorption mechanism for DOM sorption in mineral soils (Gu et al., 1994; Qualls, 2000; Münch et al., 2002) and the data in this study are consistent with this mechanism. The nature of this soil with a near absence of clay and calcium carbonate, the presence of iron and aluminum oxyhydroxides, the pH, and the almost exclusive removal of the acidic DOM fractions are also consistent. In this ligand exchange reaction, the hydroxyl group of the carboxylic acid substitutes for the coordinated hydroxyl on the Fe and Al oxyhydroxide surface (Greenland, 1971, Gu et al. 1994).

Although ligand exchange can also be associated with the release of hydroxyl ions (displacement of the hydroxyl group of the oxyhydroxide surface by the carboxylate group) in some studies (e.g. Parfitt et al., 1977), we did not detect any change in soil solution pH, even at high DOC sorption. This is probably due to the fact that negatively charged (dissociated) organic acids are preferentially electrostatically attracted and subsequently adsorbed to the protonated iron and

aluminum oxyhydroxides via ligand exchange, releasing  $\text{H}_2\text{O}$  instead of  $\text{OH}^-$  at the acidic pH of this soil (Gu et al, 1994).

This Spodosol was very acidic and the pH of the soil solution can affect this type of ligand exchange reactions in two different ways. First, the surface charge of the iron and aluminum oxyhydroxides is determined by the soil solution pH. Gu et al. (1994) pointed out that ligand exchange between DOC and iron oxides is the major adsorption process at pH values below the zero point of charge (ZPC) of iron oxide. They found that decreased DOC adsorption with an increase of pH was due to a decrease in adsorption sites on the oxide surfaces. Since the pH of the soil solution in the different horizons in our experiments is well below the ZPC of most iron and aluminum oxyhydroxides (Nagy and Kónya, 1997; Jara et al., 2005), these oxides are likely positively charged. Secondly, the degree of protonation of the acidic functional groups (carboxyl and hydroxyl) is determined by the solution pH. Since the  $pK_a$  of hydrophobic and hydrophilic acids is 4.10 and 3.70 respectively (Dai et al., 1996), the proportion of each fraction that is dissociated can be estimated at the pH of the soil solution. Ussiri and Johnson (2004) hypothesized that an adsorption maximum at pH 4 (compared to pH 3 and pH 5) was due to the lower charge density of the carboxyl groups at pH 3 and a lower charge on the oxyhydroxides at pH 5.

Since the  $pK_a$  of the hydrophilic acids fraction is slightly lower than of hydrophobic acids fraction, a higher proportion of the hydrophilic acids fraction is dissociated at a certain pH than of the hydrophobic acids fraction. The higher hydrophilicity is indicative of a higher charged functional group content and therefore higher reactivity towards metal binding (Jansen et al., 2004). Vance and David (1991) also indicated a greater content of carboxylic functional groups per C in hydrophilic acids compared to hydrophobic acids. These two facts may explain the slightly higher removal of the hydrophilic acids fraction (89 %) compared to the hydrophobic acids fraction (71 %) from the soil solution in the entire soil profile observed in our experiments.

Besides chemical adsorption to solid mineral surfaces, flocculation or precipitation of DOM can be a possible removal pathway in the soil. According to

Dahlgren and Marrett (1991), precipitation of DOM is controlled by the  $\text{DOC}/(\text{Fe}+\text{Al})$  ratio and the pH in the soil solution, with critical ratios of 10 to  $20 \text{ mol C mol}^{-1} (\text{Fe}+\text{Al})$ . The  $\text{DOC}/(\text{Fe}+\text{Al})$  ratio in the soil solution only decreased below the critical value for precipitation of 20 in the *Bs*-horizon. Precipitation is strongly and mutually pH dependent. Since polyvalent metals compete with protons for the available binding sites on the DOM, the metal complexation of DOM molecules decreases with decreasing pH. Besides, a lower pH enhances the solubility of iron and aluminum in aqueous solutions giving rise to lower  $\text{DOC}/(\text{Fe}+\text{Al})$  ratios. Since both adsorption and precipitation are strongly pH dependent, it is very difficult to discriminate between both removal mechanisms. Hayes (1986) reported that even above the critical precipitation ratios, the structural changes caused by metal saturation of fulvic acid can reduce solubility and enhance adsorption by mineral surfaces.

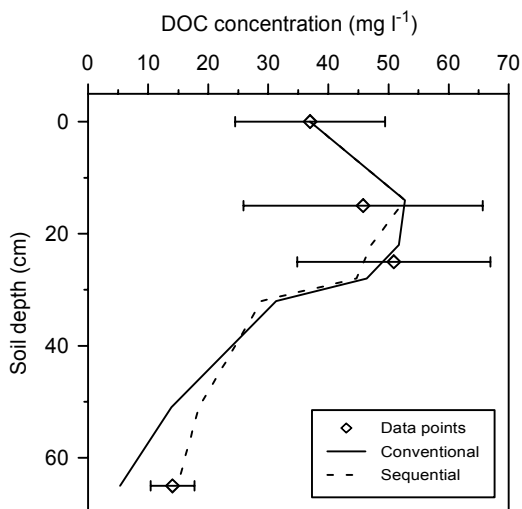
### *Field validation*

Since the major goal of this study was to design a method that better describes the changes in DOM concentration and composition when soil solution passes a layered soil profile, we compared the results of both batch experiments with the average DOM concentrations in the field (Figure 6.8). Given the fairly slow downward movement of the soil solution and the fast rate of the sorption reactions (Dahlgren and Marrett, 1991; Jardine et al., 1992; Kaiser and Zech, 1998), the soil solutions sampled in the field are considered to be in equilibrium with the surrounding mineral soil. The method used to calculate the predicted DOC concentrations as a function of soil depth based on the isotherm data was similar as described in Vandenbruwane et al. (2007b). In this method the mineral soil profile is considered to be a succession of different layers (horizons) each with their specific sorption characteristics (isotherm). The annual average DOC concentration of the litter leachate as collected in the field using zero-tension lysimeters was assumed to be a representative initial concentration of the *A*-horizon. The calculated equilibrium concentration of the horizon with this

“average litter leachate” was used in turn as the initial concentration for the underlying horizon. This procedure was repeated for all horizons.

The results obtained from the sequential batch experiments agreed very well with the field data (Figure 6.8). The predicted concentrations at all soil depths were within the standard deviation of the field measured data. The predicted values obtained using the results of the two batch methods differed considerably in the lower horizons (*BC* and *C*). This difference can be ascribed to an overestimation of sorption using the conventional batch experiments in which the litter leachate is used as initial solution for all horizons. Under these circumstances, the initial chemical composition is identical for all horizons. However, under field conditions, the passage through the above-lying horizons (esp. *Bs*) results in a shift in chemical composition of the DOM, with a reduced abundance of more reactive compounds (hydrophobic and hydrophilic acids) (Figure 6.6). Apparently, the model based on the results of the sequential batch experiments are much more realistic estimates of the true DOC concentration profile in the field. In addition, DOC concentrations in the *C*-horizon in the field over time were relatively consistent as indicated by the small standard error (Figure 6.8). This finding, together with the good similarity between measured and predicted DOC concentrations in the profile, is consistent with a sorption-determined DOC leaching in this soil.

Comparison of the chemical DOC composition of the litter leachate and of the leachate of the *C*-horizon (Figure 6.7), gives an indication of the sorption-driven shift in DOM composition both in the field and during the sequential batch experiment. The chemical composition of the artificial DOM solution (forest floor extract) was very similar to the chemical composition of the humus percolate as sampled in the field, although the artificial solution had a higher concentration of hydrophobic and hydrophilic acids and a lower concentration of hydrophilic neutrals. Therefore it can be concluded that forest floor extracts are representative for litter leachate solutions and thus can be used for the generation of sorption isotherms.



**Figure 6.8.** Field measured and simulated soil solution DOC concentrations as a function of mineral soil depth.

Although many other processes may influence the removal and fractionation of DOM, the chemical compositions of DOC in the C-horizon leachate from both field measurements and the sequential batch experiment were very similar, with some minor differences. The percentages of each fraction that could be found in the C-horizon leachate relative to the humus percolate samples were analogous under both circumstances, except for the small total bases fraction. The sequential batch experiments indicated an unaltered total bases concentration whereas the field measurements showed that 77 % of the total bases were removed during passage through the mineral soil profile. Also the field data indicate more sorption of hydrophobic acids and less sorption of the hydrophilic acids fraction compared to the batch experiment. The field measurements also show more removal of the hydrophilic neutrals fraction in the soil profile than does the sequential experiment. This difference may be attributed to the mineralization of this fraction, since this fraction has been found to be easily biodegradable (Qualls, 2004). The chemical composition of DON in the C-horizon found with the sequential adsorption experiment was also very similar as what was found in the field, except for the hydrophilic neutrals fraction.

## CONCLUSIONS

Despite the more limited range and poorer fit of the sorption isotherm data, a model based on the sequential batch experiments described DOC and DON sorption in a layered soil profile very well. As a consequence of the sequential nature of this technique it accounts for the sorption-driven fractionation of DOM as it passes through the soil profile, while conventional batch experiments do not. The IM parameters resulting from the sequential sorption experiments can thus serve as a solid base for describing the course of DOM concentrations and composition in layered soil profiles.



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## CHAPTER 7

### GENERAL DISCUSSION & CONCLUSIONS

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Illustration on p. 171:

Profile pit at the Tulderheide site displaying the sequence of the different horizons of a podzol.

The main objective of this study was to elucidate the dynamics of dissolved organic matter in forest ecosystems on sandy soils in Flanders. This chapter summarizes the major processes affecting and controlling DOM cycling as found in this work. Furthermore, a general discussion is presented on the interrelationship of these individual processes and their implications for specific environmental problems.

## **MAJOR SOURCES OF DOM**

The results of the field observations enabled the identification of the major DOM sources in forest ecosystems on acid sandy soils in Flanders. Apart from the atmospheric deposition, release from the forest canopy and forest floor were identified as the major sources of DOC and DON.

### *Atmospheric input*

The input of DOC with rainwater was rather small ( $15 \text{ kg DOC ha}^{-1} \text{ yr}^{-1}$ ) compared to the internal DOC fluxes in these forest ecosystems. Avery et al. (2006) found that the composition of DOC in rainwater is influenced by the origin of the rain events (marine versus continental), whereas Kieber et al. (2002) found that DOC concentrations and compositions varied by season. A substantial proportion (4–24 %) of the atmospheric DOC was found to be of fossil fuel origin as the result of incomplete combustion processes (Avery et al., 2006). These authors also showed that volatile organic compounds (VOC's) have relatively short residence times in the atmosphere (in the order of days), resulting in a limited though variable contribution to atmospheric DOC. Kieber et al. (2002) showed that organic acids (e.g. formic and acetic acids) comprised only a relatively small fraction of the DOC pool in rainwater.

Although the atmospheric input of DOC to the forest ecosystems was rather small compared to the internal cycling, precipitation is a significant net source of DON in the forest ecosystems in Flanders ( $6.4\text{--}7.1 \text{ kg DON ha}^{-1} \text{ yr}^{-1}$ ). We found on average 30–31 % of the dissolved nitrogen in rainwater under the form of organic species. Kieber et al. (2005) found that 17 % of rainwater DON

were free amino acids, whereas the major part (> 75 %) could be accounted for by macromolecular uncharacterized humic like substances (Cornell et al., 2003). Neff et al. (2002) suggested that possible sources of atmospheric organic nitrogen (AON) include byproducts of reactions between  $\text{NO}_x$  and hydrocarbons, marine and terrestrial sources of reduced (amino acid) N and the long-range transport of organic matter (dust, pollen etc.) and bacteria. They found that particularly dust and organic nitrates (such as peroxyacetyl nitrates (PAN)) play an important role in the overall flux of AON to the earth surface. Nakamura et al. (2006) indicated that gas to particle conversion may be the major formation process for AON.

Although DON has frequently been omitted from total nitrogen input in natural ecosystems, the substantial contribution of DON to total N deposition has important implications for the setting and assessment of critical loads (CL) for N deposition (Cape et al., 2004).

### *Throughfall*

Large quantities of DOC ( $45\text{--}92 \text{ kg DOC ha}^{-1} \text{ yr}^{-1}$ ) were released in all stands when precipitation water passed through the forest canopy as throughfall. Michalzik et al. (2001) reported that fluxes of DOC with throughfall range from 40 to  $160 \text{ kg DOC ha}^{-1} \text{ yr}^{-1}$  in temperate forests. McDowell and Likens (1988) not only found an increase in DOC concentration, but also a shift in the DOC composition. They observed an increase in the relative contribution of carbohydrates, phenolics and primary amines to DOC and a relative depletion of carboxylic acids and aldehydes. The DOC released from the forest canopy is presumably due to leaf leaching (Tukey, 1970) and leaf washing of dry deposited organic matter.

Contrary to the abundant DOC release, the forest canopy served as a net DON source only in the CPN stand. Ferm and Hultberg (1999) suggested that the DON released from the canopy may originate from the dry-deposited  $\text{NH}_x$  consumed by the canopy. Neirynck et al. (2007) emphasized the importance of the throughfall enrichment by organic N originating from deposited inorganic N in total ecosystem N inputs.

### *Forest floor material*

In accordance to many other studies (e.g. Michalzik et al., 2001), the forest floor was found to be the major source of DOM in these forest ecosystems. The release of 178, 132 and 240 kg DOC ha<sup>-1</sup> yr<sup>-1</sup> from the forest floor material corresponded with 9.5, 3.3 and 8.7 % of the annual C input with above-ground litter at the SB, CP and CPN stands, respectively. The net N release under organic form of 6.43, 3.52 and 9.07 kg DON ha<sup>-1</sup> yr<sup>-1</sup> from the SB, CP and CPN forest floor respectively, amounted to 11.1, 5.2 and 15.2 % of the organic nitrogen input via above-ground litterfall.

Our observations indicated that the resulting release of DOC from the forest floor is controlled not only by its production, but also by its consumption through microbial activity, transport and physico-chemical retention. The net production is thus a result of a complex interaction between abiotic and biotic factors, with the relative importance of each process not yet known. However, our results suggest that the release of dissolved organic matter in the forest floors is largely controlled by its colloidal properties. Factors such as pH (David et al., 1989), ionic strength and ionic composition (Kalbitz et al., 2000) determine the net charge (Dai et al., 1996) and chemical conformation of the DOM molecules and thus its solubility (Marschner and Kalbitz, 2003) and subsequent release (Tipping and Woof, 1990). This physico-chemical release mechanism has previously been identified to control the release of DOM in forest subsoils (Münch et al., 2002) and peat aggregate columns (Stutter et al., 2007).

Although substantial amounts of root-derived DOC were recently found to contribute to DOM leaching at larger depths in the mineral soil (Usselman et al., 2007), roots were not investigated as a potential source of DOM in this study.

## **MAJOR SINKS OF DOM**

The results of the field observations indicated that the mineral soil was responsible for the predominant sink mechanisms for DOC and DON in these forest ecosystems. The processes involved in the DOM removal from the

percolating water are reported to be adsorption to the mineral soil material, microbial degradation (Qualls et al., 2002) and to a minor extent plant uptake (Näsholm et al., 1998). In this study, the contribution of biodegradation and sorption to mineral soil were evaluated extensively.

### *Biodegradation*

To calculate the contribution of biodegradation to total DOM removal from the soil solution, an estimate of the mean residence time of the soil solution is needed, i.e. the time needed for the soil solution to reach the bottom of the mineral soil profile (65 cm depth). The results of bromide break-through curves resulting from unsaturated flow experiments through intact soil columns (data not shown) together with the annual field water fluxes (Chapter 3) enabled the calculation of an average residence time of water entering the mineral soil profiles. These residence times were, on average, 244, 328 and 307 days for the SB, CP and CPN stand respectively. From the results of the DOM biodegradability tests (Chapter 5), 17, 27 and 25 % of the DOC entering the mineral soil with forest floor leachate can be expected to be mineralized in the SB, CP and CPN stand respectively. The field observations (Chapter 3) indicated that 84, 78 and 76 % of the DOC and 81, 67 and 72 % of the DON entering the mineral soil profile with forest floor leachate was retained in the mineral soil. Although the results of the batch biodegradation test should be considered as rough estimates of mineralization (Schwesig et al., 2003b), these results indicate that microbial degradation is probably not the main factor responsible for the large DOC retention in the mineral soil. Since only very minor changes in DON concentrations were found during the biodegradation experiment, we suggest that biodegradation is most probably not the predominant DON removal process in the mineral soil.

Moreover, our field observations showed most DOM removal to occur between the lower boundary of the Bh horizon and that of the BC horizon, indicating most retention in the Bs and BC horizons. Fritze et al. (2000), studying the distribution of microbial biomass in podzol profiles under coniferous forest,

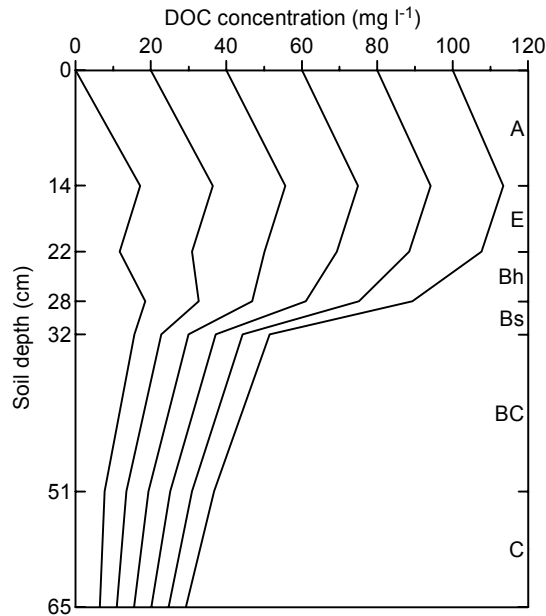
found a much higher activity in the upper A and E horizons than in the B horizons. This finding again indicates that the location of most DOM removal seen in our experiment (Bs and BC horizons), does not coincide with the horizons characterized by the highest microbial activity.

The results of the biodegradation test indicated that the degradability of DOC increased with soil depth. Since microbial interaction is generally known to preferentially consume more labile DOM (Cleveland et al., 2004), leaving behind a more refractory fraction (Kalbitz et al., 2003), biodegradation can not be the process responsible for the increased biodegradability with soil depth.

All these findings point towards the fact that mineralization is not the predominant sink or removal mechanism for DOM in mineral soils of Flemish forest ecosystems.

### *Sorption*

The results of the sorption experiments indicated that sorption to mineral soil is responsible for maintaining low DOM concentrations in water leaching from the profile. Based on the results of the sequential sorption experiments, we can calculate the course of the DOC concentrations as a function of soil depth with varying concentrations entering the mineral soil via forest floor leachate (Figure 7.1). Although the DOM concentration in the forest floor leachate fluctuates substantially as a result of external factors, sorption during passage through the entire mineral soil profile narrows the range of DOM concentrations. Consequently, only small temporal variations are observed in the DOM concentrations leaching from the mineral soil profile as was found during the field measurements. It is obvious that especially the passage through the Bh and Bs horizons, both rich in Fe and Al oxyhydroxides, are largely responsible for the smaller range of DOM leaching concentrations. This is in accordance with Jiménez and Lal (2006) who found that “*a sorption front moves downwards through the soil profile as a pedogenesis process towards a horizon where a large number of sorption sites are available*”. This also corresponds with the location of most DOM removal as observed during field measurements.



**Figure 7.1.** Course of the calculated DOC concentration as a function of soil depth with varying concentrations released from the forest floor based on the sorption isotherm data from the sequential sorption experiments (Chapter 6)

The percentage-wise reduction in DOM fluxes by sorption to mineral soil material can be calculated based on the reduction in absolute DOM concentrations together with the reduction in water fluxes (Dosskey and Bertsch, 1997). These calculations indicate that, in theory 83, 86 and 86 % of DOC and 79, 81 and 86 % of DON entering the mineral soil with forest floor leachate in the SB, CP and CPN stands respectively is retained by sorption. These values correspond very well with the 84, 78 and 76 % DOC and 81, 67 and 72 % DON retention as observed during the field experiments. This finding indicates that sorption is probably the main initial sink for DOM in the podzolic forest soils in Flanders. Sorption as a long-term sink for DOM is extensively discussed further in this chapter.

### *Removal in the deeper subsoil*

Comparison of the DOC and DON concentrations leaving the rooting zone (< 65 cm) with average concentrations in the groundwater, suggests substantial additional removal of DOM (71 - 76 %) in the vadose zone below the



rooting depth. Also Pabich et al. (2001) found considerable attenuation of surface-derived DOC in the unsaturated mineral soil below the rooting zone. Jardine et al. (2006) pointed out the importance of DOC accumulation in lower horizons for carbon sequestration. Siemens et al. (2003) found indications of sorption-induced fractionation of DOM and concluded that DOM retention in the deeper subsoil is most likely due to sorption onto mineral surfaces. Our results of the biodegradability tests indicated that the DOM sampled below the BC horizon was fairly degradable and showed a similar biodegradation rate as the DOM in forest floor leachate. Although we could not discriminate whether sorption or biodegradation is responsible for the substantial DOM removal in the deeper mineral soil of these forest ecosystems, we suggest that sorption is the main removal mechanism since microbial activity at that depth is very limited.

## **EFFECT OF FOREST TYPE ON DOM DYNAMICS**

In this study, the effect of forest type (deciduous silver birch (SB) versus coniferous Corsican pine (CP)) on the dynamics of dissolved organic matter was investigated in neighbouring stands under identical environmental (atmospheric, climatic and soil) conditions. The observed differences in DOM dynamics between both forest types can, however, be attributed to both direct (litter quantity and quality, root exudation) and indirect effects (hydrology, microclimate, interception) related to tree type. Since the number of measurements was both spatially and temporally limited, the observed differences should be interpreted with caution.

The effect of forest type was apparently most pronounced in the amounts of DOM released from the forest canopy and forest floor. There was a larger contribution of canopy release to DOC in the CP stand, whereas the forest floor was a relatively more important source of DOC in the SB stand. The differences in DOM release from both forest floors was assumed to be mainly a result of the differential physico-chemical solubilization. In the above-ground ecosystem strata, the DOC/DON ratio was highest in the coniferous forest compared to the deciduous stand, which is in accordance with the higher C/N ratio of plant derived

organic matter. However, a higher DOC/DON ratio in SB than CP was observed in the upper mineral soil horizons, probably as a result of (microbial) DON formation. Overall DIN fluxes were substantially higher in all ecosystem strata of the CP stand, especially due to the higher interception capacity for atmospheric pollutants of the coniferous forest canopy. This, together with the lower DON fluxes, resulted in considerably lower contributions of DON to TDN in the CP plot compared to the SB plot.

## **EFFECT OF N DEPOSITION AND FOREST EDGE PROXIMITY**

In order to investigate the effect of higher N deposition on DOM dynamics, two coniferous stands subjected to different N loads were selected. To obtain different N inputs under similar atmospheric conditions, we made use of the so-called forest *edge effect* described by Spangenberg and Kölling (2004). However, apart from higher N input, this edge effect also affects the input of other ions ( $\text{SO}_4^{2-}$ ,  $\text{Cl}^-$ ,  $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$ ,  $\text{K}^+$  and  $\text{Na}^+$ ) (Spangenberg and Kölling, 2004) and may affect microclimatic conditions like e.g. light intensity, relative humidity, wind velocity (Davies-Colley et al. 2000). Since these interferences have been reported to influence DOM dynamics (Kalbitz et al., 2000), the observed differences in DOM cycling between both stands should not solely be attributed to the difference in N input, but rather to a general effect of the forest edge proximity. In addition, we only found minor differences in total N input ( $9.4 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ ) via atmospheric deposition between the two stands. Since it is very difficult to discriminate between the effect of the higher N input and of other factors resulting from the edge effect on DOM dynamics, we use the term “*effect of forest edge proximity*” rather than “*N deposition*” in this discussion.

The proximity of the forest edge had the largest impact on the release of both DOC and DON from the forest floor. Results of the release experiment (Chapter 4) indicated that the differences between both forest floors are a result of a combination of several mechanisms. Firstly, the constantly high inorganic nitrogen content of the CPN forest floor material was presumably inhibiting peroxidase activity, which resulted in a suppressed DOM degradation and

accumulation of polyphenolic compounds. The latter probably inhibited proton-producing nitrification with subsequent increased DOM solubilization. Furthermore, the higher input of ions to the CPN forest floor as a result of the forest edge effect gave rise to a further increased solubilization. Also the higher input of N in the CPN stand led to the decoupling of the release of DOC and DON, probably as a result of microbial DON formation (see further).

Since there was an equal percentagewise DOC (78 and 76 % resp.) and DON (67 and 72 % resp.) retention in the mineral soil of the CP and CPN stands, we suggest that the higher DOM leaching at the forest edge is just a result of higher DOM release from the forest floor. This is however in contradiction to Smemo et al. (2006) who attributed the increased DOC leaching as a result of chronic experimental  $\text{NO}_3^-$  deposition (in the form of  $\text{NaNO}_3$ ) to a reduction of the DOC retention in the mineral soil.

The combination of higher DOM input together with an equal percentagewise retention resulted in a higher absolute DOC retention in the mineral soil profile at the forest edge, and thus a higher potential for C sequestration. This is in accordance to the finding that long-term nitrogen additions increase the carbon stocks in forest soils (Hyvönen et al., 2007). Furthermore, although the very long-term effects of nitrogen saturation on biomass production in forests are not yet known, Högberg (2007) reported an initial stimulating effect of N inputs on the growth of the living biomass which is probably much more important than the increased DOM retention. Despite the larger DON fluxes in all ecosystem strata in the stand with high N input (CPN), the relative contribution of DON to TDN was lower compared to CP stand due to the much larger DIN fluxes.

## **RELATIONSHIP BETWEEN DOC AND DON**

Since DOC and DON are both contained in the dissolved organic matter, their concentrations are usually highly correlated within the same ecosystem compartment. However, comparison of the DOC/DON ratio between different ecosystem strata in this study revealed substantial differences. The DOC/DON

ratio gradually increased as precipitation water passed the forest canopy and the forest floor, whereas the ratio decreased with depth in the mineral soil. Both sorption and microbial activity apparently impacted on the course of the DOC/DON ratio in the forest ecosystems and indicated DOC/DON fractionation mechanisms.

### *Influence of DOM sorption*

Although the DOC/DON ratio of the individual DOM fractions did not substantially change during the sequential sorption experiment (Chapter 6, Part 2), we found a continuously decreasing DOC/DON ratio of the bulk DOM solution with mineral soil depth especially between the Bh horizons and the groundwater (i.e. the layers which show the strongest adsorption). Also Kaiser and Zech (2000) found that the sorption and desorption of DOC and DON did not differ within the hydrophilic and hydrophobic fractions individually, indicating that there is no preferential binding of N-containing compounds. On the other hand, they found that the hydrophilic fraction as a whole contained more N and sorbed less than the hydrophobic fraction, resulting in a lower overall retention of DON by the mineral soil than of DOC. Also Ussiri and Johnson (2004) showed that the hydrophilic fraction contained more organic N than the hydrophobic fraction, suggesting that dissolved organic nitrogen (DON) is more mobile than dissolved organic carbon in spodosol mineral horizons. The higher mobility of DON compared to DOC is thus purely a result of the uneven distribution of N over the different DOM fractions, with a higher abundance in the more mobile hydrophilic neutrals and bases fractions.

### *Influence of microbial activity*

The release of DON from the forest floor at the CPN stand was found to be decoupled from the release of DOC (Chapter 3). Although there was no difference in the C/N ratio of the solid phase forest floor material, the DOC/DON in the forest floor leachate was slightly lower in the CPN stand compared to the CP stand. Several other studies (McDowell et al., 1998; Michalzik and Matzner,

1999; Michel and Matzner, 1999; Solinger et al., 2001) have found different release rates for DOC and DON pointing towards different mechanisms involved in the release and/or consumption of both components. Although these studies reported equal or higher DOC/DON ratios compared to the solid phase C/N ratio indicating a preferential release of DOC, we found DOC/DON ratios substantially lower than the solid phases C/N, pointing towards a stoichiometric N enrichment of DOM in all stands. Brookshire et al. (2007) also found decreasing DOC/DON ratios in water draining from forest ecosystems with increasing N deposition. Their results suggested activation of alternative pathways of DON production or direct N enrichment of DOM without prior enrichment of source pools.

Aber et al. (1998) suggested two alternative explanations for the N enrichment of DOM due to chronic nitrogen additions: (i) (abiotic) chemical reaction between inorganic nitrogen and dissolved organic matter (Davidson et al., 2003) and (ii) microbial assimilation of DIN by micro-organisms (mycorrhizae) followed by re-exudation into the soil solution as N-rich organic compounds. The results of the  $\text{HgCl}_2$  sterilization of forest floor material (Chapter 4) indicated that a microbial mechanism is responsible for the differences in release of DOC and DON. Furthermore, the results of the biodegradation experiment (Chapter 5) indicated formation of dissolved organic nitrogen from carbohydrates (glucose) and ammonium in absence of a soil matrix which was in accordance to Ogawa et al. (2001). Since nitrate was present only in very low concentrations throughout the experiment, no iron or manganese was present as catalyst and since nitrate, instead of ammonium, is thought to be used for the abiotic DON formation via nitration and nitrosation (Davidson et al., 2003), we suggest that DON was microbially produced from the incorporation in microbial biomass followed by release by or at cell death.

## **IS SORPTION A LONG-TERM SINK FOR DOM?**

Although sorption is a rapid process (Dahlgren and Marrett, 1991; Kaiser and Zech, 1998) maintaining low DOM concentrations (McDowell and Likens, 1988; Kalbitz et al., 2000) and retaining large quantities of DOM in the mineral

soil (Kaiser and Guggenberger, 2000), it is questionable if sorption is a long-term and irreversible sink for DOM. The sorption process is governed by electrostatic and chemisorptive (ligand exchange) interactions between acidic functional groups, especially carboxyl groups, and hydroxyl groups at the mineral surfaces (Gu et al., 1994; Kaiser and Zech, 2000). Gu et al. (1994) found that adsorbed DOM is very difficult to be desorbed from iron oxide at a given pH and ionic strength, indicating the presence of a so-called *hysteresis effect* in DOM sorption (Kaiser and Guggenberger, 2000).

#### *Calculated residence time versus time until saturation*

Neglecting other carbon inputs to the organic matter pool in the mineral soil, such as root litter (Buurman and Jongmans, 2005), microbial metabolites or the transfer of OM as a result of bioturbation, a simple relation of the annual DOC retention ( $\text{kg C ha}^{-1} \text{ yr}^{-1}$ ) in the mineral soil horizons to the organic carbon (OC) pool stored in the soil ( $\text{kg C ha}^{-1}$ ) provides a rough estimate for the residence time of the indigenous OC in the range of 831 - 1380 years (Table 7.1). Based on similar carbon balance calculations, Guggenberger and Kaiser (2003) found residence times of indigenous mineral-associated OC in the range of 186 - 1730 years. Also Buurman and Jongmans (2005) found  $^{14}\text{C}$  ages of organic carbon in podzol B horizons in the Netherlands and Belgium in the range of 1170-3960 years. Although the OC input in the mineral soil is underestimated in these calculations, it can be concluded that these podzolic soils store and stabilize a large amount of the incoming OC very efficiently.

The residence time can also be estimated in an alternative way. Since the sorption capacity of the different mineral horizons is limited, the annual retention of large quantities of DOC by sorption would lead to the saturation of this sorption complex within approximately 18 - 26 years (Table 7.1). These values are very similar to those reported by Guggenberger and Kaiser (2003) for a range of soils in Germany (4 - 30 years). However, no indications were found for the saturation of the sorption capacity esp. in the deeper horizons (Bs, BC and C) in our study. If we consider that these forest soils are close to a steady-state equilibrium with

respect to carbon sorption and mineralization, the mean residence time of sorbed DOC would approach the calculated time for the sorption complex to become saturated (18-26 years). Since sorption is an equilibrium reaction between the DOM in solution and the sorbed DOM, the available sorption capacity will never be completely saturated. This indicates turnover time estimated according to this second approach would even be an overestimation.

There is obviously a large discrepancy between the estimated residence times obtained in the two distinct ways.

**Table 7.1. Input-output budget for organic carbon in the mineral soil at the three forest stands**

	SB	CP	CPN
Input flux (kg DOC ha <sup>-1</sup> yr <sup>-1</sup> ) <sup>(a)</sup>	238	236	348
Output flux (kg DOC ha <sup>-1</sup> yr <sup>-1</sup> ) <sup>(b)</sup>	37	51	83
Retention (kg DOC ha <sup>-1</sup> yr <sup>-1</sup> ) <sup>(c = a - b)</sup>	201	185	265
Degradation (kg DOC ha <sup>-1</sup> yr <sup>-1</sup> ) <sup>(d)</sup>	40	64	87
Adsorption (kg DOC ha <sup>-1</sup> yr <sup>-1</sup> ) <sup>(e = c - d)</sup>	161	121	178
Adsorption capacity (kg DOC ha <sup>-1</sup> ) <sup>(f)</sup>	3157	3157	3157
Years until saturation <sup>(g = f / e)</sup>	20	26	18
Total C-stock (< 90 cm) (tons C ha <sup>-1</sup> ) <sup>(h)</sup>	134	167	148
Average residence time (yr) <sup>(i = h / e)</sup>	832	1380	831

<sup>(a)</sup> flux entering the mineral soil with forest floor leachate;

<sup>(b)</sup> flux leaving the mineral soil profile at 65 cm depth with soil solution;

<sup>(d)</sup> theoretical amount of DOM degraded calculated based on results from biodegradation tests and bromide-measurements indicating a travelling/residence time of 244, 328 and 307 days and 17, 27 and 25 % resp. degradation of the incoming DOC flux;

<sup>(e)</sup> calculated as the the difference between retention minus degradation assuming no other sinks for DOM;

<sup>(f)</sup> calculated based on the measurements of the maximum adsorption capacity in the different horizons.

### *Possible explanations*

There are two possible explanations for the large discrepancies in turnover times based on budget calculations from field studies and laboratory experiments: (i) substantial amounts of adsorbed DOM are degraded in the field and/or (ii) the ‘sorption’ capacity of the soil is largely underestimated with the batch sorption experiments because another physical mechanism apart from sorption is involved in the DOM retention and stabilization.

(i) Several studies have suggested that sorption may be responsible for the fast initial retention of DOM in the soil and that subsequent microbial degradation of this adsorbed DOM again clears the available sorption sites. McCracken et al. (2002) did only retrieve about 50 % of the retained DOC as soil OC after a 1 year experiment and concluded that microbial decomposition must be a significant factor regulating sorbed OC concentrations in soils. Guggenberger and Kaiser (2003) hypothesized that natural soil surfaces are covered by biofilms with a high affinity for DOM. They assumed that biofilms compete with inorganic surfaces for OM sorption and are probably an effective sink for DOM in the mineral soils. Furthermore, these biofilms show relatively high microbial densities and thus allow more intensive microbial interactions with DOM and its degradation products within the biofilm (Marschner and Kalbitz, 2003; Siemens et al., 2003). Also Qualls and Bridgham (2005) could not find support for the idea that increased adsorption capacity due to weathering resulted in protection of soluble organic carbon from microbial mineralization.

(ii) A second possible explanation is that the total sorption capacity of the mineral soil horizons is underestimated because another physical mechanism is involved in DOM retention. Contrary to the results of batch adsorption experiments, Zysset and Berggren (2001) did not find a maximum sorption capacity for DOC for podzol Bs horizons with a column experiment under unsaturated flow conditions, pointing towards the underestimation of sorption capacity based on batch experiments. Although the specific surface area (SSA) of mineral soil material is an important soil property determining DOM sorption (Kaiser et al., 1996), Kaiser and Guggenberger (2000) did not find a significant relationship between sorbed OC concentration and surface area (SA) of the mineral soil matrix of topsoils and illuvial subsoil horizons pointing towards another retention mechanism.

Since the retention of DOM in mineral soil generally attributed to physical sorption is a combined result of precipitation of metal-DOM-complexes and the effective sorption to mineral soil constituents, it is very difficult to determine the relative contribution of both processes to the total retention. Scheel



et al. (2007) found that between 13 and 84 % of the DOC in forest floor extracts may be precipitated, depending on pH, C/Al ratio and the type of DOM. Since especially the C/metal ratio determines the precipitation of DOM (Dahlgren and Marrett, 1991; Nierop et al., 2002) and the pH determines the solubility of Fe and Al, precipitation could be an important DOM sink in acidic sandy soils. Dahlgren and Marrett (1991) suggested that the main mechanism of DOC immobilization involved initial solubilization of metals, which lowered C/metal ratios in solution, followed by precipitation and/or adsorption of the organo-metal complexes. Also Jansen et al. (2005) found that the solubility of organic matter in podzol B horizons is mainly controlled by precipitation as organo-metal complexes and/or by adsorption on freshly precipitated solid Al- and Fe-phases.

The lower soil/solution ratio (generally 1/10) at which batch experiments are performed compared to field conditions, results in low metal concentrations in the solution due to dilution, which prevent DOC/metal ratios in the solution to drop below the critical value of 10 to 20 needed for precipitation (Dahlgren and Marrett, 1991). As a result, batch experiments will probably underestimate the DOM retention in acidic mineral soils. Since these metal-organic complexes were found to be much more stable (up to 28 times) than the respective DOM solutions (Schwesig et al., 2003a; Scheel et al., 2007), the mechanism of precipitation might explain the accumulation of large quantities of stable organic matter in the mineral soil without the complete saturation of the sorption complex.

### *Implications for C sequestration*

In times of growing concern about global climate change, increasing attention is paid to the role of different ecosystems as source or sink for atmospheric carbon dioxide (CO<sub>2</sub>). Apart from the storage of carbon in the living biomass, the mineral soil has been characterized as an important sink for organic carbon in forest ecosystems. Vande Walle et al. (2005) found an overall mean C-stock of 101 ton C ha<sup>-1</sup> in the living biomass of Belgian forests whereas soil organic matter generally makes up 41.5 – 47.5 % of the total C stocks in Belgian forest ecosystems (Janssens et al., 1999; Vande Walle et al., 2001). Furthermore,

Buurman and Jongmans (2005) found  $^{14}\text{C}$  ages of organic carbon in podzol B horizons in the Netherlands and Belgium in the range of 1170-3960 years, indicating the very long-term C storage potential of these mineral soils. The potential of DOM for C sequestration in mineral soils depends on (i) the total C input with DOM; (ii) the efficiency of the retention mechanism(s); and (iii) the efficiency of the organic carbon stabilization. Further research is needed to elucidate the exact mechanisms and processes involved in the carbon stabilization in these sandy soils.

## DOM AND PODZOLISATION

Podzolisation is the pedogenic process responsible for the formation of podzols. Podzols are characterized by a bleached eluvial E horizon from which organic matter, Al and Fe are leached, overlying one or more illuvial B horizons in which organic matter, Al and in most cases Fe are deposited (FAO-UNESCO, 1990). Podzols and podzolisation have been the subject of research for many decades, but there is no consensus about the exact mechanisms governing the mobility of Al, Fe and organic matter in them (Jansen et al., 2005). Several podzolization theories currently exist (Buurman and Jongmans (2005):

- Mobilization of unsaturated metal-organic complexes in the forest floor and the A and E horizons, followed by precipitation in the B horizon upon saturation of organic molecules through metal complexation: the *fulvate theory* (e.g. McKeague et al., 1978).
- Transport of sols of (proto-)imogolite/allophane, precipitation of these sols in the B horizon and subsequent adsorption of mobile humus: the *proto-imogolite theory* (e.g. Anderson et al., 1982).
- Two stages of profile development which occur sequentially or simultaneously: (a) in situ formation of imogolite/allophane in the Bs horizon by a carbonic acid weathering process, and (b) precipitation of fulvic acid on the Al-rich precipitates in the Bs horizon. This is known as the *fulvate-bicarbonate theory* (Ugolini and Dahlgren, 1987).

Although the role of DOM in the translocation of Al and Fe from the E horizon is not clear (Jansen et al., 2004), the retention, by adsorption and/or precipitation, of large quantities of DOM with Fe and Al as found in this study aids the formation of the typical humus accumulation (Bh) horizon (Jansen et al., 2005). Buurman and Jongmans (2005) found that in podzols developed on nutrient-poor parent materials characterized by slow organic matter dynamics as found in the podzols in this study, there is a larger abundance of DOC-derived, monomorphous organic matter coatings in the B horizons and a lower contribution of root-derived, polymorphous organic matter. These findings point out that the DOM dynamics found in the forest ecosystem in Flanders contribute substantially to podzolisation of these poor sandy soil.

## **ORGANIC ACIDS AND THE INORGANIC ANION DEFICIT**

Charge properties of DOC in soil solutions and natural waters provide important information on organic acidity. Vance and David (1991) found that the organic acids account for 97 % of the charge balance deficit in forest floor leachates. Consequently, the abundance of organic anions can be estimated by the anion deficit (Lundström, 1993). The forest floor leachates under the three forest floors had significant amounts of organic anions, ranging between 17 and 27 % of the total sum of negative charge. The annual anion deficit in the forest floor leachates of the three forest stands showed good correlation with the total amount of DOC. The ratio of the anion deficit (as a measure for total organic anions) to the amount of DOC provides an estimate of the net negative charge of the DOM molecules; namely 48, 42 and 75 mmol (-) mol<sup>-1</sup> C in the forest floor leachates of the SB, CP and CPN stands respectively. Since the major part of the organic anions are of carboxylic acids origin (Vance and David, 1991), the anionic density on the DOM molecules is determined by the degree of dissociation, the strength of the organic acids and the ratio of carboxylic carbon atoms over total C atoms in the DOM molecules (Dijkstra et al., 2001). Using a dissociation constant (*pK<sub>a</sub>*) of 4.23 (Vance and David, 1991) together with the average proton concentration in the forest floor leachate, the percentage dissociation of the organic acids was

estimated to be 35, 19 and 48 % in the SB, CP and CPN forest floor leachates respectively. Thus, the proton concentration largely determines the contribution of organic acids to the anion deficit. Furthermore, since biodegradation of organic acids contribute for a substantial part of total soil respiration (van Hees et al., 2002) and microbial decarboxylation of organic anions leads to a decreased organic acidity (Marschner and Noble, 2000; Nätcher and Schwertmann, 1991), microbial activity might reduce the anionic density on DOM molecules and thus the contribution of organic acids to the anion deficit. As a result, the leaching of organic acids in combination with their dissociation state, also determines the base cation leaching from forest floors (Dijkstra et al., 2001).

## ECOSYSTEM N BALANCES

### *Input-output N balances in the forest floor and mineral soil*

The input-output N balances for the forest floors and the mineral soils of the three stands were calculated for the period March 2005 till March 2006 (Table 7.2). The forest floor of the SB and CP stand functioned as net N sink as a result of litter accumulation and/or root uptake. This is in accordance with the findings of Hart and Firestone (1991) that the forest floor acts both as a source and sink for N in the mineral soil. On the contrary, the CPN forest floor released more N than was brought in with throughfall and litterfall. This is in accordance to McDowell et al. (2004) who found that the large excess of N outputs over inputs in the forest floor of a pine plot with high N addition points towards “*mining*” of N from soil organic matter. In our study, nitrogen outputs in excess of inputs are due to an increased N mineralization of the forest floor material. The mineral soil at the three forest stands showed to serve as a net N sink due to immobilization of DIN, root uptake and/or DON retention (Johnson et al., 2000). These findings indicate that the forest edge proximity results in a shift of the major sink for nitrogen from the forest floor to the mineral soil in the coniferous stands. This is in accordance with the findings of Vestgarden et al. (2004) in *Pinus sylvestris* stands exposed to different nitrogen inputs.

Subtraction of ecosystem TDN loss via leaching from the ecosystem TDN input via throughfall indicated a net ecosystem retention of 16.5 and 17.8 kg N ha<sup>-1</sup> yr<sup>-1</sup> in the SB and CP stand respectively. On the contrary, the CPN ecosystem showed a net N release of 8.1 kg N ha<sup>-1</sup> yr<sup>-1</sup>, pointing towards a larger ecosystem N availability than total plant and microbial nutritional demand. This is probably a result of higher N release through mineralization of previously accumulated N (Falkengren-Grerup et al., 1998). Although we found a fairly low throughfall N deposition rate (46.5 kg N ha<sup>-1</sup> yr<sup>-1</sup>) at this site compared to previous studies in the same region under similar conditions (96.5 kg N ha<sup>-1</sup> yr<sup>-1</sup>) (De Schrijver et al., 2000), we suggest that N deposition has been larger in the past (i.e. in the previous 30-50 years). This is in accordance to measurements of the Flemish Environmental Agency (VMM) that N deposition in Flanders decreased with 18 % between 1990 and 2004 (MIRA, 2006). This previously higher N input has probably resulted in accumulation of large amounts of nitrogen in the forest floor, which now seem to be steadily released.

**Table 7.2. Annual input-output N balances (kg N ha<sup>-1</sup> yr<sup>-1</sup>) of the forest floor, mineral soil and entire ecosystem of the SB, CP and CPN stands between March 2005 and March 2006. Positive values mean addition, negative values indicate losses.**

	SB	CP	CPN
<i>Forest floor</i>			
Input with throughfall <sup>1</sup>	24.4 (5.4)	37.1 (6.3)	46.5 (8.8)
Input with fresh litter <sup>2</sup>	57.7	67.6	59.7
Output with forest floor leachate <sup>1</sup>	33.2 (11.6)	49.2 (9.8)	116.8 (17.5)
Balance	48.9 (-6.2)	55.5 (-3.5)	-10.6 (-8.7)
<i>Mineral soil (&lt; 65 cm)</i>			
Input with forest floor leachate <sup>1</sup>	33.2 (11.6)	49.2 (9.8)	116.8 (17.5)
Output with BC horizon leachate <sup>1</sup>	7.9 (2.2)	19.3 (3.3)	54.6 (4.9)
Balance	25.3 (9.4)	29.9 (6.5)	62.2 (12.6)
<i>Ecosystem N retention</i>	16.5 (3.2)	17.8 (3.0)	-8.1 (3.9)
<i>Soil N stock (&lt; 90 cm) (ton N ha<sup>-1</sup>)</i>	4.44	6.19	4.25

<sup>1</sup> Data from Chapter 3;

<sup>2</sup> Data from Chapter 4.

### *Role of DON in N retention*

The annual DON retention in the mineral soil amounted to 9.4, 6.5 and 12.6 kg N ha<sup>-1</sup> yr<sup>-1</sup> in the SB, CP and CPN stand i.e. 37, 22 and 20 % respectively of total N retention in the mineral soils. If DON were not considered in these ecosystem N budgets, both TN input and output would be underestimated. As a result, the overall net ecosystem N retention would be underestimated by 3.2, 3.0 and 3.9 kg N ha<sup>-1</sup> yr<sup>-1</sup> in the SB, CP and CPN stand respectively. However, the relative importance of DON in N retention is probably still underestimated, since the biotic and/or abiotic conversion of highly mobile DIN (especially nitrate) to DON followed by immediate sorptive retention was not accounted for in this study.

### *Role of DON in TDN leaching*

Although Perakis and Hedin (2002) found that dissolved organic nitrogen was the major form through which N is lost from unpolluted South American forests, we found only a relatively limited contribution of DON to TDN leaching (9-28 %). The absolute fluxes of DON leaching followed the order CPN>CP>SB, whereas the relative contribution of DON to total nitrogen leaching followed the order SB>CP>CPN. This indicates that the relative contribution of DON to TDN leaching is mainly determined by the extent of the DIN fluxes. These DIN fluxes, in turn, are controlled by the overall throughfall N input and by the ecosystem N status (Gundersen et al., 1998; MacDonald et al., 2002). This is in accordance with the findings of Park and Matzner (2006) that the relative contribution of nitrate increased whereas that of DON decreased with increasing throughfall nitrogen input.

### *N saturation*

We found considerable nitrate leaching at the three forest stands during the entire year indicating that nitrogen is available in excess of total combined plant and microbial nutritional demand. This is a definition for nitrogen saturation of forest ecosystems (Aber et al., 1989).

Several experimental N addition studies in North America (e.g. Aber et al., 1998) indicated that temperate forests initially retain most of the added N without impact on N leaching. Long-term N additions, however, lead to substantially increased leaching losses (Magill et al., 1997). In European forests receiving high atmospheric N deposition, nitrate losses typically represented 30 % of inorganic N deposition (Dise and Wright, 1995). Van der Salm et al. (2007) estimated that approximately 40 % of European forests are N saturated, whereas Galloway et al. (2004) predicted that this percentage will inevitably increase as a result of the growth of the world population. Emmett et al. (1995) found that N input to N output ratios approached 1 at sites receiving high atmospheric N inputs for long periods. In our study, this ratio amounted to 0.32, 0.52 and 1.17 in the SB, CP and CPN stands respectively. The ratio of 1.17 in the CPN stand indicates that previously retained N is released and thus that the ecosystem is clearly oversaturated.

## **SCOPE FOR FURTHER RESEARCH**

In the view of the debate on global warming, one of the most important findings of this study is that large quantities of dissolved organic carbon are transported to and retained in these poor sandy soils. Further research is needed to investigate the mechanisms of C accumulation and C stabilization in these soils. In particular, the relative importance of precipitation to total DOC immobilization must be investigated. Besides, laboratory incubation and OC characterizations should be performed to determine the stability and the mechanisms protecting this carbon.

Biological activity and the input of potentially acidifying deposition (Vogt et al., 2006) leads to the continuous acidification of mineral soils in forest ecosystems (De Schrijver et al., 2006). Due to its amphoteric (Dai et al., 1996) and colloidal properties (Münch et al., 2002), DOM cycling both might influence and might be influenced by soil acidification (Zech et al., 1994). Increased input of protons may lead to a suppressed solubilization of DOM (David et al., 1989), increased precipitation (due to higher solubilization of Al) (Nilsson and Bergkvist,

1983), lower DOM sorption and a shift in chemical DOM composition towards the hydrophilic fractions (Kaiser, 1998). Besides, since organic acids contribute to the total acidity in forest soil solutions (van Hees et al., 2000), their biodegradation (decarboxylation) leads to acid neutralisation (Nätscher and Schwertmann, 1991; Marschner and Noble, 2000) and their leaching leads to losses of base cations (Dijkstra et al., 2001). The complexation of Al with DOM molecules lowers its bioavailability (and toxicity) and thus buffers one of the most serious consequences of soil acidification. Therefore, more research is needed to determine the absolute effect of acidification on DOM cycling and vice versa.

Comparison of the results of the sorption experiments with field data revealed that the sorption isotherms are a sound basis for modelling DOM transport through layered soil profiles. In the current DOM transport models, sorption is generally quantified by use of only the partition coefficient ( $K_d$ ) (Michalzik et al., 2003) or the linear initial mass isotherm (Neff and Asner, 2001). The use of sorption isotherm data generated by the sequential experiments together with (non-linear) isotherms accounting for the presence of initially adsorbed DOM could further increase the performance of these models. In addition, the introduction of chemical DOM composition data could improve the modelling efficiency.

Since DOM is an important carrier of heavy metals and organic pollutants (Ravichandran, 2004), DOM dynamics considerably influence the retention and mobility of these pollutants (Zhao et al., 2007). However, the total DOC concentration in soil was found to be an insufficient predictor for heavy metal mobilization (Avery et al., 2007). Since different chemical DOM fractions exhibit different affinities for sorption to mineral soil, the determination of heavy metal distribution over the different DOM fractions may lead to a better understanding and estimation of their mobility, bio-availability and leachability. The chemical fractionation and dialysis techniques used in this study may contain potential as alternative heavy metal speciation tools (Buzier et al., 2006).



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## SUMMARY

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Dissolved organic matter (DOM) is the organic matter dissolved in the aqueous phase. Although this organic matter pool constitutes only a small fraction of the total OM pool, it is certainly the most mobile and probably the most reactive of all pools. The high inherent mobility of DOM contains potential for the translocation of organic material and associated nutrients within and between ecosystems. The downward transport of DOM with percolating water in mineral soils also plays a crucial role in soil formation, nutrient loss and eutrophication of ground and surface waters. The main objective of this study was to determine the dynamics of DOM in forest ecosystems under long-term nitrogen deposition on sandy soils in Flanders.

The first part of this work focussed on the analytical methodology of dissolved organic nitrogen (DON) determinations. Aqueous field samples originating from forest ecosystems in Flanders are characterized by high inorganic nitrogen contents (nitrate and ammonium). Since DON can only be determined by subtraction of the dissolved inorganic nitrogen (DIN) concentration from the total dissolved nitrogen (TDN) concentration, analytical variance accumulates in the DON determination, exceeding the DON concentration in a substantial amount of samples. The alkaline persulfate oxidation (PO) showed higher TDN recoveries compared to the commonly used high temperature catalytic oxidation (HTCO) method and was further used in this study. The potential of a refined dialysis pretreatment to increase the precision of DON determinations was assessed and approved, though the method is labour-intensive and thus difficult to use routinely.

The second part of this work aimed to quantify dissolved organic carbon (DOC) and DON concentrations and fluxes in the different ecosystem strata of three forest stands in Flanders. The fluxes were determined based on the results of one year intensive monitoring and could be used to localise the major sources and sinks of DOM in these ecosystems. Atmospheric deposition was the major input of DON in these ecosystems, whereas most DOC was brought in solution during passage through the forest canopy and the forest floor. Although substantial amounts of DOC and DON were retained in the vadose zone below the rooting zone, most DOM retention took place in the upper 90 cm of the mineral soil. As a

result of the efficient DOM retention in the vadose zone, the absolute annual amounts of DOM reaching the groundwater were fairly low.

Since the forest floor was identified as the major source of DOM in these forest ecosystems in Flanders and since substantial differences in the DOM release from forest floor were found between the three forest stands, the factors affecting the DOM release were investigated into further detail. The combination of field and laboratory observations together with literature data allowed the design of a conceptual model for the mechanisms involved in the release of DOM from forest floors. The differences in DOM release from the forest floors of the three stands were explained using the conceptual model. Furthermore, microbial activity showed to be (partially) responsible for the different release rates and patterns between DOC and DON.

Although the mineral soil was identified as the major sink for DOM, the results of the field measurements did not allow determination of the processes involved in this retention. Therefore, laboratory experiments were performed to quantify the importance of individual removal mechanisms.

The first mechanism tested under controlled laboratory conditions was the microbial degradation of DOM. Therefore, DOM solutions of various origins were inoculated with a mixture of indigenous microorganisms and incubated for one year. The results showed that limited fractions of DOM in forest floor leachates were mineralized (27-34 %) during the experiment with minor influences of its origin. Furthermore, the DOM biodegradability tended to increase with increasing soil depth. These findings indicated that microbial degradation is probably not the main mechanism responsible for the high retention of DOM in the mineral soil.

Beside microbial degradation, physical sorption to the mineral soil was investigated as a potential mechanism responsible for the retention of DOM. In a first experiment, the isotherm model yielding the best fit and the most reliable and field-representative parameter estimates was determined. The results indicated that DOM concentration estimated based on sorption isotherm data were in good accordance with the concentrations sampled under field conditions. A second

experiment was performed in an attempt to better predict DOM concentrations in layered soil profiles by approaching the field conditions. Therefore, a sequential batch method was designed in which the DOM solution was equilibrated with the succession of the different horizons. This new method was found to even better predict DOM, especially DOC, concentrations with soil depth compared to the conventional method. Furthermore, this new method was found to account for the sorption-induced fractionation of dissolved organic matter in these podzol profiles underlining its applicability for DOM transport models.

In conclusion, this work revealed the importance of dissolved organic matter in a variety of environmental issues. Firstly, DOM forms an important carrier in the transport of carbon from aboveground sources to the mineral soil. Adsorption to the mineral soil constituents together with its inherent refractoriness or external stabilization mechanisms, emphasizes the potential role of DOM in belowground C sequestration in forest ecosystems. The inclusion of DON in the nitrogen cycle of the eutrophic forest ecosystems puts ecosystem N input, output and retention in another light.

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## SAMENVATTING

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Opgelost organisch materiaal (DOM) is het organisch materiaal (OM) opgelost in de waterige fase. Hoewel deze DOM pool slechts een beperkt deel uitmaakt van de totale OM pool, toch is het de meest mobiele en hoogstwaarschijnlijk de meest reactieve van alle pools. De hoge mobiliteit van DOM maakt transport van organisch materiaal en nutriënten mogelijk zowel binnenin als tussen verschillende ecosystemen. Het neerwaarts transport van DOM en geassocieerde elementen met het percolerend water speelt daarnaast een belangrijke rol bij bodemvorming, verlies van nutriënten en eutrofiëring van grond- en oppervlaktewater. De hoofdbedoeling van deze studie was het begroten van de dynamiek van opgelost organisch materiaal in bosccosystemen onder historisch hoge stikstofdeposities op zandige bodems in Vlaanderen.

Het eerste deel van dit werk focuste op de analytische methode voor het bepalen van opgeloste organische stikstof (DON). Waterige stalen afkomstig uit Vlaamse bosccosystemen worden gekenmerkt door hoge anorganische stikstofconcentraties (nitraat en ammonium). Gezien DON enkel kan bepaald worden uit het verschil tussen de totale opgeloste stikstofconcentratie (TDN) en de opgeloste anorganische stikstofconcentratie (DIN), accumuleren analytische fouten op deze DON bepaling. Deze fout overschrijdt zelfs de absolute DON concentratie in een aanzienlijk aantal van de gevallen. Systematisch hogere TDN concentraties werden teruggevonden aan de hand van de basische persulfaat oxidatie (PO) in vergelijking met de vaak gebruikte hoge temperatuur katalytische oxidatie (HTCO) methode. Daarom werd de PO verder gebruikt voor de TDN bepalingen in deze studie. Het potentieel van een verfijnde dialyse-voorbehandeling om de precisie van de DON bepalingen te verhogen werd bepaald en positief bevonden, hoewel de methode arbeidsintensief is en dus moeilijk routinematig toe te passen valt.

Het tweede deel van dit werk had de bedoeling om de concentraties en fluxen van opgeloste organische koolstof (DOC) en stikstof (DON) te begroten in de verschillende ecosysteemniveaus van drie bosbestanden in Vlaanderen. De fluxen werden bepaald aan de hand van de resultaten van een intensieve, éénjarige meetcampagne en liet toe om de belangrijkste bronnen en verliesposten van DOM

in deze ecosystemen te lokaliseren. Atmosferische depositie was de belangrijkste bron van DON in deze ecosystemen, terwijl de boomkruinen en de strooisellaag de meeste DOC in oplossing brachten. Hoewel aanzienlijke hoeveelheden DOC en DON in de onverzadigde zone onder de wortelzone werden weerhouden, greep de meeste DOM retentie plaats in de bovenste 90 cm van de minerale bodem. Als gevolg van de efficiënte DOM retentie in de volledige onverzadigde zone bereikten vrij lage hoeveelheden aan DOM jaarlijks het grondwater.

Aangezien de strooisellaag als belangrijkste bron van DOM werd geïdentificeerd in deze bosccosystemen in Vlaanderen en aangezien aanzienlijke verschillen in DOM vrijstelling uit de strooisellaag werden gevonden tussen de drie bosbestanden, werden de factoren die deze DOM vrijstelling beïnvloeden verder in detail onderzocht. De combinatie van observaties tijdens de veldmetingen en tijdens labo-experimenten, samen met gegevens uit de literatuur, liet toe om een conceptueel model op te stellen voor de mechanismen die betrokken zijn bij de vrijstelling van DOM uit de strooisellaag. De verschillen in DOM vrijstelling uit de strooisellagen van de drie bosbestanden werden uitgelegd aan de hand van dit conceptueel model. Bovendien werd gevonden dat microbiële activiteit (gedeeltelijk) verantwoordelijk is voor de verschillen in snelheid en verloop tussen DOC en DON.

Hoewel de minerale bodem als belangrijkste verliespost voor DOM werd geïdentificeerd, toch lieten de resultaten van de veldmetingen niet toe om de processen die hiervoor verantwoordelijk zijn te bepalen. Daarom werden een aantal laboratoriumexperimenten opgezet om het belang van de individuele verwijderingsprocessen te kwantificeren.

Het eerste mechanisme dat onder gecontroleerde laboratorium omstandigheden werd getest, was microbiële afbraak van DOM. Daarvoor werden DOM oplossingen van uiteenlopende origine geïnoculeerd met een mengsel van aanwezige microorganismen en geïncubeerd voor een totale duur van 1 jaar. De resultaten lieten zien dat slechts een beperkt deel van de DOM in strooiselwater werd gemineraliseerd (27-34 %) gedurende het experiment, waarbij ook slechts een beperkt invloed te merken was van de oorsprong van de oplossing. Bovendien

neigde de afbreekbaarheid van DOM toe te nemen met toenemende diepte in de bodem. Deze bevindingen duiden aan dat microbiële afbraak hoogstwaarschijnlijk niet het belangrijkste mechanisme is dat instaat voor de hoge DOM retentie in de minerale bodem.

Naast microbiële afbraak werd fysische sorptie op de minerale bodemdeeltjes onderzocht als mogelijk mechanisme verantwoordelijk voor het weerhouden van DOM. In een eerste experiment werd bepaald welk van de courantste modellen voor het beschrijven van sorptie-isothermen het beste fit en de meest betrouwbare en realistische parameterschattingen oplevert. De resultaten gaven aan dat de DOM concentraties geschat op basis van de sorptie-isothermen goed overeenkwamen met de gemeten concentraties in het veld. Een tweede experiment werd uitgevoerd in een poging om de DOM concentraties in gelaagde bodemprofielen beter te voorspellen door de veldomstandigheden beter te benaderen. Daarvoor werd een sequentiele batch methode ontwikkeld waarbij de DOM oplossing wordt in evenwicht gebracht met de opeenvolgende minerale bodemhorizonten. Er werd gevonden dat deze nieuwe methode in staat is om de DOM en in het bijzonder DOC concentraties in functie van de diepte beter te voorspellen dan de conventionele methode. Bovendien werd gevonden dat deze nieuwe methode de sorptie-geïnduceerde fractionatie van DOM in deze podzolprofielen zeer goed beschrijft, wat de bruikbaarheid van deze method voor DOM transport modellen onderstreept.

Samengevat onthult dit werk het belang van opgelost organisch materiaal in een waaier van milieukwesties. Eerst en vooral vormt DOM een belangrijke drager van koolstof bij het transport van bovengrondse bronnen naar de minerale bodem. Adsorptie aan de bodemdeeltjes samen met zijn inherente recalcitrantie of externe stabilisatiemechanismen, benadrukken de mogelijke rol van DOM bij de ondergrondse koolstofsequestratie in bosesystemen. Het in rekening brengen van DON in de totale stikstofbalans van deze eutrofe bosesystemen geeft een ander en beter beeld van de totale N input, output en retentie.



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## REFERENCES

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- Aber J.D. (1992). Nitrogen cycling and nitrogen saturation in temperate forest ecosystems. *Trends in Ecology and Evolution* 7: 220-223.
- Aber J.D., Goodale C.L., Ollinger S.V., Smith M.-L., Magill A.H., Martin M.E., Hallett R.A. & Stoddard J.L. (2003). Is nitrogen deposition altering the nitrogen status of northeastern forests? *BioScience* 53: 375-389.
- Aber J.D., McDowell W., Nadelhoffer K., Magill A., Bernston G., Kamakea M., McNulty S., Currie W., Rustad L. & Fernandez I. (1998). Nitrogen saturation in temperate forest ecosystems: hypotheses revisited. *BioScience* 48: 921-934.
- Aber J.D., Nadelhoffer K.J., Steudler P. & Melillo J.M. (1989). Nitrogen saturation in Northern Forest Ecosystems. *BioScience* 39: 378-386.
- Agiv (2007). [www.agiv.be](http://www.agiv.be) (Accessed September 2007).
- Aiken G.R. (2002). Organic matter in ground water. In: Aiken G.R. & Kuniandy E.L. (Eds.), *US Geological Survey Artificial Recharge Workshop Proceedings*, April 2-4, 2002, Sacramento, California, p. 88.
- Akimoto H. (2003). Global air quality and pollution. *Science* 302: 1716-1719.
- Alcock M.R. & Morton A.J. (1985). Nutrient content of throughfall and stemflow in woodland recently established on heathland. *Journal of Ecology* 73: 625-632.
- Ambonguilat S., Gallard H., Garron A., Epron F. & Croué J.P. (2006). Evaluation of the catalytic reduction of nitrate for the determination of dissolved organic nitrogen in natural waters. *Water Research* 40: 675-682.
- Amery F., Degryse F., Degeling W., Smolders E. & Merckx R. (2007). The copper-mobilizing-potential of dissolved organic matter in soils varies 10-fold depending on soil incubation and extraction procedures. *Environmental Science & Technology* 41: 2277-2281.
- Ammann A.A., Rüttimann T.B. & Bürgi F. (2000). Simultaneous determination of TOC and TN<sub>B</sub> in surface and wastewater by optimized high temperature catalytic combustion. *Water Research* 34: 3573-3579.
- Amon R.M.W. & Benner R. (1996). Bacterial utilization of different size classes of dissolved organic matter. *Limnology and Oceanography* 41: 41-51.

- Andersen B.R. & Gundersen P. (2000). Nitrogen and carbon interactions of forest soil water. In: Schulze E.-D. (Ed.), Carbon and nitrogen cycling in European forest ecosystems. Springer-Verlag Berlin Heidelberg 2000, Germany, pp. 332-340.
- Anderson H.A., Berrow M.L., Farmer V.C., Hepburn A., Russell J.D. & Walker A.D. (1982). A reassessment of podzol formation processes. *Journal of Soil Science* 33: 125-136.
- Andersson M., Kj  ller A. & Struwe S. (2004). Microbial enzyme activities in leaf litter, humus and mineral soil layers of European forests. *Soil Biology & Biochemistry* 36: 1527-1537.
- Andersson S., Nilsson S.I. & Saetre P. (2000). Leaching of dissolved organic carbon (DOC) and dissolved organic nitrogen (DON) in mor humus as affected by temperature and pH. *Soil Biology & Biochemistry* 32: 1-10.
- Asman W.A.H., Drukker B. & Janssen A.J. (1988). Modelled historical concentrations and depositions of ammonia and ammonium in Europe. *Atmospheric Environment* 22: 725-735.
- Avery G.B. Jr, Willey J.D. & Kieber R.J. (2006). Carbon isotopic characterization of dissolved organic carbon in rainwater: Terrestrial and marine influences. *Atmospheric Environment* 40: 7539-7545.
- Baldwin I.T., Olson R.K. & Reiners W.A. (1983). Protein binding phenolics and the inhibition of nitrification in subalpine balsam fir soils. *Soil Biology & Biochemistry* 15: 419-423.
- Banaitis M.R., Waldrip-Dail H., Diehl M.S., Holmes B.C., Hunt J.F., Lynch R.P. & Ohno T. (2006). Investigating sorption-driven dissolved organic matter fractionation by multidimensional fluorescence spectroscopy and PARAFAC. *Journal of Colloid and Interface Science* 304: 271-276.
- Barkle G., Clough T. & Stenger R. (2007). Denitrification capacity in the vadose zone at three sites in the Lake Taupo catchment, New Zealand. *Australian Journal of Soil Research* 45: 91-99.

- Bernoux M., Arrouays D., Cerri C.C. & Bourennane H. (1998). Modeling the vertical distribution of carbon in oxisols of the western Brazilian Amazon (Rondonia). *Soil Science* 163: 941-951.
- Blackwood C.B., Waldrop M.P., Zak D.R. & Sinsabaugh R.L. (2007). Molecular analysis of fungal communities and laccase genes in decomposing litter reveals differences among forest types but no impact of nitrogen deposition. *Environmental Microbiology* 9: 1306-1316.
- Blagodatskaya E.V. & Anderson T.-H. (1998). Interactive effects of pH and substrate quality on the fungal-to-bacterial ratio and QCO<sub>2</sub> of microbial communities in forest soils. *Soil Biology & Biochemistry* 30: 1269-1274.
- Blight E.G. & Dyer W.J. (1959). A rapid method of total lipid extraction and purification. *Canadian Journal of Biochemistry and Physiology* 37: 911-917.
- Bolan N.S., Adriano D.C. & de-la-Luz M. (2004). Dynamics and environmental significance of dissolved organic matter in soil. In: Singh, B (Ed.), *Supersoil 2004: Proceedings of the 3rd Australian New Zealand Soils Conference*, University of Sydney, Australia, 5 – 9 December 2004.
- Bond W.J. & Phillips I.R. (1990). Cation-exchange isotherms obtained with batch and miscible-displacement techniques. *Soil Science Society of America Journal* 54: 722-728.
- Bossio D.A. & Scow K.M. (1998). Impacts of carbon and flooding on soil microbial communities: phospholipid fatty acid profiles and substrate utilization patterns. *Microbial Ecology* 35: 265-278.
- Brierley E.D.R., Shaw P.J.A. & Wood M. (2001). Nitrogen cycling and proton fluxes in an acid forest soil. *Plant and Soil* 229: 83-96.
- Brookshire E.N.J., Valett H.M., Thomas S.A. & Webster J.R. (2007). Atmospheric N deposition increases organic N loss from temperate forests. *Ecosystems* 10: 252-262.
- Brown A.H.F. & Iles M.A. (1991). Water chemistry profiles under four tree species at Gisburn, NW England. *Forestry* 64: 169-187.

- Bundt M., Jäggi M., Blaser P., Siegwolf R. & Hagedorn F. (2001). Carbon and nitrogen dynamics in preferential flow paths and matrix of a forest soil. *Soil Science Society of America Journal* 65: 1529-1538.
- Burton A.J., Pregitzer K.S., Crawford J.N., Zogg G.P. & Zak D.R. (2004). Simulated chronic  $\text{NO}_3^-$  deposition reduces soil respiration in northern hardwood forests. *Global Change Biology* 10: 1080-1091.
- Buurman P. & Jongmans A.G. (2005). Podzolisation and soil organic matter dynamics. *Geoderma* 125: 71-83.
- Buzier R., Tusseau-Vuillemin M.-H. & Mouchel J.-M. (2006). Evaluation of DGT as a metal speciation tool in wastewater. *Science of the Total Environment* 358: 277-285.
- Cabrera M.L. & Beare M.H. (1993). Alkaline persulfate oxidation for determining total nitrogen in microbial biomass extracts. *Soil Science Society of America Journal* 57: 1007-1012.
- Calace N. & Petronio B.M. (2004). The role of organic matter on metal toxicity and bio-availability. *Annali Di Chimica* 94: 487-493.
- Cape J.N., Anderson M., Rowland A.P. & Wilson D. (2004). Organic nitrogen in precipitation across the United Kingdom. *Water, Air, and Soil Pollution: Focus*, 4: 25-35.
- Carreiro M.M., Sinsabaugh R.L., Repert D.A. & Parkhurst D.F. (2000). Microbial enzyme shifts explain litter decay responses to simulated nitrogen deposition. *Ecology* 81: 2359-2365.
- Chen C.R., Xu Z.H., Keay P. & Zhang S.L. (2005). Total soluble nitrogen in forest soils as determined by persulfate oxidation and by high temperature catalytic oxidation. *Australian Journal of Soil Research* 43: 515-523.
- Christ M.J. & David M.B. (1996a). Dynamics of extractable organic carbon in spodosol forest floors. *Soil Biology & Biochemistry* 28: 1171-1179.
- Christ M.J. & David M.B. (1996b). Temperature and moisture effects on the production of dissolved organic carbon in a spodosol. *Soil Biology & Biochemistry* 28: 1191-1199.

- Chung H., Zak D.R., Reich P.B. & Ellsworth D.S. (2007). Plant species richness, elevated CO<sub>2</sub>, and atmospheric nitrogen deposition alter soil microbial community composition and function. *Global Change Biology* 13: 980-989.
- Cleveland C.C. & Liptzin D. (2007). C:N:P stoichiometry in soil: is there a "Redfield ratio" for the microbial biomass? *Biogeochemistry* 85: 235-252.
- Cleveland C.C., Neff J.C., Townsend A.R. & Hood E. (2004). Composition, dynamics and fate of leached dissolved organic matter in terrestrial ecosystems: results from a decomposition experiment. *Ecosystems* 7: 275-285.
- Colman B.P., Fierer N. & Schimel J.P. Abiotic nitrate incorporation in soil: is it real? *Biogeochemistry* 84: 161-169.
- Cook B.D. & Allan D.L. (1992). Dissolved organic carbon in old field soils: compositional changes during the biodegradation of soil organic matter. *Soil Biology & Biochemistry* 24: 595-600.
- Cornell S.E., Jickells T.D., Cape J.N., Rowland A.P. & Duce R.A. (2003). Organic nitrogen deposition on land and coastal environments: a review of methods and data. *Atmospheric Environment* 37: 2173-2191.
- Cotrufo M.F., Miller M. & Zeller B. (2000). Litter decomposition. In: Schulze E.-D. (Ed.), *Carbon and Nitrogen cycling in European Forest Ecosystems*. Ecological Studies 142, Springer-Verlag Berlin Heidelberg, pp. 276-296.
- Cottenie A., Verloo M., Kiekens L., Velghe G. & Camerlynck R. (1982). Cation exchange capacity. In: *Chemical Analysis of Plants and Soils*, Ghent, Belgium, pp. 38-39.
- Currie W.S., Aber J.D., McDowell W.H., Boone R.D. & Magill A.H. (1996). Vertical transport of dissolved organic C and N under long-term N amendments in pine and hardwood forests. *Biogeochemistry* 35: 471-505.
- Dahlgren R.A. & Marrett D.J. (1991). Organic carbon sorption in arctic and subalpine spodosol B horizons. *Soil Science Society of America Journal* 55: 1382-1390.
- Dai K.H., David M.B. & Vance G.F. (1996a). Characterization of solid and dissolved carbon in a spruce-fir spodosol. *Biogeochemistry* 35: 339-365.

- Dai K.H., David M.B., Vance G.F., McLaughlin J.W. & Fernandez I.J. (1996b). Acidity characteristics of soluble organic substances in spruce-fir forest floor leachates. *Soil Science* 161: 694-704.
- David M.B., Vance G.F., Rissing J.M. & Stevenson F.J. (1989). Organic carbon fractions in extracts of O and B horizons from a New England spodosol: effects of acid treatment. *Journal of Environmental Quality* 18: 212-217.
- Davidson E.A., Chorover J. & Dail B.D. (2003). A mechanism of abiotic immobilization of nitrate in forest ecosystems: the ferrous wheel hypothesis. *Global Change Biology* 9: 228-236.
- Davies-Colley R.J., Payne G.W. & van Elswijk M. (2000). Microclimate gradients across a forest edge. *New Zealand Journal of Ecology* 24: 111-121.
- DeForest J.L., Zak D.R., Pregitzer K.S. & Burton A.J. (2004). Atmospheric nitrate deposition, microbial community composition, and enzyme activity in northern hardwood forests. *Soil Science Society of America Journal* 68: 132-138.
- DeForest J.L., Zak D.R., Pregitzer K.S. & Burton A.J. (2005). Atmospheric nitrate deposition and enhanced dissolved organic carbon leaching: test of a potential mechanism. *Soil Science Society of America Journal* 69: 1233-1237.
- D'Elia C.F., Steudler P.A. & Corwin N. (1977). Determination of total nitrogen in aqueous samples using persulfate digestion. *Limnology and Oceanography* 22: 760-764.
- De Schrijver A. (2007). Acidification and eutrophication of forests on sandy soil: effects of forest type and deposition load. Ph.D. thesis, Ghent University, Belgium, 189p.
- De Schrijver A., Devleaminck R., Mertens J., Wuyts K., Hermy M. & Verheyen K. (2007a). On the importance of incorporating forest edge deposition for evaluating exceedance of critical pollutant loads. *Applied Vegetation Science* 10: 293-298.

- De Schrijver A., Geudens G., Augusto L., Staelens J., Mertens J., Wuyts K., Gielis L. & Verheyen K. (2007b). The effect of forest type on throughfall deposition and seepage flux: a review. *Oecologia* 153: 663-674.
- De Schrijver A., Mertens J., Geudens G., Staelens J., Campforts E., Luysaert S., De Temmerman L., De Keersmaecker L., De Neve S. & Verheyen K. (2006). Acidification of forested podzols in northern Belgium during the period 1950-2000. *Science of the Total Environment* 361: 189-195.
- De Schrijver A., Van Hoydonck G., Nachtergale L., De Keersmaecker L., Mussche S. & Lust N. (2000). Comparison of nitrate leaching under Silver Birch (*Betula pendula*) and Corsican Pine (*Pinus nigra* ssp. *Laricio*) in Flanders (Belgium). *Water, Air, and Soil Pollution* 122: 77-91.
- Dijkstra F.A., Geibe C., Holmström S., Lundström U.S. & van Breemen N. (2001). The effect of organic acids on base cation leaching from the forest floor under six North American tree species. *European Journal of Soil Science* 52: 205-214.
- DiLuca T.H., Nilsson M.-C. & Zackrisson, O. (2002). Nitrogen mineralization and phenol accumulation along a fire chronosequence in northern Sweden. *Oecologia* 133: 206-214.
- Dise N.B. & Wright R.F. (1995). Nitrogen leaching from European forests in relation to nitrogen deposition. *Forest Ecology and Management* 71: 153-161.
- Don A. & Kalbitz K. (2005). Amounts and degradability of dissolved organic carbon from foliar litter at different decomposition stages. *Soil Biology & Biochemistry* 37: 2171-2179.
- Dosskey M.G. & Bertsch P.M. (1997). Transport of dissolved organic matter through a sandy forest soil. *Soil Science Society of America Journal* 61: 920-927.
- Doyle A., Weintraub M.N. & Schimel J.P. (2004). Persulfate digestion and simultaneous colorimetric analysis of carbon and nitrogen in soil extracts. *Soil Science Society of America Journal* 68: 669-676.



- Draaijers G.P.J. (1993). The variability of atmospheric deposition to forests: the effects of canopy structure and forest edges. Ph.D. thesis, Utrecht, Utrecht University, 200 p.
- Dunnivant F.M., Jardine P.M., Taylor D.L. & McCarthy J.F. (1992). Transport of naturally occurring dissolved organic carbon in laboratory columns containing aquifer material. *Soil Science Society of America Journal* 56: 437-444.
- EFI (2007). [www.efi.fi](http://www.efi.fi) (Accessed September 2007).
- Emmett B.A., Brittain A., Hughes S., Gorres J., Kennedy V. Norris D., Rafarel R.K., Reynolds B. & Stevens P.A. (1995). Nitrogen additions ( $\text{NaNO}_3$  and  $\text{NH}_4\text{NO}_3$ ) at Aber forest, Wales: I. Response of throughfall and soil water chemistry. *Forest Ecology and Management* 71: 45-60.
- Erisman J.W. & Draaijers G. (2003). Deposition to forests in Europe: most important factors influencing dry deposition and models used for generalisation. *Environmental Pollution* 124: 379-388.
- Evans A., Zelazny L.W. & Zipper C.E. (1988). Solution parameters influencing dissolved organic carbon in 3 forest soils. *Soil Science Society of America Journal* 52: 1789-1792.
- Falkengren-Grerup U., Brunet J. & Diekmann, M. (1998). Nitrogen mineralization in deciduous forest soils in south Sweden in gradients of soil acidity and deposition. *Environmental Pollution* 102 S1: 415-420.
- Falkengren-Grerup U. & Tyler G. (1993). The importance of soil acidity, moisture, exchangeable cation pools and organic matter solubility to the cationic composition of beech forest (*Fagus sylvatica* L.) soil solution. *Journal of Plant Nutrition and Soil Science* 156: 365-370.
- FAO-UNESCO (1990). Soil map of the world, revised legend. World Soil Resources Report 60, FAO, Rome.
- Ferm M. & Hultberg H. (1999). Dry deposition and internal circulation of nitrogen, sulphur and base cations to a coniferous forest. *Atmospheric Environment* 33: 4421-4430.

- Freeman C., Ostle N. & Kang H. (2001). An enzymatic latch on a global carbon store. *Nature* 409: 149.
- Frey S.D., Knorr M., Parrent J.L. & Simpson R.T. (2004). Chronic nitrogen enrichment affects the structure and function of the soil microbial community in temperate hardwood and pine forests. *Forest Ecology and Management* 196: 159-171.
- Fritze H., Pietikäinen J. & Pennanen T. (2000). Distribution of microbial biomass and phospholipids fatty acids in podzol profiles under coniferous forest. *European Journal of Soil Science* 51: 565-573.
- Fröberg M., Berggren-Kleja D., Bergkvist B., Tipping E. & Mulder J. (2005). Dissolved organic carbon leaching from a coniferous forest floor – a field manipulation experiment. *Biogeochemistry* 75: 271-287.
- Fröberg M., Berggren-Kleja D. & Hagedorn F. (2007). The contribution of fresh litter to dissolved organic carbon leached from a coniferous forest floor. *European Journal of Soil Science* 58: 108-114.
- Fröstegård A., Tunlid A. & Baath E. (1993). Phospholipid fatty acid composition and activity of microbial communities from two soil types experimentally exposed to different heavy metals. *Applied and Environmental Microbiology* 59: 3605-3617.
- Gallo M., Amonette R., Lauber C., Sinsabaugh R.L. & Zak D.R. (2004). Microbial community structure and oxidative enzyme activity in nitrogen-amended north temperate forest soils. *Microbial Ecology* 48: 218-229.
- Galloway J.N., Dentener F.J., Capone D.G., Boyer E.W., Howarth R.W., Seitzinger S.P., Asner G.P., Cleveland C.C., Geen P.A., Holland E.A., Karl D.M., Michaels A.F., Porter J.H., Townsend A.R. & Vorosmarty C.J. (2004). Nitrogen cycles: past, present and future. *Biogeochemistry* 70: 153-226.
- Galloway J.N., Schlesinger W.H., Levy H., Michaels A. & Schnoor J.L. (1995). Nitrogen fixation: anthropogenic enhancement – environmental response. *Global Biogeochemical Cycles* 9: 235-252.

- Gash J.H.C., Lloyd C.R. & Lachaud G. (1995). Estimating sparse forest rainfall interception with an analytical model. *Journal of Hydrology* 170: 79-86.
- Gee G.W. & Bauder J.W. (1986). Particle-size analysis. In: Klute A. (Ed.), *Methods of Soil Analysis, Part I: Physical and mineralogical methods*, 2nd ed., ASA & SSSA Madison WI USA, pp. 283-411.
- Genouw G., Coenen C., Sioen G., Neiryneck J. & Roskams P. (2006). Bosgezondheid in Vlaanderen. Onderzoeksresultaten in het kader van het ICP-Forests Programma (Level I & II, meetstation luchtverontreiniging), meetjaar 2005. INBO.R.2006.17. Instituut voor Natuur- en Bosonderzoek, Brussel.
- Gödde M., David M.B., Christ M.J., Kaupenjohann M. & Vance G.F. (1996). Carbon mobilization from the forest floor under red Spruce in the Northeastern U.S.A. *Soil Biology & Biochemistry* 28: 1181-1189.
- Green R.N., Klinka K. & Trowbridge R.L. (1993). Towards a taxonomic classification of humus forms. *Forest Science Monographs* 29: 1-49.
- Greenland D.J. (1971). Interactions between humic and fulvic acids and clays. *Soil Science* 3: 34-41.
- Gu B.H., Schmitt J., Chen Z.H., Liang L.Y. & McCarthy J.F. (1994). Adsorption and desorption of natural organic matter on iron-oxide – Mechanisms and models. *Environmental Science & Technology* 28: 38-46.
- Guggenberger G. (1994). Acidification effects on dissolved organic matter mobility in spruce forest ecosystems. *Environmental International* 20: 31-41.
- Guggenberger G. & Kaiser K. (2003). Dissolved organic matter in soil: challenging the paradigm of sorptive preservation. *Geoderma* 113: 293-310.
- Guggenberger G., Kaiser K. & Zech W. (1998). Mobilization and immobilization of dissolved organic matter in forest soils. *Journal of Plant Nutrition and Soil Science* 161: 401-408.
- Guggenberger G. & Zech W. (1992). Retention of dissolved organic carbon and sulfate in aggregated forest soils. *Journal of Environmental Quality* 21: 643-653.

- Guggenberger G., Zech W. & Schulten H.-R. (1994). Formation and mobilization pathways of dissolved organic matter: evidence from chemical structural studies of organic matter fractions in acid forest floor solutions. *Organic Geochemistry* 21: 51-66.
- Gundersen P., Callesen I. & de Vries W. (1998). Nitrate leaching in forest ecosystems is related to forest floor C/N ratios. *Environmental Pollution* 102: 403-407.
- Hagedorn F., Saurer M. & Blaser P. (2004). A  $^{13}\text{C}$  tracer study to identify the origin of dissolved organic carbon in forested mineral soils. *European Journal of Soil Science* 55: 91-100.
- Hagedorn F. & Schleppi P. (2000). Determination of total dissolved nitrogen by persulfate oxidation. *Journal of Plant Nutrition and Soil Science* 163: 81-82.
- Halstead J.A., Edwards J., Soracco R.J. & Armstrong R.W. (1999). Potential for chlorate interference in ion chromatographic determination of total nitrogen in natural waters following alkaline persulfate digestion. *Journal of Chromatography A* 857: 337-342.
- Hart S.C. & Firestone M.K. (1991). Forest floor-mineral soil interactions in the internal nitrogen cycle of an old-growth forest. *Biogeochemistry* 12: 103-127.
- Harter R.D. & Baker D.E. (1977). Applications and misapplications of Langmuir equation to soil adsorption phenomena. *Soil Science Society of America Journal* 41: 1077-1080.
- Hayes M.H.B. (1986). Extraction of humic substances from soil. In: Aiken G.R., McKnight D.M., Wershaw R.L. & McCarthy P. (Eds.), *Humic substances in soil, sediment, and water – Geochemistry, isolation, and characterization*. Wiley-Interscience, New York, pp. 329-362.
- Hedin L.O., Armesto J.J. & Johnson A.H. (1995). Patterns of nutrient loss from unpolluted, old-growth temperate forests: Evaluation of biogeochemical theory. *Ecology* 76: 493-509.
- Högberg P. (2007). Nitrogen impacts on forest carbon. *Nature* 447:781-782.

- Huygens D., Boeckx P., Vermeulen J., De Paepe X., Park A., Barker S., Pullan C. & Van Cleemput O. (2005). Advances in coupling a commercial total organic carbon analyser with an isotope ratio mass spectrometer to determine the isotopic signal of the total dissolved nitrogen pool. *Rapid Communications in Mass Spectrometry* 19: 3232-3238.
- Huygens D., Boeckx P., Vermeulen J., De Paepe X., Park A., Barker S. & Van Cleemput O. (2005). On-line technique to determine the isotopic composition of total dissolved nitrogen. *Analytical Chemistry* 79: 8644-8649.
- Hyvönen R., Persson T., Andersson S., Olsson B., Ågren G.I. & Linder S. (2007). Impact of long-term nitrogen addition on carbon stocks in trees and soils in northern Europe. *Biogeochemistry* (DOI: 10.1007/s10533-007-9121-3).
- Jackson M.L., Lim C.H. & Zelazny L.W. (1986). Oxides, hydroxides, and aluminosilicates. In: Klute A. (Ed.), *Methods of Soil Analysis, Part I: Physical and mineralogical methods*, 2nd ed., ASA & SSSA Madison WI USA, pp 101-150.
- Jaffe R. (1991). Fate of hydrophobic organic pollutants in the aquatic environment – A review. *Environmental Pollution* 69: 237-257.
- Jansen B., Nierop K.G.J. & Verstraten J.M. (2004). Mobilization of dissolved organic matter, aluminium and iron in podzol eluvial horizons as affected by formation of metal-organic complexes and interactions with solid soil material. *European Journal of Soil Science* 55: 287-297.
- Jansen B., Nierop K.G.J. & Verstraten J.M. (2005). Mechanisms controlling the mobility of dissolved organic matter, aluminium and iron in podzol B horizons. *European Journal of Soil Science* 56: 537-550.
- Jansen J.J., Sevenster J. & Faber P.J. (1996). *Opbrengsttabellen voor belangrijke boomsoorten in Nederland*. IBN rapport nr 221, pp. 1-240.
- Janssens I.A., Sampson D.A., Cermak J., Meiresonne L., Riguzzi F., Overloop S. & Ceulemans R. (1999). Above- and belowground phytomass and carbon storage in a Belgian Scots pine stand. *Annals of Forest Science* 56: 81-90.

- Jara A.A., Goldberg S. & Mora M.L. (2005). Studies of the surface charge of amorphous aluminosilicates using surface complexation models. *Journal of Colloid and Interface Science* 292: 160-170.
- Jardine P.M., Dunnivant F.M., Selim H.M. & McCarthy J.F. (1992). Comparison of models for describing the transport of dissolved organic carbon in aquifer columns. *Soil Science Society of America Journal* 56: 393-401.
- Jardine P.M., Mayes M.A., Mulholland P.J., Hanson P.J., Tarver J.R., Luxmoore R.J., McCarthy J.F. & Wilson G.V. (2006). Vadose zone flow and transport of dissolved organic carbon at multiple scales in humid regimes. *Vadose Zone Journal* 5: 140-152.
- Jardine P.M., Weber N.L. & McCarthy J.F. (1989). Mechanisms of dissolved organic carbon adsorption on soil. *Soil Science Society of America Journal* 53: 1378-1385.
- Jiménez J.J. & Lal, R. (2006). Mechanisms of C sequestration in soils of Latin America. *Critical Reviews in Plant Sciences* 25: 337-365.
- Johnson D.W., Cheng W. & Burke I.C. (2000). Biotic and abiotic nitrogen retention in a variety of forest soils. *Soil Science Society of America Journal* 64: 1503-1514.
- Johnson J.A. & Farmer W.J. (1993). Batch versus column method for determining distribution of organics between soil and water phases. *Soil Science* 155: 92-99.
- Jones D.L. & Willet V.B. (2006). Experimental evaluation of methods to quantify dissolved organic nitrogen (DON) and dissolved organic carbon (DOC) in soil. *Soil Biology & Biochemistry* 38: 991-999.
- Kaiser K. (1998). Fractionation of dissolved organic matter affected by polyvalent metal cations. *Organic Geochemistry* 28: 849-854.
- Kaiser K. (2003). Sorption of natural organic matter fractions to goethite ( $\alpha$ -FeOOH): effect of chemical composition as revealed by liquid-state  $^{13}\text{C}$  NMR and wet-chemical analysis. *Organic Geochemistry* 34: 1569-1579.

- Kaiser K. & Guggenberger G. (2000). The role of DOM sorption to mineral surfaces in the preservation of organic matter in soils. *Organic Geochemistry* 31: 711-725.
- Kaiser K., Guggenberger G. & Haumaier L. (2004). Changes in dissolved lignin-derived phenols, neutral sugars, uronic acids, and amino sugars with depth in forested Haplic Arenosols and Rendzic Leptosols. *Biogeochemistry* 70: 135-151.
- Kaiser K., Guggenberger G., Haumaier L. & Zech W. (1997). Dissolved organic matter sorption on subsoils and minerals studied by  $^{13}\text{C}$ -NMR and DRIFT spectroscopy. *European Journal of Soil Science* 48: 301-310.
- Kaiser K., Guggenberger G., Haumaier L. & Zech W. (2001). Seasonal variations in the chemical composition of dissolved organic matter in organic forest floor layer leachates of old-growth Scots pine (*Pinus sylvestris* L.) and European beech (*Fagus sylvatica* L.) stands in northeastern Bavaria, Germany. *Biogeochemistry* 55: 103-143.
- Kaiser K., Guggenberger G. & Zech W. (1996). Sorption of DOM and DOM fractions to forest soils. *Geoderma* 74: 281-303.
- Kaiser K., Guggenberger G. & Zech W. (2001). Organically bound nutrients in dissolved organic matter fractions in seepage and pore water of weakly developed forest soils. *Acta Hydrochimica et Hydrobiologica* 28: 411-419.
- Kaiser K., Kaupenjohann M. & Zech W. (2001). Sorption of dissolved organic carbon in soils: effects of soil sample storage, soil-to-solution ratio, and temperature. *Geoderma* 99: 317-328.
- Kaiser K. & Zech W. (1997). Competitive sorption of dissolved organic matter fractions to soils and related mineral phases. *Soil Science Society of America Journal* 61: 64-69.
- Kaiser K. & Zech W. (1998). Rates of dissolved organic matter release and sorption in forest soils. *Soil Science* 163: 714-725.
- Kaiser K. & Zech W. (2000). Sorption of dissolved organic nitrogen by acid subsoil horizons and individual mineral phases. *European Journal of Soil Science* 51: 403-411.

- Kalbitz K., Kaiser K., Bargholz J. & Dardenne P. (2006). Lignin degradation controls the production of dissolved organic matter in decomposing foliar litter. *European Journal of Soil Science* 57: 504-516.
- Kalbitz K., Schmerwitz J., Schwesig D. & Matzner E. (2003). Biodegradation of soil-derived dissolved organic matter as related to its properties. *Geoderma* 113: 273-291.
- Kalbitz K., Schwesig D., Schmerwitz J., Kaiser K., Haumaier L., Glaser B., Ellerbrock R. & Leinweber P. (2003). Changes in properties of soil-derived dissolved organic matter induced by biodegradation. *Soil Biology & Biochemistry* 35: 1129-1142.
- Kalbitz K., Solinger S., Park J.-H., Michalzik B. & Matzner E. (2000). Controls on the dynamics of dissolved organic matter in soils: A review. *Soil Science* 165: 277-304.
- Kalra Y.P. & Maynard D.G. (1991). *Methods manual for forest soil and plant analysis*. Forestry Canada, Northwest Region, Northern Forestry Centre, Edmonton, Alberta, pp. 84-94.
- Kanerva S., Kitunen V., Kiikkilä O., Lojonen J. & Smolander A. (2006). Response of soil C and N transformations to tannin fractions originating from Scots pine and Norway spruce needles. *Soil Biology & Biochemistry* 38: 1364-1374.
- Kang H. & Lee D. (2005). Inhibition of extracellular enzyme activities in a forest soil by additions of inorganic nitrogen. *Communications in Soil Science and Plant Analysis* 36: 2129-2135.
- Kaplan L.A. (1994). A field and laboratory procedure to collect, process, and preserve freshwater samples for dissolved organic carbon analysis. *Limnology and Oceanography* 39, 1470-1476.
- Kawahigashi M., Kaiser K., Rodionov A. & Guggenberger G. (2006). Sorption of dissolved organic matter by mineral soils of the Siberian forest tundra. *Global Change Biology* 12: 1868-1877.



- Kieber R.J., Long M.S. & Willey J.D. (2005). Factors influencing nitrogen speciation in coastal rainwater. *Journal of Atmospheric Chemistry* 52: 81-99.
- Kieber R.J., Peake B., Willey J.D. & Avery G.B. (2002). Dissolved organic carbon and organic acids in coastal New Zealand rainwater. *Atmospheric Environment* 36: 3557-3563.
- Kiikkilä O., Kitunen V. & Smolander A. (2005). Degradability of dissolved soil organic carbon and nitrogen in relation to tree species. *FEMS Microbiology Ecology* 53: 33-40.
- Klyosov A.A. (1990). Trends in biochemistry and enzymology of cellulose degradation. *Biochemistry* 47: 10577-10585.
- Koprivnjak J.F., Perdue E.M. & Pfromm P.H. (2006). Coupling reverse osmosis with electrodialysis to isolate natural organic matter from fresh waters. *Water Research* 40: 3385-3392.
- Koroleff F. (1983). Simultaneous oxidation of nitrogen and phosphorus compounds by persulphate. In: Grasshoff K., Eberhardt M. & Kremling K. (Eds.), *Methods of seawater analysis*. Verlag Chemie, Weinheim, Germany, 1983, pp. 168-169.
- Kowalenko C.G. & Babuin D. (2003). Re-evaluation of factors affecting total nitrogen measurements in soil extract solutions using microwave-assisted persulfate oxidation. *Communications in Soil Science and Plant Analysis* 34: 2745-2762.
- Kozdroj J. & van Elsas J.D. (2001). Structural diversity of microorganisms in chemically perturbed soil assessed by molecular and cytochemical approaches. *Journal of Microbiological Methods* 43: 197-212.
- Kristensen H.L. & McCarty G.W. (1999). Mineralization and immobilization of nitrogen in heath soil under intact *Calluna*, after heather beetle infestation and nitrogen fertilization. *Applied Soil Ecology* 13: 187-198.
- Kumar K.V. & Sivanesan S. (2005). Comparison of linear and non-linear method in estimating the sorption isotherm parameters for safranin onto activated carbon. *Journal of Hazardous Materials B* 123: 288-292.

- Lajtha K., Crow S.E., Yano Y., Kaushal S.S., Sulzman E., Sollins P. & Spears J.D.H. (2005). Detrital controls on soil solution N and dissolved organic matter in soils: a field experiment. *Biogeochemistry* 76: 261-281.
- Langmuir I. (1918). The adsorption of gasses on plane surfaces of glass, mica and platinum. *Journal of the American Chemical Society* 40: 1361-1382.
- Langouche D., Wiedemann T., Van Ranst E., Neiryneck J. & Langohr R. (2002). Berekening en kartering van kritische lasten en overschrijdingen voor verzuring en eutrofiëring in bosccosystemen in Vlaanderen. In: Neiryneck J., de Ridder K., Langouche D., Wiedemann T., Kowalski A., Ceulemans R., Mensinck C., Roskams P. & Van Ranst E. (Eds.). *Bepaling van de verzurings- en vermestingsgevoeligheid van Vlaamse bossen met gemodelleerde depositiefluxen. Eindverslag van project VLINA 98/01, studie uitgevoerd voor rekening van de Vlaamse Gemeenschap binnen het kader van het Vlaamse Impulsprogramma Natuurontwikkeling.*
- Lee W. & Westerhoff P. (2005). Dissolved organic nitrogen measurement using dialysis pretreatment. *Environmental Science & Technology* 39: 879-884.
- Leenheer J.A. (1981). Comprehensive approach to preparative isolation and fractionation of dissolved organic carbon from natural waters and waste water. *Environmental Science & Technology* 15: 578-587.
- Lennon J.T. & Pfaff L.E. (2005). Source and supply of terrestrial organic matter affects aquatic microbial metabolism. *Aquatic Microbial Ecology* 39: 107-119.
- Lensi R., Lescure C., Steinberg C., Savoie J.M. & Faurie G. (1991). Dynamics of residual enzyme activities, denitrification potential, and physico-chemical properties in a  $\gamma$ -sterilized soil. *Soil Biology & Biochemistry* 23: 367-373.
- Li Z. & Shuman L.M. (1997). Estimation of retardation factor of dissolved organic carbon in sandy soils using batch experiments. *Geoderma* 78: 197-206.
- Lilienfein J., Qualls R.G., Uselman S.M. & Bridgham S.D. (2004a). Adsorption of dissolved organic carbon and nitrogen in soils of a weathering chronosequence. *Soil Science Society of America Journal* 68: 292-305.

- Lilienfein J., Qualls R.G., Uselman S.M. & Bridgham S.D. (2004b). Adsorption of dissolved organic and inorganic phosphorus in soils of a weathering chronosequence. *Soil Science Society of America Journal* 68: 620-628.
- Lilleskov E.A., Fahey T.J. & Lovett G.M. (2001). Ecotmycorrhizal fungal aboveground community change over an atmospheric nitrogen deposition gradient. *Ecological Applications* 11: 397-410.
- Loague K. & Green R.E. (1991). Statistical and graphical methods for evaluating solute transport models: Overview and application. *Journal of Contaminant Hydrology* 7: 51-73.
- Lovett G.M. & Lindberg S.E. (1993). Atmospheric deposition and canopy interactions of nitrogen in forests. *Canadian Journal of Forest Research* 23: 1603-1616.
- Lovley D.R., Fraga J.L., Blunt-Harris E.L., Hayes L.A., Philips E.J.P. & Coates J.D. (1998). Humic substances as a mediator for microbially catalyzed metal reduction. *Acta Hydrochimica et Hydrobiologica* 26: 152-157.
- Lu Y.H., Wassmann R., Neue H.U. & Huang C.Y. (2000). Dynamics of dissolved organic carbon and methane emissions in a flooded rice soil. *Soil Science Society of America Journal* 64: 2011-2017.
- Lundström U.S. (1993). The role of organic acids in the soil solution chemistry of a podzolized soil. *Journal of Soil Science* 44: 121-133.
- Lundström U.S., van Breemen N. & Bain D. (2000). The podzolization process. A review. *Geoderma* 94: 91-107.
- Lundström U.S., van Breemen N. & Iongmans A.G. (1995). Evidence for microbial decomposition of organic acids during podzolization. *European Journal of Soil Science* 46: 489-496.
- Magill A.H. & Aber J.D. (2000). Dissolved organic carbon and nitrogen relationships in forest litter as affected by nitrogen deposition. *Soil Biology & Biochemistry* 32: 603-613.
- Magill A.H., Aber J.D., Berntson G.M., McDowell W.H., Nadelhoffer K.J., Melillo J.M. & Steudler P. (2000). Long-term nitrogen additions and nitrogen saturation in two temperate forests. *Ecosystems* 3: 238-253.

- Magill A.H., Aber J.D., Currie W.S., Nadelhoffer K.J., Martin M.E., McDowell W.H., Melillo J.M. & Steudler P. (2004). Ecosystem response to 15 years of chronic nitrogen additions at the Harvard Forest LTER, Massachusetts, USA. *Forest Ecology and Management* 196: 7-28.
- Magill A.H., Aber J.D., Hendricks J.J., Bowden R.D., Melillo J.M. & Steudler P.A. (1997). Biogeochemical response of forest ecosystems to simulated chronic nitrogen deposition. *Ecological Applications* 7: 402-415.
- Marchand P. & Houle G. (2006). Spatial patterns of plant species richness along a forest edge: What are their determinants? *Forest Ecology and Management* 223: 113-124.
- Marschner B. & Bredow A. (2002). Temperature effects on release and ecologically relevant properties of dissolved organic carbon in sterilized and biologically active soil samples. *Soil Biology & Biochemistry* 34: 459-466.
- Marschner B. & Kalbitz K. (2003). Controls of bioavailability and biodegradability of dissolved organic matter in soils. *Geoderma* 113: 211-235.
- Marschner P., Kandeler E. & Marschner B. (2003). Structure and function of the soil microbial community in a long-term fertilizer experiment. *Soil Biology & Biochemistry* 35: 453-461.
- Marschner B. & Noble A.D. (2000). Chemical and biological processes leading to the neutralisation of acidity in soil incubated with litter materials. *Soil Biology & Biochemistry* 32: 805-813.
- Matzner E. (1988). Der Stoffumsatz zweier Waldökosysteme im Solling. Ber. d. Forschungszentrums Waldökosysteme/Waldsterben d. Uni Göttingen, Reihe A, Bd. 1988; 40:1-217.
- Mayer O. (1993). Functional groups of microorganisms. In: Schulze E.-D. & Mooney H.A. (Eds.). *Biodiversity and ecosystem function*. Ecological Studies 99, Springer, Berlin Heidelberg, New York, pp. 67-96.
- McCracken K.L., McDowell W.H., Harter R.D. & Evans C.V. (2002). Dissolved organic carbon retention in soils: comparison of solution and soil measurements. *Soil Science Society of America Journal* 66: 563-568.

- McDonald J.A., Dise N.B., Matzner E., Armbruster M., Gundersen P. & Forsius M. (2002). Nitrogen input together with ecosystem nitrogen enrichment predict nitrate leaching from European forests. *Global Change Biology* 8: 1028-1033.
- McDowell W.H. (2003). Dissolved organic matter in soils – Future directions and unanswered questions. *Geoderma* 113: 179-186.
- McDowell W.H., Currie W.S., Aber J.D. & Yano Y. (1998). Effects of chronic nitrogen amendments on production of dissolved organic carbon and nitrogen in forest soils. *Water, Air, and Soil Pollution* 105: 175-182.
- McDowell W.H. & Likens G.E. (1988). Origin, composition and flux of dissolved organic carbon in the Hubbard Brook Valley. *Ecological Monographs* 58:177-195.
- McDowell W.H., Magill A.H., Aitkenhead-Peterson J.A., Aber J.D., Merriam J.L. & Kaushal S.J. (2004). Effects of chronic nitrogen amendment on dissolved organic matter and inorganic nitrogen in soil solution. *Forest Ecology and Management* 196: 29-41.
- McDowell W.H., Zsolnay A., Aitkenhead-Peterson J.A., Gregorich E.G., Jones D.L., Jödemann D., Kalbitz K., Marschner B. & Schwesig D. (2006). A comparison of methods to determine the biodegradable dissolved organic carbon from different terrestrial sources. *Soil Biology & Biochemistry* 38: 1933-1942.
- McGill W.B. & Cole C.V. (1981). Comparative aspects of cycling of organic C, N, S and P through soil organic matter. *Geoderma* 26: 267-286.
- McKeague J.A., Ross G.J. & Gamble D.S. (1978). Properties, criteria of classification and genesis of podzolic soils in Canada. In: Mahaney W.C. (Ed.), *Quaternary Soils*. Geo Abstracts, Norwich, pp. 27-60.
- McKenna J.H. & Doering P.H. (1995). Measurement of dissolved organic carbon by wet chemical oxidation with persulfate: influence of chloride concentration and reagent volume. *Marine Chemistry* 48: 109-114.

- McNamara N.P., Black H.I.J., Beresford N.A. & Parekh N.R. (2003). Effect of acute gamma irradiation on chemical, physical and biological properties of soils. *Applied Soil Ecology* 24: 117-132.
- Mendoza R.E. & Barrow N.J. (1987). Characterizing the rate of reaction of some Argentinian soils with phosphate. *Soil Science* 143: 105-112.
- Menyailo O.V., Hungate B.A. & Zech W. (2002). Tree species mediated soil chemical changes in a Siberian artificial afforestation experiment – Tree species and soil chemistry. *Plant and Soil* 242: 171-182.
- Menzel D.W. & Vaccaro R.F. (1964). The measurement of dissolved organic and particulate carbon in seawater. *Limnology and Oceanography* 9: 138-142.
- Mercer D.K., Iqbal M., Miller P.G.G. & McCarthy A.J. (1996). Screening actinomycetes for extracellular peroxidase activity. *Applied and Environmental Microbiology* 62: 2186-2190.
- Merckx R., Brans K. & Smolders E. (2001). Decomposition of dissolved organic carbon after soil drying and rewetting as an indicator of metal toxicity in soils. *Soil Biology & Biochemistry* 33: 235-240.
- Merriam J., McDowell W.H. & Currie W.S. (1996). A high-temperature catalytic oxidation technique for determining total dissolved nitrogen. *Soil Science Society of America Journal* 60: 1050-1055.
- Metting F.B. (1993). Structure and physiological ecology of soil microbial communities. In: Metting F.B. (Ed.), *Soil Microbia Ecology – Application in Agricultural and Environmental Management*. Marcel Dekker, New York, pp 3-24.
- Michalzik B., Kalbitz K., Park J.-H., Solinger S. & Matzner E. (2001). Fluxes and concentrations of dissolved organic carbon and nitrogen – a synthesis for temperate forests. *Biogeochemistry* 52: 173-205.
- Michalzik B. & Matzner E. (1999). Dynamics of dissolved organic nitrogen and carbon in a Central European Norway spruce ecosystem. *European Journal of Soil Science* 50: 579-590.
- Michalzik B., Tipping E., Mulder J., Gallardo J.F., Lancho E., Matzner E., Bryant C.L., Clarke N., Lofts S. & Vicente Esteban M.A. (2003). Modelling the

- production and transport of dissolved organic carbon in forest soils. *Biogeochemistry* 66: 241-264.
- Michel K. & Matzner E. (1999). Release of dissolved organic carbon and nitrogen from forest floors in relation to solid phase properties, respiration and N-mineralization. *Journal of Plant Nutrition and Soil Science* 162: 645-652.
- Michel K. & Matzner E. (2002). Nitrogen content of forest floor Oa layers affects carbon pathways and nitrogen mineralization. *Soil Biology & Biochemistry* 34: 1807-1813.
- Michel K., Matzner E., Dignac M.-F. & Kögel-Knabner I. (2006). Properties of dissolved organic matter related to soil organic matter quality and nitrogen additions in Norway spruce forest floors. *Geoderma* 130: 250-264.
- Miller D.M., Summer M.E. & Miller W.P. (1989). A comparison of batch- and flow-generated anion adsorption isotherms. *Soil Science Society of America Journal* 53: 373-380.
- MIRA (2006). Milieu- en natuurrapport Vlaanderen, Achtergronddocument 2006 Vermesting. In: Overloop S., Bossuyt M., Buysse M., Ducheyne S., Dumortier M., Eppinger R., Genouw G., Stengée K., Van Gijseghe D., Van Hoof K., Vogels N., Vanden Auweele W., Wustenberghs H., D'hooghe J. & Fernagut B. (Eds.) Vlaamse Milieumaatschappij, [www.milieurapport.be](http://www.milieurapport.be).
- Moliner-Martínez Y., Campíns-Falcó P. & Herráez-Hernández R. (2006). Influence of the presence of surfactants and humic acids in waters on the indophenol-type reaction method for ammonium determination. *Talanta* 69: 1038-1045.
- Møller J., Miller M. & Kjoller A. (1999). Fungal-bacterial interaction in beech leaves: influence on decomposition and dissolved organic carbon quality. *Soil Biology & Biochemistry* 31: 367-374.
- Moore T.R. & Matos L. (1999). The influence of source on the sorption of dissolved organic carbon by soils. *Canadian Journal of Soil Science* 79: 321-324.

- Moran W.M., Denton J., Wilson K., Williams M. & Runge S.W. (1999). A simple, inexpensive method for teaching how membrane potentials are generated. *Advances in Physiology Education* 22: S51-S59.
- Münch J.-M., Totsche K.U. & Kaiser K. (2002). Physicochemical factors controlling the release of dissolved organic carbon from columns of forest subsoils. *European Journal of Soil Science* 53: 311-320.
- Nagy N.M. & Kónya J. (2007). Study of pH-dependent charges of soils by surface acid-base properties. *Journal of Colloid and Interface Science* 305: 94-100.
- Nakamura T., Ogawa H., Maripi D.K. & Uematsu M. (2006). Contribution of water soluble organic nitrogen to total nitrogen in marine aerosols over the East China Sea and western North Pacific. *Atmospheric Environment* 40: 7259-7264.
- Näsholm T., Ekblad A., Nordin A., Giesler R., Högberg M. & Högberg P. (1998). Boreal plants take up organic nitrogen. *Nature* 392:914-916.
- Nätscher L. & Schwertmann U. (1991). Proton buffering in organic horizons of acid forest soils. *Geoderma* 48: 93-106.
- Neff J.C. & Asner G.P. (2001). Dissolved organic carbon in terrestrial ecosystems: synthesis and a model. *Ecosystems* 4: 29-48.
- Neff J.C., Holland E.A., Dentener F.J., McDowell W.H. & Russell K.M. (2002). The origin, composition and rates of organic nitrogen deposition: A missing piece of the nitrogen cycle? *Biogeochemistry* 57/58: 99-136.
- Neiryck J., Genouw G., Coenen S. & Roskams P. (2004). Depositie en luchtkwaliteit in Vlaamse bosgebieden. Instituut voor Bosbouw & Wildbeheer, mededelingen 2004 – 1; pp. 71.
- Neiryck J., Kowalski A.S., Carrara A., Genouw G., Berghmans P. & Ceulemans R. (2007). Fluxes of oxidised and reduced nitrogen above a mixed coniferous forest exposed to various nitrogen emission sources. *Environmental Pollution* 149: 31-43.
- Neiryck J., Maddelein D., de Keersmaecker L., Lust N. & Muys B. (1998). Biomass and nutrient cycling of a highly productive Corsican pine stand on



- former heathland in northern Belgium. *Annales des Sciences Forestières* 55: 389-405.
- Neiryneck J., Van Ranst E., Roskams P. & Lust N. (2002). Impact of decreasing throughfall depositions on soil solution chemistry at coniferous monitoring sites in northern Belgium. *Forest Ecology and Management* 160: 127-142.
- Nierop K.G.J., Jansen B. & Verstraten J.M. (2002). Dissolved organic matter, aluminium and iron interactions: precipitation induced by metal/carbon ratio, pH and competition. *Science of the Total Environment* 300: 201-211.
- Nilsson J. & Grennfelt P. (1988). Critical loads for sulphur and nitrogen. Report from a workshop held at Sklokloster, Sweden, March 19-24, Copenhagen, Denmark: Miljø report 15.
- Nilsson S.I. & Bergkvist B. (1983). Aluminium chemistry and acidification processes in a shallow podzol on the Swedish westcoast. *Water, Air, and Soil Pollution* 20: 311-329.
- Nodvin S.C., Driscoll C.T. & Likens G.E. (1986). Simple partitioning of anions and dissolved organic carbon in a forest soil. *Soil Science* 142: 27-35.
- Norman R.J., Kurtz L.T. & Stevenson F.J. (1987). Solubilization of soil organic matter by liquid anhydrous ammonia. *Soil Science Society of America Journal* 51: 809-812.
- Ogawa H., Amagai Y., Koike I., Kaiser K. & Benner R. (2001). Production of refractory dissolved organic matter by bacteria. *Science* 292: 917-920.
- Ogawa H. & Tanoue E. (2003). Dissolved organic matter in oceanic waters. *Journal of Oceanography* 59: 129-147.
- Pabich W.J., Valiela I. & Hemond H.F. (2001). Relationship between DOC concentration and vadose zone thickness and depth below water table in groundwater of Cape Cod, USA. *Biogeochemistry* 55: 247-268.
- Parfitt R.L., Fraser A.R. & Farmer V.C. (1977). Adsorption of hydrous oxides. III. Fulvic and humic acid on goethite, gibbsite, and imogolite. *Journal of Soil Science* 28: 289-296.

- Park J.-H., Kalbitz K. & Matzner E. (2002). Resource control on the production of dissolved organic carbon and nitrogen in a deciduous forest floor. *Soil Biology & Biochemistry* 34: 813-822.
- Park J.-H. & Matzner E. (2003). Controls on the release of dissolved organic carbon and nitrogen from a deciduous forest floor investigated by manipulations of aboveground litter inputs and water flux. *Biogeochemistry* 66: 265-286.
- Park J.-H. & Matzner E. (2006). Detrital control on the release of dissolved organic nitrogen and dissolved inorganic nitrogen (DIN) from forest floor under chronic N deposition. *Environmental pollution* 143: 178-185.
- Pennanen T., Fröstegård A., Fritze H. & Bååth E. (1996). Phospholipid fatty acid composition and heavy metal tolerance of soil microbial communities along two heavy metal-polluted gradients in coniferous forests. *Applied and Environmental Microbiology* 62: 420-428.
- Pennanen T., Liski J., Bååth E., Kitunen V., Uotila J., Westman C.J. & Fritze H. (1999). Structure of the microbial communities in coniferous forest soils in relation to site fertility and stand development stage. *Microbial Ecology* 38: 168-179.
- Perakis S.S. & Hedin L.O. (2002). Nitrogen loss from unpolluted South American forests mainly via dissolved organic compounds. *Nature* 415: 416-419.
- Perdue E.M. & Ritchie J.D. (2003). Dissolved organic matter in freshwaters. In: Drever J.I., Holland H.D. & Turekian K.K. (Eds.), *Surface and Ground Water, Weathering, and Soils*, Vol. 5, *Treatise on Geochemistry*. Elsevier-Pergamon, Oxford, pp. 273-318.
- Persson T. & Wiren A. (1995). Nitrogen mineralization and potential nitrification at different depths in acid forest soils. *Plant and Soil* 168: 55-65.
- Pilkington M.G., Caporn S.J.M., Carroll J.A., Cresswell N., Lee J.A., Ashenden T.W., Brittain S.A., Reynolds B. & Emmett B.A. (2005). Effects of increased deposition of atmospheric nitrogen on an upland moor: leaching of N species and soil solution chemistry. *Environmental Pollution* 135: 29-40.

- Pizzeghello D., Nicolini G. & Nardi S. (2002). Hormone-like activities of humic substances in different forest ecosystems. *New Phytologist* 155: 393-402.
- Prechtel A., Alewell C., Michalzik B. & Matzner E. (2000). Different effect of drying on the fluxes of dissolved organic carbon and nitrogen from a Norway spruce forest floor. *Journal of Plant Nutrition and Soil Science* 163: 517-521.
- Pregitzer K.S., Zak D.R., Burton A.J., Ashby J.A. & MacDonald N.W. (2004). Chronic nitrate additions dramatically increase the export of carbon and nitrogen from northern hardwood ecosystems. *Biogeochemistry* 68: 179-197.
- Qualls R.G. (2000). Comparison of the behavior of soluble organic and inorganic nutrients in forest soils. *Forest Ecology and Management* 138: 29-50.
- Qualls R.G. (2004). Biodegradability of humic substances and other fractions of decomposing leaf litter. *Soil Science Society of America Journal* 168: 1705-1712.
- Qualls R.G. & Bridgman S.D. (2005). Mineralization rate of  $^{14}\text{C}$ -labelled dissolved organic matter from leaf litter in soils of a weathering chronosequence. *Soil Biology & Biochemistry* 37: 905-916.
- Qualls R.G. & Haines B.L. (1991). Geochemistry of dissolved organic nutrients in water percolating through a forest ecosystem. *Soil Science Society of America Journal* 55: 1112-1123.
- Qualls R.G. & Haines B.L. (1992a). Biodegradability of dissolved organic matter in forest throughfall, soil solution and stream water. *Soil Science Society of America Journal* 56: 578-586.
- Qualls R.G. & Haines B.L. (1992b). Measuring adsorption isotherms using continuous, unsaturated flow through intact soil cores. *Soil Science Society of America Journal* 56: 456-460.
- Qualls R.G., Haines B.L. & Swank W.T. (1991). Fluxes of dissolved organic nutrients and humic substances in a deciduous forest. *Ecology* 72: 254-266.
- Qualls R.G., Haines B.L., Swank W.T. & Tyler S.W. (2002). Retention of soluble organic nutrients by a forested ecosystem. *Biogeochemistry* 61: 135-171.

- Raastad I.A. & Mulder J. (1999). Dissolved organic matter (DOM) in acid forest soils at Gårdsjön (Sweden): natural variabilities and effects of increased input of nitrogen and of reversal of acidification. *Water, Air, and Soil Pollution* 114: 199-219.
- Raulund-Rasmussen K., Borggaard O.K., Hansen H.C.B. & Olsson M. (1998). Effect of natural organic soil solutes on weathering rates of soil minerals. *European Journal of Soil Science* 49: 397-406.
- Ravichandran M. (2004). Interactions between mercury and dissolved organic matter – a review. *Chemosphere* 55: 319-331.
- Reemtsma T., Bredow A. & Gehring M. (1999). The nature and kinetics of organic matter release from soil by salt solutions. *European Journal of Soil Science* 50: 53-64.
- Rogora M., Minella M., Orrù A. & Tartari G.A. (2006). A comparison between high-temperature catalytic oxidation and persulphate oxidation for the determination of total nitrogen in freshwater. *International Journal of Environmental Analytical Chemistry* 86: 1065-1078.
- Rothe A., Huber C., Kreutzer K. & Weis W. (2002). Deposition and soil leaching in stands of Norway spruce and European beech: results from the Höglwald research in comparison with other European case studies. *Plant and Soil* 240: 33-45.
- Runge S.W., Shelton K.R., Melton S.A. & Moran W.M. (2005). Maintaining the ionic permeability of a cellulose ester membrane. *Journal of Biochemical and Biophysical Methods* 64: 200-206.
- Saiya-Cork K.R., Sinsabaugh R.L. & Zak D.R. (2002). The effects of long term nitrogen deposition on extracellular enzyme activity in an *Acer saccharum* forest soil. *Soil Biology & Biochemistry* 34: 1309-1315.
- Scheel T., Dörfler C. & Kalbitz K. (2007). Precipitation of dissolved organic matter by aluminum stabilizes carbon in acidic forest soils. *Soil Science Society of America Journal* 71: 64-74.
- Schmidt S.K., Costello E.K., Nemergut D.R., Cleveland C.C., Reed S.C., Weintraub M.N., Meyer A.F. & Martin A.M. (2007). Biogeochemical

- consequences of rapid microbial turnover and seasonal succession in soil. *Ecology* 88: 1379-1385.
- Schröter D., Wolters V. & De Ruiter P.C. (2003). C and N mineralisation in the decomposer food webs of a European forest transect. *Oikos* 102: 294-308.
- Schulten H.-R. & Schnitzer M. (1998). The chemistry of soil organic nitrogen: a review. *Biology and Fertility of Soils* 26: 1-15.
- Schulze E.-D. (2000). The carbon and nitrogen cycle of forest ecosystems. In: Schulze E.-D. (Ed.). *Carbon and Nitrogen cycling in European forest ecosystems*. Springer-Verlag Berlin Heidelberg, pp. 3-13.
- Schwesig D., Kalbitz K. & Matzner E. (2003a). Effects of aluminium on the mineralization of dissolved organic carbon derived from forest floors. *European Journal of Soil Science* 54: 311-322.
- Schwesig D., Kalbitz K. & Matzner E. (2003b). Mineralization of dissolved organic carbon in mineral soil solution of two forest soils. *Journal of Plant Nutrition and Soil Science* 166: 585-593.
- Serkiz S.M. & Perdue E.M. (1990). Isolation of dissolved organic matter from the Suwannee River using reverse osmosis. *Water Research* 24: 911-916.
- Shapiro J. (1957). Chemical and biological studies on the yellow organic acids of lake water. *Limnology and Oceanography* 2: 161-179.
- Sharp J.H. (1997). Marine dissolved organic carbon: are the older values correct? *Marine Chemistry* 56: 265-277.
- Sharp J.H. (2002). Analytical methods for dissolved organic carbon, nitrogen, and phosphorus. In: Hansell D.A. & Carlson C.A. (Eds.). *Biogeochemistry of Marine Dissolved organic matter*, Academic Press, San Diego, pp. 35-90.
- Sharp J.H., Beauregard A.Y., Burdige D., Cauwet G., Curless S.E., Lauck R., Nagel K., Ogawa H., Parker A.E., Primm O., Pujo-Pay M., Savidge W.B., Seitzinger S., Spyres G. & Styles R. (2004). A direct instrument comparison for measurement of total dissolved nitrogen in seawater. *Marine Chemistry* 84: 181-193.
- Siemens J., Haas M. & Kaupenjohann M. (2003). Dissolved organic matter induced denitrification in subsoils and aquifers? *Geoderma* 113: 253-271.

- Siemens J. & Kaupenjohann M. (2002). Contribution of dissolved organic nitrogen to N leaching from four German agricultural soils. *Journal of Plant Nutrition and Soil Science* 165: 675-681.
- Sinsabaugh R.L., Carreiro M.M. & Repert D.A. (2002). Allocation of extracellular enzymatic activity in relation to litter composition, N deposition, and mass loss. *Biogeochemistry* 60: 1-24.
- Sinsabaugh R.L., Zak D.R., Gallo M., Lauber C. & Amonette R. (2004). Nitrogen deposition and dissolved organic carbon production in northern temperate forests. *Soil Biology & Biochemistry* 36: 1509-1515.
- Sjöberg G., Bergkvist B., Berggren D. & Nilsson S.I. (2003). Long-term N addition effects on the C mineralization and DOC production in mor humus under spruce. *Soil Biology & Biochemistry* 35: 1305-1315.
- Skyllberg U. & Magnusson T. (1995). Cations adsorbed to soil organic matter – A regulatory factor for the release of organic carbon and hydrogen ions from soils to waters. *Water, Air, and Soil Pollution* 85: 1095-1100.
- Smart M.M., Rada R.G. & Donnermeyer G.N. (1983). Determination of total nitrogen in sediments and plants using persulfate digestion - an evaluation and comparison with the Kjeldahl procedure. *Water Research* 17: 1207-1211.
- Smart M.M., Reid F.A. & Jones J.R. (1981). A comparison of a persulfate digestion and the Kjeldahl procedure for determination of total nitrogen in fresh-water samples. *Water Research* 15: 919-921.
- Smemo K.A., Zak D.R. & Pregitzer K.S. (2006). Chronic experimental  $\text{NO}_3^-$  deposition reduces the retention of leaf litter DOC in a northern hardwood forest soil. *Soil Biology & Biochemistry* 38: 1340-1347.
- Smemo K.A., Zak D.R., Pregitzer K.S. & Burton A.J. (2007). Characteristics of DOC exported from northern hardwood forests receiving chronic experimental  $\text{NO}_3^-$  deposition. *Ecosystems* 10: 369-379.
- Smith W. (1981). *Air pollution and forests: interactions between air contaminants and forest ecosystems*. New York: Springer Verlag, 397 p.

- Smolander A. & Kitunen V. (2002). Soil microbial activities and characteristics of dissolved organic C and N in relation to tree species. *Soil Biology & Biochemistry* 34: 651-660.
- Solinger S., Kalbitz K. & Matzner E. (2001). Controls on the dynamics of dissolved organic carbon and nitrogen in a Central European deciduous forest. *Biogeochemistry* 55: 327-349.
- Søndergaard M. & Worm J. (2001). Measurement of biodegradable dissolved organic carbon (BDOC) in lake water with a bioreactor. *Water Research* 35: 2505-2513.
- Spangenberg A. & Kölling C. (2004). Nitrogen deposition and nitrate leaching at forest edges exposed to high ammonia emissions in Southern Bavaria. *Water, Air, and Soil Pollution* 152: 233-255.
- Ste-Marie C. & Paré D. (1999). Soil, pH and N availability effects on net nitrification in the forest floors of a range of boreal forest stands. *Soil Biology & Biochemistry* 31: 1579-1589.
- Stevens P.A. & Wannop C.P. (1987). Dissolved organic nitrogen and nitrate in an acid forest soil. *Plant and Soil* 102: 137-139.
- Stutter M.I., Lumsdon G. & Thoss V. (2007). Physico-chemical and biological controls on dissolved organic matter in peat aggregate columns. *European Journal of Soil Science* 58: 646-657.
- Suberkropp K. & Weyers H. (1996). Application of fungal and bacterial production methodologies to decomposing leaves in streams. *Applied and Environmental Microbiology* 62: 1610-1615.
- Sugimura Y. & Suzuki Y. (1988). A high temperature catalytic oxidation method for the determination of no-volatile dissolved organic carbon in seawater by direct injection of a liquid sample. *Marine Chemistry* 41: 105-131.
- Sun L., Perdue E.M. & McCarthy J.F. (1995). Using reverse osmosis to obtain organic matter from surface and ground waters. *Water Research* 29: 1471-1477.
- Suominen K., Kitunen V. & Smolander A. (2003). Characteristics of dissolved organic matter and phenolic compounds in forest soils under silver birch

- (*Betula pendula*), Norway spruce (*Picea abies*) and Scots pine (*Pinus sylvestris*). European Journal of Soil Science 54: 287-293.
- Tiktak A. & Bouten W. (1994). Soil water dynamics and long-term water balances of a Douglas fir stand in The Netherlands. Journal of Hydrology 156:265-283.
- Tipping E. (1998). Modelling the properties and behaviour of dissolved organic matter in soils. Mitteilungen der Deutsche Bodenkundliche Gesellschaft 87: 237-252.
- Tipping E. & Woof C. (1990). Humic substances in acid organic soils – modeling their release to the soil solution in terms of humic charge. Journal of Soil Science 41: 573-586.
- Tipping E., Woof C., Rigg E., Harrison A.F., Inneson P., Taylor K., Benham D., Poskitt J., Rowland A.P., Bol R. & Harkness D.D. (1999). Climatic influences on the leaching of dissolved organic matter from upland UK moorland soils investigated by a field manipulation experiment. Environment International 25: 83-95.
- Travis C.C. & Etnier E.L. (1981). A survey of sorption relationships for reactive solutes in soil. Journal of Environmental Quality 10: 8-17.
- Trigo C. & Ball A.S. (1994). Is the solubilized product from the degradation of lignocellulose by actinomycetes a precursor of humic substances? Microbiology-UK 140: 3145-3152.
- Trulleyová S. & Rulík M. (2004). Determination of biodegradable dissolved organic carbon in waters: comparison of batch methods. Science of the Total Environment 332: 253-260.
- Tukey H.B. (1970). The leaching of substances from plants. Annual Review of Plant Physiology 21: 305-324.
- Ugolini F.C. & Dahlgren R.A. (1987). The mechanism of podzolisation as revealed through soil solution studies. In: Righi D. & Chauvel A. (Eds.), Podzols et podzolisation, Comptes Rendus de la Table Ronde Internationale. Institut National de la Recherche Agronomique, France, pp. 195-203.



- Uselman S.M., Qualls R.G. & Lilienfein J. (2007). Contribution of root vs. leaf litter to dissolved organic carbon leaching through soil. *Soil Science Society of America Journal* 71: 1555-1563.
- Ussiri D.A.N. & Johnson C.E. (2004). Sorption of organic carbon fractions by spodosol mineral horizons. *Soil Science Society of American Journal* 68: 253-262.
- van Breemen N. (2002). Natural organic tendency. *Nature* 415: 381-382.
- Vance G.F. & David M.B. (1989). Effect of acid treatment on dissolved organic carbon retention by a spodic horizon. *Soil Science Society of America Journal* 53: 1242-1247.
- Vance G.F. & David M.B. (1991). Chemical characteristics and acidity of soluble organic substances from a northern hardwood forest floor, central Maine, USA. *Geochimica et Cosmochimica Acta* 55: 3611-3625.
- Vance G.F. & David M.B. (1992). Dissolved organic carbon and sulfate sorption by spodosol mineral horizons. *Soil Science* 154, 136-144.
- Van Dam J.C. (2000). Field-scale water flow and solute transport. SWAP model concepts, parameter estimation, and case studies. PhD-thesis, Wageningen University, Wageningen, The Netherlands, 167 p., English and Dutch summaries.
- Van Dam J.C., Huygen J., Wesseling J.G., Feddes R.A., Kabat P., Van Walsum P.E.V., Groendendijk P. & Van Diepen C.A. (1997). Simulation of water flow, solute transport and plant growth in the soil-water-atmosphere-plant environment. Theory of SWAP version 2.0. SC-DLO, Technical Document 45, Wageningen, The Netherlands.
- Vandenbruwane J., De Neve S., Qualls R.G., Salomez J. & Hofman G. (2007a). Optimization of dissolved organic nitrogen (DON) measurements in aqueous samples with high inorganic nitrogen concentrations. *Science of the Total Environment* 386: 103-113.
- Vandenbruwane J., De Neve S., Qualls R.G., Sleutel S. & Hofman G. (2007b). Comparison of different isotherm models for dissolved organic carbon

- (DOC) and nitrogen (DON) sorption to mineral soil. *Geoderma* 139: 144-153.
- van der Salm C., de Vries W., Reinds G.J. & Dise N.B. (2007). N leaching across European forests: Derivation and validation of empirical relationships using data from intensive monitoring plots. *Forest Ecology and Management* 238: 81-91.
- Vande Walle I., Mussche S., Samson R., Lust N. & Lemeur R. (2001). The above- and belowground carbon pools of two mixed deciduous forest stands located in East-Flanders (Belgium). *Annals of Forest Science* 58: 507-517.
- Vande Walle I., Van Camp N., Perrin D., Lemeur R., Verheyen K., Van Wesemael B. & Laitat E. (2005). Growing stock-based assessment of the carbon stock in the Belgian forest biomass. *Annals of Forest Science* 62: 853-864.
- van Genuchten M.Th. (1980). A closed-form equation for predicting the hydraulic conductivity of unsaturated soils. *Soil Science Society America Journal* 44: 892-898.
- van Hees P.A.W., Jones D.L. & Godbold D.L. (2002). Biodegradation of low molecular weight organic acids in coniferous forest podzolic soils. *Soil Biology & Biochemistry* 34: 1261-1272.
- van Hees P.A.W., Lundström U.S. & Giesler R. (2000). Low molecular weight organic acids and their Al-complexes in soil solution – composition, distribution and seasonal variation in three podzolized soils. *Geoderma* 94: 173-200.
- Vervaet H. (2003). Interaction between ecosystems: The influence of intensive agriculture on  $\text{NH}_x$  deposition and N transformation processes in forest soils. Ph.D. thesis, Ghent University, Belgium, 213 p.
- Vestgarden L.S. (2001). Carbon and nitrogen turnover in the early stage of Scots pine (*Pinus sylvestris* L.) needle litter decomposition: effects of internal and external nitrogen. *Soil Biology & Biochemistry* 33: 465-474.
- Vestgarden L.S., Nilsen P. & Abrahamsen G. (2004). Nitrogen cycling in *Pinus sylvestris* stands exposed to different nitrogen inputs. *Scandinavian Journal of Forest Research* 19: 38-47.

- Vestgarden L.S., Selle L.T. & Stuanes A.O. (2003). *In situ* nitrogen mineralization in a Scots pine (*Pinus sylvestris* L.) stand: effects of increased nitrogen input. *Forest Ecology and Management* 176: 205-216.
- VMM (Vlaamse Milieumaatschappij) (2004). MIRA Achtergronddocument 2004 Vermesting. Milieu- en Natuurrapport Vlaanderen, 2004; pp. 117.
- Vogt R.D., Seip H.M., Larssen T., Zhao D., Xiang R., Xiao J., Luo J. & Zhao Y. (2006). Potential acidifying capacity of deposition: Experiences from regions with high  $\text{NH}_4^+$  and dry deposition in China. *Science of the Total Environment* 367: 394-404.
- von Wandruszka R. (2000). Humic acids: Their detergent qualities and potential use in pollution remediation. *Geochemical Transactions* 1: 10.
- Voroney R.P., Winter J.P. & Beyaert R.P. (1993). Soil microbial biomass C and N. In: Carter, M.R. (Ed.), *Soil Sampling and Methods of Analysis*. Lewis Publishers, pp. 277-286.
- Waldrop M.P. & Zak D.R. (2006). Response of oxidative enzyme activities to nitrogen deposition affects soil concentrations of dissolved organic carbon. *Ecosystems* 9: 921-933.
- Waldrop M.P., Zak D.R. & Sinsabaugh R.L. (2004). Microbial community response to nitrogen deposition in northern forest ecosystems. *Soil Biology & Biochemistry* 36: 1443-1451.
- Westerhoff P. & Mash H. (2002). Dissolved organic nitrogen in drinking water supplies: a review. *Journal of Water Supply Research and Technology-Aqua* 51: 415-448.
- Wolf D.C., Dao T.H., Scott H.D. & Lavy T.L. (1989). Influence of sterilization methods on selected soil microbiological, physical and chemical properties. *Journal of Environmental Quality* 18: 39-44.
- Wolf D.C. & Skipper H.D. (1994). Soil sterilization. In: Weaver R.W., Angle J.S. & Bottomley P.S. (Eds.). *Methods of soil analysis, part 2. Microbiological and biochemical properties*. Soil Science Society of America, Madison, WI, pp 41-51.

- Wolters V., Pflug A., Taylor A.R. & Schroeter D. (2000). Diversity and role of the decomposer food web. In: Schulze E.-D. (Ed.), Carbon and Nitrogen Cycling in European Forest ecosystems, Springer-Verlag Berlin Heidelberg pp. 366-381.
- Wuyts K., De Schrijver A., Staelens J., Gielis L., Geudens G. & Verheyen K. Patterns of throughfall deposition along a transect in forest edges of silver birch and Corsican pine. *Canadian Journal of Forest Research*, in press.
- Yano Y., McDowell W.H. & Aber J.D. (2000). Biodegradable dissolved organic carbon in forest soil solution and effects of chronic nitrogen deposition. *Soil Biology & Biochemistry* 32: 1743-1751.
- Yano Y., McDowell W.H. & Kinner N.E. (1998). Quantification of biodegradable dissolved organic carbon in soil solution with flow-through bioreactors. *Soil Science Society of America Journal* 62: 1556-1564.
- Zak D.R., Holmes W.E., Tomlinson M.J., Pregitzer K.S. & Burton A.J. (2006). Microbial cycling of C and N in northern hardwood forests receiving chronic atmospheric  $\text{NO}_3^-$  deposition. *Ecosystems* 9: 242-253.
- Zech W., Guggenberger G. & Schulten H.-R. (1994). Budgets and chemistry of dissolved organic carbon in forest soils: effects of anthropogenic soil acidification. *Science of the Total Environment* 152:49-62.
- Zhao L.Y.L., Schulin R., Weng L. & Nowack B. (2007). Coupled mobilization of dissolved organic matter and metals (Cu and Zn) in soil columns. *Geochimica et Cosmochimica Acta* 71: 3407-3418.
- Zsolnay A. (1996). Dissolved humus in soil waters. In: Piccolo A. (Ed.), Humic substances in terrestrial ecosystems, Elsevier, Amsterdam: pp 171-223.
- Zsolnay A. (2003). Dissolved organic matter: artefacts, definitions, and functions. *Geoderma* 113: 187-209.
- Zysset M. & Berggren D. (2001). Retention and release of dissolved organic matter in Podzol B horizons. *European Journal of Soil Science* 52: 409-421.

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## APPENDICES

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## APPENDIX 1:

### CALCULATION OF HYDROLOGICAL FLUXES

The fortnightly water fluxes of precipitation and canopy throughfall (mm) were determined using the measured water volumes at each sampling occasion. Because of the high temporal and spatial variability of the water volumes collected with the zero-tension lysimeters, forest floor leachate fluxes could not be quantified using the measured volumes.

Water balances and fluxes in the forest floor and mineral soil were calculated separately for the three forest stands and for each fortnightly sampling period of the one year monitoring program. The water balance can be described as (Equation A.1):

$$P = E + R + D + Q + \Delta S \quad [A.1]$$

where  $P$  is the precipitation,  $E$  the evapotranspiration,  $R$  the runoff,  $D$  the lateral drainage,  $Q$  the leaching flux and  $\Delta S$  the change in the water storage in the soil profile. The various terms of the water balance were calculated using the SWAP model (Van Dam et al., 1997; Van Dam, 2000).

The calculation of actual evapotranspiration in SWAP is based on the two-step approach. In the first step, potential evapotranspiration is calculated using the minimum value of the canopy resistance and the actual air resistance. In the second step, the actual evapotranspiration is calculated using the root water uptake reduction due to water and/or salinity stress and evaporation reduction. Within the SWAP model, the potential evapotranspiration is calculated using the Penman-Monteith equation. The potential evapotranspiration is divided over interception evaporation, potential soil evaporation and potential transpiration. The total amount of interception in the forest stands was calculated according to physically based formula of Gash et al. (1995) in which rainfall is considered to occur as a series of discrete events, each comprising a period of wetting up, a period of saturation and a period of drying out after rainfall ceases. The potential soil evaporation is calculated using empirical evaporation functions. Potential transpiration is calculated from the potential evapotranspiration by reducing the

evapotranspiration during rainfall with the calculated interception evaporation and potential soil evaporation.

To calculate the hydrological fluxes, the application of the SWAP model requires (i) meteorological data, (ii) abiotic characteristics of the site (soil physical and drainage characteristics) and (iii) vegetation dependent parameters (including crop resistance, LAI or soil cover, storage capacity of the crown, tree height, rooting depth and root distribution).

### *Meteorological data*

The model requires daily meteorological data of precipitation, net radiation, temperature, wind speed and relative humidity. These data were not collected on the site, but were obtained from the nearest meteorological station of the Royal Meteorological Institute of Belgium (KMI), i.e. at Retie (17-19 km south of the research sites). Precipitation may vary considerably over short distances, possibly leading to large differences between data from the meteorological station and the actual precipitation at the site. To obtain the best estimates of the daily precipitation at the two sites, daily precipitation data from the meteorological station were multiplied with the ratio of the fortnightly precipitation measured in the open field at the site, to the cumulative fortnightly precipitation at the meteorological station.

### *Abiotic characteristics*

Water retention curves of the forest soils at different depths were determined in the lab by measuring the gravimetric water content of intact soil cores at six different matric potentials (-3, -5, -7, -10, -33 and -100 kPa). This was done by means of the sandbox apparatus (Eijkelkamp Agrisearch Equipment, Giesbeek, The Netherlands) for matric potentials between -3 and -10 kPa, and with pressure chambers (Soilmoisture Equipment, Santa Barbara, CA) for matric potentials of -33 and -100 kPa. The model of van Genuchten (1980) was fitted to these data points to obtain the water retention parameters (Table A.1). The saturated hydraulic conductivity ( $K_{sat}$ ) was measured in the lab on intact soil cores

using a constant head permeameter (Eijkelkamp Agrisearch Equipment, Giesbeek, The Netherlands).

**Table A.1. Soil hydrological characteristics ( $\theta_{\text{res}}$  = residual water content;  $\theta_{\text{sat}}$  = saturated water content;  $\alpha$  = shape parameter;  $n$  = shape parameters;  $K_{\text{sat}}$  = saturated hydraulic conductivity;  $\lambda$  = exponent in the Mualem hydraulic conductivity function) for the three sites under investigation**

Depth (cm)	$\theta_{\text{res}}^a$ ( $\text{cm}^3 \text{ cm}^{-3}$ )	$\theta_{\text{sat}}^a$ ( $\text{cm}^3 \text{ cm}^{-3}$ )	$\alpha^a$ $\text{cm}^{-1}$	$n^a$ (-)	$K_{\text{sat}}^b$ ( $\text{cm d}^{-1}$ )	$\lambda^c$ (-)
<i>SB</i>						
5-0 <sup>d</sup>	0.000	0.500	0.100	1.25	800	-0.255
0-17	0.068	0.405	0.021	1.82	336	7.75
17-21	0.076	0.409	0.020	2.83	404	7.75
21-58	0.034	0.332	0.017	3.92	321	7.75
> 58	0.031	0.315	0.016	3.16	334	7.75
<i>CP</i>						
3-0 <sup>d</sup>	0.000	0.500	0.100	1.25	800	-0.0281
0-30	0.074	0.432	0.024	2.21	533	4.08
30-37	0.063	0.450	0.020	2.25	534	4.08
37-61	0.044	0.357	0.018	3.56	440	4.08
> 61	0.034	0.316	0.016	3.31	348	4.08
<i>CPN</i>						
2-0 <sup>d</sup>	0.000	0.500	0.100	1.25	800	16.3
0-29	0.078	0.405	0.029	1.89	217	0.49
29-42	0.066	0.383	0.027	1.87	251	0.49
42-73	0.025	0.303	0.023	3.57	658	0.49
> 73	0.039	0.300	0.019	2.86	386	0.49

<sup>a</sup> Parameters resulting from the fit of the formula of van Genuchten to the datapoints of the measured water retention curves;

<sup>b</sup> Parameter resulting from laboratory measurements;

<sup>c</sup> Parameter estimated using PEST;

<sup>d</sup> data for the forest floor are based on Tiktak and Bouten (1994).

Since the groundwater level was consistently below a depth of 1.5 m throughout the year, free drainage of the soil profile at a depth of 90 cm was assumed. Run-off and run-on were set to zero since the study sites were located on flat terrain with sandy soils. No lateral drainage was calculated for the three sites



since actively draining ditches and canals were absent in the immediate proximity of the study plots.

### *Stand characteristics*

The most important stand parameters used by the model are tree height, soil cover fraction ( $SC$ ), storage capacity of the canopy ( $S$ ), minimum crop resistance ( $r_{canopy}$ ), rooting depth ( $D_{root}$ ) and root distribution ( $\ell_{root}$ ). Tree height (Table 3.1) was measured using an ultrasonic height meter (Vertex III, Haglöf Sweden AB), whereas the other stand parameters (Table A.2) were estimated using the parameter estimation package PEST (see further).

**Table A.2. The most important stand and crop characteristics used in the SWAP model calculations**

Stand	$D_{root}^{a,f}$	$r_{canopy}^{b,f}$	$\ell_{root}^{c,f}$	$SC^{d,f}$	Interception parameters	
	(cm)	(s/m)	(-)	(m <sup>2</sup> m <sup>-2</sup> )	$p^{e,f}(-)$	$S^{e,f}$ (cm)
SB	87	149	0.00 / 8.69 / 0.00 / 0.77	0.93 / 0.92 / 0.90 <sup>g</sup>	0.70 / 0.98 / 0.70 <sup>g</sup>	0.40 / 0.45 / 0.57 <sup>g</sup>
CP	71	138	0.09 / 3.34 / 0.00 / 1.70	1.00 / 1.00 / 0.93	0.69 / 0.69 / 0.62	0.47 / 0.39 / 0.23
CPN	71	138	0.09 / 3.34 / 0.00 / 1.70	0.91 / 1.00 / 0.77	0.63 / 0.58 / 0.40	0.37 / 0.35 / 0.29

<sup>a</sup> Rooting depth;

<sup>b</sup> Minimum canopy resistance;

<sup>c</sup> Relative root density at four relative rooting depths (0.00; 0.20; 0.50; 1.00);

<sup>d</sup> Soil cover fraction;

<sup>e</sup> Parameters for Gash model to simulate interception for forests:  $p$  = free throughfall coefficient;  $S$  = storage capacity of canopy;

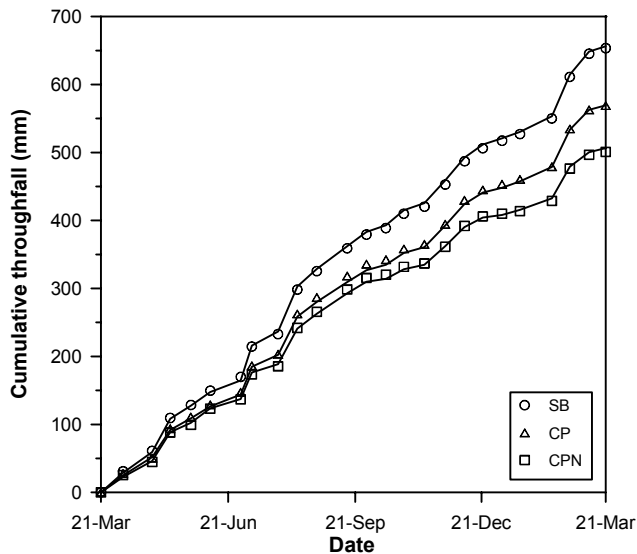
<sup>f</sup> SWAP parameters estimated using PEST;

<sup>g</sup> Parameter values at three crop development stages (DVS) (0.00; 1.00; 2.00).

### *Parameter estimation*

Several parameters of the SWAP model were estimated using the model-independent parameter estimation package PEST (Watermark Numerical Computing, USEPA). In a first parameter estimation run, the crop-related parameters soil cover fraction ( $SC$ ), free throughfall coefficient ( $p$ ) and storage capacity of the canopy ( $S$ ) for three development stages ( $DVS$ ) were estimated for each of the three forest stands (Table A.2). For this model calibration, the fortnightly throughfall volumes measured on-site (Figure A.1) in the three stands were used.

In a second parameter estimation run, the exponent ( $\lambda$ ) of the hydraulic conductivity function (van Genuchten-Mualem equation) for the forest floor and the mineral soil, the rooting depth ( $D_{root}$ ), the minimum canopy resistance ( $r_{canopy}$ ) and the relative root density ( $\ell_{root}$ ) as a function of relative rooting depth (Tables A.1 and A.2) were calibrated using the measured matric potentials (at 3 depths and 14 occasions) and soil moisture contents (at 4 depths and 4 occasions).



**Figure A.1.** Cumulative throughfall volume (mm) for the three forest stands in Ravels as measured on site from 21 March 2005 till 21 March 2006. The data points represent field measurements, whereas the solid lines represent the SWAP simulations optimized by PEST.

### *Calculation of solute fluxes*

The SWAP model generated fortnightly water fluxes in the different ecosystem strata of the three forest stands. The sum of these fortnightly water fluxes is the annual water flux passing through each ecosystem stratum (Table A.3). Fortnightly solute fluxes were calculated by multiplying the fortnightly water fluxes by the fortnightly solute concentration. The sum of these fortnightly solute fluxes over the 1 year monitoring program yielded the annual solute flux for each stratum.

**Table A.3. Water fluxes ( $\text{mm yr}^{-1}$ ) in the different strata of the three forest stands in Flanders**

Stratum	SB	CP	CPN
Precipitation <sup>a</sup>	784	784	792
Throughfall <sup>a</sup>	654	569	504
Humus percolate <sup>b</sup>	649	564	497
E-horizon <sup>b</sup>	468	420	423
Bh-horizon <sup>b</sup>	376	358	368
BC-horizon <sup>b</sup>	281	210	224
Groundwater depth <sup>c</sup>	182	209	213

<sup>a</sup> results from the actual measured volumes at the site.

<sup>b</sup> results from the SWAP model calculations.

<sup>c</sup> Average groundwater depth measured in field (cm).



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# CURRICULUM VITAE

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## SCIENTIFIC PUBLICATIONS

### *Publications in international Journals with peer review (A1)*

- D'Haene K., Sleutel S., De Neve S., Gabriels D., Vandenbruwane J. & Hofman G. (2008). The influence of reduced tillage on carbon dynamics in silt loam soils. *Soil and Tillage Research* (Submitted).
- D'Haene K., Van den Bossche A., Vandenbruwane J., De Neve S., Gabriels D. & Hofman G. (2008). The effect of reduced tillage on dinitrogen oxide losses from silt loam soils. *Biology and Fertility of Soils* (Submitted).
- D'Haene K., Vandenbruwane J., De Neve S., Gabriels D., Salomez J. & Hofman G. (2008). The effect of reduced tillage on nitrogen dynamics in silt loam soils. *European Journal of Agronomy* (Accepted).
- Gielis L., De Schrijver A., Wuyts K., Staelens J., Vandenbruwane J. & Verheyen K. (2008). Comparison of nutrient cycling in two continuous cover scenarios for forest conversion of pine plantations on sandy soil. II. Throughfall deposition and seepage flux in regeneration stands of silver birch and Scots pine. *Forest Ecology and Management* (Submitted).
- Sleutel S., Moeskops B., De Neve S., Leroy B., Vandenbossche A., Vandenbruwane J., Salomez J. & Hofman G. (2008). Modeling soil moisture effects on the evolution of mineral nitrogen concentrations in loamy wetland soils. *Wetlands* (Submitted).
- Vandenbruwane J., De Neve S., De Schrijver A., Geudens G., Verheyen K. & Hofman G. (2008). Laboratory comparison of ceramic and PTFE/Quartz suction cups for sampling inorganic ions in the soil solution. *Communications in Soil Science and Plant Analysis* (In Press).
- Vandenbruwane J., De Neve S., De Schrijver A. & Hofman G. (2008). Sampling soil solution for dissolved organic carbon (DOC) measurements: comparison of ceramic and PTFE/Quartz porous suction cups. *Communications in Soil Science and Plant Analysis* (Submitted).
- Vandenbruwane J., De Neve S., Qualls R.G., Salomez J. & Hofman G. (2007). Optimization of dissolved organic nitrogen (DON) measurements in

aqueous samples with high inorganic nitrogen concentrations. *Science of the Total Environment* 386: 103-113.

Vandenbruwane J., De Neve S., Qualls R.G., Sleutel S. & Hofman G. (2007). Comparison of different isotherm models for dissolved organic carbon (DOC) and nitrogen (DON) sorption to mineral soil. *Geoderma* 139: 144-153.

Vandenbruwane J., Leroy B., Van den Bossche A., De Neve S. & Hofman G. (2008). Factors affecting dissolved organic matter (DOM) release from forest floor material receiving chronic atmospheric nitrogen deposition in Flanders. *European Journal of Soil Science* (Submitted).

Vandenbruwane J., Sleutel S., De Neve S., Qualls R.G. and Hofman G. (2008). Biodegradability of dissolved organic carbon (DOC) and nitrogen (DON) from different origins and soil depths. *Soil Biology & Biochemistry* (Submitted).

Vandenbruwane J., Sleutel S., Qualls R.G., De Neve S. & Hofman G. (2008). Sequential sorption experiments for describing dissolved organic matter (DOM) sorption in layered soil profiles. *Soil Science Society of America Journal* (Submitted).

Vandenbruwane J., Wuyts K., Gielis L., De Neve S., De Schrijver A., Verheyen K. & Hofman G. (2008). Fluxes and concentrations of dissolved organic carbon (DOC) and nitrogen (DON) in deciduous and coniferous forests under long-term nitrogen deposition. *Biogeochemistry* (Submitted).

Wuyts K., De Schrijver A., Staelens J., Gielis L., Vandenbruwane J. & Verheyen K. (2008). Comparison of forest edge effects on throughfall deposition in different forest types. *Environmental Pollution* (Submitted).

### *Other Publications*

Vandenbruwane J., De Neve S. & Hofman G. (2006). Leaching of dissolved organic carbon (DOC) and nitrogen (DON) under different tree species and with elevated atmospheric deposition in Flanders, Belgium. In: van Aarssen



- B., The Origin and Fate of Naturally Occurring Organic Matter: Conference Program and Abstracts, Curtin University of Technology, pp. 35-36.
- Vandenbruwane J., De Neve S. & Hofman G. (2006). Fluxes and concentrations of dissolved organic carbon (DOC) and nitrogen (DON) in coniferous and deciduous forests in Flanders, Belgium. In: Crow S. Biogeomon, 5<sup>th</sup> International Symposium on Ecosystem Behavior, Conference Program and Abstracts, Villanova University, Santa Cruz, CA, USA, p. 227.
- Vandenbruwane J., De Neve S., Qualls R.G. & Hofman G. (2006). Optimization of total nitrogen (TN) analysis for dissolved organic nitrogen measurements: Recommendations and limitations. In: Crow S. Biogeomon, 5<sup>th</sup> International Symposium on Ecosystem Behavior, Conference Program and Abstracts, Villanova University, Santa Cruz, CA, USA, p. 228
- Vandenbruwane J., De Neve S., Qualls R.G. & Hofman G. (2007). Sorption processes control DOC and DON concentrations and fluxes in a sandy forest soil in Flanders. Day of the Young Soil Scientists, The Royal Academies of Belgium for Science and the Arts, Brussels, 21 February 2004, Abstracts of oral presentations and posters, p. 25.

## **POSTER AND ORAL PRESENTATIONS**

- Vandenbruwane J., De Neve S. & Hofman G. Leaching of dissolved organic carbon (DOC) and nitrogen (DON) under different tree species and with elevated atmospheric deposition in Flanders, Belgium. The Origin and Fate of Naturally Occurring Organic Matter. Combined National Conference of the Australian Organic Geochemists and the NOM Interest Group, 12-15 February 2006, Perth, Western Australia. *Oral presentation.*
- Vandenbruwane J., De Neve S. & Hofman G. Fluxes and concentrations of dissolved organic carbon (DOC) and nitrogen (DON) in coniferous and deciduous forests in Flanders, Belgium. Biogeomon, 5<sup>th</sup> International Symposium on Ecosystem Behavior, June 25-30, 2006, Santa Cruz, CA, USA. *Oral presentation.*

- Vandenbruwane J., De Neve S., Qualls R.G. & Hofman G. (2006). Optimization of total nitrogen (TN) analysis for dissolved organic nitrogen measurements: Recommendations and limitations. Biogeomon, 5<sup>th</sup> International Symposium on Ecosystem Behavior, June 25-30, 2006, Santa Cruz, CA, USA. *Poster presentation.*
- Vandenbruwane J., De Neve S., Qualls R.G. & Hofman G. (2007). Sorption processes control DOC and DON concentrations and fluxes in a sandy forest soil in Flanders. Day of the Young Soil Scientists, February 21, 2004, Brussels, Belgium. *Poster presentation.*
- Vandenbruwane J., De Neve S., De Schrijver A. & Hofman G. Sampling soil solution for dissolved organic carbon measurements: comparison of ceramic and PTFE/quartz porous suction cups. 10<sup>th</sup> International Symposium on Soil and Plant Analysis, June 11-15, 2007, Budapest, Hungary. *Poster presentation.*



