PRODUCTION AND BIODEGRADATION OF DISSOLVED CARBON, NITROGEN AND PHOSPHORUS FROM CANADIAN FOREST FLOORS

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"An increasing number of scientists are starting to emphasize the extent to which soil – even more than petroleum or water or air – is a limited and fragile resource. Managing it better, and even improving it, will be vital to any equation that allows the earth to support more than 9 billion people the UN estimates will live on the planet by midcentury. [...] Nevertheless, progress in the science of soil has the potential to be truly transformative, and to help solve some of the biggest problems the planet faces. [...] Ultimately, it may be the issue of climate change that drives the public interest in soil."

Drake Bennett, April 27, 2008, The Boston Globe.

Abstract

Dissolved organic matter (DOM) is operationally defined as soluble/colloidal material passing through a 0.45μm filter paper. The importance of DOM in soils relies on its role in soil formation and weathering processes, plant and microbial assimilation and soil and water acidification. However, the scientific community studying DOM still disagrees on whether fresh or humified material is the major source of DOM within the forest floor. One of the factors that could influence the overall importance of DOM production by organic horizons is its potential for biodegradability. In addition, the interaction occurring between the nutrients (i.e. nitrogen (N) and phosphorus (P)) and carbon (C) substrate is believed to be of major importance.

To acquire more knowledge on the production and biodegradation of dissolved C, N and P during decomposition of organic matter (OM), I performed laboratory incubations to evaluate rates of production and transformation, the influence of the degree of OM decomposition and stand type on these rates, and the stoichiometric relationships of the different quotients during the incubations. First, I performed a 30-day incubation of coniferous and deciduous OM from 10 Canadian forest floors representing various degrees of OM decomposition and subsequently measured the amount of: dissolved organic carbon (DOC), total dissolved nitrogen (TDN), nitrate (NO_3-N), ammonium (NH_4-N), dissolved organic nitrogen (DON), total dissolved phosphorus (TDP) and carbon dioxide (CO_2-C). I performed water extractions with the same set of samples to evaluate the biodegradability of DOC and DON and the transformations of TDN, NO_3-N and NH_4-N.

Fresh material produces more DOM than humified material; material in the mid-point of decomposition (F horizon) produced the largest amount of DIN. Coniferous and deciduous samples did not display different rates of DOM production, most likely because of the overshadowing effect of OM degree of decomposition. I found strong links between the organic matter and dissolved phase C and N content and C:N quotient. The biodegradation, measured as DOC disappearance and mineralization of CO_2-C, showed a discrepancy, reflecting the importance of increasing microbial biomass at the beginning of the incubation in response to priming effect. The sharp decrease of TDN and DON observed in the first few days of the incubation, in addition to increasing

amount of dissolved inorganic N as waste products during decomposition of DON, supports this hypothesis. A better understanding of the dynamics of dissolved C, N and P in soil is essential to further understand their role in global elemental cycles, including climate change, forest management and pollution.

Résumé

La matière organique dissoute (DOM) est composée de particules dissoutes et colloïdales passant au travers un filtre de 0.45 μm. L'importance de DOM dans les sols est liée à son rôle dans la pédogenèse, les processus d'altération des minéraux, l'assimilation par les plantes et microbes, ainsi que l'acidification des plans d'eau et des sols. Présentement, un désaccord existe dans la communauté scientifique étudiant les DOM. Ce désaccord porte sur la source majoritaire de DOM dans les horizons organiques d'un sol forestier : litière fraîche *versus* humus. Le potentiel de biodégradation est un facteur d'importance affectant la production du DOM. De plus, nous croyons que l'interaction entre les nutriments (i.e. azote (N) et phosphore (P)) et le substrat (i.e. carbone (C)) dans les sols a une importance majeure.

La matière organique de forêt de conifères et de feuillus provenant de 10 couverts forestiers Canadien, présentant divers degrés de décomposition, a été incubée afin de mesurer la production de carbone organique dissous (DOC), d'azote total dissous (TDN), de nitrate (NO_3-N), d'ammonium (NH_4-N), d'azote organique dissous (DON), de phosphore total dissous (TDP) et de dioxide de carbone (CO_2-C) durant 30 jours. Des extractions à l'eau ont été fait avec ces échantillons afin d'évaluer le potentiel de biodégradation du DOC et DON, et la transformation de TDN, NO_3-N et NH_4-N.

Les conclusions majeures de la recherche montrent une production de DOM significativement plus élevée provenant de la litière fraîche comparativement à l'humus. L'horizon F, qui représente un point milieu de degrés de décomposition, a produit significativement plus d'azote dissous que les autres horizons. Le type de végétation n'a pas permis de différencier les taux de production, probablement dissimulé derrière l'effet majeur du degré de décomposition. Des liens stoichiométriques ont été mesurés entre le ratio C:N de la matière organique et les taux de production de C, N et P et leurs ratios. Les expériences de biodégradation ont mesuré la disparition du DOC et la minéralisation du CO_2-C durant l'incubation, présentant une dichotomie. Ces résultats ont montré l'importance de la biomasse microbienne au début de l'incubation étant donnée la stimulation par les matières dissoutes labile. Une diminution du TDN et DON durant les premiers jours de l'incubation appuie la dernière hypothèse, suivi d'une augmentation d'azote inorganique dissous, un sous-produit de décomposition du DON. Il est important

de mieux comprendre le comportement du carbone, de l'azote et du phosphore dissous dans le sol pour une meilleure compréhension de leur rôle dans les cycles globaux des éléments, donc leurs effets sur les changements climatiques, la gestion des forêts et la pollution.

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The bricked walls are there to show you how badly you want something (Randy Pausch, Last lecture)

Authors' contributions

Manuscript #1 (Chapter 3) **"Canadian forest floor production of dissolved organic carbon and carbon dioxide during laboratory incubations"** by Julie M.L. Turgeon, Tim R. Moore and Edward G. Gregorich (*submitted*). Tim Moore, as a supervisor, contributed intellectually and financially to the research and is the co-author of all manuscripts. Edward Gregorich contributed to the intellectual brainstorming and the writing and editing of the first, third and fourth manuscripts.

Manuscript #2 (Chapter 4) **"Nitrogen and phosphorus release from decomposing litter and organic matter in Canadian forest floors"** by Julie M.L. Turgeon and Tim R. Moore (*submitted*). Tim Moore helped with the writing, editing of the manuscript and formatting of the figures.

Manuscript #3 (Chapter 5) "**Biodegradation of dissolved organic carbon in water-extracts from Canadian forest floors"** by Julie M.L. Turgeon, Edward G. Gregorich and Tim R. Moore. Edward Gregorich helped with the development and modification of the biodegradation assay (also applied in manuscript #4) and writing. Tim Moore assisted with writing and editing.

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To my family and friends...
Pour ma famille et mes amis...

Chapter 1. Thesis introduction and Objectives

1.1. Context of research

Dissolved organic matter (DOM) is defined as any organic compound passing through a 0.45μm filter (Kalbitz et al., 2000). Dissolved organic matter is recognised to play a major role in many biogeochemical soil processes such as: 1) providing energy and nutrients for plant and microbes growth (Stevenson and Cole, 1999); 2) interacting with other elements during pedogenetic processes and mineral weathering (Kaiser et al., 2000; Michalzik et al., 2001); 3) taking part in the transport of nutrients; trace metals and contaminants (Kalbitz et al., 2000); 4) influencing soil acidity; and 5) providing a means of transport for elements contributing to surface water acidification and eutrophication (Likens et al., 1981). Within a global context, soils are the central point for the interactions between atmosphere, biosphere and hydrosphere during the production, retention and transformations of DOM and dissolved inorganic nutrients (i.e. N and P). Also, according to some studies, the dissolved phase is a prerequisite for the diffusion of the substrate, hence absorption by the plant and microbial biomass (Zsolnay and Steindl, 1991; Marschner and Kalbitz, 2003; Kemmitt et al., 2008).

The dissolved elements found in soils originates from throughfall, plant litter and humus decomposition and leaching, and microbial and root exudates (Michalzik and Matzner, 1999; Kalbitz et al., 2000). Knowledge of dissolved organic carbon (DOC) in soil has been summarised in various studies (i.e. Michalzik and Matzner, 1999; Kalbitz et al. 2000; Michalzik et al., 2001). However, our knowledge of DOC dynamics is still incomplete and studies have neglected the dissolved N and P components in soils, despite their role as major, and possibly limiting, nutrients in many ecosystems (Stevenson and Cole, 1999; Lovett et al., 2004; Gradowski and Thomas, 2006).

Dissolved organic matter is mainly composed of organic carbon (C) and nitrogen (N) in a suspended or dissolved phase, but also consists of other elements such as phosphorus (P) and sulphur (S) (Stevenson and Cole, 1999). The main forms of DOM studied in the literature are dissolved organic C (DOC) and dissolved organic N (DON). In this study, the focus will also be on dissolved inorganic N, particularly nitrate (NO_3-N) and ammonium (NH_4-N), as well as total dissolved N (TDN) and total dissolved

phosphorus (TDP) due to their important role in nutrient cycling. Unlike dissolved N, the emphasis of the study will be on total dissolved P (TDP) because dissolved P is found in very low concentration in soils. Additionally, dissolved organic phosphorus (DOP), calculated as the difference between TDP and inorganic P, has a low accuracy of computation (i.e. Qualls et al., 1991).

1.2. Thesis structure and objectives

The thesis is divided into two major sections: 1) production and 2) biodegradation. The first two manuscripts focus on the production of both DOC and CO_2-C (Chapter 3) and the production of TDN, NO_3-N, NH_4-N and TDP (Chapter 4) during the laboratory incubation of organic matter (OM). The third and fourth manuscripts focus on the biodegradability of DOC (Chapter 5), and transformations of TDN, DON, NO_3-N and NH_4-N (Chapter 6) from water extractions of OM samples.

The objective of this thesis is to evaluate the potential rates of production and biodegradation of dissolved C, N and P compounds. In addition to comparing production and biodegradation rates of the OM found across Canadian forests, this work aims to determine various predictors for the production and biodegradation of the dissolved components. Among these predictors, we evaluated the role of initial C, N and P content, their quotients, as well as microbial biomass C and N and their quotients in determining DOM production and biodegradation. The quotients were calculated to evaluate if the stoichiometric relationships were applicable in understanding the interactions between soils (here OM) and dissolved phase elements and quotients. Spectrophotometry was also used to characterize the biodegradability of the different OM samples.

Chapter 2. Literature Review

2.1.Production of DOM and dissolved nutrients

One of the conflicting issues regarding DOC (or DOM) is its major source of production within the soil system. A number of studies suggest that the litter (L horizon and fresh litter, such as litterfall) is the major source of DOC (e.g. Qualls et al., 1991; Huang and Schoenau, 1998; Michalzik and Matzner, 1999) while other studies suggest that old, humified organic matter (OM) is the main contributor to the production of DOC (e.g. Kalbitz et al., 2000; Fröberg et al., 2003). Table 2.1 provides a review of studies with their results concerning this debate.

Discrepancy between studies can be explained by differences in methodology. Different methods of measurements (i.e. water-extraction vs. tension or zero-tension lysimetry), the size of filtration (varying between 1.0 to 0.2 μm) and the conceptualization and definition of DOM can all lead to different results. For the latter, it is important to remember that the dissolved elements measured, either from laboratory or field experiments, are the net result from processes that produce DOM (i.e. leaching, desorption or microbial exudation) and processes that retain DOM (i.e. adsorption, precipitation, uptake or leaching to another spatial entity) (summarised in Kalbitz et al., 2000). In short, DOM production is the result of biological, physical and chemical processes that control the production and retention of dissolved elements, contributing to the cycling and recycling of these elements (Michalzik et al., 2001; Gregorich et al., 2003).

As stated above, N and P are essential nutrients for both plant and microbes growth. Our knowledge of dissolved N is incomplete for forested ecosystems (Michel and Matzner, 1999; Gundersen et al., 2006) but the knowledge of dissolved P is even more limited (McDowell, 2003). These elements are intensively measured in surface water studies, and recent advances in chemical analysis have allowed measurement of the different species composing both TDN (i.e. NO_3-N and NH_4-N) and TDP (i.e. phosphates, PO_4-P). Studies have reported their importance in forest floor nutrient availability and dynamics (e.g. Yavitt and Fahey, 1986; Wardle et al., 2003; Ganjegunte et al., 2004; Moore et al., 2006) and have shown them to be important in decomposition

studies (e.g. Prescott 1996; Prescott et al., 2000). Despite the few studies on dissolved N and P, the literature seems to agree that nutrients are released first through a rapid leaching, and then by tissue breakdown/decomposition mediated by microbial activity (Prescott, 2005). For this reason, the debate concerning the major source of DOM would apply to both dissolved organic N (DON) and dissolved organic P (DOP) but also to dissolved inorganic nutrients.

The literature reports that DON and DOP also originate mainly from the decomposition of OM, leaching of plant material, roots and microbe exudates and chemical processes. In the case of dissolved inorganic N, even though decomposition is believed to be a major source, it mainly originates from atmospheric precipitation in field studies (Turgeon et al., submitted, Kalbitz et al., 2004b). Many studies suggested that DON behaviour mimics that of DOC, but the transformations between inorganic N and organic N probably suggest that different factors control its cycling. Park and Matzner (2003) suggested that the debate concerning the major source of DOC also applies to DON. Qualls et al. (1991), Michalzik and Matzner (1999) and Magill and Aber (2000) reported that fresh litter was the major source of DON and inorganic N, along with throughfall inputs significant for field studies (Michalzik et al., 2001). Other studies, however suggest that older OM is the major source of organic and inorganic N (McDowell and Likens, 1988; Kalbitz et al., 2000).

Few studies have looked at dissolved P in soils and little is known about its chemistry within the soil system. Dissolved P initially originates from rock weathering, but the contemporary cycling results from its release from OM during decomposition. This contemporary cycling is continuous and recycles dissolved P, with an insignificant amount being lost through hydrological leaching (Stevenson and Cole, 1999).

2.2. Biodegradability of dissolved C and N

There is a growing desire to understand the biodegradability of DOM in soils (Zsolnay, 2003; McDowell, 2003; Marschner and Kalbitz, 2003) because of its role in the rate of nutrient cycling. While there are many terms defining biodegradability (i.e. mineralization, lability, bioavailability) in the literature (e.g. Servais et al., 1987; Volk et al., 1997; Gregorich et al., 2003; Kiikkila et al., 2005; Young et al., 2005), many of those

terms are not appropriate. Unfortunately, many of the papers use these terms interchangeably.

Biodegradation is often compared to mineralization and decomposition. Mineralization represents the conversion of an element from an organic to an inorganic state as the result of microbial activity. Mineralization is not a synonym of DOM biodegradability because the end-products of DOM biodegradation can also be organic, like storage in microbial biomass or formation of more complex DOM compounds. On the other hand, decomposition is defined as a loss of OM mass and the breakdown or decay of OM. In that sense, DOM biodegradation could be considered as the decomposition of DOM in solution. In that sense, decomposition and biodegradation can be considered synonyms.

Servais et al. (1987) defines biodegradability as the disappearance of DOM from the solution by biological processes. During this process, DOM is either assimilated into biomass or metabolized by heterotrophic microflora. However, in a laboratory experiment, plant uptake of DOM is excluded. Because of this exclusion of plants, biodegradability of DOM (BDOM) can be defined, for laboratory studies, as the fraction of DOM that is biologically transformed (Gregorich et al., 2003; Kiikkila et al., 2005). The BDOM could then be defined as the portion of DOM in the solution that is either mineralized to carbon dioxide (CO_2-C) or to dissolved inorganic nutrients (for N, P and others nutrients), stored in the microbial biomass or transformed into new compounds of DOM (more or less degradable [Young et al., 2005]).

Sondergaard and Worm (2001) suggest that the proportion of BDOM is defined as the concentration of DOM measured at a somewhat arbitrary end-point in time. A different time would give a different proportion of BDOM (measured as the difference in initial and final DOM concentration) because micro-organisms keep on degrading the DOM in the solution through time.

Lability is another term that is often used as a synonym for biodegradability. However, lability refers to the portion of DOM readily transformed by the micro-organisms or readily available for plants (Volk et al., 1997; Gregorich et al., 2003; Young et al., 2005). There is a difference between lability and biodegradability: "biodegradability" represents the actual utilisation of DOM during a period of time while

"lability" is the material preferentially used by the micro-organisms, but it does not mean that it is the only fraction of DOM used. More recalcitrant materials are also degraded, but at a slower rate. The DOM that is biodegraded is composed of a mixture of heterogeneous molecules that range from the very labile to near refractory (Qualls and Haines, 1992; Kaplan and Newbold, 1995). When the labile material is degraded or when competition between communities arises, microbes produce specific enzymes to help degrade the more recalcitrant material (Qualls and Haines, 1992; Paul and Clark, 1996; Schimel and Weintraub, 2003; Trulleyova and Rulik, 2004).

> "Most substances can be degraded by some micro-organisms under at least some, perhaps peculiar, conditions (Qualls and Haines, 1992)".

Bioavailability is often used as a surrogate for biodegradability. However, bioavailability means that DOM compounds are accessible both physically and chemically for further processing by biological processes. The physical accessibility refers to the pore size, aggregation or water content and drought of soils (Marschner and Kalbitz, 2003) while the chemical accessibility refers to reactions that change the chemical state of DOM (i.e. sorption or complexity of the compounds) that results from any transformation of the DOM compounds. When DOM is available, it can be either biologically transformed or leached. Specifically, bioavailability refers to the potential of micro-organisms and plants to interact with DOM. The bioavailability of DOM is a prerequisite for biodegradation of DOM because the biomass needs to interact with the solution to be able to degrade DOM (Ribas et al., 1991; Boyer and Groffman, 1996; Huesemann et al., 2004).

Recent studies have stated the importance of looking at both labile and recalcitrant fractions of DOM (Marschner and Kalbitz, 2003; McDowell et al., 2006). Recalcitrant and refractory are terms used to define the material that offers a resistance to degradation or decomposition, which is different from non-degradable fractions (Marschner and Kalbitz, 2003).

Kalbitz et al. (2003) stated that up to 80% of total DOC from fresh material can be biodegraded within a few weeks, and DOM biodegradation decreases with increasing degrees of decomposition (Boyer and Groffman, 1996). In general, very little is known about the biodegradability of DOM in forest soils and its significance (Kiikkila et al.,

2005). The biodegradability of DOM has not been studied thoroughly for different types of forest (coniferous, deciduous). And again, our knowledge about biodegradability of dissolved N needs further studies.

2.3. Stoichiometry

One of the goals of the present thesis is to investigate the possible use of the stoichiometry approach for soil/solution dynamics and interactions. The nutrient composition of ecosystems, including organisms and vegetation, and the interactions between "chemical systems" are part of biogeochemistry (Schlesinger, 2004). Knowing about the distribution, transformation, cycling, interaction and limitation of nutrients in soils is important because of their role on global cycling of elements. One key element to the understanding of ecosystem processes is the relative abundance of essential elements and their distribution in the environment. Researchers use an approach called "stoichiometry" to study this balance between nutrients, among the organisms and environments (Sterner and Elser, 2002; Hessen et al., 2004).

Stoichiometry is strictly defined as the relative proportion of elements that form compounds and biomass. Many authors use related concepts such as nutrient interactions and limitations as examples of stoichiometric relationships. This approach allows the researchers to focus on more than one element, stressing the importance of element interactions (Sterner and Elser, 2002). Melillo et al. (2003) noted that nutrient interactions can be direct (i.e. chelate, immobilize or catalyze a reaction involving other nutrients) or indirect (i.e. nutrient limitation will influence the rate at which the other nutrients cycle in the ecosystem). Nutrient limitation is a direct result of stoichiometry, as the organisms (i.e. plants, bacteria, fungi and animals) constantly face imbalanced mixtures of nutrients, represented by the C:N:P quotient of the available food (Cross et al., 2003; Frost et al., 2005a, b).

One of the major concerns about the use of stoichiometry in terrestrial vs. aquatic ecosystems is mainly related to the type of medium. While water offers "free" movement, the soil is composed of a solid matrix that offers a more static and immobile medium for the flows of nutrients. This immobility promotes soil heterogeneity which affects the nutrient cycling (McGroddy et al., 2004). Furthermore, the turnover of elements tends to be slower in soils because of the heterogeneity (Hessen et al., 2004).

Soil nutrient cycling is defined by the movement and transformation of elements essential for organisms (plants and microorganisms) for their growth, reproduction and maintenance. During nutrient cycling, the speciation of elements is transferred from an organism to another and to the environment, between the soil's above- and below-ground biomass. Nutrients are constantly redistributed in the different components of the ecosystems (i.e. litterfall, soil water flow, forest-atmosphere exchanges, etc.) (McGroddy et al., 2004).

In soil science, the approach of stoichiometry with respect to nutrient cycling, is represented by the use of soil C:N:P quotients to infer soil quality. Litter or OM quality (represented as the C:N quotients) have a major influence on microbially mediated processes such as decomposition, mineralization, and nitrification (Dodds et al., 2004; Wang et al., 2004; McGroddy et al., 2004). The balance of the elemental composition of this OM and the organisms that consume the material will influence the rate at which nutrients are released and consumed, hence cycled within the soils.

If the C:element quotient is high in the OM, there will be less ingestion of the elements relative to C, leading to low efficiency in the storage of C, and potential release of C compounds (Agren et al., 2003; Frost et al., 2005a, b). In short, elements present in excess will be released into the environment or recycled. Elements that are limiting will be retained (Cross et al., 2003). In that matter, C:N:P quotients can be used as predictors of soil quality, nutrient availability, and maybe its response to disturbance (direction and maybe even its magnitude, see Schlesinger, 2004).

Some studies demonstrated that the nutrients and elements contained in dissolved phase are a prerequisite for the uptake by plants and microbes in soils (e.g. Schimel and Bennett, 2004). Other research has looked at the C:N:P quotients from the DOM solution to evaluate the extent of the leaching of elements to surface water (Qualls et al., 1991; Neff et al., 2000; Cleveland et al., 2004). Qualls et al. (1991) stated that the study of DOC:DON:DOP quotients could be a convenient way to look at DOM as a vector for N and P through soils, by comparing it to the transport of DOC. However, some studies have found that quotients can not solely explain the transport of DOM, since there are both biotic and abiotic factors affecting the transport of DOM in a forested watershed (Qualls et al., 1991; Neff et al., 2000; Turmel et al., 2005).

Obtaining data on the production and biodegradation of dissolved C, N and P and their stoichiometry will allow to either: adequately understand the role of vegetation and decomposition on the dissolved element dynamics, help model global cycling by including the dissolved components to predict changes occurring with the forecasted climate change and/or help forest managers take decisions for a better forest productivity and resilience. The dataset, and understandings provided by this thesis will be a tremendous addition to the current knowledge of Canadian ecosystems. The extended dataset that has been gathered in this study alone will allow the verification of previously reported relationships related to dissolved C, N and P. Hence, the 42 litter type represents a large spectrum of organic matter samples produced from the Canadian forested area.

Table 2.1. Synthesis on the source of DOC in forest floor from Canadian sites.

Authors	Method	Notes
Qualls et al. (1991)	Zero-tension lysimeters	**L > H (Oi > Oa)** Throughfall and fresh material as major contributor of DOC
Michalzik and Matzner (1999)	Suction lysimeters (300 hPa)	**L > H (Oi > Oa) -** Oi as the major contribution during the initial decomposition stage of the litter.
Michel and Matzner (1999)	Incubation, extraction by percolation	**F > H (Oe > Oa)** DOC results (as well as DON) showed a larger range within the Oe (1.4 to 12.4 mg gC^{-1}) than in the Oa (0.86 to 7.2 m g gC^{-1}). These results are generally significant for all study sites.
Kalbitz et al. (2000)	Review	Suggest that the amount of litter in soil increase the concentrations and fluxes of DOC
Magill and Aber (2000)	Incubation of litter	Experiment on litter only, concluded that litter is the greatest contributor to DOM production.
Moore and Dalva (2001)	Laboratory incubations with fresh and old maple leaves, under different conditions	**Fresh > litter -** Importance of the degree of decomposition, with smaller amounts of DOC released from better decomposed materials
Solinger *et al.* (2001)	Suction lysimeters (100-200 hPa) Mean concentration of bi-weekly sampling	**F/H (Oe/Oa) larger contribution** European beech (*Fagus sylvatica* L.) and Sessile oak (*Quercus petraea*), 1997 and 1998 data. Concluded that Oa was a larger contributor. Oa included Oi and Oe solution. The Oa horizon was not always present, so it seems to be mostly Oe horizon.
Park *et al.* (2002)	Laboratory incubation, 15°C, 98 d, bi-weekly leaching with vaccum, *Material was air-dried, and sieved at 5 mm (Oi previously shopped in smaller pieces before sieving)* *Values are cumulative DOC production*	**H (Oa) larger contribution** European beech (*Fagus sylvatica* L.) and Sessile oak (*Quercus petraea*). Both treatments (Oe + Oa and Oi + Oa) are statistically different from the control. They estimated that the Oa was the major contributor of the control leaching with 46% contribution. Kalbitz et al. 2004b and Park and Matzner 2003 suggested that this study show equal contribution.
Park and Matzner 2003	Zero-tension lysimeters under the Oi and Oa horizons. (control, no-litter, double litter and glucose treatments)	In the control plot, the Oa gave significantly larger DOC values, but the authors suggest, all results taken together, that all horizons of the organic layer have an equal importance as a DOM source.
Fröberg *et al.* (2003) Fröberg (2004)	Water extraction, NMR Spectrophotometry and ^{14}C measurement	**L > F > H (Oi > Oe > Oa)** Concluded that the Oi was not a major source of DOC because of its rapid degradation, and that Oe would be the important contribution of WEOC.

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Cleveland et al. (2004)	Deionised water extraction of foliage and litter samples	**Foliage > litter** - Results vary depending on the type of vegetation (temperate and tropical ecosystems). In general, foliage produced more DOM than litter of the same species.
Hagedorn et al. (2004)	^{13}C and ^{14}C experiments from DOC (lysimetry) and WEOC	Up to 95% of the DOC originated from old organic matter (older than 4 years)
Kalbitz et al. (2004b)	Field exclusion / addition experiment. Tension lysimeters at the coniferous site, zero-tension lysimeters at the deciduous site.	**H (Oa) larger contribution** Coniferous (Norway spruce) and deciduous (beech and oak) forest stand. Generally larger concentrations of DOC in Oa. But Oi is the largest contributor when subtracting the Oi from the Oa concentration (called net release in the paper). Coniferous stand Oi larger contribution. Observed a significantly higher DOM at the double litter treatment. Suggesting a similar importance of every organic horizons to the DOM release
Yano et al. 2005	Water extraction	**L ≥ F ≥ H (Oi ≥ Oe ≥ Oa)** Oi being statistically different from Oa, but not from Oe, which is also not statistically different from Oa from coniferous stand
Ganjegunte et al. 2006	Extraction with water	**L > F+H (Oi > Oe+Oa)** Measured significantly larger values of TOC from the Oi horizon than the Oe+Oa horizons

Chapter 3 – Canadian forest floor production of dissolved organic carbon and carbon dioxide during laboratory incubations (manuscript #1)

A brief overview – Context within the thesis

The literature review presented in Chapter 2 clearly showed the state of knowledge regarding the production and biodegradation of dissolved C, N and P within forest floors and the ongoing debate over the major source of dissolved organic carbon in soils. While some studies reported a larger production of DOC from fresh material, others supported a larger DOC release from humified material. The first step towards a better understanding of dissolved element cycling in soil is to resolve the major sources of soil DOC and to identify possible controlling variables. In order to address this objective, samples representing varying degrees of OM decomposition were incubated and subsequently analyzed for DOC and CO_2 production.

Chapter 3 focuses on the partitioning of OM decomposition into DOC and CO_2, and measured microbial biomass C to evaluate its importance in DOC production. Furthermore, we investigated gross DOC production and attempted to identify a method of quantifying its importance during laboratory incubations.

The degree of OM decomposition is the best predictor for DOC and CO_2-C production: both DOC and CO_2-C production from fresh material are statistically greater than that from older material. Initial C content (%) and C:N quotient explained only a small percent of the variation in DOC and CO_2-C production. Microbial biomass C did not change during the incubation and it seems that microbial activity plays a larger role in CO_2 production than DOC production, the latter being mostly the result of biotic (i.e. decomposition) and abiotic (i.e. leaching, enzymatic breakdown) processes. The simultaneous microbial biomass production and consumption of DOC render the characterization and measurement of gross DOC production nearly impossible.

3.1. Introduction

In soils, dissolved organic matter (DOM) mainly originates from throughfall, plant litter, humus, microbial biomass and root exudates (Michalzik and Matzner, 1999; Kalbitz et al., 2000). The majority of DOM is composed of dissolved organic carbon (DOC), which plays an important role in the growth of plants and micro-organisms (Stevenson and Cole, 1999), pedogenetic processes and mineral weathering (Kaiser et al., 2000), and soil and water acidification (Likens et al., 1981). Within a global context, soils are the central point of production and retention of DOM and contribute to the global carbon (C) and nitrogen (N) cycles (McDowell and Likens, 1988; Qualls and Haines, 1991). The importance of DOC within the soil resides in its complex internal cycling and recycling (McDowell and Likens, 1988; Qualls et al., 1991; Currie et al., 1996; Jandl and Solins, 1997). The interaction of these biological, physical and chemical processes controls the internal soil DOC turnover that influences C sequestration in soils (Kalbitz et al., 2000; Michalzik et al., 2001; Kemmitt et al., 2008).

Knowledge of DOC dynamics in soils has been summarised in numerous studies (e.g., Michalzik et al., 2001, Kalbitz et al. 2000, Michalzik and Matzner, 1999), but few studies have examined the role of vegetation type and degree of organic matter (OM) decomposition on the production of DOC (e.g. Bauhus et al., 1998; Côté et al., 2000; Kiikkila et al., 2005). Don and Kalbitz (2005) noted the absence of studies that looked at both tree species and degree of OM decomposition and their year-long litterbag decomposition experiment included five litter types retrieved periodically and subsequently leached in a lab. The study showed that the fresh material (with few or no signs of decomposition) produced larger amounts of DOC, and that the patterns of DOC release through time were different between tree species and environmental conditions. Bauhus et al. (1998) suggested that microbial biomass, varying with the dominant tree species, plays a large role in DOC production as more CO_2-C was produced under coniferous than deciduous stands. Kiikkila et al. (2005) suggested that the production of DOC is affected by the OM quality and Solinger et al. (2001) observed the limited number of studies of deciduous OM, which comprised only one-third of the studies reviewed by Michalzik et al. (2001).

Although several studies, using varied sites, techniques and methods, have gathered a large dataset on DOC production dynamics, there is an ongoing debate about the major source of DOC in the forest floor (Kalbitz et al., 2000; Qualls, 2000; Neff and Asner, 2001; Park et al., 2002). Several studies have suggested that fresh litter (L horizon) was the major source of DOC (e.g. Qualls et al., 1991; Huang and Schoenau, 1998; Michalzik and Matzner, 1999), while others identified older and more decomposed OM (F and H horizons) as the dominant source of DOC (e.g. Kalbitz et al., 2000; Park et al., 2002; Fröberg et al., 2003). The disagreement may be related to the method of measurement, for example water-extraction and tension or zero-tension lysimetry, or the different definitions of DOC. In these field and laboratory studies, net DOC is measured, representing the balance between the inputs, (i.e. by-products of OM decomposition, microbial and root exudates, hydrological transport of accumulated soluble C on litter and humus surfaces), and the outputs (i.e. microbial consumption, sorption onto minerals, C mineralization into CO_2-C) as summarised in Kalbitz et al. (2000). Gross DOC production is more difficult to measure (McDowell et al., 2006) but we may assume, as proposed by Zsolnay and Steindl (1991), Marschner and Kalbitz (2003) and Kemmitt et al. (2008), that the dissolved phase of OM is a prerequisite for the diffusion of substrate to the biomass and that the CO_2-C emitted is only the result of consumption of DOC, neglecting the fraction of C mineralized from the solid pool. Measuring the net DOC produced by leaching, CO_2-C emitted and the change in microbial biomass C (MBC) from pre- to post-incubation would then allow an estimation of gross DOC production.

To determine the rates of DOC and CO_2-C production and partitioning of DOC and CO_2-C production, we incubated 42 samples of organic material from forest floors, varying by species and degree of decomposition, for 30 days under laboratory conditions to control for temperature, moisture and water fluxes. We addressed the following questions:

1) What are the rates of DOC and CO_2-C production and to what degree can the variation be explained by plant species / vegetation type and degree of decomposition?
2) What characterizes the partitioning between DOC and CO_2-C production?

3) Does initial C content and C:N quotient of the OM influence the production of DOC and CO_2-C?
4) Do changes in microbial biomass contribute to DOC and CO_2-C cycling?

3.2. Materials and Methods

3.2.1. Sites

Samples were collected in summer 2005, from 10 sites across Canada, chosen to represent a range in forest floors found across Canadian forests. Details are presented in Table 3.1.

3.2.2. Samples

The forest floor from each site was sampled from two soil pits after horizon designation (Agriculture Canada Expert Committee on Soil Survey, 1987). The two samples were mixed to form one composite sample. Where available, fresh litter (leaves or needles fallen during the previous year), litterfall (collected in the autumn) and fresh canopy needles were collected. The 42 samples were stored at 4°C and field moisture content was preserved. The samples were divided into two categories based on stand type and degree of OM decomposition. Group 1 (stand type) was divided into a) coniferous, b) deciduous and c) other (Table 3.2a). The boreal mixed wood site was considered deciduous, because of the predominance of deciduous material in the L horizon samples. In the category "other" of the vegetation type, we included feather moss (*Pleurozium schreberi*), *Sphagnum* moss (*Sphagnum capillifolium*), lichen (*Cladina stellaris*), *Dicranum* moss (*Dicranum scoparium*) and small roots (< 1 mm, hereafter referred to as 'roots'). Group 2 (degree of decomposition) was divided into a) fresh (such as recent litterfall, roots and needles cut from trees), b) relatively fresh material (L horizon), 3) partly decomposed (F horizon) and 4) decomposed (H horizon) material (Table 3.2b). The "fresh" category included samples taken directly from the trees (fresh needles), the yearly production (litterfall and small roots) or plants growing on the ground at the site (lichen and mosses). The material grouped under the "L" category included the L horizon as well as litter from the previous year litterfall (sampled from the forest floor and named fresh litter) or the needles collected on the floor (old needles). Finally, the partly decomposed material is represented by the F horizon, while the decomposed (humified)

material is the H horizon (Agriculture Canada Expert Committee on Soil Survey, 1987).

3.2.3. Incubation

The chamber method used for the incubation was modified from Nadelhoffer (1990) and Park et al. (2002), using Falcon® 150ml bottle top filters (Figure 3.1a). The membrane was removed, the chambers were washed with deionised water, and fitted with a glass microfibre filter (Whatman GF/A, 47 mm diameter), fibre-glass wool and an O-ring gasket (Figure 3.1b). The upper and lower parts of the chamber were screwed together and sealed with silicon. The weight of sample added to the chamber varied depending on the type of material and the volume of the samples, equivalent to 1 to 10 g of dry material. The chambers were incubated open and in the dark at room temperature (~ 20°C). Each sample was incubated in triplicate.

Production of DOC was determined by leaching on days 1, 7, 14, 21 and 28. At each leaching, 90-ml of deionised water (equivalent to 25 mm of precipitation weekly) was added to the chamber and allowed to equilibrate with the sample for 30 min and then drained by removing the rubber stopper followed by further removal of water with a 60-ml syringe. The leachate was filtered through a 0.45 μm filter paper (Macherey-Nagel 85/90 BF, 25 mm diameter) and stored at 4°C before analysis. Concentration of DOC was measured on a Shimadzu VSN TOC/TN analyzer.

The production of CO_2-C was measured twice a week at days 2, 6, 8, 12, 15, 19, 22, 26 and 29 by tightly closing the chambers with the lid. Headspace air was sampled after zero and 300 minutes with a 1-ml syringe, and CO_2-C concentrations were determined on a Shimadzu Mini2 gas chromatograph with methanizer. Mass of CO_2-C produced was calculated as the change in CO_2-C concentration, using the headspace volume determined at the end of the 30-day incubation. Two days after the final CO_2-C analysis, the incubated samples were frozen for MBC analysis (post-incubation samples).

3.2.4. Chemical analysis

Samples were dried, ground and C and N concentrations were determined on an Elemental Analyzer Carlo Erba™ (instrument model NC2500). Dissolved organic carbon and CO_2 production rates were normalized to C content in the initial sample. Microbial biomass C from the initial litter samples (pre-incubation) and the material

incubated for 30-d (post-incubation) was determined using a chloroform ($CHCl_3$) fumigation-extraction method modified from Voroney et al. (2008). All samples were frozen for 3 to 4 months, allowed to thaw at 4°C for 2 days, and then equilibrated at room temperature for 2 days before analysis. A sub-sample (approximately 1 g of dry material equivalent) was placed in a vacuum dessicator and fumigated with ethanol-free $CHCl_3$ (Basiliko et al., 2006; Voroney et al., 2008) in the dark for 24 h. Samples were transferred to a sealed container with 0.5 M K_2SO_4 solution, gently shaken for one hour on an oscillating shaker, centrifuged at 200 rpm and filtered through Whatman ® GF 934-AH filter paper. The filtrate was analysed for total C concentration in the Shimadzu VSN TOC/TN analyzer. The same extraction and analysis procedure was also done on non-fumigated sub-samples. Following Joergensen (1996) and Park et al. (2002), we used an efficiency coefficient of 0.45 to estimate the MBC.

The values obtained from the fumigation-extraction method are presented as the average of triplicate of non-fumigated samples, and the average value for the individual samples for the post-incubation samples (incubated in triplicates).

3.2.5. Statistical analysis

Prior to statistical analysis, we tested the normality of the distribution of our dataset using a Kolmogorov-Smirnov following a Lilliefors test. When needed, values were log-transformed to achieve normality before analysis. Where it was not possible to get a normal distribution, we used non-parametric tests. Normality of regression residuals was tested using the same Kolmogorov-Smirnov test.

We used a one-way ANOVA to evaluate the differences between the measurements from the incubation, in respect to individual and cumulative samples, stand types and degree of OM decomposition. Post-hoc comparisons were done using the Tukey procedure. We used a Mann-Whitney test to compare the MBC between the pre- and post-incubation material and between stand type and degree of OM decomposition. The programs used to do the statistical analyses are SPSS v. 15.0 and SYSTAT 10.0.

3.3. Results

3.3.1. DOC production

There was some variation in the rates of DOC production through the 30-d incubation. In 18 of the 42 samples, there was no statistically significant variation (slope not different from 0) of DOC concentrations with time, but in 8 of these, there was an initial increase followed by a decrease. In 5 samples there was a significant increase ($P < 0.05$) of DOC concentration through time (mainly plant materials and fresh litters), and in the remaining 19 samples there was a decrease ($P < 0.05$) (mainly coniferous litters).

Production of DOC (cumulative curves showed in Figure 3.2) over the 30-d incubation (ΣDOC_{30d}) ranged from 1.1 to 78.0 mg DOC g C^{-1} with a mean of 11.7 ± 13.8 mg DOC g C^{-1} (Figure3.3a). For all but two sites, the ΣDOC_{30d} from fresh material was always significantly greater ($P < 0.05$) than from the more decomposed F / H horizon material (Table 3.3). In the white pine and jack pine stands, there was no significant decrease in ΣDOC_{30d} with depth and the F/H horizons were not significantly different from the L horizon.

The five largest ΣDOC_{30d} values were hazelnut litterfall, roots, *Sphagnum*, balsam fir fresh litter and Douglas fir L horizon. The five smallest ΣDOC_{30d} values were the H horizons of the mixed woods, Douglas fir, maple and black spruce stands and the jack pine needles. There were no significant differences in ΣDOC_{30d} between coniferous and deciduous stands, but ΣDOC_{30d} were significantly larger in the "other" group, which includes feather moss, *Sphagnum* and *Dicranum* mosses, lichen and roots. The total amount of DOC produced from fresh materials and L horizons is not statistically different from one another, but significantly larger than older material (F and H horizons), which are also significantly different from one another (Fresh ≈ L > F > H; ANOVA, $P < 0.05$; statistical data not shown). When separated into stand type and degree of OM decomposition, the only significant difference was that fresh deciduous litters produced more DOC than the fresh coniferous litters (Table 3.4).

Spruce needles had a very small DOC production rate at the beginning of the experiment, followed by a much larger value starting from week three. As Don and Kalbitz (2005) suggested, the chemical composition of the needles could protect the needles from fast DOC release, explaining the lag before the maximum release of DOC.

Two problems arise from this hypothesis: 1) the time scale of our incubation and 2) the absence of similar behaviour for the other fresh needles in our study (jack pine, white pine and Douglas fir). Further investigation needs to be done in order to understand the pattern of DOC release from needles compared to leaves.

For the Douglas fir stand, the older needles and L horizon produced more DOC than fresh needles, but the F and H layers produced significantly less. Furthermore, for the white pine and jack pine stands more decomposed horizons produced larger amount of DOC than fresh material. These patterns were not observed in the deciduous stands, generally being L > F > H.

3.3.2. CO_2 production

There were no major changes in CO_2-C production through time for the majority of the 42 samples (P >0.05). The production of CO_2-C (cumulative curves shown in Figure 3.4) during the 30-d incubation ($\Sigma CO_2\text{-}C_{30d}$) ranged between 1.4 and 104.2 mg CO_2-C g C^{-1} with a mean of 18.4 ± 16.9 mg CO_2-C g C^{-1} (Figure 3.3b). The largest $\Sigma CO_2\text{-}C_{30d}$ values are for roots, aspen litterfall, L horizons from aspen, fresh balsam fir litter and L horizon of maple / beech, where only the roots and the aspen litterfall are not statistically different. The $\Sigma CO_2\text{-}C_{30d}$ values are smallest for the H horizons from the mixed woods, maple / beech, Douglas fir, maple and spruce/pine, and these values are not statistically different from one another. The same statistical results were observed for $\Sigma CO_2\text{-}C_{30d}$ as for ΣDOC_{30d} with respect to stand type and degree of OM decomposition. The L horizon samples have higher $\Sigma CO_2\text{-}C_{30d}$ values for the deciduous than the coniferous samples, while the H horizons from coniferous samples produced ($P < 0.10$) more $\Sigma CO_2\text{-}C_{30d}$ than the deciduous H horizons.

3.3.3. Partitioning between DOC and CO_2-C production

Over the 30-d incubation, the total DOC + CO_2-C release ranged from 0.2 to 14.8% of the initial C content, with a mean of 3.0% ± 2.7. Of this, DOC represented an average of 1.2% ± 1.4 (0.1 to 7.8%) and CO_2-C an average of 1.8% ± 1.7 (0.1 to 10.4%) of the initial C content. There was no significant difference between coniferous and deciduous vegetation types, but the "other" type of vegetation was significantly different.

The total C released is not statistically different between the fresh group and L horizon, but follow the trend: L > F > H.

The CO_2-C:DOC quotient calculated using $\Sigma CO_2\text{-}C_{30d}$:$\Sigma DOC_{30d}$ ranged from 0.3 to 8.5 with a mean of 2.1 ± 1.7 (Figure 3.3c). The quotients are not significantly different according to stand type. The fresh and L horizon quotients are not statistically different, but the latter are different from F and H horizons which are not statistically different from one another.

Of the 42 samples, 19% produced more DOC than CO_2-C (CO_2-C:DOC < 1), 30% produced approximately the same amount of DOC and CO_2-C (quotient 1.0 to 1.5), 17% had a CO_2-C:DOC quotient between 1.5 and 2, 10% between 2 and 2.5 and only 24% produced more than 2.5 times more CO_2-C than DOC (Table 3.3). Most of the coniferous litter types were in the 1 to 1.5 CO_2-C:DOC quotient category, while deciduous litter was distributed almost evenly between categories. There was no relationship between the degree of OM decomposition and the CO_2-C:DOC quotient.

3.3.4. Initial C concentration and C:N quotient

The C concentration of the initial samples ranged between 22.2 and 52.2% (average 45.1%, SD = 5.8%) and the C:N quotient varied between 16.5 and 86.2 (average 37.2 ± 16.7, Table 3.3). Using linear regression analysis we found significant positive relationships between sample C concentration (%C) and ΣDOC_{30d} ($P < 0.01$, $r^2 = 0.11$), $\Sigma CO_2\text{-}C_{30d}$ ($P < 0.01$, $r^2 = 0.17$, after removing the values from the roots which are outliers) and CO_2-C:DOC ($P = 0.01$, $r^2 = 0.05$). We also found a significant regression between C:N quotient and ΣDOC_{30d} ($P = 0.04$, $r^2 = 0.03$) and with $\Sigma CO_2\text{-}C_{30d}$ ($P = 0.02$, $r^2 = 0.05$) (Figure 3.5). There was no significant relationship between C:N quotient of the initial samples and the CO_2-C:DOC quotient (Figure 3.5f).

When dividing the dataset into stand type, in the deciduous stand there was a significant positive relationship between %C and ΣDOC_{30d}, $\Sigma CO_2\text{-}C_{30d}$ and CO_2-C:DOC quotient ($P < 0.05$; $r^2 = 0.41$, 0.51 and 0.19, respectively). In the coniferous stand there was a significant positive relationship between %C and $\Sigma CO_2\text{-}C_{30d}$ ($P = 0.01$, $r^2 = 0.10$). In the 'other' vegetation group %C was significantly related to ΣDOC_{30d} and $\Sigma CO_2\text{-}C_{30d}$ ($P < 0.05$, $r^2 = 0.70$ and 0.36 respectively). The C:N quotient of the initial samples had a significant regression with ΣDOC_{30d} and $\Sigma CO_2\text{-}C_{30d}$ for both divisions into deciduous and

coniferous ($P < 0.00$, $r^2 = 0.10$ to 0.60), but not with the CO_2-C:DOC quotient. On the other hand, the other type of vegetation was only significantly related to the CO_2-C:DOC quotient ($P = 0.01$, $r^2 = 0.46$). When dividing into degree of decomposition (fresh, L, F and H), the only significant relationship was in the F horizon between %C and ΣCO_2-C_{30d} ($P = 0.04$, $r^2 = 0.16$).

3.3.5. Microbial biomass C

The MBC in the pre-incubation samples ranged from 0.1 to 10.5 mg C g C^{-1}, with an average of 2.5 ± 2.6 mg C g C^{-1} and the post-incubation samples ranged from 0.03 to 8.85 mg C g C^{-1}, with an average of 2.5 ± 2.4 mg C g C^{-1} (Figure 3.6). Pre- and post-incubation MBC decreased with increasing degree of OM decomposition (Mann-Whitney, $P < 0.05$), except there was no difference between fresh and L horizons within the pre-incubation samples. As for the division into vegetation type, the type of vegetation 'other' was significantly different from both deciduous and coniferous (Mann-Whitney, $P < 0.05$), but no significant difference was observed between deciduous and coniferous.

Differences between the pre- and post-incubation MBC were never significant (Mann-Whitney, $P = 0.12$), but the numbers suggest a general decline in the MBC. Coniferous and 'other' showed a significant decrease of MBC (Mann-Whitney, $P = 0.02$ and 0.00 respectively) with a decrease from 2.08 to 1.69 mg MBC g C^{-1} (coniferous) and from 6.65 to 4.07 mg MBC g C^{-1} for the other vegetation type, with *Sphagnum* losing nearly half the MBC during incubation. However, when divided by stand type, deciduous showed a significant increase in MBC (Mann-Whitney, $P = 0.00$, from average 1.91 to 3.25 mg MBC g C^{-1}). Division into degree of OM decomposition did not show a significant difference between pre- and post-incubation MBC (Mann-Whitney, $P > 0.05$). Taken together, our MBC results suggest that there is no overall, unidirectional change in the size of the MBC during the 30-d incubation. For this reason, further analysis using MBC will be done with the average values between pre- and post-incubation and MBC assumed to be at steady-state.

The %C of the initial samples was not strongly related ($P = 0.02$, $r^2 = 0.05$) to the MBC values, despite the regression slope value being significantly different from zero (P

< 0.02). Similarly, there was a weak relationship between C:N quotient of the initial sample and MBC ($P < 0.05$, $r^2 = 0.08$).

The positive regression slopes between the average MBC and ΣDOC_{30d}, ΣCO_2-C_{30d} and CO_2-C:DOC quotients are significantly different from zero for all variables ($P < 0.05$), but the ΣDOC_{30d} and ΣCO_2-C_{30d} present larger r^2 values (0.28 and 0.35 respectively) than for the quotient, which has an r^2 of only 0.02 (Figure 3.7).

To estimate C turnover by the microbial community, we calculated the amount of DOC or CO_2-C produced per unit of MBC per day. The average DOC:MBC quotient was 191 ± 177 mg DOC g MBC^{-1} day^{-1} (max: 891, min: 20) and was 301 ± 281 mg CO_2-C g MBC^{-1} day^{-1} (max: 1401, min: 40) for the average CO_2-C:MBC quotient (metabolic quotient, qCO_2). The values of DOC turnover did not show any statistical difference according to stand type or degree of OM decomposition, but qCO_2 were significantly different between stand types ($P < 0.05$).

3.4. Discussion

3.4.1. Rates of DOC production and their controls

Our DOC production rates at 20°C over 30 days ranged from 1.1 to 78.0 mg DOC g C^{-1}, with an average 11.7 ± 13.8 mg DOC g C^{-1}, respectively. These results are similar to those obtained by others over a similar duration: <25 mg DOC g C^{-1} from a beech stand (Hagedorn and Machwitz, 2007); 6.2 to 15 mg DOC g C^{-1} from maple, beech, spruce and pine (Don and Kalbitz, 2005); 2 to 12 mg DOC g C^{-1} for the F horizon and 1 to 7 mg DOC g C^{-1} for the H horizon of a Norway spruce stand (Michel and Matzner, 1999); 5 to 55 mg DOC g C^{-1} (estimation from figure, incubated at 30°C, for 360 days – equivalent 0.4 to 17 mg DOC g C^{-1} for 30 days, Neff and Hooper, 2002); and 10 to 200 mg DOC g $soil^{-1}$, for a 15 week period – representing around 1.4 to 27 mg DOC g C^{-1} for a 4 week period (Magill and Aber, 2000).

We conclude that degree of OM decomposition is the most important control on DOC production (Figure 3.3), with the L horizon showing largest values, followed by those of F and H horizons. This finding is supported by many studies (Qualls et al., 1991; Huang and Schoenau, 1998; Michalzik and Matzner, 1999; Moore and Dalva, 2001;

Cleveland et al., 2004). Others, however, have found the largest production of DOC in more decomposed material (Kalbitz et al., 2000; Park et al., 2002; Fröberg et al., 2003).

In our incubation, there were no significant differences in DOC and CO_2-C between vegetation types (coniferous v. deciduous forest), and this may reflect the greater importance of the degree of OM decomposition, thus overshadowing the possible effect of tree species in the DOC production observed in other studies (e.g. Bauhus et al., 1998; Kiikkila et al., 2005).

3.4.2. Rates of CO_2 production and their controls

Carbon dioxide production varied between 1.4 and 104.2 mg CO_2-C g C^{-1} with an average of 18.4 ± 16.9 mg CO_2-C g C^{-1} respectively, with little variation through the incubation, for most of our samples. These rates are similar to those reported by others. Hazlett et al. (2007) determined CO_2 production rates of 2.7 to 4.2 mg CO_2-C g C^{-1} for 30 days; Kanerva and Smolander (2007) reported 50 to 60 mg CO_2-C g^{-1} over 8 weeks for L (12.5 to 15 mg CO_2-C g C^{-1} for 4 weeks) and 20 to 30 (5 to 7.5 mg CO_2-C g C^{-1} for 4 weeks), and 8 to 10 mg CO_2-C g^{-1} for H horizons (2 to 2.5 mg CO_2-C g C^{-1} for 4 weeks), respectively; Park and Matzner (2003) observed rates that ranged between 0.48 to 1.08 mg CO_2-C g C^{-1} day^{-1} (estimated to 14.4 to 32.4 mg CO_2-C g C^{-1} for 30 days); Neff and Hooper (2002) reported production rates that were from 50 to 425 mg CO_2-C g C^{-1} for 360 days (estimated to 4.2 to 35.4 mg CO_2-C g C^{-1} for 30 days).

Our results showed a pattern of CO_2-C production similar to that of DOC production, with larger production rates from fresh material compared to older OM. Incubation of fresh material produced more CO_2-C during the month of the incubation than the more decomposed OM. Gödde et al. (1996) suggested that the degree of OM decomposition might have a larger effect on CO_2-C than DOC production, but this does not seem to be the case in our experiment.

3.4.3. Partitioning into DOC and CO_2-C

Few studies have examined both DOC and CO_2-C lost during OM decomposition (e.g. Moore and Dalva, 2001; Neff and Hooper, 2002). Our range of CO_2-C: DOC quotient is between 0.3 and 8.5 with an average 2.1 ± 1.7. Moore and Dalva (2001) and Moore et al. (submitted) reported CO_2-C: DOC quotients ranging from 1 to 100 and Neff

and Hooper (2002) found values from 0.5 to 3. We observed more DOC than CO_2-C production in 8 out of the 42 samples, mainly F or H horizons, suggesting that the material might be composed of recalcitrant compounds, slowing down decomposition (hence C mineralization), but still releasing considerable amount of DOC. This release is probably the result of physico-chemical leaching or enzymatic activity (Kemmett et al., 2008). Our results suggest that factors controlling the DOC release are different and not solely related to the C decomposition, supported by the results of Neff and Hooper (2002), Park et al. (2002) and Hagedorn and Machwitz (2007). Other studies, however, suggest that similar factors control production of both DOC and CO_2-C (e.g. Christ and David, 1996; Kalbitz et al., 2000).

About 62% of our samples had a CO_2-C:DOC quotient below 2, highlighting the importance of DOC as being a large contributor to C cycling within soils. As both labile and recalcitrant fractions of DOC percolate through the soil profile, a large proportion will be mineralized into CO_2-C (Neff and Hooper, 2002), leading to a large contribution to the atmosphere. This is particularly true for the labile compounds in DOC which will be mineralized, while the most recalcitrant compounds will be preferentially sequestered in the soil organic matter (SOM) through organo-mineral complex formation and sorption (i.e. Qualls et al., 1991; Courchesne and Hendershot, 1997; Michalzik et al., 2001). In the field, other factors such as soil moisture status (Christ and David, 1996; Neff and Hooper, 2002), temperature and frequency of precipitation (Gödde et al., 1996) will influence the partitioning between the DOC and CO_2-C production.

Carbon dioxide and DOC may be produced from different pools. While DOC can be produced by both biotic, such as decomposition, and abiotic processes, such as chemical oxidation and hydrolysis, diffusion through pores, desorption and action of extracellular enzymes (Kemmitt et al., 2008), CO_2-C production is believed to be mainly the result of microbial activity, which consumes the C from soil once it passes into the dissolved phase (e.g. Marschner and Kalbitz, 2003; Kemmitt et al., 2008).

The total amount of C lost during the 30-d incubation varied between 0.27 to 13 % of the initial C content of the litter. Over 1 year, Don and Kalbitz (2005) measured a maximum of 6.2% loss (~0.5% loss per month in field conditions), and Park and Matzner (2003) reported a total loss of 13% yearly. Cleveland et al. (2004) reported a loss of

between 5 to 15% of C content from McDowell and Likens (1988) and Zsolnay and Steindl (1991).

3.4.4. Sample quality

Although ΣDOC_{30d}, $\Sigma CO_2\text{-}C_{30d}$ or CO_2-C:DOC quotient values were significantly related ($p < 0.05$) to sample C concentration and C:N quotient, r^2 values were small. We obtained stronger results when dividing the database into vegetation type, but not when dividing into degree of OM decomposition. Gödde et al. (1996) found a significant positive correlation between DOC production and soil C:N quotient, while Neff and Hooper (2002) and Michel and Matzner (1999) were unable to find a significant relationship. Moore et al. (submitted) were unable to relate DOC or CO_2-C production in laboratory incubations to C:N quotient and proximate analyses (i.e. lignin, cellulose). They also found that CO_2-C production was more dependent on temperature than DOC production, supporting the idea of a larger control of microbial activity on the production of CO_2-C than DOC. However, Kemmitt et al. (2008) suggest that mineralization of C occurs at a relatively constant rate independent of microbial activity and that the microbial community adjusts its production to the amount of available dissolved substrate.

Our results, along with others, suggest that the degree of OM decomposition is the best predictor of DOC (and CO_2-C) production during laboratory incubations, within the range of samples analyzed. Dissolved organic C production seems to be the result of a far too complex process to rely only on quality index for prediction purpose.

3.4.5. The role of microbial activities

We were unable to detect a significant change in MBC during our 30-day incubations, similar to the findings of Hazlett et al. (2007) during their 90-day incubation of F horizons. The *Sphagnum* moss lost half of its MBC during the incubation and had one of the largest DOC and CO_2-C production rates (29.9 mg DOC g C^{-1} and 23.8 mg CO_2-C g C^{-1}, respectively) and one of the highest initial C:N quotients (77). In their study of a black spruce forest, Wickland et al. (2007) found that leachates of *Sphagnum* had a large biodegradability, contrasting with the slow decomposition rate of *Sphagnum* moss. Thus, the decrease in MBC in the *Sphagnum* sample may be related to the exhaustion of

the biodegradable DOC pool, and the consequent loss of MBC. This issue needs further investigation.

The average DOC:MBC quotient was 191 ± 177 mg DOC g MBC^{-1} day^{-1} (max: 891, min: 20) and was 301 ± 281 mg CO_2-C g MBC^{-1} day^{-1} (max: 1401, min: 40). Our metabolic quotients (CO_2-C:MBC) varied between 40 to 1401 with an average of 301 ± 281 mg CO_2-C g MBC^{-1} day^{-1}. Other studies reported 52 for L horizon, between 32 and 47 for the F horizon and between 17 and 27 for H horizon (Kanerva and Smolander, 2007) and between 2 to 6 for lodgepole pine F/H horizon (Thirukkumaran and Parkinson, 2000). The lower range of our data is within the reported results. The DOC:MBC for our study are between 20 and 891, with average of 191 ± 177 and we are unaware of any comparable data. Given that DOC production can be partially abiotic, through simple leaching, there should not be as strong a relationship with MBC as for CO_2 production (Schimel and Wieintraub, 2003; Bengtson and Bengtsson, 2007; Hagedorn and Machwitz, 2007; Kemmitt et al., 2008).

3.4.6. Gross DOC production

Gross DOC production is the sum of all DOC inputs (i.e. microbial exudates and lysis, enzymatic breakdown of OM and physical solubilization/leaching), before any DOC uptake by microbes (Figure 3.8). Many studies support the idea that the C respired as CO_2 can only result from microbial DOC uptake (Zsolnay and Steindl, 1991; Marschner and Kalbitz, 2003; Bengtson and Bengtsson, 2007; Kemmitt et al., 2008), so that none of the C would be taken up directly from the solid OM. Our results support another assumption that the microbial biomass is constant. Furthermore, as proposed by Bengtson and Bengtsson (2007), since the microbial biomass is constant, the amount of C assimilated would be equal to the amount of C exuded back to the DOC pool. The microbial biomass uses about half of the C for respiration and the other half for maintenance. Bengtson and Bengtsson (2007) estimated maintenance as 42% of microbial C uptake in a beech/oak forest soil. Thus, the amount of DOC mobilized by microbes should be approximately equal to the CO_2-C produced. Bengtson and Bengtsson (2007) also proposed, using ^{13}C analyses, that 60% of the DOC pool was from microbial mobilization, and the 40% left was from enzymatic breakdown of OM (which would also include the leaching).

A problem with this previous view of DOC cycling is that the amount of DOC needed for uptake is larger than the total amount of DOC input. Bengtson and Bengtsson (2007) and Kemmitt et al. (2008) suggest that DOC is turned over quickly, so this component is not measured in conventional DOC analyses.

If we assume a steady state in MBC, combining CO_2-C and net DOC production is the first approximation of gross DOC production. One of the challenges in calculating gross DOC production is that microbial biomass is a collection of a myriad of individual microbes that intake and release simultaneously DOC rather than a large entity that consume and then exude DOC.

3.4.7. Conclusions

Our results showed that DOC and CO_2-C production is generally related to the degree of decomposition of soil organic matter. Deciduous and coniferous materials resulted in similar production rates, but live tissues, such as *Sphagnum*, and *Dicranum* mosses, lichen and roots, produced significantly larger amounts of DOC than the other forms of organic matter. DOC production results from both abiotic and biotic processes and some samples produced more DOC than CO_2-C. Initial C content and C:N quotients were not good predictors of DOC or CO_2-C production, over our range of samples. MBC did not change during most of the incubations and the production of DOC and CO_2-C per MBC was variable. DOC production is the result of microbial mobilization, enzymatic breakdown of OM and leaching, so that numerous factors and interactions can influence the production of DOC.

Table 3.1. Characteristics of the 10 sites from which samples were taken.

Site	Vegetation	Elevation (m)	Mean annual Temperature (° C)	Mean annual Precipitation (mm)
Douglas fir, Campbell River, BC (49.51°N, 125.19°W)	57 years old Douglas fir (*Pseudotsuga mensiesii*); red cedar (*Thuja plicata*) and western hemlock (*Tsuga heterophylla*)	300	8.3	1461
Black spruce, Waskesiu, SK (53.99°N, 105.12°W)	111 years old black spruce (*Picea mariana*) with feather moss ground cover	629	0.4	467
Jack pine, Waskesiu, SK (53.91°N, 104.69°W)	91 years old jack pine (*Pinus banksiana*); lichen ground cover	579	0.4	467
Aspen, Waskesiu, SK (53.63°N, 106.20°W)	83 years old aspen (*Populus tremuloïdes*); thick hazel (*Corylus spp.*) understorey	600	0.4	467
Boreal mixed wood, Groundhog River, ON (48.22°N, 82.16°W)	74 years old boreal mixed wood: aspen (*P. tremuloïdes*), black spruce (*P. mariana*), white spruce (*Picea glauca*), white birch (*Betula papyrifera*) and balsam fir (*Abies balsamea*)	340	2.1	834
White pine, Turkey Point, ON (42.22°N, 80.36°W)	65 years old white pine (*Pinus strobus*) plantation	784	8.1	832
La Tirasse Lake, St-Félicien, QC (49.12°N, 73.29°W)	66-85 years old black spruce (*P. mariana*) and jack pine (*P. strobus*)	400	0	817
Laflamme Lake, Québec, QC (47.17°N' 71.14°W)	55-60 years old balsam fir (*A. balsamea*)	800	-0.6	
Hermine Watershed, St-Hippolyte, QC (45.59°N, 74.01°W)	85 years old sugar maple (*Acer saccharum*) with american beech (*Fagus grandifolia*) and yellow birch (*Betula alleghaniensis*)	400	3.9	1150
Mont St-Hilaire, Saint-Hilaire, QC (45.31°N; 73.08°W)	Up to 400 years old sugar maple (*A. saccharum*) and american beech (*F. grandifolia*)	350	5.9	1017

Table 3.2. Samples divided into: a) vegetation type and b) degree of decomposition.

a. Vegetation type

Coniferous (n = 22)	**Deciduous** (n = 15)	**Other** (n = 5)
Douglas fir fresh needles	aspen leaves litterfall	feather moss
Douglas fir old needles	hazel leaves litterfall	*Sphagnum*
Douglas fir L	aspen L	lichen
Douglas fir F	aspen F	*Dicranum*
Douglas fir H	aspen H	roots
black spruce fresh needles	mixed wood L	
black spruce L	mixed wood F	
black spruce F	mixed wood H	
black spruce H	maple fresh litter	
jack pine fresh needles	maple L	
jack pine old needles	maple F	
jack pine H	maple H	
white pine fresh needles	maple / beech L	
white pine L	maple / beech F	
white pine F	maple / beech H	
spruce / pine L		
spruce / pine F		
spruce / pine H		
balsam fir fresh litter		
balsam fir L		
balsam fir F		
balsam fir H		

b. Degree of decomposition

Fresh (n = 11)	**L** (n = 13) relatively fresh	**F** (n = 9) partly decomposed	**H** (n = 9) decomposed
Douglas fir fresh needles	Douglas fir old needles	Douglas fir F	Douglas fir H
black spruce fresh needles	Douglas fir L	black spruce F	black spruce H
feather moss	black spruce L	aspen F	jack Pine H
Sphagnum	jack pine old needles	mixed wood F	aspen H
jack pine fresh needles	aspen L	white pine F	mixed wood H
lichen	mixed wood L	spruce / pine F	spruce / pine H
aspen leaves litterfall	white pine L	balsam fir F	balsam fir H
hazel leaves litterfall	spruce / pine L	maple F	maple H
white pine fresh needles	balsam fir fresh litter	maple / beech F	maple / beech H
Dicranum	balsam fir L		
roots	maple fresh litter		
	maple L		
	maple / beech L		

Table 3.3. Production of DOC and CO_2-C during the 30-d incubation (average and standard deviation), CO_2-C + DOC produced as % of initial C, CO_2-C:DOC quotient and C:N quotient and C content of the initial sample. Standard deviation is in parentheses. (*see next page*)

Sample	DOC* (mg DOC g C^{-1})	CO_2-C* (mg CO_2-C g C^{-1})	CO_2-C + DOC (% of initial C)	CO_2-C:DOC	C :N sample	C %
Douglas fir, Campbell River						
Douglas fir fresh needles	2.9 (0.7)^{ad}	22.9 (3.5)^{a}	2.6	7.9^{a}	35.1	51.2
Douglas fir old needles	8.2 (1.5)^{b}	10.5 (1.0)^{b}	1.9	1.3^{b}	35.4	44.3
Douglas fir L	23.7 (3.1)^{c}	28.6 (4.2)^{a}	5.2	1.2^{b}	53.9	43.7
Douglas fir F	3.5 (0.6)^{a}	4.8 (0.7)^{c}	0.8	1.4^{b}	60.7	46.7
Douglas fir H	2.0 (0.1)^{d}	1.8 (0.3)^{d}	0.4	0.9^{c}	78.4	49.4
Black spruce, Waskesiu						
black spruce fresh needles	13.7 (2.9)^{a}	24.1 (3.5)^{a}	3.8	1.8^{ad}	51.7	52.3
feather moss	14.1 (2.5)^{a}	14.5 (4.4)^{ab}	2.9	1.0^{bc}	45.5	46.4
Sphagnum	29.9 (5.6)^{b}	23.8 (3.9)^{a}	5.4	0.8^{bc}	76.9	44.6
black spruce L	10.8 (2.1)^{a}	16.1 (1.7)^{ab}	2.7	1.6^{a}	43.8	46.0
black spruce F	4.3 (0.1)^{c}	5.5 (0.5)^{c}	1.0	1.3^{ac}	42.7	48.3
black spruce H	2.3 (0.4)^{d}	6.3 (0.6)^{c}	0.9	2.8^{d}	27.0	22.1
Jack pine, Waskesiu						
jack pine fresh needles	2.0 (0.1)^{a}	10.5 (1.3)^{a}	1.3	5.2^{a}	36.9	51.3
jack pine old needles	3.7 (0.5)^{ac}	14.6 (3.4)^{a}	1.8	3.9^{ac}	37.9	50.7
Lichen	15.6 (7.0)^{b}	17.0 (6.6)^{a}	3.3	1.1^{b}	86.2	43.1
jack pine H	4.3 (0.7)^{c}	13.6 (1.5)^{a}	1.8	3.3^{c}	40.7	42.7
Aspen, Waskesiu						
aspen litterfall	14.8 (6.4)^{a}	49.2 (7.4)^{a}	6.4	3.8^{a}	49.2	49.7
hazel litterfall	78.0 (0.3)^{b}	33.1 (5.8)^{b}	11.1	0.4^{b}	40.2	50.2
aspen L	14.5 (1.3)^{a}	43.6 (6.3)^{a}	5.8	3.0^{ac}	43.8	48.2
aspen F	6.5 (0.4)^{c}	13.6 (2.6)^{c}	2.0	2.1^{ac}	19.6	42.0
aspen H	4.3 (0.1)^{c}	6.8 (1.1)^{d}	1.1	1.6^{c}	24.3	37.4
Boreal mixed wood, Groundhog River						
mixed woods L	6.3 (1.5)^{a}	33.0 (6.0)^{a}	3.9	5.3^{a}	22.3	48.1
mixed woods F	4.5 (0.2)^{a}	6.4 (0.6)^{b}	1.1	1.4^{b}	16.5	44.5
mixed woods H	1.1 (0.1)^{b}	1.5 (0.2)^{c}	0.3	1.4^{b}	17.1	38.4
White pine, Turkey Point						
white pine fresh needles	5.6 (1.2)^{a}	36.0 (2.4)^{a}	4.2	6.6^{a}	26.7	51.0
Dicranum	5.0 (0.5)^{a}	11.4 (2.3)^{b}	1.6	2.3^{b}	36.1	36.4
white pine L	16.3 (1.1)^{b}	23.3 (4.3)^{c}	4.0	1.4^{bc}	29.2	49.0
white pine F	19.3(2.5)^{b}	17.3 (2.5)^{c}	3.7	0.9^{c}	27.2	43.1
Black spruce and jack pine, Tirasse Lake						
spruce / pine L	5.6 (1.5)^{a}	9.9 (2.4)^{a}	1.6	1.8^{a}	47.3	48.3
spruce / pine F	7.3 (0.5)^{a}	10.8 (1.2)^{a}	1.8	1.5^{a}	54.5	48.5
spruce / pine H	2.5 (0.1)^{b}	4.1 (0.3)^{b}	0.7	1.6^{a}	48.8	42.9
Balsam fir, Laflamme Lake						
balsam fir fresh litter	29.1 (2.6)^{a}	40.2 (6.4)^{a}	6.9	1.4^{a}	30.2	50.1
balsam fir L	14.2 (1.0)^{b}	18.2 (2.3)^{b}	3.2	1.3^{a}	24.1	49.6
balsam fir F	11.1 (2.7)^{b}	8.6 (0.9)^{c}	2.0	0.8^{b}	19.1	48.6
balsam fir H	4.9 (0.4)^{c}	4.2 (0.8)^{d}	0.9	0.9^{b}	27.7	42.6
Sugar maple, Hermine - Saint-Hippolyte						
maple fresh litter	9.8 (1.6)^{a}	27.2 (10.9)^{a}	3.7	2.7^{a}	32.4	45.4
maple L	7.2 (0.4)^{a}	15.6 (2.5)^{a}	2.3	2.2^{a}	22.8	45.7
maple F	4.9 (0.6)^{b}	8.9 (1.1)^{b}	1.4	1.8^{b}	19.9	44.9
maple H	2.3 (0.3)^{c}	2.8 (0.2)^{c}	0.5	1.2^{c}	22.7	40.9
roots	42.5 (3.6)^{d}	88.2 (13.9)^{d}	13.1	2.1^{a}	37.7	48.3
Sugar maple and american beech, Mont Saint-Hilaire						
maple / beech L	22.3 (2.4)^{a}	37.9 (1.0)^{a}	6.0	1.7^{a}	37.2	45.3
maple / beech F	7.3 (0.4)^{b}	4.2 (0.9)^{b}	1.2	0.6^{b}	18.6	43.7
maple / beech H	3.9 (0.6)^{c}	1.8 (0.2)^{c}	0.6	0.5^{b}	20.1	29.3

$^{a, b, c, d}$ Statistical analysis ANOVA, post-hoc Tukey was performed. Different letters within a study site represent significant differences between the sample value for a given variable at $\alpha = 0.05$. Statistical analysis of DOC, CO_2-C, and CO_2-C :DOC quotient was done on log-transformed data.

Table 3.4. Average (standard deviation in parentheses) of samples within groups of Fresh, L, F and H decomposition degree divided between coniferous and deciduous stand types.

	Coniferous			Deciduous		
	DOC (mg DOC g C^{-1})	CO_2-C (mg CO_2-C g C^{-1})	CO_2-C:DOC	DOC (mg DOC g C^{-1})	CO_2-C (mg CO_2-C g C^{-1})	CO_2-C:DOC
Fresh +	10.7 (10.5)	26.7 (11.3)	4.6 (2.8)**	34.2 (33.1)**	36.5 (12.3)	2.3 (1.8)
L	11.8 (6.7)	17.3 (6.9)	1.8 (1.0)	12.6 (6.9)	32.5 (11.6)**	3.0 (1.5)
F	8.4 (5.8)	9.3 (4.4)	1.3 (0.4)	6.1 (1.3)	8.1 (4.5)	1.4 (0.7)
H	3.2 (1.2)	6.0 (4.2)*	1.9 (1.1)	2.9 (1.4)	3.2 (2.3)	1.2 (0.5)

+ Fresh material includes new needles, litterfall, and fresh L horizons of needles from the forest floor. This table exclude all fresh plants and roots considered as others in Table 3

* Significantly higher (coniferous vs. deciduous) at $\alpha = 0.10$, ANOVA, post-hoc Tukey, done on log-transformed values.

** Significantly higher (coniferous vs. deciduous) at $\alpha = 0.05$, ANOVA, post-hoc Tukey, done on log-transformed values.

Figure 3.1. Incubation chamber view a) Falcon © schematic of the chamber and b) cross-section of the set-up of the incubation chambers.

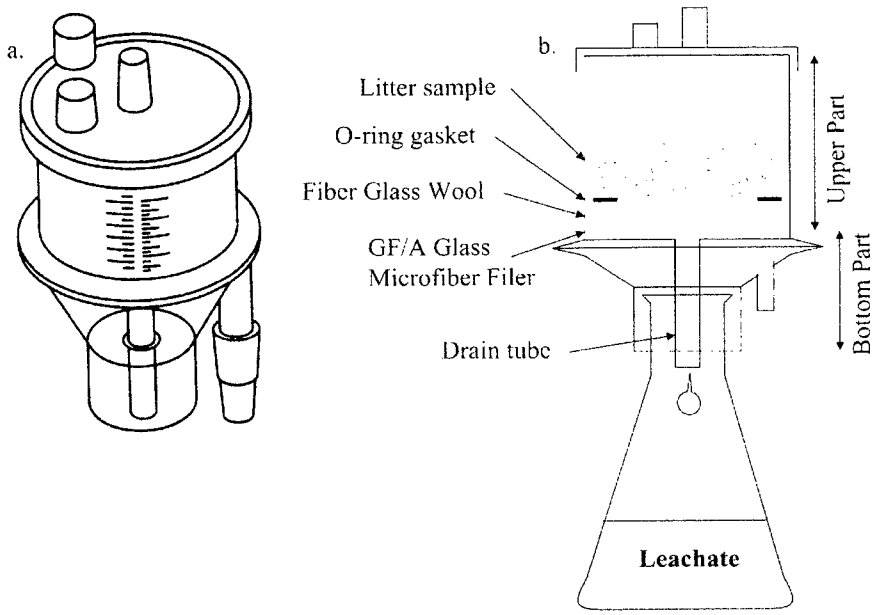


Figure 3.2. Mean cumulative DOC produced (mg DOC g C^{-1}) during the incubation, organized by site.

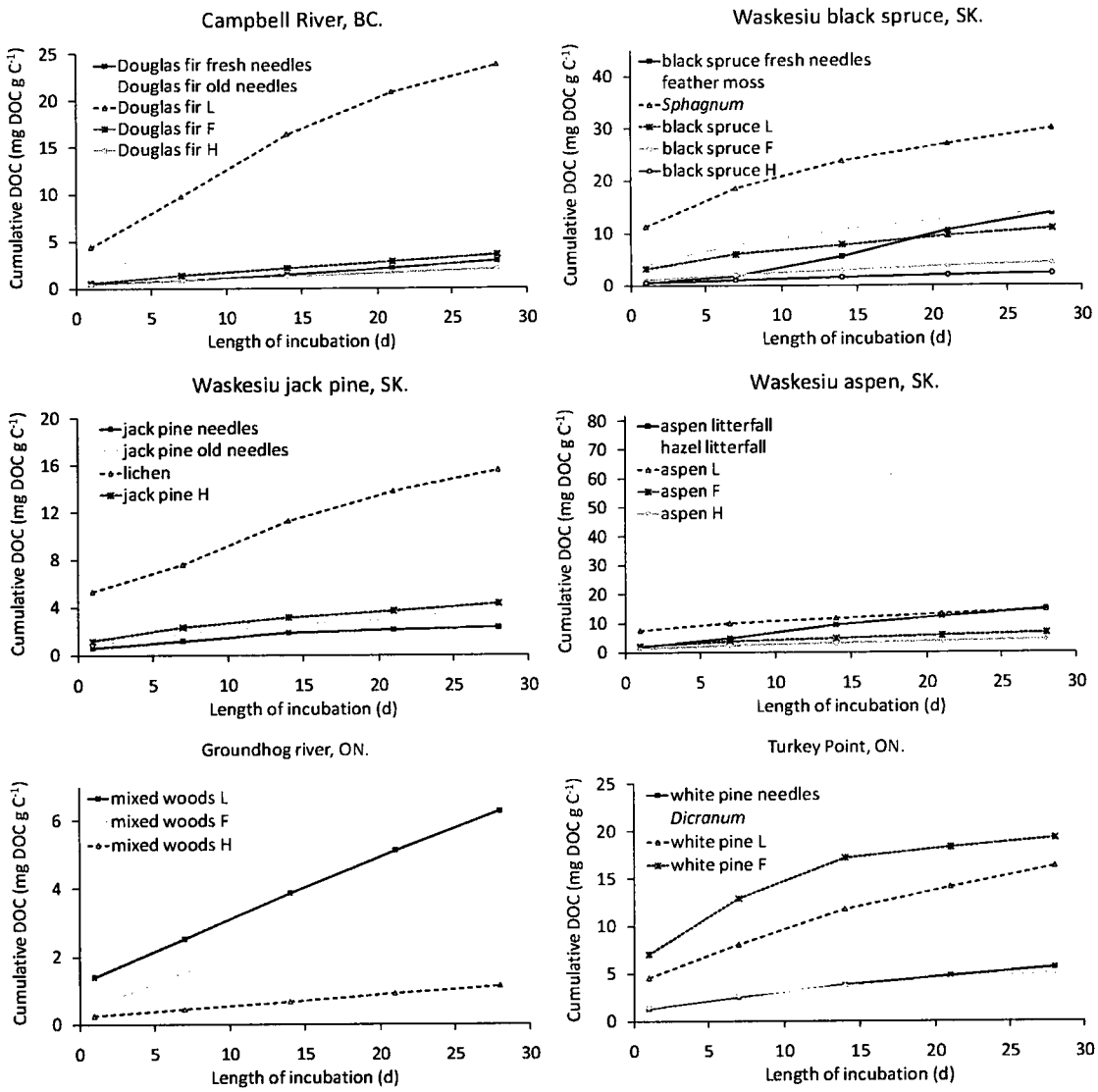


Figure 3.2. (continued) Mean cumulative DOC produced (mg DOC g C^{-1}) during the incubation, organized by site.

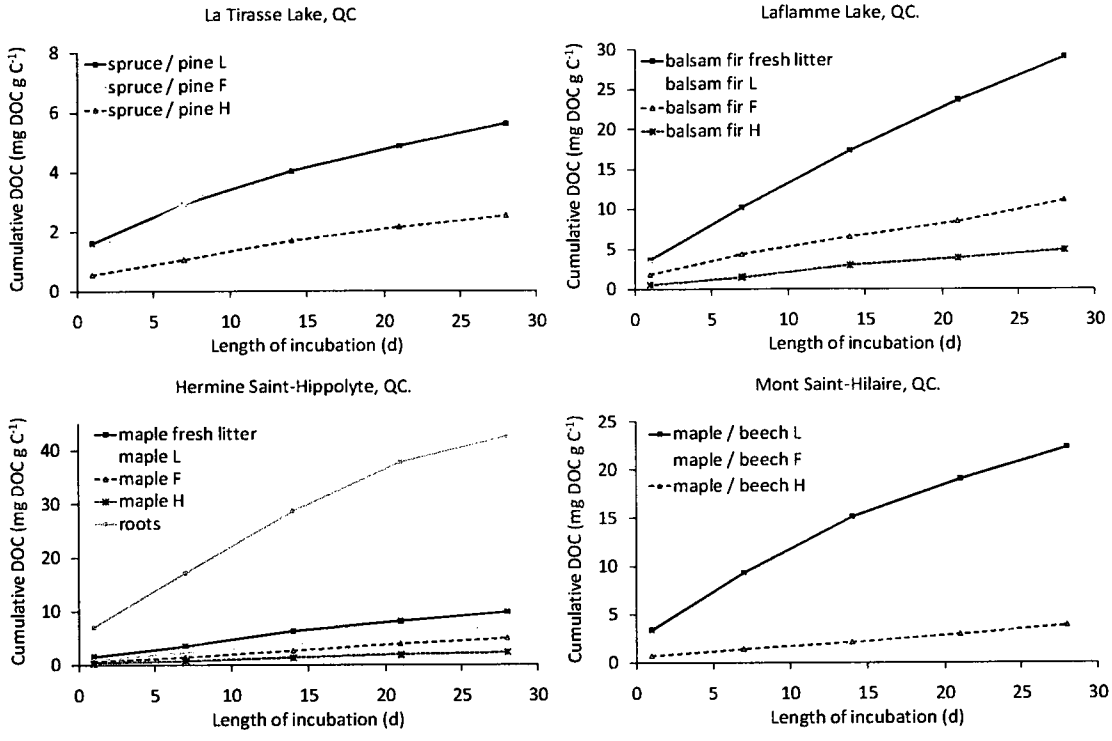


Figure 3.3. Box-plot of the a) DOC, b) CO_2-C total production and c) CO_2-C:DOC quotient divided into degree of decomposition.

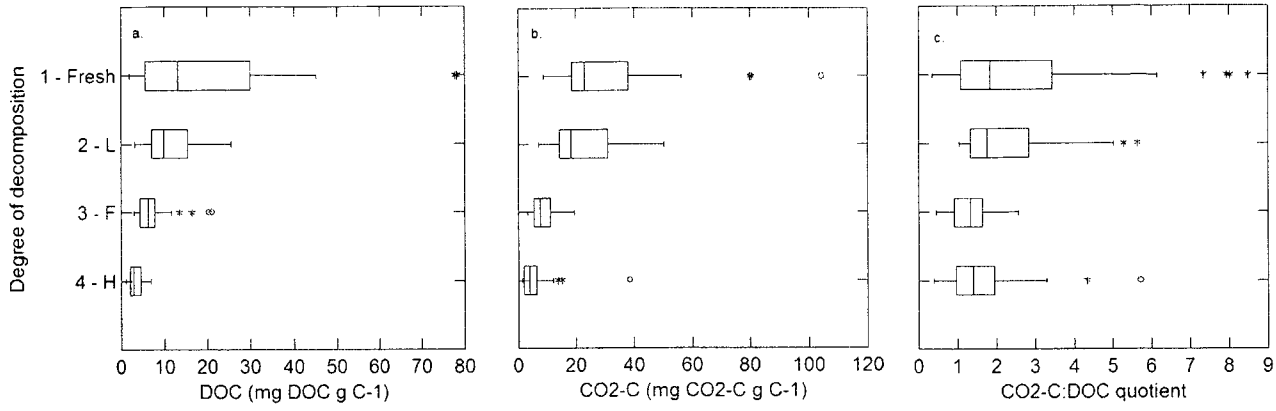


Figure 3.4. Mean cumulative CO_2-C produced (mg CO_2-C g C^{-1}) during the incubation, organized by site.

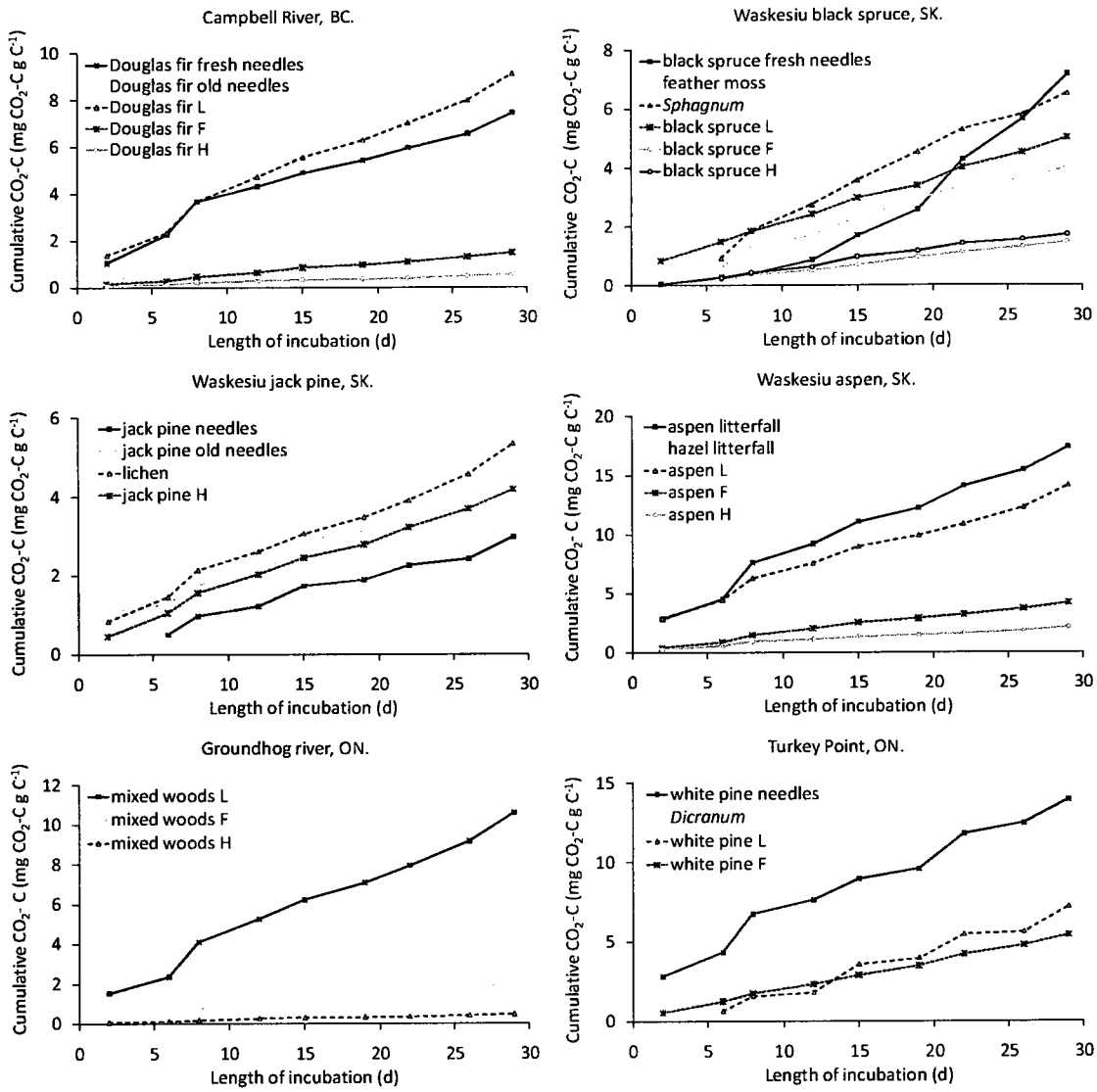


Figure 3.4. (continued) Mean cumulative CO_2-C produced (mg CO_2-C g C^{-1}) during the incubation, organized by site.

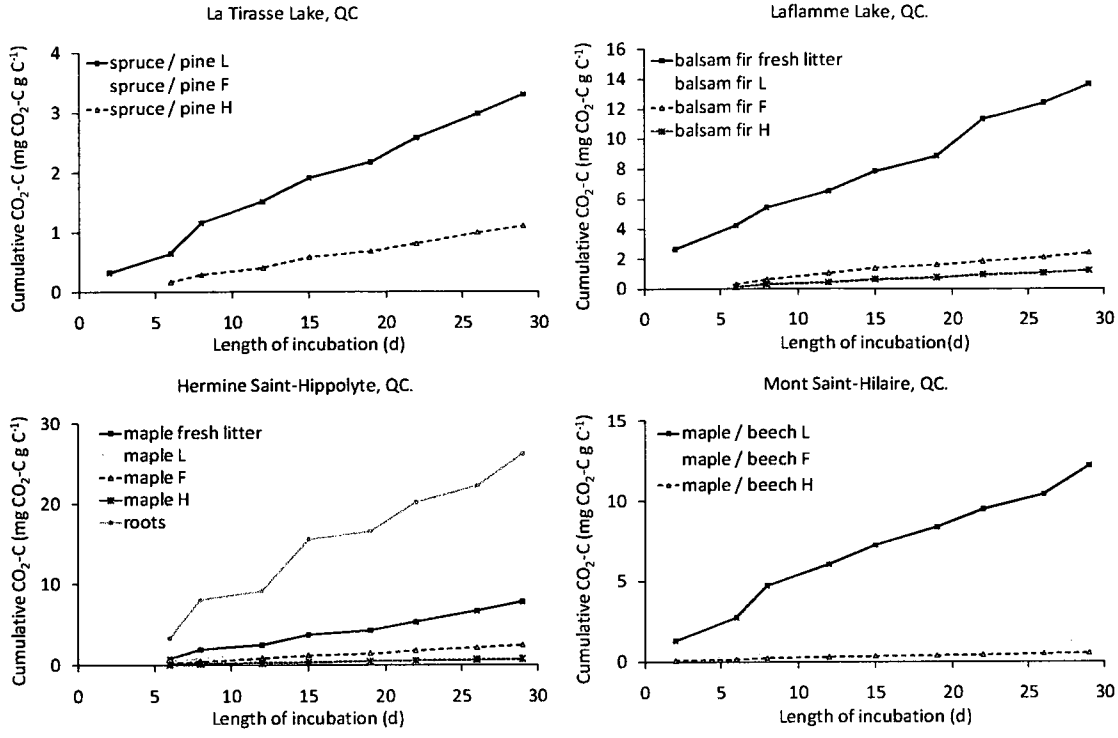


Figure 3.5. Scatterplot between initial sample C concentration and DOC production (a), CO_2-C production (b) and CO_2-C:DOC quotient (c); and between initial sample C:N quotient and DOC production (d), C:N quotient and CO_2-C (e) and CO_2-C:DOC quotient. The empty dots in b) represent the outliers that were removed for the regression analysis

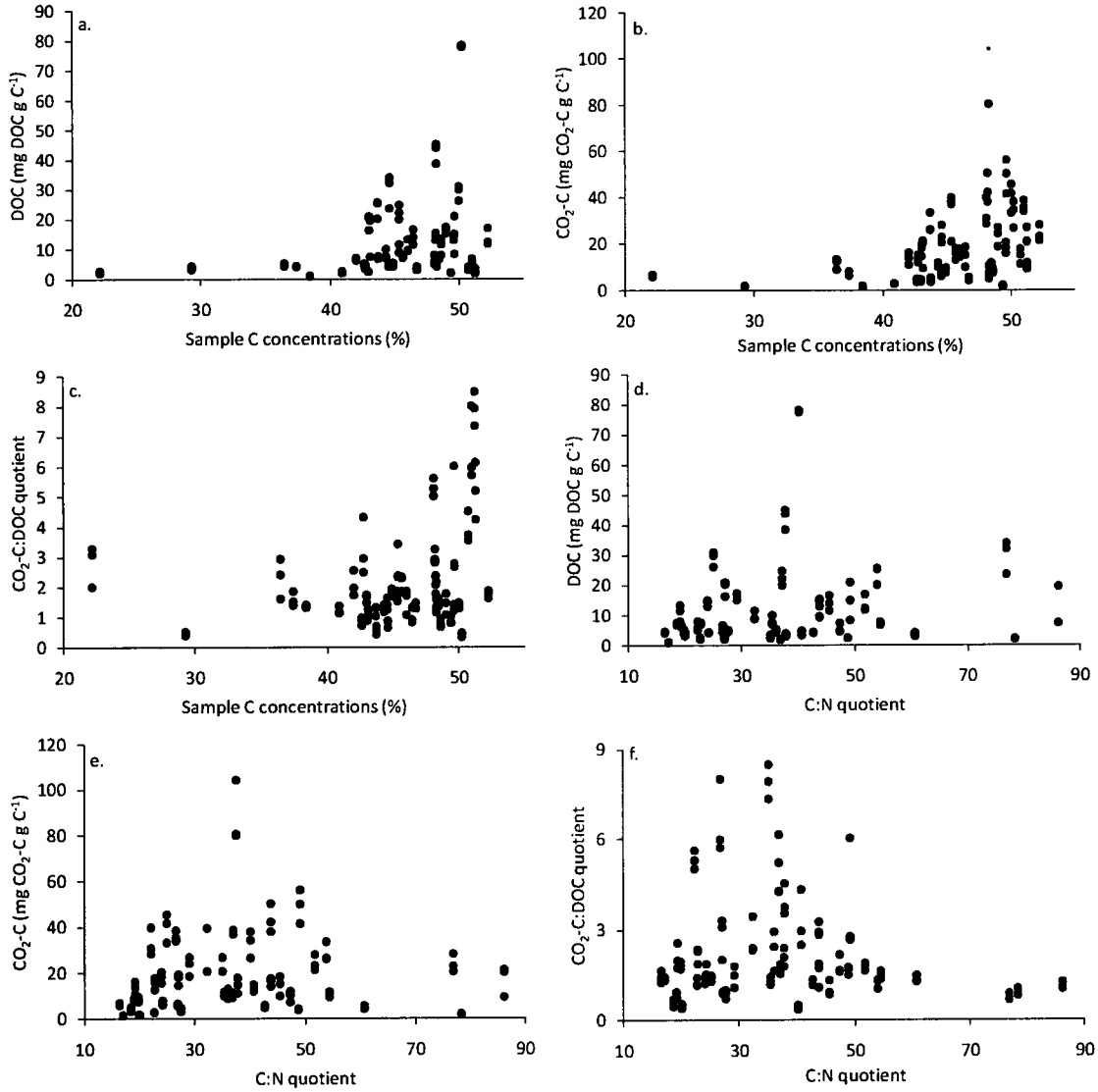


Figure 3.6. Box-plot of microbial biomass C (MBC) divided into degree of decomposition, and between pre- (left) and post-incubation (right). The asterisks indicate extreme values. The hash symbols are placed between the groups that are statistically different (Mann-Whitney, $p < 0.05$).

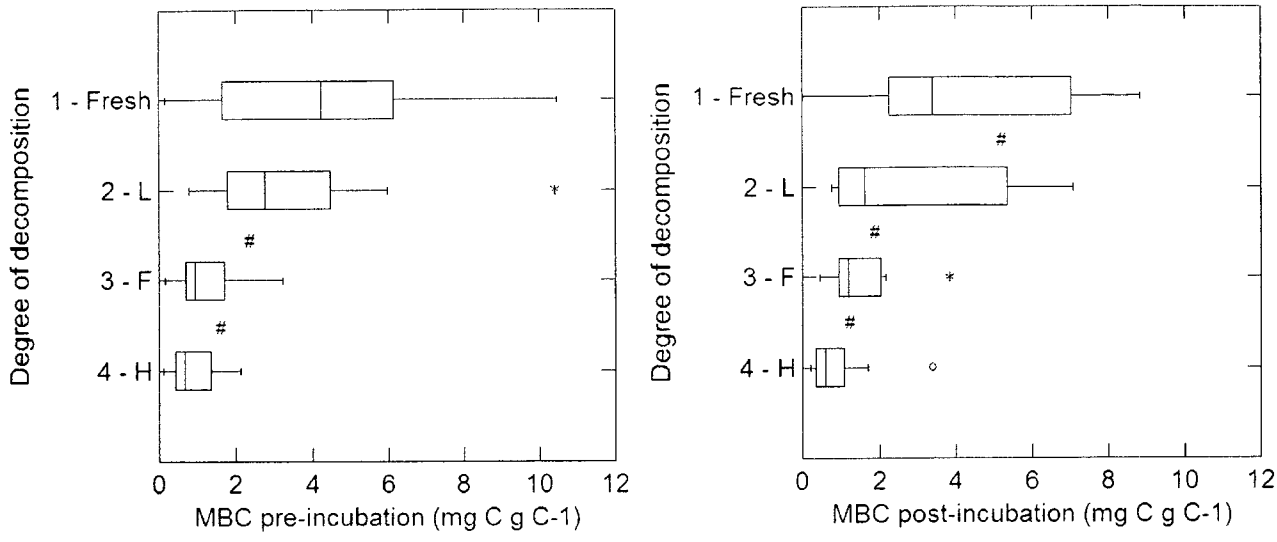


Figure 3.7. Scatter plot between the average microbial biomass C (MBC) and a) DOC, b) CO_2-C and c) CO_2:DOC quotient.

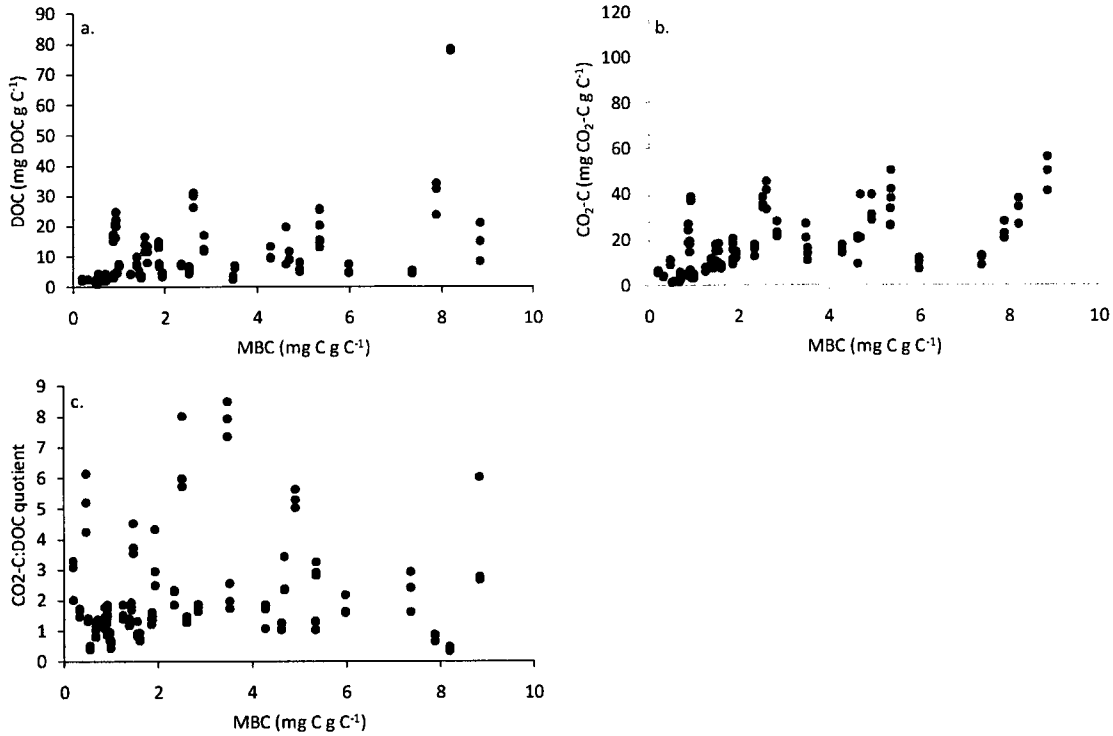
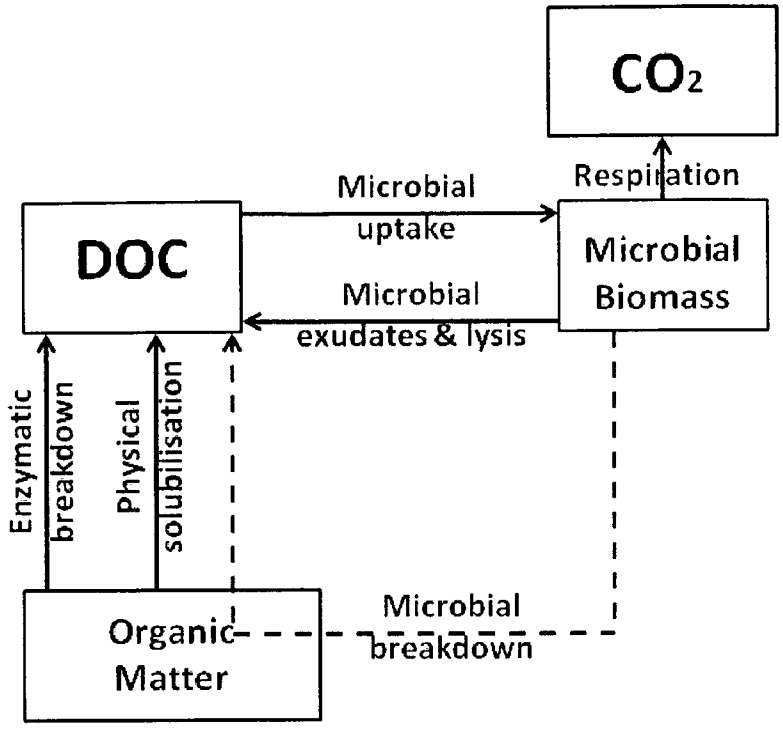


Figure 3.8. Conceptual model of C cycling (derived from Bengtson and Bengtsson, 2007).



Chapter 4 - Nitrogen and phosphorus release from decomposing litter and organic matter in Canadian forest floors (manuscript #2)

A brief overview – Context within the thesis

Having investigated the controlling factors of DOC and CO_2-C production in the first manuscript (Chapter 3), it is logical to further understand the release of dissolved elements during decomposition. The role of degree of OM decomposition and stand type during the production of nutrients such as N and P was evaluated because of their dynamic interactions. It is well known that plant and microbes need both energy substrate (i.e. C) and nutrients for food requirements (i.e. N and P) for their growth, maintenance, and general activity. As previously observed for DOC and CO_2-C production, dissolved N and P are released in solution as a result of biotic and abiotic processes occurring in soils.

In Chapter 4, the production of dissolved organic carbon, nitrate, ammonium, dissolved organic nitrogen and total dissolved phosphorus were simultaneously measured in order to identify stoichiometric links between solid, dissolved and microbial phase. In general, dissolved organic nitrogen (DON), nitrate (NO_3-N), ammonium (NH_4-N) and total dissolved nitrogen (TDN) were mainly produced in the F horizon, and total dissolved phosphorus (TDP) was almost equally produced by the L and F horizons. DON did not always account for the major proportion of TDN, particularly in the soil horizons. These results suggest a two-stage decomposition process: 1) an initial release of easily soluble material that triggers microbial activity and N uptake which release DON as a waste-product of decomposition, followed by 2) net mineralization. Significant links were observed between the tissues and dissolved C, N and P quotients, supporting the stoichiometry of nutrients released

4.1. Introduction

Dissolved organic matter (DOM) influences nitrogen (N) and phosphorus (P) cycling, storage and export in soils (Sollins and McCorison 1981; Qualls and Haines 1991; Michalzik and Matzner 1999). Until recently, studies have focused primarily on the role of the carbon (C) component on processes such as plant and microbial uptake, pedogenetic and weathering processes and soil/water acidification (Stevenson and Cole 1999; McDowell 2003). The roles of dissolved organic nitrogen (DON) and dissolved organic phosphorus (DOP) have been largely ignored (Williams and Edwards 1998) despite their importance as part of DOM components and for microbial and plant uptake pathways (for example, Huang and Schoenau 1998; Näsholm et al. 1998; Smith et al. 1998). Furthermore, DON is believed to comprise the majority of total N export from undisturbed forested catchments (for example, Perakis and Hedin 2002). While several studies have examined the loss of N and P, along with C, as litters decompose in the field (Prescott et al. 2004; Moore et al. 2006), few studies have examined the release of dissolved N and P, along with dissolved organic carbon (DOC), as litter and soil organic matter decompose under laboratory incubations.

In forest soils, dissolved inorganic N (DIN; NO_3-N and NH_4-N) originates mainly from atmospheric deposition and organic matter decomposition (for example, Arheimer et al. 1996; Turgeon, 2004) while DON originates from the decomposition of organic matter (OM) as well as root and microbial exudates, which can also be a source and sink for DIN. For DOC production, there is disagreement on the main source, such as fresh *versus* old organic matter (for example, Qualls 2000; Neff and Asner 2001; Park et al. 2002) and Park and Matzner (2003) suggested that the major source of DON was also part of this debate, though dissolved N dynamics is complicated by the numerous transformations occurring between organic and inorganic forms of N.

Fresh litter was the major source of DON and inorganic N in forest soils in some studies (for example, Qualls et al. 1991; Michalzik and Matzner 1999; Magill and Aber 2000) but other studies reported that dissolved N mainly results from the decomposition of older OM present in the forest floor (McDowell and Likens 1988). Fröberg et al. (2003) in a Norway spruce stand in Sweden showed that the DOM produced and leached from the fresh litter and canopy rapidly disappears during its translocation throughout the

soil, resulting in a larger contribution from the older, more decomposed material. Nutrient release results mainly from the decomposition of OM, with major controlling factors being litter quality, which mostly depends on tree species (for example, Hobbie, 1992; McGroddy et al. 2004; Kanerva and Smolander 2007). Hence, Lovett et al. (2004) suggested that N cycling would likely be controlled by the dominant tree species, though few studies have looked at this issue for dissolved N or P.

Phosphorus release also occurs during OM decomposition, and is either recycled through the reuptake by plants and microbes, or added to litter and organic debris pools for further decomposition in a semi-closed system (Stevenson and Cole 1999). Decomposition studies have shown both an initial release and storage of P in the litter (Moore et al. 2006) depending on the initial N:P quotient. Phosphorus is mainly present as dissolved inorganic P forms available for plants in most forest systems, with a small proportion being DOP (Huang and Schoenau 1998; Stevenson and Cole 1999), though Qualls et al. (1991) reported that two third of dissolved P from the forest floor was organic. Few studies have examined P cycling, with 4 reported by Michalzik et al. (2001) and we know little about the factors controlling its release, such as the effect of the degree of OM decomposition and stand species.

The interaction, distribution, transformation, cycling and limitation of N and P nutrients and C substrate in soils are key elements to the understanding of ecosystem processes through stoichiometry (Sterner and Elser 2002; Hessen et al. 2004). Many studies use related concepts such as nutrient interactions and limitations as examples of stoichiometric relationships, allowing a focus on more than one element (Sterner and Elser 2002). Nutrient limitation is a direct result of stoichiometry, as the organisms constantly face imbalanced mixtures of nutrients, represented by the C:N:P quotient of the available food (Cross et al. 2003; Frost et al. 2005a, b). In soil science, C:N:P quotients are used to infer soil quality. Litter or OM qualities have a major influence on microbial mediated processes such as decomposition, mineralization, and nitrification (see, for example, Dodds et al. 2004; Wang et al. 2004; McGroddy et al. 2004; Moore et al. 2006). The balance of the elemental composition of this OM and the organisms that consume the material should influence the rates at which nutrients are released and consumed, but this issue have not been thoroughly studied, and we have only few

examples of those controlling the release of dissolved C, N and P in soils and forest floors.

The objectives of this study are 1) to determine the production of dissolved N species and total P as varying types of forest litter and organic matter decomposes, 2) to establish the role of vegetation type and degree of OM decomposition on these production rates and 3) to examine the links between C, N and P in OM, microbial biomass and dissolved forms. To do this, we conducted laboratory incubations of 42 samples of litter and soil organic matter from 10 forest sites across Canada and determined C, N and P in the leachates and original tissues and microbial biomasses C and N.

4.2. Materials and Methods

4.2.1.Study sites and sampling

Samples of OM were collected from 10 forest stands across Canada, whose characteristics are described in details in Turgeon et al. (Chapter 3). The stand types are: 1) Douglas fir (*Pseudotsuga mensiesii*), 2) black spruce (*Picea mariana*), 3) jack pine (*Pinus banksiana*), 4) aspen (*Populus tremuloïdes*) with hazel (*Corylus spp.*) understory, 5) boreal mixed woods: (aspen (*P. tremuloïdes*), black spruce (*P. mariana*), white spruce (*Picea glauca*), white birch (*Betula papyrifera*) and balsam fir (*Abies balsamea*)), 6) white pine (*Pinus strobus*), 7) black spruce (*P. mariana*) and jack pine (*P. banksiana*) 8), balsam fir (*A. balsamea*), 9) sugar maple (*Acer saccharum*) and 10) sugar maple (*A. saccharum*) and american beech (*F. grandifolia*). After horizon designation (Agriculture Canada Expert Committee on Soil Survey 1987), two soil pits were sampled, and mixed to create a representative composite of the forest floor for the L, F and H horizons. If available, fresh litter, characterized as the leaves or needles fallen the previous year was also collected, as well as fresh canopy needles and plants growing on the ground. We collected 42 samples, which were stored at 4°C, preserving field moisture content, shortly after collection.

The samples were divided into groups representing either vegetation stand type (deciduous, coniferous and others) or degree of OM decomposition. The latter category included: 1) fresh (plants and yearly production such as senescent leaves, roots and needles from the canopy), 2) relatively fresh material (L horizon), 3) partly decomposed

OM (F horizon) and 4) humified OM (H horizon). A complete list of the samples in each category can be found in Turgeon et al. (Chapter 3).

4.2.2.Incubations

The 42 samples were incubated in triplicate for 30 days in dark chambers (Falcon® 150ml bottle top filters) at room temperature (~ 20°C), using a modification of the technique used by Nadelhoffer (1990) and Park et al. (2002). The Falcon chambers were washed with deionised water, the membrane removed and replaced with a Whatman GF/A membrane, followed by fibre-glass wool and an O-ring gasket. Opaque tape was used to block light on the upper part of the chamber; silicon gel was used to seal the upper to the bottom part of the chamber to avoid possible leaks. Because of the diversity of samples, the weight added to the chambers varied between 1 to 10 g of dry material. All production rates were standardized to the dry mass of the initial sample (expressed in mg/g soil).

The production of dissolved N and P from the laboratory incubations was measured by pouring 90-ml (representing the equivalent of 25 mm of rain) of deionised water weekly into each chamber, allowing equilibration for 30 min and then draining, using a 60-ml syringe to pull the water from the drain tube at the bottom of the chambers. The solutions were filtered through 0.45 μm paper (Macherey-Nagel 85/90 BF) and stored at 4°C before chemical analysis. The leaching was done on days 1, 7, 14, 21 and 28; 3 days after the last leaching, the tissue samples were frozen for microbial biomass C and N analysis (post-incubation samples).

4.2.3. Chemical analysis

The solutions were analysed for TDN and DOC on a Shimadzu VSN TOC/TN analyzer. Nitrate and NH_4-N were analysed by colorimetry on a Flow Injection Analyser (FIA, Lachat). Total dissolved phosphorus (TDP) was analysed by colorimetry following a persulfate digestion (Rowland and Haygarth 1997). We did not separate TDP into inorganic and organic forms because of the low P concentration, resulting in analytical problems (Qualls et al. 1991).

The C and N concentration of the initial tissues was measured on dried sub-samples, using an Elemental Analyzer Carlo Erba™ (instrument model NC2500) and P

concentration was determined by a wet-oxidation procedure modified from Parkinson and Allen (1975) (Lalande, personal communication).

Microbial biomass C and N was determined on the pre- and post-incubation tissue samples. We used a chloroform ($CHCl_3$) fumigation-extraction method modified from Voroney and others (2008) and Basiliko and others (2006) (see Turgeon et al. Chapter 3). Before the fumigation procedure, all samples were allowed to thaw at 4°C for 2 days and then 2 days at room temperature. Total C and N content of the 0.5 M potassium sulfate (K_2SO_4) extraction of the fumigated and non-fumigated samples was measured with a Shimadzu VSN TOC/TN analyzer.

4.2.4. Statistical analysis

To verify for the normality of the distribution of our dataset and of the residuals of our regressions, we used a Kolmogorov-Smirnov test following a Lilliefors procedure. Where the distribution was not considered statistically normal, we used log-transformed data. Significance for the analysis was determined using a p value below 0.05. Non-parametric tests were used on raw data when the log-transformation did not resulted in normal distribution.

4.3. Results

4.3.1. Production of dissolved N and P

During the 30-day incubation, the overall mean (± standard deviation) N production rates were: 0.54 (±0.79) mg TDN/g soil, 0.19 (±0.36) mg NH_4-N/g soil, 0.18 (±0.48) mg NO_3-N/g soil and 0.20 (±0.27) mg DON/g soil (Table 4.1). Degree of OM decomposition was significant (Kruskall-Wallis, $p < 0.05$) in separating production rates, with TDN, NO_3-N and NH_4-N following this trend: Fresh = L < F > H. For the production rates of DON, the trend was: Fresh > L < F > H. For the differences according to stand type (Kruskall-Wallis, $p < 0.05$), the production of TDN and NO_3-N followed coniferous > deciduous = others and NH_4-N and DON production showed this trend: coniferous = deciduous < others. Within each site, there was no overall pattern of production between samples, but the most common feature was the largest production from the F horizon, especially for the deciduous sites (Table 4.1).

The speciation of dissolved N species is presented in Table 4.2 as the percentage of TDN for each species (NO_3-N, NH_4-N and DON). Dissolved organic N was the statistically dominant proportion of TDN for the three stand groups, as well as the fresh material and L horizon. No statistical difference was observed between species proportion for the overall database, the F and H horizons.

The overall P production rate averaged 0.08 (±0.16) mg TDP/g soil, with significantly (Kruskall-Wallis, $p < 0.05$) larger production in the Fresh > L = F = H and: other > coniferous = deciduous by stand type. Within each profile, the L and/or F horizons generally released the largest amount of P, though sometimes there were no statistically significant differences (Table 4.1).

4.3.2. Tissue chemistry and the release of dissolved N and P

We examined the relationship between the concentrations of C, N and P in the original tissues (Table 4.3) with the release of the N species and TDP (Table 4.1). Overall, C was positively correlated with the production of NH_4-N, DON and TDP, while both N and P were positively correlated with all the dissolved N species and TDP (Table 4.4). When divided into groups based on degree of OM decomposition or stand type (data not shown), only a few cases gave significant correlations, the most noteworthy being the F horizon and deciduous groups for which C, N and P content were significantly correlated to almost all species of N and P released. Also, within the "other vegetation" category, there were significant correlations between C and N content with all release rates, but there was no significant correlation between P content and dissolved N and P release.

Although there was no significant difference between pre- and post-incubation microbial biomass N (MBN) ($p > 0.05$), there was an almost halving in the overall mean from pre-incubation (1.3 ± 2.3 mg N / g soil) to post-incubation (0.7 ± 0.6 mg N / g soil) (Figure 4.1). We used the average of pre- and post-incubation (1.0 ± 1.2 mg N / g soil) for our statistical analyses. The microbial biomass C (MBC) averages were pre-incubation: 6.3 ± 7.7 mg C / g soil and post-incubation: 5.4 ± 5.2 mg C / g soil with an pre- and post-average of 6.0 ± 5.9 mg C / g soil (see Turgeon et al. Chapter 3 for details).

Using the whole database, significant positive correlations were observed between both MBC and MBN, and the production of DON and TDP, with MBN being also

correlated to TDN production (Table 4.4). Here again, the division into degree of OM decomposition or stand type did not improve the strength or number of significant correlations. Only the F and H horizon groups presented significant correlations between MBN and all dissolved species, while the group of deciduous samples presented significant correlations between both MBC and MBN and all dissolved N and P except NO_3-N.

4.3.3. Stoichiometric relationships between C, N and P in solid, dissolved and microbial components

To assess whether the C:N:P stoichiometry of the original tissues affects that of the production of the dissolved forms, we compared the C:N, C:P and N:P quotients of the OM samples, the MBC:MBN quotient and the dissolved released and quotients. Relationships between tissue and dissolved quotients are presented in Figure 4.2. We found significant positive correlations between the C:N quotient of the whole database and all dissolved N species, except DON and with all dissolved quotients. The division into degree of OM decomposition did not result in better correlations, except the F horizon group which had strong correlations between C:N quotient and the production of dissolved N and P and TDN:TDP quotient. On the other hand, the coniferous group showed significant correlations with all dissolved species and quotients, except the TDN:TDP quotient (Table 4.5).

The C:P quotient is correlated to TDN, NO_3-N and TDP, as well as DOC:TDN, DOC:DON and DOC:TDP quotients. Here again, the F and H horizon C:P quotients resulted in good correlations with dissolved N and P production. However, contrary to the C:N quotient, the C:P quotient division into stand type highlighted the deciduous group, with correlations being significant for all dissolved N and P and with the DOC:TDN, DOC:DON and DOC:TDP quotients.

Finally, the N:P quotient was correlated with all dissolved elements and quotients except DON and DOC:DON. In this case, none of the division into degree of OM decomposition and stand type resulted in better correlation than when using the whole database. We did a regression tree analysis to find threshold values in the production of dissolved N and P, a concept proposed by decomposition studies (Prescott, 2005, Moore

et al. 2006). We obtained threshold N:P values of 15 for the production of DON and NH_4-N, 23 for the production of TDN and NO_3-N and 7 for the production of TDP.

The MBC:MBN quotient averaged 7.8 ± 7.4 with the fresh material being statistically larger than the other groups (Figure 4.3). For the overall database, we observed significant correlations between MBC:MBN quotient with all dissolved elements and quotients except DOC:TDP (Table 4.5). When divided into degree of OM decomposition, MBC:MBN quotient was always correlated to TDN and DON in all groups, correlated to NH_4-N for the groups of fresh material, L, and F horizons and correlated to TDN:TDP quotient for the fresh material and the F and H horizon. Within the stand group division, MBC:MBN quotient was significantly correlated to TDN in all groups. The quotients were all correlated to MBC:MBN in the coniferous group, while only to DOC:TDN for deciduous and DOC:TDP for others. MBC:MBN is significantly correlated to NO_3-N for coniferous and others, and to NH_4-N for coniferous and deciduous, and only in the deciduous group that DON and TDN were significantly correlated to MBC:MBN.

4.4. Discussion

4.4.1. Production of dissolved N and P

Few studies of N and P release from decomposing litter and soil organic matter are similar enough in methods to provide a meaningful comparison. Our TDN production rates ranged from 0.0 and 3.5 mg N/g soil, whereas other studies reported values ranging from 0.3 to 0.4 mg/g with KCl extractions (Kranabetter et al. 2007). The only study to which our data are really comparable in terms of method gives values between 0 to 25 mg N for 15 weeks incubation (Magill and Aber 2000, representing approximately 0 to 1.75 mg N/g soil for 4 weeks).

We found that DON formed between 9 and 98% of the TDN produced, compared to the 93 to 100% (laboratory incubations, Magill and Aber 2000), 17 to 24% (field study, Michalzik and Matzner 1999), 19 to 28% (field study, Park and Matzner 2006), 70 to 93% (field study, Sollins and McCorrison 1981; field study, Qualls et al. 1991) and 61 to 97% (field study, Perakis and Hedin 2002). Our production of DON did not always resulted in the larger proportion of TDN as sometimes observed in other studies (for

example, Sollins and McCorison 1981; Qualls et al. 1991; Magill and Aber 2000; Hagedorn and Machwitz 2007). However, for the fresh material and L horizon, the DON produced represented respectively 4 and 58% of TDN. In our study, the release of inorganic N was between 0.0 to 2.7 mg NO_3-N /g soil and 0.0 to 2.8 mg NH_4-N/g soil. Magill and Aber (2000) reported between 0.0 to 21 mg NO_3-N and 0.0 to 18 mg NH_4-N (data presented in mg N released, approximately between 0 to 1.4 mg NO_3-N /g soil for 4 weeks).

The two-stage model of decomposition of Prescott (2005) could help explain this difference between Fresh / L horizon and F /H horizons. During the early stage, the OM loses mainly the easily soluble compounds, such as available C and nutrients. These substrates could then increase microbial activity, resulting in the immobilization of nutrients (i.e. NO_3-N and NH_4-N) and the release of DON as a waste product of decomposition. In the second stage of decomposition, Prescott (2005) reported the net mineralization of nitrogen, which explains our observed large contribution of DIN to the total N produced in the F and H horizon. The discrepancy between the different results presented in the literature obviously results from different methodology: field *versus* laboratory incubation, individual samples *versus* cascading contribution between samples. Because of the continuous production and consumption of dissolved N (Park and Matzner, 2003), the conditions of the experiment can be a major factor in interpreting the results. In our study, we can conclude that DON is not always the major form of total dissolved N.

We found few studies reporting on dissolved P production. While Smith et al. (1998) reported similar values to ours (estimated from their graphs), McComb et al. (2007) observed, for their 30-d incubation, average values of 0.2 mg P/g litter, an order of magnitude higher than our average value (0.08 mg P/g soil).

4.4.2. Litter and organic matter composition

There were significant correlations between the OM composition (C, N and P content, MBC and MBN) and the N and P production rates. A single component of the litter or humus cannot predict the release of dissolved N species and P. It was impossible for us to perform multi-variate analyses due to the non-normal distribution of the data and model residuals. Prescott (2005) noted that nutrient release appears to be controlled by

the initial N and P content of the OM, which are the only two sample characteristics that correlated with all dissolved N species and TDP release in our study. Division into stand type and degree of OM decomposition gave uneven results. Some division gave better correlations, while others resulted in no correlation all together. Production of dissolved nutrients such as N and P are most probably controlled by more variables than OM composition and microbial biomass C and N. Taken together, these results support the importance of the study of nutrient interaction during the processes of OM decomposition and nutrient release. Our correlations with microbial biomass C and N support the findings from Kemmitt et al. (2008) suggesting that the size of microbial biomass is not the primary control on the release of nutrients.

4.4.3. Role of the degree of organic matter decomposition and stand type

The most important finding in respect to division into degree of OM decomposition was the larger production rates in the F horizon, and for most of our correlation analyses, the results were stronger for the F horizon group than the overall database or other groups. We hypothesise that because the F horizon is the mid-point in terms of degree of OM decomposition, its behaviour is the results of the processes happening in both the initial and late stage of decomposition as proposed by Prescott (2005). Because there is still partly fresh material, soluble and biodegradable compounds are still released, which triggers further decomposition of the more decomposed material present in the horizon. However, the MBC (see Turgeon et al. Chapter 3) and MBN data do not support an increase in microbial biomass in the F horizon. In terms of stoichiometry, the F horizon could also represent a middle point. While the composition of fresh litter and L horizons are mostly influenced by the stand species (Côté et al. 2000; Prescott et al. 2004), the H horizon is believed to represent the convergence of the different quotient, particularly true for N:P (e.g. Prescott et al. 2004; Prescott, 2005; Moore et al. 2006) and chemistry. This is explained by the loss of elements during decomposition, which is believed to results in a somewhat homogenous humus material at the end of the process (Moore et al. 2006). We were unable to find differences according to stand type (coniferous vs. deciduous) for most of our production rates as proposed by Lovett et al. (2004).

4.4.4. Stoichiometry of C, N and P

The quotients of the OM composition (i.e. C:N, C:P and N:P) were all positively correlated with their analogues measured in the dissolved phase (i.e. DOC:TDN, DOC:TDP and TDN:TDP), with Spearman coefficients of 0.78, 0.30 and 0.38, respectively. There were generally lower quotients in the dissolved phase compared to the OM quotient (below the 1:1 line), invoking a loss of C over N and P, and N over P during production. These observations were still true when dividing the database into groups of stand type and degree of OM decomposition, except for the correlation between C:P and DOC:TDP and N:P and TDN:TDP in the F and H horizons.

As reported in other studies (for example, Gundersen et al. 1998; Currie 1999; Qualls et al. 2000; Mitchell et al. 2001), the C:N quotient of the OM was negatively correlated to the leaching of NO_3-N, also observed in our study. However, when divided into degree of OM decomposition and stand type, only the F and H horizons and the coniferous group provided the same negative correlation between C:N and NO_3-N release.

A threshold value of 15 or 16 for the N:P quotient was proposed in decomposition studies (for example, Prescott, 2005; Moore et al. 2006) to partition between N and P release/retention. They suggested that below this quotient, N was released and P retained, and when above the N was retained and P released. Our regression tree analysis confirmed that for NH_4-N and DON, the N:P threshold value of 15 seems appropriate. In the case of TDN and NO_3-N, the threshold reported by the regression tree analysis was around 23. Above these quotients, we observed larger production while below these quotient, production was smaller. However, our analyses showed that for the release of P, the critical quotient was close to 7, with larger values below this threshold and *vice versa*. The software used did not allowed to derive statistical significance for the regression tree analyses, however, the similar thresholds obtained from our study and the ones proposed in Prescott (2005) and Moore et al. (2006) stress the importance of nutrients interactions and stoichiometric relationships during decomposition processes and nutrient release/retention.

4.4.5. Conclusions

From our determination of the release of N and P during the decomposition of litter and OM, we have observed that initial sample concentrations of C, N and P can be used to predict production rates. On the other hand, microbial biomass C and N were not good indicators of the production rates. The process of decomposition takes a heterogeneous litter and modifies it to obtain a somewhat homogenous humus material resulting in different rates of production between our degrees of OM decomposition. It also seems that the nutrients interaction, stoichiometric relationship, plays a large role in the release of nutrients during decomposition. In this case, the links between the litter/OM composition quotients and dissolved quotient stress the importance of these interactions between organisms, food and nutrients release. Furthermore, not only the interaction is of major importance, but some studies raised the issue of factors limiting decomposition. They suggested that the nutrients availability is suspected to affect the C availability, while C availability might affect the actual consumption of limiting nutrients. Which raise the question of which is more limiting in an ecosystem: C availability or nutrient quantity. The importance of dissolved N and P is too often ignored due to the small numbers reported in the literature. However, combined with others researchers results, we support the overall importance of dissolved N, P and C in the ecosystem functioning such as biological growth, decomposition and soil development among others. Through decomposition, both dissolved N and P are released and obtained, as part of their global cycles. Within a global climate change scenario, biotic processes, affecting decomposition, are believed to be modified by changes in temperature and precipitation. Hence, a better understanding of the factors that control dissolved N and P production will help our prediction and mitigation of future environmental changes.

Table 4.1. Average production of TDN, NO_3-N, NH_4-N, DON and TDP during the 30-d incubation.

Sample	TDN	NO_3-N	NH_4-N	DON	TDP
			(mg/g soil)		
Douglas fir, Campbell River (BC)					
Douglas fir fresh needles	0.04 (0.01)^{a}	0.01 (0.01)^{ab}	0.01 (0.00)^{a}	0.02 (0.00)^{a}	0.03 (0.00)^{a}
Douglas fir old needles	0.24 (0.03)^{b}	0.04 (0.01)^{a}	**0.09 (0.00)^{b}**	0.11 (0.03)^{b}	0.03 (0.00)^{a}
Douglas fir L	**0.52 (0.12)^{c}**	0.04 (0.02)^{a}	**0.14 (0.05)^{b}**	**0.35 (0.05)^{c}**	**0.09 (0.03)^{b}**
Douglas fir F	0.04 (0.01)^{a}	0.01 (0.01)^{ab}	0.01 (0.00)^{a}	0.03 (0.00)^{a}	0.02 (0.00)^{a}
Douglas fir H	0.03 (0.00)^{a}	0.01 (0.00)^{b}	0.01 (0.00)^{a}	0.02 (0.00)^{a}	0.03 (0.00)^{a}
Black Spruce, Waskesiu (SK)					
Black spruce fresh needles	0.04 (0.00)^{a}	0.00 (0.00)^{a}	0.00 (0.00)^{a}	0.03 (0.00)^{a}	0.02 (0.00)^{ad}
Feather Moss	**0.23 (0.04)^{b}**	0.01 (0.00)^{b}	0.08 (0.03)^{b}	**0.15 (0.03)^{b}**	0.03 (0.00)^{ac}
Sphagnum	**0.27 (0.03)^{b}**	0.01 (0.00)^{b}	0.05 (0.02)^{ab}	**0.19 (0.03)^{b}**	**0.04 (0.01)^{b}**
Black spruce L	**0.20 (0.03)^{b}**	0.01 (0.00)^{b}	0.07 (0.04)^{b}	**0.15 (0.02)^{b}**	0.03 (0.00)^{cd}
Black spruce F	0.08 (0.03)^{c}	0.00 (0.00)^{a}	0.04 (0.03)^{b}	0.04 (0.00)^{a}	0.02 (0.00)^{d}
Black spruce H	0.07 (0.00)^{ac}	0.02 (0.01)^{b}	0.03 (0.01)^{b}	0.02 (0.01)^{a}	0.01 (0.00)^{e}
Jack Pine, Waskesiu (SK)					
Jack pine fresh needles	0.06 (0.01)^{a}	0.00 (0.00)^{a}	0.01 (0.00)^{a}	0.05 (0.01)^{a}	0.02 (0.00)^{ac}
Jack pine old needles	**0.10 (0.02)^{ab}**	0.00 (0.00)^{a}	**0.04 (0.00)^{b}**	0.06 (0.02)^{ab}	0.03 (0.00)^{b}
Lichen	**0.15 (0.09)^{b}**	0.00 (0.00)^{a}	0.00 (0.00)^{c}	0.15 (0.08)^{b}	0.02 (0.00)^{ab}
Jack pine H	**0.14 (0.02)^{b}**	0.00 (0.00)^{a}	**0.07 (0.02)^{b}**	0.06 (0.00)^{ab}	0.01 (0.00)^{c}
Aspen, Waskesiu (SK)					
Aspen litterfall	0.19 (0.07)^{a}	0.00 (0.00)^{a}	0.01 (0.00)^{a}	0.18 (0.06)^{a}	0.07 (0.03)^{a}
Hazel litterfall	0.51 (0.09)^{b}	0.02 (0.01)^{b}	0.03 (0.00)^{a}	**0.45 (0.08)^{b}**	**0.41 (0.09)^{b}**
Aspen L	0.19 (0.04)^{a}	0.00 (0.00)^{a}	0.03 (0.01)^{a}	0.16 (0.04)^{ac}	0.08 (0.01)^{ac}
Aspen F	**2.92 (0.28)^{c}**	**2.35 (0.35)^{c}**	**0.26 (0.02)^{b}**	**0.41 (0.02)^{b}**	0.11 (0.00)^{a}
Aspen H	1.12 (0.11)^{d}	0.68 (0.14)^{d}	**0.26 (0.02)^{b}**	0.19 (0.08)^{bc}	0.03 (0.00)^{c}
Boreal Mixed wood, Groundhog River (ON)					
Mixed woods L	0.22 (0.04)^{a}	0.04 (0.03)^{a}	**0.08 (0.01)^{a}**	0.11 (0.03)^{a}	0.03 (0.00)^{a}
Mixed woods F	**2.06 (0.10)^{b}**	**1.51 (0.02)^{b}**	**0.11 (0.04)^{a}**	**0.45 (0.10)^{b}**	0.03 (0.00)^{a}
Mixed woods H	0.21 (0.00)^{a}	0.17 (0.01)^{c}	0.01 (0.00)^{b}	0.04 (0.01)^{c}	0.02 (0.00)^{b}
White Pine, Turkey Point (ON)					
White pine fresh needles	0.33 (0.17)^{a}	0.01 (0.00)^{a}	0.15 (0.09)^{a}	0.16 (0.06)^{a}	0.06 (0.00)^{a}
Dicranum	0.09 (0.02)^{b}	0.00 (0.00)^{a}	0.02 (0.00)^{b}	0.07 (0.01)^{a}	0.03 (0.00)^{b}
White pine L	0.28 (0.03)^{a}	0.01 (0.00)^{a}	0.08 (0.00)^{a}	0.19 (0.01)^{a}	0.09 (0.00)^{a}
White pine F	**2.95 (0.13)^{c}**	**1.21 (0.02)^{b}**	**1.03 (0.24)^{c}**	**0.98 (0.21)^{b}**	0.07 (0.00)^{a}
Black spruce and Jack Pine, la Tirasse Lake (QC)					
Spruce / pine L	**0.15 (0.04)^{a}**	0.00 (0.00)^{ab}	0.07 (0.02)^{a}	**0.09 (0.01)^{a}**	0.04 (0.00)^{a}
Spruce / pine F	**0.14 (0.03)^{a}**	0.01 (0.00)^{a}	**0.13 (0.01)^{a}**	**0.08 (0.01)^{a}**	0.01 (0.00)^{b}
Spruce / pine H	0.06 (0.01)^{b}	0.00 (0.00)^{b}	0.03 (0.00)^{a}	0.03 (0.00)^{b}	0.01 (0.00)^{b}
Balsam fir, Laflamme Lake (QC)					
Balsam fir fresh litter	**0.78 (0.01)^{ab}**	0.01 (0.00)^{a}	**0.39 (0.14)^{a}**	**0.38 (0.05)^{a}**	**0.16 (0.00)^{a}**
Balsam fir L	**0.90 (0.18)^{a}**	0.01 (0.00)^{a}	**0.63 (0.13)^{a}**	**0.26 (0.26)^{ab}**	**0.18 (0.00)^{a}**
Balsam fir F	0.54 (0.15)^{b}	0.01 (0.00)^{a}	**0.37 (0.09)^{a}**	0.21 (0.06)^{b}	0.03 (0.00)^{b}
Balsam fir H	0.25 (0.02)^{c}	0.01 (0.00)^{a}	0.16 (0.04)^{b}	0.07 (0.02)^{c}	0.03 (0.00)^{b}
Sugar Maple, Hermine – Saint-Hippolyte (QC)					
Maple fresh litter	**0.23 (0.01)^{a}**	0.00 (0.00)^{a}	0.07 (0.01)^{a}	**0.15 (0.01)^{a}**	0.02 (0.00)^{ac}
Maple L	**0.33 (0.03)^{a}**	0.01 (0.00)^{b}	0.23 (0.03)^{b}	**0.10 (0.00)^{ab}**	0.02 (0.00)^{ab}
Maple F	**0.21 (0.02)^{ab}**	0.01 (0.00)^{a}	0.14 (0.02)^{b}	0.06 (0.00)^{b}	0.03 (0.00)^{b}
Maple H	0.15 (0.01)^{b}	**0.05 (0.01)^{c}**	0.08 (0.03)^{a}	0.02 (0.00)^{c}	0.01 (0.00)^{c}
Roots	**2.58 (0.85)^{c}**	**0.07 (0.01)^{c}**	**1.93 (0.74)^{c}**	**1.36 (0.39)^{d}**	**0.74 (0.00)^{d}**
Sugar Maple and American Beech, Mont Saint-Hilaire (QC)					
Maple / beech L	0.38 (0.07)^{a}	0.01 (0.01)^{a}	0.05 (0.03)^{a}	0.32 (0.04)^{a}	0.06 (0.01)^{a}
Maple / beech F	**2.09 (0.14)^{b}**	**1.15 (0.13)^{b}**	**0.70 (0.07)^{b}**	**0.60 (0.01)^{a}**	**0.13 (0.05)^{b}**
Maple / beech H	0.50 (0.03)^{a}	0.21 (0.03)^{c}	0.18 (0.02)^{c}	0.10 (0.02)^{b}	0.02 (0.00)^{c}

Standard deviation in parentheses

$^{a, b, c, d, e}$ different letters represent statistical differences between the samples within a site (Kruskall-Wallis, $p < 0.05$)

Bold values indicate the statistically larger production than other samples within the site profile

Table 4.2. Average speciation of total dissolved N in the leachate.

Grouping	NO_3-N	NH_4-N	DON
Overall	15.8 (23.2)	33.3 (20.2)	50.8 (24.6)
Deciduous	29.9 (30.5)	26.3 (21.3)	**43.7 (30.3)**
Coniferous	8.9 (12.5)	39.7 (17.2)	**51.4 (17.7)**
Other	2.2 (0.6)	26.6 (21.0)	**71.2 (21.1)**
Fresh	4.4 (7.0)	22.0 (18.7)	**73.6 (20.2)**
L	4.7 (6.3)	37.3 (17.1)	**57.9 (17.9)**
F	29.4 (29.8)	36.3 (23.1)	34.3 (15.8)
H	31.1 (27.8)	37.7 (18.9)	31.2 (17.0)

Standard deviation in parentheses
Significantly larger percentages (Kruskall-Wallis $p < 0.05$) are indicated in **bold**

Table 4.3. Concentrations (%) of C, N and P and their quotients in the 42 samples.

Sample	C	N	P	C:N	C:P	N:P
Douglas fir, Campbell River (BC)						
Douglas fir fresh needles	51.2	1.5	0.20	35.1	251.2	7.2
Douglas fir old needles	44.3	1.3	0.12	35.4	357.3	10.1
Douglas fir L	43.7	0.8	0.12	53.9	366.8	6.8
Douglas fir F	46.7	0.8	0.08	60.7	583.9	9.6
Douglas fir H	49.4	0.6	0.05	78.4	1007.6	12.9
Black spruce, Waskesiu (SK)						
Black spruce fresh needles	52.3	1.0	0.15	51.7	360.3	7.0
Feather moss	46.4	1.0	0.07	45.5	703.5	15.5
Sphagnum	44.6	0.6	0.04	76.9	1173.4	15.3
Black spruce L	46.0	1.1	0.07	43.8	676.5	15.4
Black spruce F	48.3	1.1	0.04	42.7	1122.8	26.3
Black spruce H	22.2	0.8	0.04	27.0	568.2	21.0
Jack pine, Waskesiu (SK)						
Jack pine fresh needles	51.3	1.4	0.10	36.9	512.7	13.9
Jack pine old needles	50.7	1.3	0.11	37.9	457.0	12.1
Lichen	43.1	0.5	0.03	86.2	1267.4	14.7
Jack pine H	42.7	1.1	0.09	40.7	480.1	11.8
Aspen, Waskesiu (SK)						
Aspen litterfall	49.7	1.0	0.15	49.2	333.3	6.8
Hazel litterfall	50.2	1.3	0.21	40.2	239.2	6.0
Aspen L	48.2	1.1	0.16	43.8	308.9	7.1
Aspen F	42.0	2.2	0.18	19.4	230.8	11.9
Aspen H	37.4	1.5	0.13	24.3	285.4	11.8
Boreal mixed wood, Groundhog River (ON)						
Mixed woods L	48.1	2.2	0.11	22.3	458.0	20.6
Mixed woods F	38.4	2.7	0.10	16.5	468.5	28.4
Mixed woods H	43.1	2.2	0.21	17.4	184.6	10.8
White pine, Turkey Point (ON)						
White pine fresh needles	51.0	1.9	0.19	26.7	267.1	10.0
Dicranum	36.4	1.0	0.13	36.1	271.9	7.5
White pine L	49.0	1.7	0.12	29.2	395.2	13.5
White pine F	43.0	1.6	0.11	27.2	405.7	14.9
Black spruce and Jack pine, la Tirasse Lake (QC)						
Spruce / pine L	48.3	1.0	0.09	47.3	519.1	11.0
Spruce / pine F	48.5	0.9	0.06	54.5	757.5	13.9
Spruce / pine H	42.9	0.9	0.06	48.8	692.6	14.2
Balsam fir, Laflamme Lake (QC)						
Balsam fir fresh litter	50.0	2.0	0.15	25.1	324.7	12.9
Balsam fir L	49.6	2.1	0.15	24.1	322.1	13.4
Balsam fir F	48.6	2.5	0.19	19.4	254.5	13.3
Balsam fir H	42.6	1.5	0.15	27.7	278.5	10.1
Sugar maple, Hermine - Saint-Hippolyte (QC)						
Maple fresh litter	45.4	1.4	0.08	32.4	539.9	16.7
Maple L	45.7	2.0	0.09	22.8	518.9	22.7
Maple F	44.9	2.3	0.10	19.9	435.9	21.9
Maple H	40.9	1.8	0.14	22.7	284.0	12.5
Roots	48.2	1.3	0.09	37.7	561.0	14.9
Sugar maple and American beech, Mont Saint-Hilaire (QC)						
Maple / beech L	45.3	1.2	0.08	37.2	539.6	14.5
Maple / beech F	43.7	2.4	0.11	18.6	400.9	21.6
Maple / beech H	29.3	1.5	0.11	20.1	261.6	13.0

Table 4.4. Spearman correlation coefficients (* $p < 0.05$) between organic matter, leachate and microbial biomass composition.

	C	N (%)	P	MBC (mg C/g)	MBN (mg N/g)	C:N	C:P	N:P	MBC:MBN
TDN (mg N/g)	0.105	0.566*	0.297*	0.148	0.376*	-0.429*	-0.221*	0.181*	-0.480*
NO_3-N (mg N/g)	0.039	0.501*	0.237*	-0.143	-0.078	-0.466*	-0.215*	0.177*	-0.313*
NH_4-N (mg N/g)	0.151	0.610*	0.178*	-0.046	0.167	-0.509*	-0.137	0.316*	-0.475*
DON (mg N/g)	0.237*	0.328*	0.223*	0.401*	0.599*	-0.121	-0.091	0.086	-0.322*
TDP (mg P/g)	0.379*	0.252*	0.419*	0.432*	0.530*	-0.004	-0.219*	-0.211*	-0.225*
DOC:TDN	-	-	-	-	-	0.783*	0.302*	-0.305*	0.390*
DOC:DON	-	-	-	-	-	0.639*	0.322*	-0.165	0.369*
DOC:TDP	-	-	-	-	-	0.395*	0.423*	0.239*	0.148
TDN:TDP	-	-	-	-	-	-0.164	0.144	0.375*	-0.227*

Figure 4.1. Box-plot of microbial biomass N (MBN) results for pre- and post-incubation samples. ^{x} represents extreme values, the boxes represent the values within the 25th and 75th percentile, median is represented by the line in the boxes and whiskers represent the lower and upper adjacent values.

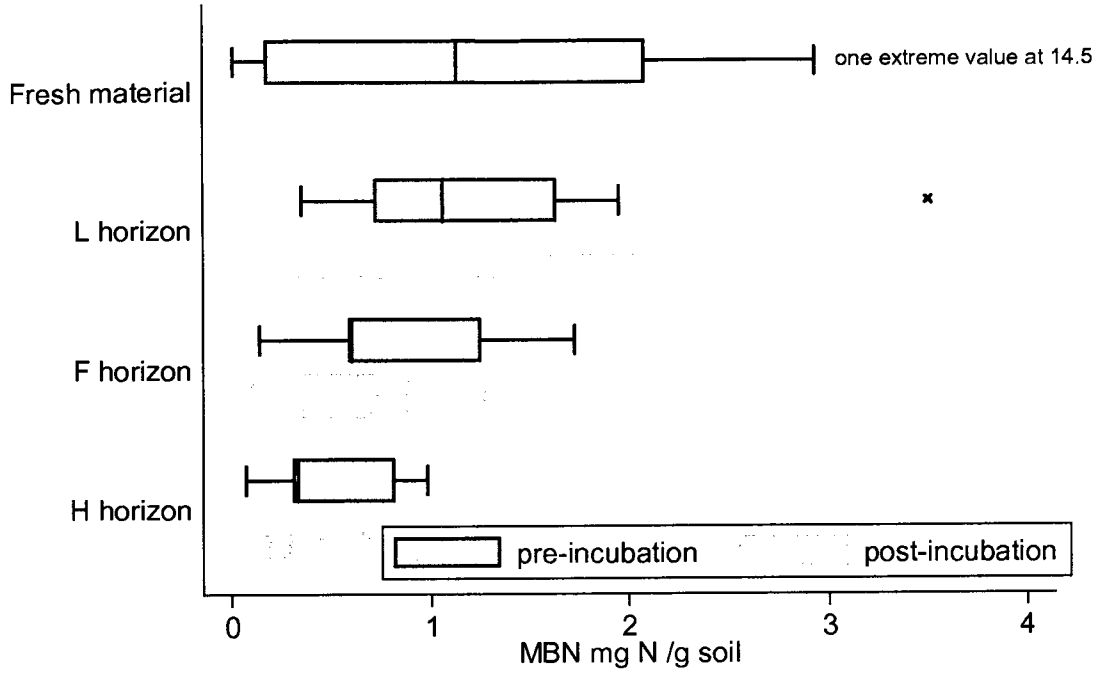


Figure 4.2. Scatterplots between a) leachate DOC:TDN quotient and OM C:N quotient, b) leachate DOC: TDN quotient and MBC:MBN quotient, c) leachate TDN:TDP quotient and OM N:P quotient, d) leachate DOC:DON quotient and OM C :N quotient, e) leachate DOC :DON quotient and MBC:MBN quotient, f) leachate DON:TDP quotient and OM N:P quotient. Spearman correlation coefficients and regressions significant at $p <$ 0.05. Dash line represents the 1:1 line, solid line represent the regression line.

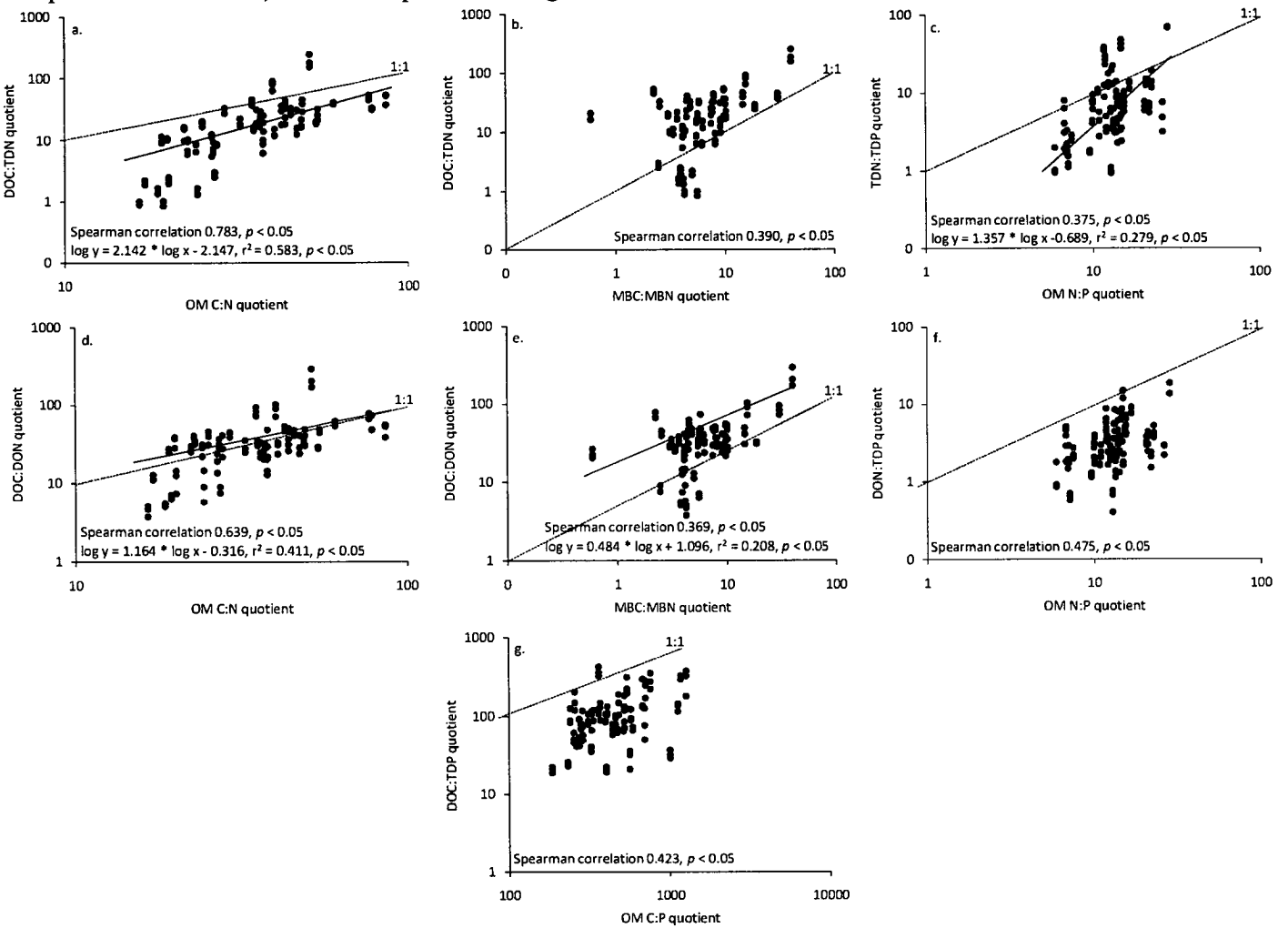
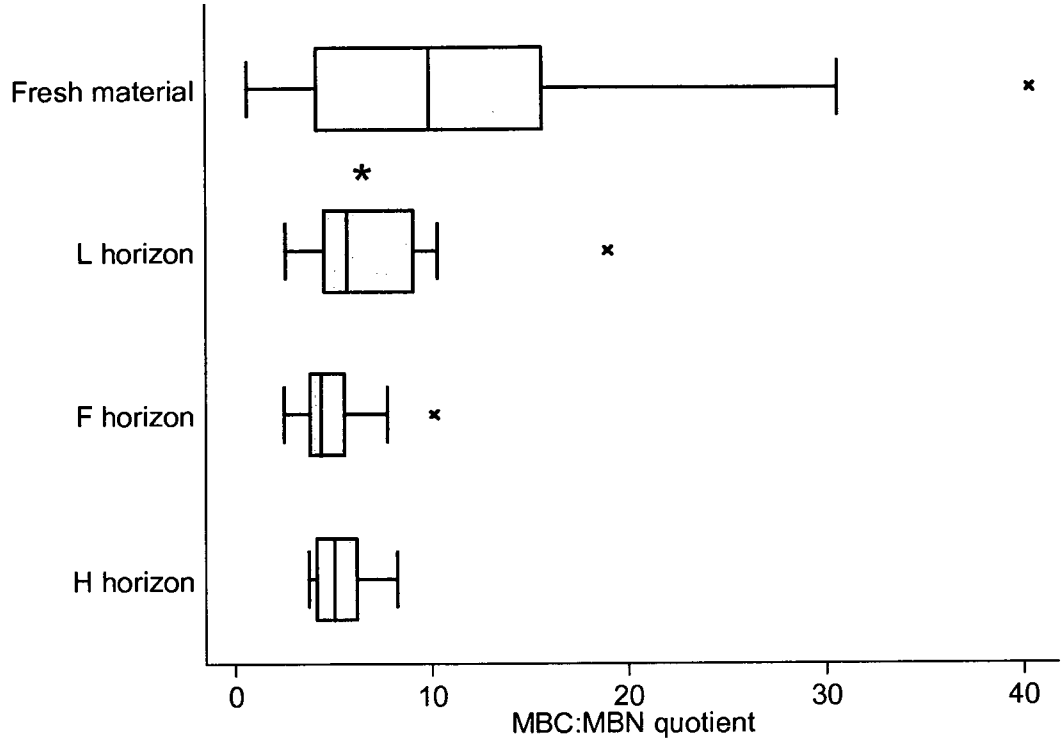


Figure 4.3. Box-plot of MBC :MBN quotient separated by degree of organic matter decomposition. x represents extreme values, * represents a statistical difference at $p < 0.05$. The boxes represent the values within the 25th and 75th percentile, median is represented by the line in the boxes and whiskers represent the lower and upper adjacent values.



Chapter 5 - Biodegradation of dissolved organic carbon in water-extracts from Canadian forest floors (manuscript #3)

A brief overview – Context within the thesis

The first 2 manuscripts (Chapters 3 and 4) clearly described the role of the degree of organic matter decomposition on the production of dissolved C, N and P. While the production of DOM and TDP mainly occurred in the fresher material, the NO_3 was primarily produced in the F horizon. These results clearly reflect the balance between production, mainly through decomposition and mineralization, and assimilation by microbes (plant uptake being excluded in this laboratory condition). In order to obtain a better understanding of the cycling of dissolved C and N in forest floors, DOC and DON biodegradation and NO_3-N and NH_4-N transformations were measured. Water extractions were performed using the same set of samples as used in the production incubations included in Chapter 3 and 4, and the water extraction concentrations correlated with the results of the production experiments. During the bioassays, concentrations of DOC, TDN, NO_3-N, NH_4-N and DON were measured in solution along with CO_2-C evolution.

In Chapter 5, the biodegradability of DOC was quantified over a 28-day period by measuring both DOC concentration loss and CO_2-C mineralization. The results of DOC loss as percent remaining in solution, followed the double-exponential decay model, provided k values (decomposition rates d^{-1}), pool size (% of initial concentration) and mean residence time (MRT; $1/k$) for both fast and slow decomposing pools. Furthermore, losses of DOC after 3 and 28 days were calculated.

None of the parameters derived from the double-exponential decay model used to characterize the kinetics of biodegradation showed significant differences among degree of OM decomposition and stand type of the samples. In general, more DOC in solution was lost than mineralized into CO_2, suggesting a prominent role of microbial biomass in the uptake and storage of DOC during its biodegradation. Of the five spectrophotometric parameters of the extracts, only the specific ultra-violet absorbance (SUVA) was a good predictor of biodegradability of DOC, allowing a possible evaluation without a long bioassay experiment.

5.1. Introduction

Dissolved organic carbon (DOC) plays a key role in pedogenetic and weathering processes, biological growth and acidification processes (Michalzik and Matzner, 1999; Kalbitz et al., 2000; Michalzik et al., 2001; Gregorich et al., 2003; McDowell et al., 2006). Dissolved organic C is the energy substrate for biological growth (Marschner and Bredow, 2002; Marschner and Kalbitz, 2003; Kemmitt et al., 2008) which is controlled by its biodegradability, defined as the fraction of DOM that is labile and readily available for biological uptake (Gregorich et al., 2003; Kiikkila et al., 2005) and/or its bioavailability, which refers to the potential of microorganisms to interact with DOC (Ribas et al., 1991; Boyer & Groffman, 1996; Huesemann et al., 2004). The current knowledge about biodegradability mainly originates from aquatic science and drinking water and waster water treatment studies (Servais et al. 1987, 1989; Ribas et al., 1991; Block et al. 1992). The biodegradability of DOM has a striking role in nutrient cycling, C sequestration, water quality, greenhouse gas emission (Boyer and Groffman, 1996; Gregorich et al., 2003) and allocation of energy for plants and microbes (Cook and Allan, 1992; Wagai and Sollins, 2002).

When it is biologically degraded, some of the DOC is taken up and immobilized in the microbial biomass, mineralized into carbon dioxide (CO_2) and/or released as metabolites back into solution. Kalbitz et al. (2003) concluded that up to 80% of total DOC from fresh material can be biodegraded within a few weeks, and DOC biodegradation decreases with increasing degree of organic matter (OM) decomposition (Boyer & Groffman, 1996). In general, very little is known about the biodegradability of DOC in forest soils (Marschner and Kalbitz, 2003; Kiikkila et al., 2005).

Two kinetically distinct pools of biodegradable DOC (BDOC) have been recognized as fast and slow (Qualls and Haines 1992; Gregorich et al. 2003; Kalbitz et al. 2003). Marschner and Kalbitz (2003) concluded that the major factors affecting DOC biodegradation, and the size of these pools, included its molecular size, chemical composition (i.e. quantities of carbohydrates, lignin, etc.), polarity and acidity, as well as the chemical characteristics of the solution itself, such as pH, nutrient content, oxygen and metal concentrations.

Dissolved organic C is a mixture of compounds, ranging from simple to very complex, that vary in their potential for biodegradation (Kaplan and Newbold, 1995; Kiikkila et al., 2005). Chemical composition (i.e. carbohydrates, proteins, lignin etc.) might determine the potential for biodegradability of DOC. However, the biodegradable pool can be composed of both simple and more complex compounds (e.g. Qualls and Haines, 1992; Qualls, 2005; Kim et al., 2006). In that sense, chemical analysis of DOC seems inappropriate for estimating the size of DOC pools (e.g. Servais et al., 1987). Chemical analysis such as ^{13}C-NMR, TMH thermochemolysis GC-MS, ^{14}C labelling and molecular weight fractionation, among others, are often costly, time consuming, destructive and sometimes requires a large volume for analysis. Spectrophotometric analysis, however, is rapid, needs little preparation, and requires only a small volume of solution sample (Jaffrain et al. 2007).

In our study, 5 of the indices obtained by spectrophotometer analysis were determined: E2:E3, E4:E6, LMW:HMW ratios, the ratio of slopes (Rs) and the specific ultraviolet absorbance (SUVA). The E2:E3 ratio gives a relative indication of the molecular size of the samples, with increasing molecular size detected by decreasing ratio (e.g. Thomsen et al., 2002; Hunt and Ohno, 2007; Minero et al. 2007).The E4:E6 ratio is inversely proportional to the aromaticity of DOC (e.g. Thomsen et al., 2002). The abundance of low molecular weight (LMW) relative to the high molecular weight DOC (HMW) is given by the LMW:HMW ratio (e.g. Trulleyova and Rulik, 2004; Helms unpublished). The ratio of slopes (Rs) is a dimensionless parameter (see Helms 2006). The SUVA is believed to correlate with the aromaticity of the DOC, representing a negative relationship between SUVA (λ = 254 or 280) and biodegradability of DOC (Marschner and Kalbitz, 2003; McDowell et al. 2006), suggesting that aromatic compounds are more stable and recalcitrant to biological degradation (Kalbitz et al. 2003). The change in SUVA during biodegradation assays both results from the decrease in DOC concentration and the change in UV-absorbance (Akagi et al., 2007).

In this study, we had the following 3 objectives:

1. To determine the rates of DOC biodegradation of leachates (or water extracts) from organic matter samples obtained from forest floors across Canada;

2. To establish the controls on these biodegradation rates, such as stand type and degree of decomposition of the original organic matter; and
3. To examine whether simple spectrophotometric characteristics of the extract could be used to predict DOC biodegradation rates.

5.2. Materials and methods

5.2.1. Samples

In summer 2005, we gathered a total of 40 samples from forest floors across Canada. Study sites were chosen to represent part of the variability of forest found in Canada. Forest type included 1) Douglas fir (*Pseudotsuga mensiesii*), 2) black spruce (*Picea mariana*), 3) jack pine (*Pinus banksiana*), 4) aspen (*Populus tremuloïdes*) with hazel (*Corylus spp.*) understory, 5) boreal mixed woods (aspen (*P. tremuloïdes*), black spruce (*P. mariana*), white spruce (*Picea glauca*), white birch (*Betula papyrifera*) and balsam fir (*Abies balsamea*)), 6) white pine (*Pinus strobus*), 7) black spruce (*P. mariana*) and jack pine (*P. banksiana*) 8), balsam fir (*A. balsamea*), 9) sugar maple (*Acer saccharum*) and 10) sugar maple (*A. saccharum*) and American beech (*Fagus grandifolia*). Details about the sites can be found in Turgeon et al. (Chapter 3). At each site, two soil pits were dug and L, F and H horizons sampled according to the horizon designation with the criteria from the Agriculture Canada Expert Committee on Soil Survey (1987). At some sites, needles were cut from the trees and fresh litter, needles and plants were collected from the ground. Furthermore, litterfall from the aspen site was collected in the fall and separated into aspen and hazel leaves. Once collected, the samples were stored at 4°C, preserving field moisture conditions. For the purpose of this study, samples were divided into groups representing the stand type (coniferous, deciduous and others) or the degree of OM decomposition (Fresh material, L, F and H horizons). A complete list of the samples in each category can be found in Turgeon et al. (Chapter 3) (excluded from the list are old Douglas fir needles and lichen, which were not available for the current study).

5.2.2. Extraction of water soluble C

The water-extraction of the 40 OM samples was done using a 1:100 (dry weight) soil:solution suspension, mixing gently for 30 min on a rotating shaker, then centrifuging

at 4500 rpm for 20 min. The supernatant was filtered through a 0.45 μm filter paper (Macherey-Nagel 85/90 BF) and immediately frozen.

5.2.3. Biodegradation incubation

We modified the incubation method of Gregorich et al. (2003). Three d before the beginning of the incubation, the samples were allowed to thaw at 4°C. An inoculum was prepared with a composite of all 40 OM samples mixed with deionised water (4 g of OM for 45 ml of deionised water), shaken vigorously and incubated for 24 hrs at ~20°C. Prior to inoculation, the inoculum solution was passed through fibre glass wool to avoid any particulate matter being added to the incubation.

Each sample was incubated in triplicate, with 200 ml of water-extract in a 300 ml glass Erlenmeyer flask, diluted to between 10 and 20 mg DOC L^{-1} to avoid excessive growth of microorganisms (Kalbitz et al., 2003; McDowell et al., 2006). Two ml of inoculum and 2 fibre-glass filter papers cut in half were added to the flask, the paper providing a medium for microbial growth. Blanks were run to evaluate whether any contamination occurred during the incubation. No nutrients were added to the solution, because we also measured dissolved organic nitrogen (DON) biodegradation and nitrogen (N) species during mineralization / immobilization (Turgeon et al., Chapter 6). The flasks were gently shaken every other day and incubated at ~20°C in the dark. A 30 ml aliquot was sampled from the flasks at days 1, 3, 7, 14 and 28, filtered through a 0.45 μm filter paper (Macherey-Nagel 85/90 BF) and analysed for DOC on a Shimadzu VSN TOC/TN analyzer. Spectrophotometer analyses were done on each aliquot, by pouring 1 ml of the filtered sample in the cuvette, and placed in the GENESYS 10uv (Thermo Electron Corporation) spectrophotometer for analysis. The range of wavelength covered in our study was between 250 to 750 nm.

On days 3 and 7, carbon dioxide (CO_2-C) emission was measured by sealing the flasks with a rubber septum, and sampling the headspace with a syringe at time 0 and 240 min. Carbon dioxide concentrations were determined on a Shimadzu Mini2 gas chromatograph with methanizer. The CO_2-C emitted was calculated as the change in CO_2-C concentration between the sampling at time 0 and 240 min, adjusted for the headspace volume in the flask.

5.2.4. Spectrometer indices calculation

The E2:E3 ratio is calculated by dividing the absorbance at λ 250 nm by λ 365 nm (Thomsen et al., 2002; Hunt and Ohno, 2007; Minero et al. 2007). The E4:E6 ratio is calculated by dividing the absorbance at λ 465nm by λ 665 nm (Thomsen et al., 2002). The LMW:HMW ratio is estimated by the absorbance at λ 250 divided by λ 450 (Trulleyova and Rulik, 2004 (used 250:400); Helms, 2006). To compute the Rs parameter, we need to calculate the natural logarithm (ln) of the absorption coefficient (a) of the different absorbance (equation 1):

$$a = 2.303\ A/l \qquad (1)$$

where a is the absorption coefficient at a given wavelength (m^{-1}), A is the absorbance, and l is the path length (m) of the cuvette used for the analysis. The slopes of interest are between λ 260-340 nm and λ 340 450 nm. This parameter as the advantage of being independent of DOC concentrations, and is able to track the shift in molecular weight (see Helms unpublished). The SUVA is the quotient of the absorbance at λ 254 nm (or sometimes λ 280 nm) divided by the DOC concentration in mg l^{-1} (Kalbitz et al. 2003; Marschner and Kalbitz, 2003; McDowell et al. 2006; Akagi et al., 2007).

5.2.5. Statistical analyses

Normality of the dataset was tested using a Shapiro-Wilk test (Stata v. 10.0). When not normal, the data were log-transformed before further analysis. When normality was not obtained with the log-transformation, we used non-parametric tests such as Kruskall-Wallis to compare groups or Spearman correlation (Stata v.10.0 and SYSTAT v.10.0). For normal distribution, we used paired t-test to evaluate the link between initial and final values, and ANOVA (post-hoc Tukey's) to evaluate differences between groups. Regression analysis residuals were tested for normality using the same Shapiro-Wilk test. SigmaPlot 2001 (v.7.0) was used to fit the data remaining in solution to double-exponential decay models (% DOC remaining = $[(100-b)e^{-K1t}] + [\ be^{-K2t}]$, where 10-$b$ is the size of the fast pool, k_1 is the decomposing rate constant of the fast pool, b is the size of the slow pool and k_2 is the decomposing rate constant of the slow pool).

5.3. Results

5.3.1. Biodegradation of DOC

The initial DOC concentration in our water extracts ranged from 7 (spruce/pine H) to 950 (hazel litterfall) mg DOC l^{-1} with an average of 100 ± 157 mg DOC l^{-1} (Table 5.1). The DOC concentrations were significantly smaller ($p < 0.05$) for the H horizons than the fresh and L horizons, while the F horizon is not significantly different from fresh, L and H horizons. The DOC concentration in the water-extracts was significantly correlated (Spearman correlation coefficient = 0.70, $p < 0.05$) with those obtained from the first leaching of the production experiment performed with the same samples (Turgeon et al., Chapter 3), representing less than 5% of the initial sample C content. The initial DOC concentration was not correlated with any of the biodegradation parameters presented later in this paper ($p > 0.05$).

The kinetics of mass DOC loss, as measured by the changes in the DOC remaining in the solution (average of the triplicates), are presented in Figure 5.1. We characterized the loss of DOC with a double exponential model, with r^2 values ranging between 0.85 and 0.99, $p < 0.05$ (Table 5.2), by estimating the relative size of the fast and slow pools (as a proportion of the initial DOC concentration), their decomposition rate constant (k) and mean residence time (MRT; $1/k$). The size of the fast pool varied between 11 (maple/beech F) and 59% (Douglas fir needles), with an average value of 29 ± 11%. The decomposition rate constant of the fast pool (k_1) ranged between 0.4 (white pine F) to 3.4 d^{-1} (jack pine H) with an average of 1.0 ± 0.5 d^{-1}. We obtained values between 0.3 (jack pine H) to 2.8 d (white pine F) for the MRT of the fast pool (MRT_1), with an average of 1.2 ± 0.5 d.

The size of the slow pool varied between 41 (Douglas fir needles) and 89% (balsam fir L) with an average of 71 ± 11%. The decomposition rate constant for the slow pool (k_2) varied between 0.001 (L horizons of maple/beech, spruce/pine and black spruce) to 0.014 d^{-1} (white pine needles) with an average of 0.005 ± 0.004 d^{-1}. The MRT of the slow pool (MRT_2) varied between 69 (white pine needles) to 1427 d (maple F) with an average of 322 ± 270 d. None of the k values, pool size or MRT show significant differences between degree of OM decomposition or stand type ($p > 0.05$). Even though not statistically different, the coniferous needles seem to consistently have a larger fast

pool size than any other litter type or horizons (Douglas fir 59%, black spruce 44%, jack pine 55% and white pine 40%).

During the first 3 d of the incubation, the water extracts lost between 8 (maple/beech F) and 52% (jack pine needles) with an average of 24 ± 10% of their initial DOC concentration (Table 5.2). The fresh material lost significantly larger relative amounts of DOC ($p < 0.05$) than the F and H horizons. No statistical differences were observed between stand types ($p > 0.05$) (Table 5.3). During the 28-d biodegradation assay, the mass loss of DOC ranged between 9 (maple/beech F) to 73% (Douglas fir needles) with an average of 36 ± 14% of the initial DOC concentration (Table 5.3). Dissolved organic matter extracted from fresh material lost significantly more DOC ($p < 0.05$) than the L, F or H horizons during 28 days (Table 5.3), but no differences were observed between stand types.

We found a significant positive relationship between the size of the fast pool and the percentage lost during the first 3 d and a significant negative relationship between the size of the slow pool and the total relative C lost from solution during the 28 d incubation (Figure 5.2).

5.3.2. Spectrophotometric properties

The SUVA of the initial DOC solutions varied between 0.1 (jack pine needles) and 6.3 l mg DOC^{-1} m^{-1} (balsam fir fresh) with an average of 1.7 ± 1.3 l mg DOC^{-1} m^{-1}. The SUVA of the samples incubated for 28 d ranged between 0.9 (white pine needles and jack pine old needles) to 7.5 l mg DOC^{-1} m^{-1} (hermine fresh) with an average of 2.5 ± 1.7 l mg DOC^{-1} m^{-1}. The SUVA of the initial and final samples was strongly correlated and there was an overall increase during the incubation (Figure 5.3). Although there were no statistically significant differences by degree of OM decomposition or stand type ($p > 0.05$), the increase in SUVA was greater for the coniferous stand samples (0.9 ± 0.5 l mg DOC^{-1} m^{-1}) than for the deciduous stand samples (0.7 ± 0.3 l mg DOC^{-1} m^{-1}) ($p < 0.05$).

The initial E2:E3 values varied between 2.6 (spruce/pine F) to 7.4 (white pine needles) with an average of 4.7 ± 0.9 with a statistical decline in the values (paired *t*-test, $p < 0.05$) to reach final values varying between 2.6 (spruce/pine F) to 5.7 (balsam fir H), with an average of 4.3 ± 0.7. Neither the initial nor final value was different according to the degree of OM decomposition, but the coniferous samples were always lower than the

deciduous samples ($p < 0.05$). The values obtained for the E4:E6 ratios are not statistically different between the initial and final samples (paired t-test, $p < 0.05$). The values ranged between 0.1 to 20 with an initial average of 4.3 ± 2.7 and a final average of 4.1 ± 3.4. There is no difference according to degree of OM decomposition or stand type (ANOVA, $p > 0.05$).

No statistical differences according to degree of OM decomposition and stand type were found for LMW:HMW or Rs parameters ($p > 0.05$, ANOVA), nor did the initial values differed from the final values (paired t-test, $p > 0.05$). The LMW:HMW ratio varied between 5.8 to 53, with an average of 17.4 ± 7.6 and 15.5 ± 8.4, respectively for initial and final solution. The Rs parameter varied between 0.5 and 2.2 and had average values of 0.9 ± 0.2 for both initial and final solutions. The Rs parameter, as stated in Helms (2006) is not correlated with the DOC concentration ($p > 0.05$).

Significant Spearman correlations ($p < 0.05$) were found between E2:E3 and E4:E6 (Spearman coefficient = 0.332), between E2:E3 and LMW:HMW (Spearman coefficient = 0.761), between E4:E6 and LMW:HMW (Spearman coefficient = 0.365) and between LMW:HMW and Rs (Spearman coefficient = -0.454) ($p < 0.05$). The initial SUVA value was correlated with the initial E2:E3 values (Spearman coefficient = -0.383; $p < 0.05$) but no correlation was found between the final values. Correlations were found between final SUVA and final Rs (Spearman coefficient = -0.195; $p < 0.05$) and final E4:E6 and final Rs (Spearman coefficient = -0.230; $p < 0.05$), but not within the initial values.

5.3.3. Link between spectrophotometer parameters and biodegradation results

Only the SUVA values from the initial solution were correlated with parameters from the biodegradation assay and the total percentage lost of DOC for 3 and 28 d. The scatter plots of the significant correlations between initial SUVA and slow pool size, fast pool size, MRT_2, k_2, DOC lost during 3 days and 28 days are presented in Figure 5.4. The strongest correlations are observed between SUVA values and the slow pool size (Spearman coefficient 0.730), the fast pool size (Spearman coefficient -0.728) and the total lost of DOC in 28 d (Spearman coefficient -0.765). The three other correlations are

still strong, with coefficient of -0.663, 0.551 and -0.465 for the correlations of SUVA with lost of DOC in 3d, MRT_2 and k_2 values respectively.

5.3.4. CO_2 emisssion

As an alternative to the loss of DOC in solution, we assessed the biodegradation of DOC by measuring the emission of CO_2-C over the first 7 d of the incubation. The relationships between the mass loss of DOC in solution and that lost as CO_2-C after 3 days and 7 days are significant (Figure 5.5), being slightly stronger for the 7 days (r^2 = 0.73) than the 3 days (r^2 = 0.71). Note that, after a Kruskall-Wallis analysis, the values of CO_2-C mineralization are not statistically different between the 2 periods (p = 0.684), while the DOC lost are statistically different between the 2 periods ($p < 0.001$).

During the first 3 days, the average cumulative loss C as CO_2-C was 4 ± 6 mg CO_2-C (range between 0.1 (spruce/pine L) and 34 mg CO_2-C (hazel litterfall)). The fresh material produced significantly larger amount of CO_2-C (7 ± 8 mg CO_2-C) compared to the F (1 ±0.8 mg CO_2-C) and H (1.1 ±0.6 mg CO_2-C) horizons ($p < 0.05$). No statistical differences were observed for the stand types ($p > 0.05$). The mass loss DOC in solution for the first 3 days varied between 0.2 (balsam fir fresh) to 57 mg DOC (hazel litterfall) (average 6 ± 10 mg DOC). The fresh material lost statistically more DOC than other samples: Fresh 10 ± 14 mg DOC > F horizon 2 ± 0.8 mg DOC and H horizon 1 ± 0.6 mg DOC). However, the amount of DOC lost during the first 3 days for the coniferous group (10 ± 16 mg DOC) was larger ($p < 0.05$) than that for the deciduous group (3 ± 2 mg DOC).

The loss of CO_2-C during the first 7 days varied between 0.3 (maple/beech H) to 147 mg CO_2-C (hazel litterfall) (average 14 ± 28 mg CO_2-C). Values are not statistically different according to the degree of OM decomposition ($p > 0.05$) but coniferous loss (28 ± 44 mg CO_2-C) are statistically larger than deciduous (7 ± 7 mg CO_2-C). As for the DOC, the average loss is 8 ± 16 mg DOC, ranging between 0.3 (spruce/pine H) to 81 mg DOC (hazel litterfall). The same statistical differences were observed: fresh (10 ± 14 mg DOC) > F horizon (2 ± 0.8 mg DOC) and H horizon (1 ± 6 mg DOC) and loss from coniferous samples (10 ± 6 mg DOC) was larger than that from deciduous samples (3 ± 2 mg DOC) ($p < 0.05$).

5.4. Discussion

5.4.1. Biodegradation parameters

The biodegradation results from all 40 samples fit the double-exponential decay model as proposed in the literature (e.g. Qualls and Haines, 1992; Gregorich et al., 2003; Kiikkila et al., 2006). The range of our parameters (size pool, k values and MRT for both fast and slow decomposing pools) are in agreement with those reported in the literature (e.g. Gregorich et al., 2003; Wickland et al., 2007) except our k_1 values are one order of magnitude larger than in Kalbitz et al. (2003) and Qualls and Haines (1992). We believe that these differences are due to the type of solution / extraction used for these studies (varying ratios of extraction or lysimeter solutions).

Despite the overall absence of statistical differences related to degree of OM decomposition and stand type, fresh needles gave consistently larger fast pool sizes than other samples. Our hypothesis is that large fraction of the C in needles is water-soluble, and that water-extracted DOC is the most labile/biodegradable one.

The total DOC disappearance during the 28 d of the bioassay ranged from 9 to 73%, similar to what is found in other studies: 0 to 45% (Boyer and Groffman, 1996), 5 to 93% (Kalbitz et al., 2003), 11 and 17% (Kiikkila et al., 2005), 11 to 98% (Wickland et al., 2007), 1 to 75% (Sun et al., 1997), 10 to 45 % (Yano et al., 2000) and 4 to 44% (Bowen et al., in prep.). Variability of the range of values reported could be related to the type of solution used (lysimeter *versus* water-extraction; ratios of extraction; conditions of samples preservation), the method used (bioreactor *versus* bioassays; DOC disappearance *versus* CO_2 mineralization) or the type of samples used (mineral *versus* organic). It is not surprising that our results, representing such a large type of samples obtained a large range of % BDOC values.

Only one study reported losses during the first 4 d of the incubation (Weigner and Seitzinger, 2004) and found similar values to those that we measured: 7 to31% for 4 d, similar to our values of 8 to 52% measured for 3d. Most of the DOC disappearance occurs during the first 3 d of the incubation (up to 85% reported by Wagai and Sollins 2002, Gregorich et al., 2003 and Kalbitz et al., 2003). Our measurements of the loss of initial DOC showed statistically larger values for the fresh material than the L, F and H horizons, similar to Kalbitz et al. (2003) and Kiikkila et al. (2006), but contrary to Qualls

and Haines (1992) and Boyer and Groffman (1996) who observed larger BDOC in the H (Oa) horizon. As suggested in OM decomposition studies, fresh material is composed of relatively large amounts easily decomposable material. Moore et al. (2006) suggested that the process of decomposition transforms a rather heterogeneously composed material (i.e. leaves, needles, plants) into a more homogeneous composition, probably rich in recalcitrant material, but labile material being still present. During decomposition, microbes tend to use labile material first, leaving behind the recalcitrant material. Hence, during water extractions, more of this labile material is soluble compared to humified OM, resulting in larger proportion of the water-extraction that is biodegradable from fresh material. Similar to other studies (Kaiser et al., 2001; Kalbitz et al., 2003; Kiikkila et al., 2006), we found significant differences between the BDOC parameters according to stand type: coniferous samples had larger total lost in 28 d (%) than deciduous samples. Here again, the importance of the soluble pool (which is dissolved during the water extraction) compared to the OM composition could be the key to explain this difference between types of trees. For a better understanding of the dynamic between organic matter samples and water-extracts, it would be necessary to characterize the quality of both litter / soil organic matter samples and water-extractions to compared their relative importance, and contribution to the biodegradable pool. We need a better understanding of what fraction of the samples is water-soluble because the water-extracts could be both similar or different to the initial material composition, influencing the importance of the biodegradation parameters measured.

5.4.2. Linkage between biodegradation and SUVA

To our knowledge, very few studies in the literature reported systematic and simultaneous analysis of biodegradation and spectrophotometric properties of DOC (e.g. Kalbitz et al., 2003). In our study, we took advantage of our large number of samples (40) representing different types of OM to test the usefulness of spectrophotometric properties to predict DOC biodegradation rates. Of all the indices tested (E2:E3, E4:E6, LMW:HMW quotients, Rs and SUVA), only 2 seemed to trace the subtle change in the composition of DOC during the bioassay: SUVA and E2:E3 quotient. On one hand, the E2:E3 quotient showed a general decline, suggesting an increase in molecular size during the bioassay (e.g. Thomsen et al., 2002; Hunt and Ohno, 2007; Minero et al. 2007).

These results support the idea that DOC compounds with small molecular size are preferentially degraded during decomposition (e.g. Trulleyova and Rullik, 2004). However, the E2:E3 quotient was not related to any of the biodegradation parameters reported in this study and hence could not be used to predict the biodegradation of DOC in the water extracts.

On the other hand, the SUVA showed a general increase during the incubation, as also observed by Saadi et al. (2006). It has been suggested that SUVA can be used to estimate the aromatic content of DOC, with increasing SUVA values resulting lower rate of biodegradation (e.g. Kalbitz et al., 2003; Marschner and Kalbitz, 2003; Weishaar et al., 2003; Hur et al., 2006; McDowell et al., 2006). Our results suggest that the solution microbial activity degrades selectively less resistant components (e.g. Saadi et al., 2006) leaving behind the recalcitrant/aromatic compounds resulting in an increase of the SUVA, consistent with other biodegradation studies and reviews (e.g. McDowell et al., 2003; Kalbitz et al., 2003; Trulleyova and Rullik, 2004). Furthermore, the SUVA values of the initial water extractions was strongly correlated with DOC loss during 3 and 28 d, slow and fast pool size and the k_2 (also observed in Kalbitz et al. 2003) and MRT values of the slow pool. These findings support the notion that SUVA is the best predictor of DOC biodegradation, obtaining strong correlations (Kalbitz et al., 2003; Marschner and Kalbitz, 2003; McDowell et al., 2006).

5.4.3. DOC vs. CO_2-C measurements

Only a few studies have measured both DOC loss and CO_2 emission during incubations but our results agreed with theirs in showing generally larger losses of DOC in solution compared to mineralization into CO_2 (Strottman et al., 1995; Kiikkila et al., 2005; McDowell et al., 2006; Bowen et al., in prep.). The discrepancy between DOC losses from solution and those by mineralization (Figure 5.6 shows the dynamic of biodegradation during the bioassay, the start of the incubation is showed in figure 6a) was larger for the 3 d period than for 7 d period, consistent with other studies (e.g. Bowen et al., in prep.) and suggests that during the first d of the incubation, part of the DOC is stored in the microbial biomass which increases during the initial stage of the bioassay (Figure 5.6b). Later in the incubation, after about 7 d, the size of the microbial biomass stabilizes (Young et al., 2005), resulting in less uptake and immobilization, and more of

the DOC is mineralized to CO_2 (Figure 5.6c), which would explain the smaller difference compared to the 3 days results. The coefficient of determination obtained for both regressions between DOC and CO_2-C was about 0.70, was similar to that reported by Kiikkila et al. (2005). Dissolved organic C lost measures both the portion that is stored in the microbial biomass and the amount mineralized into CO_2-C. The emission of CO_2-C represents C mineralization/ microbial respiration, not accounting for C storage in the microbial population. Both methods are meaningful depending on the objective of the study: measuring internal DOC cycling *versus* calculating C gas fluxes.

5.4.4. Conclusions

The biodegradation pathway of DOC includes the microbial biomass uptake of the available substrate C for storage, the exudation of new DOC compounds as waste products, and the loss of C by mineralization into CO_2. We found a wide range of DOC biodegradation, defined by either DOC loss from solution or CO_2 emission, similar to those reported by others. However, we did not find strong relationships between biodegradation parameters and degree of OM decomposition or stand type of the material used for the water extraction, except for the fresh material and coniferous samples having greater lost of DOC in 28 d (%) than other groups of samples. Bioassays measuring either DOC disappearance or CO_2-C mineralization, although good indication of biodegradation, probably measure different dynamics, and their respective contribution needs further analysis and understanding. One of the key findings of our study is the overall importance of the SUVA for the estimation of biodegradation parameters. Taken together, our 40 organic matter samples, supported by the findings of other studies allow to use the SUVA index, a fast measurement, to estimate biodegradation parameters such as the % DOC loss during 3 and 28 d, the slow and fast pool size, the k_2 or the MRT_2 values, that are usually obtained through long bioassays. Unless someone needs a specific number, SUVA values of water extractions can be used to estimate biodegradation allowing to save considerable amount of time in research.

Table 5.1. Initial DOC concentration of the water-extracts from the organic matter samples and loss of DOC after 3 and 28 d of incubation. Within a site, means with different letters are significantly different (Kruskall-Wallis, $p < 0.05$).

Samples	Initial DOC (mg L^{-1})	DOC loss after 3 d (%)	DOC loss after 28 d (%)
Douglas fir, Campbell River			
Douglas fir fresh needles	57	46[a]	70[a]
Douglas fir L	92	17[b]	30[b]
Douglas fir F	33	27[c]	37[c]
Douglas fir H	23	27[c]	37[c]
Black spruce, Waskesiu			
Black spruce fresh needles	82	31[a]	42[a]
Feather moss	220	14[b]	19[b]
Sphagnum	125	29[a]	36[ab]
Black spruce L	152	22[a]	28[ab]
Black spruce F	39	33[a]	42[a]
Black spruce H	22	20[a]	31[ab]
Jack pine, Waskesiu			
Jack pine fresh needles	47	51[a]	68[a]
Jack pine old needles	83	33[b]	48[b]
Jack pine H	61	17[c]	31[c]
Aspen, Waskesiu			
Aspen litterfall	221	35[a]	59[a]
Hazel litterfall	950	29[a]	50[b]
Aspen L	362	43[b]	58[a]
Aspen F	58	21[c]	31[c]
Aspen H	39	14[c]	34[c]
Boreal mixed wood, Groundhog River			
Mixed woods L	174	28[a]	51[a]
Mixed woods F	64	23[a]	42[a]
Mixed woods H	16	27[a]	43[a]
White pine, Turkey Point			
White pine fresh needles	100	35[a]	60[a]
Dicranum	127	37[a]	49[b]
White pine L	233	15[b]	26[c]
White pine F	93	13[b]	26[c]
Black spruce and jack pine, Tirasse Lake			
Spruce / pine L	117	23[a]	31[a]
Spruce / pine F	29	22[a]	33[a]
Spruce / pine H	7	18[a]	23[b]
Balsam fir, Laflamme Lake			
Balsam fir fresh litter	76	13[a]	20[ab]
Balsam fir L	19	11[a]	18[b]
Balsam fir F	23	24[b]	29[a]
Balsam fir H	13	19[ab]	25[ab]
Sugar maple, Hermine - Saint-Hippolyte			
Maple fresh litter	33	16[a]	28[a]
Maple L	40	29[b]	35[a]
Maple F	16	21[ab]	31[a]
Maple H	19	27[ab]	35[a]
Roots	13	22[ab]	29[a]
Sugar maple and American beech, Mont Saint-Hilaire			
Maple / beech L	88	15[a]	18[a]
Maple / beech F	32	10[a]	12[a]
Maple / beech H	15	38[b]	41[b]

Table 5.2. Predicted parameters from the double-exponential decay model. Size is the proportion of the original DOC in the 2 pools. The double exponential model uses the following equation: % C remaining = $[(100\text{-}b)e^{-K1t}] + [\ be^{-K2t}]$. [a] equivalent to $(100 - b)$, [b] MRT = mean residence time = $(1/K)$, [c] equivalent to b.

Samples	Fast pool			Slow pool		
	Size[a] (%)	K_1 (d^{-1})	MRT[b] (d)	Size[c] (%)	K_2 (d^{-1})	MRT[b](d)
Douglas fir, Campbell River						
Douglas fir fresh needles	58	0.73	1.4	42	0.010	100
Douglas fir L	23	0.65	1.7	77	0.004	245
Douglas fir F	32	0.92	1.1	68	0.003	425
Douglas fir H	29	1.21	0.9	71	0.004	227
Black spruce, Waskesiu						
Black spruce fresh needles	45	0.34	2.9	55	0.004	222
Feather moss	17	0.62	2.6	83	0.001	776
Sphagnum	33	0.88	1.2	67	0.003	393
Black spruce L	25	1.06	0.9	75	0.001	2475
Black spruce F	35	1.21	0.8	65	0.005	227
Black spruce H	21	1.20	0.9	79	0.006	176
Jack pine, Waskesiu						
Jack pine fresh needles	55	1.17	0.9	45	0.012	81
Jack pine old needles	34	2.13	0.7	66	0.010	113
Jack pine H	21	1.84	3.0	79	0.008	144
Aspen, Waskesiu						
Aspen litterfall	43	0.75	1.4	57	0.013	78
Hazel litterfall	40	0.65	1.7	60	0.006	202
Aspen L	50	1.90	0.9	50	0.006	220
Aspen F	26	0.79	1.3	74	0.004	294
Aspen H	28	0.55	2.3	72	0.009	138
Boreal mixed wood, Groundhog River						
Mixed woods L	34	0.95	1.3	66	0.011	125
Mixed woods F	28	0.77	1.3	72	0.008	132
Mixed woods H	27	0.66	1.6	73	0.004	323
White pine, Turkey Point						
White pine fresh needles	39	1.00	1.1	61	0.015	67
Dicranum	42	1.06	1.0	58	0.004	252
White pine L	21	0.58	1.8	79	0.003	343
White pine F	21	0.52	2.4	79	0.003	691
Black spruce and jack pine, Tirasse Lake						
Spruce / pine L	27	1.12	1.1	73	0.003	669
Spruce / pine F	32	0.69	1.8	68	0.005	188
Spruce / pine H	21	0.96	1.1	79	0.003	346
Balsam fir, Laflamme Lake						
Balsam fir fresh litter	18	0.69	2.0	82	0.002	645
Balsam fir L	11	1.19	0.9	89	0.004	302
Balsam fir F	25	1.42	0.7	75	0.003	525
Balsam fir H	20	1.48	0.7	80	0.004	284
Sugar maple, Hermine - Saint-Hippolyte						
Maple fresh litter	23	0.60	1.8	77	0.003	634
Maple L	31	1.23	0.8	69	0.002	530
Maple F	28	0.64	1.8	72	0.002	957
Maple H	31	0.98	1.0	69	0.003	361
Roots	24	1.34	0.8	76	0.003	465
Sugar maple and American beech, Mont Saint-Hilaire						
Maple / beech L	15	1.58	0.7	85	0.002	792
Maple / beech F	10	1.23	0.8	90	0.002	599
Maple / beech H	47	1.86	0.5	53	0.002	516

Table 5.3. Mean (± standard deviation) percentage (%) loss of DOC after 3 and 28 d, categorized by degree of decomposition and stand type of the original organic matter. Within an OM decomposition and stand type category, means with different letters are significantly different ($p < 0.05$) by Kruskall-Wallis test.

Sample	3 d	28 d
Degree of OM decomposition		
Fresh	$30^{a} \pm 12$	$44^{a} \pm 18$
L horizon	$24^{ab} \pm 10$	$34^{b} \pm 14$
F horizon	$21^{b} \pm 7$	$31^{b} \pm 9$
H horizon	$22^{b} \pm 8$	$33^{b} \pm 7$
Stand type		
Coniferous	$25^{a} \pm 10$	$38^{a} \pm 14$
Deciduous	$25^{a} \pm 11$	$36^{a} \pm 15$
Others	$25^{a} \pm 9$	$33^{a} \pm 12$

Figure 5.1. Percentage of dissolved organic carbon (DOC) remaining in the solution during the 28 days of the incubation, organized by site. Each data point represents the average of triplicates.

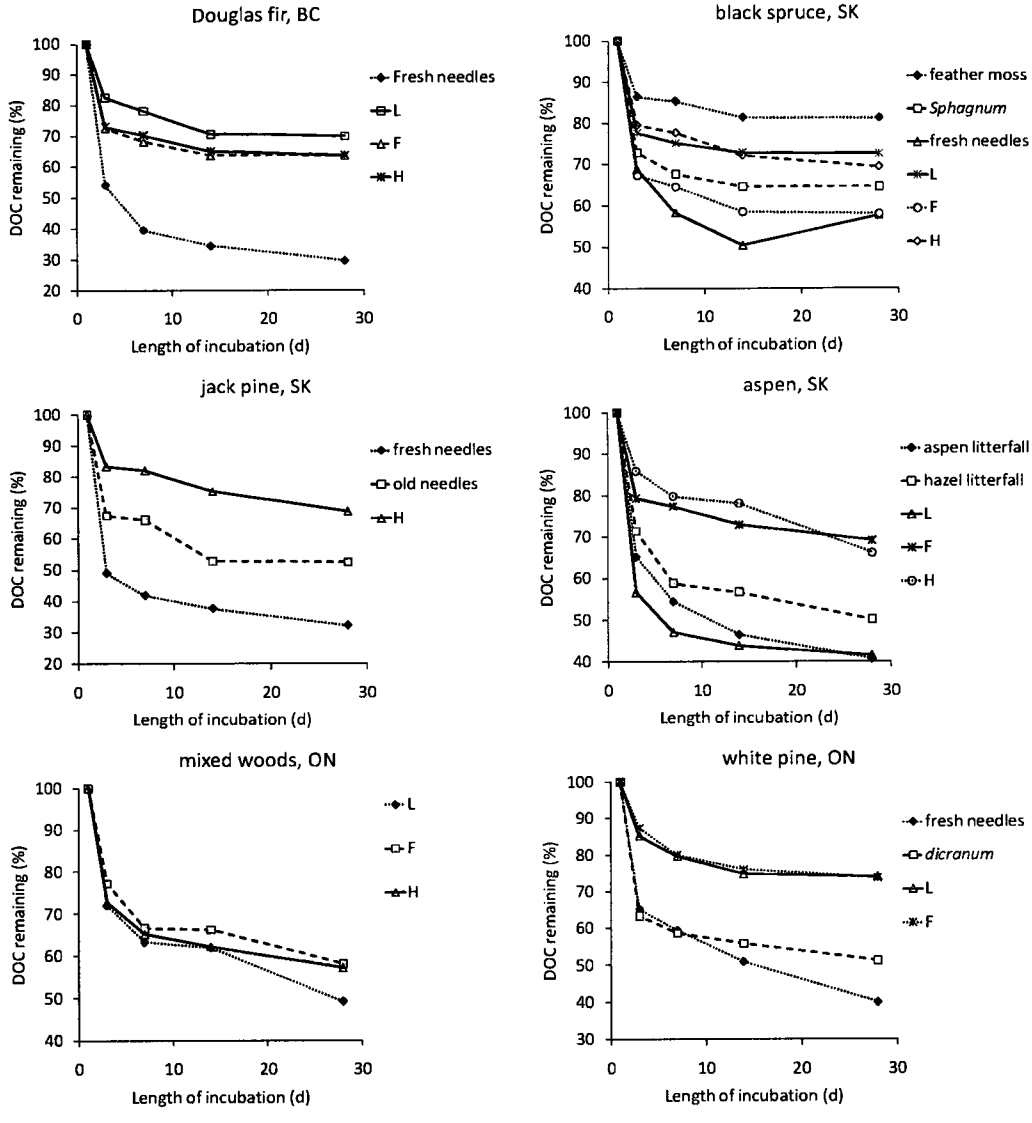


Figure 5.1. (continued) Percentage of dissolved organic carbon (DOC) remaining in the solution during the 28 days of the incubation, organized by site. Each data point represents the average of triplicates.

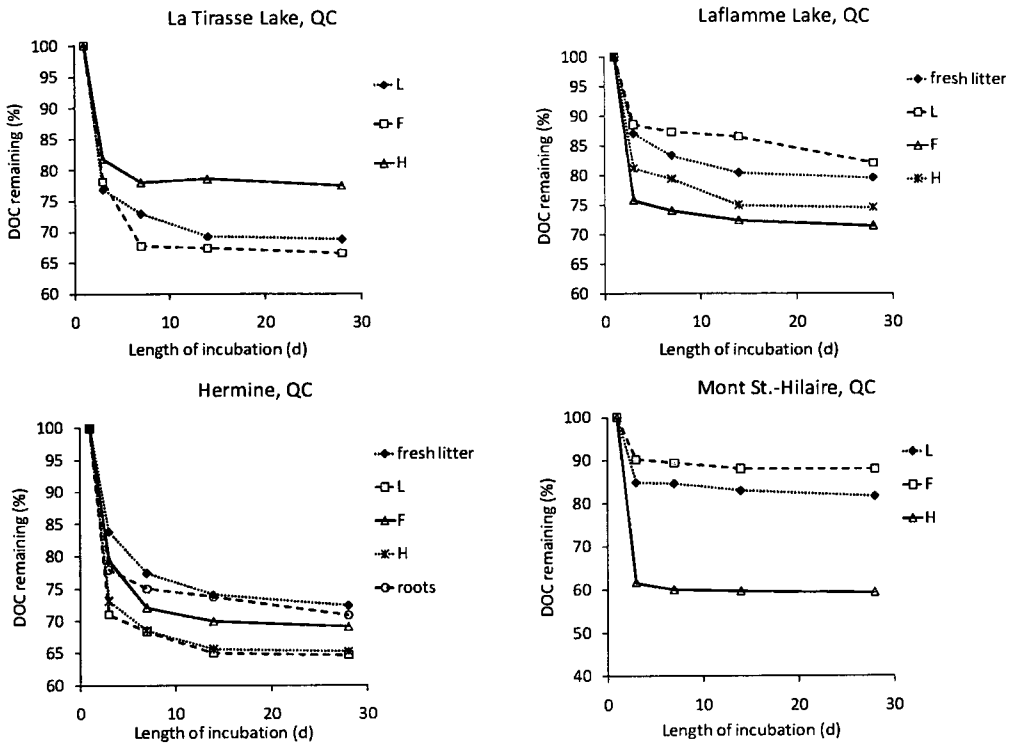


Figure 5.2. Relationships between the fast and slow pool sizes in the DOC extracts and the loss of DOC after 3 and 28 d, respectively.

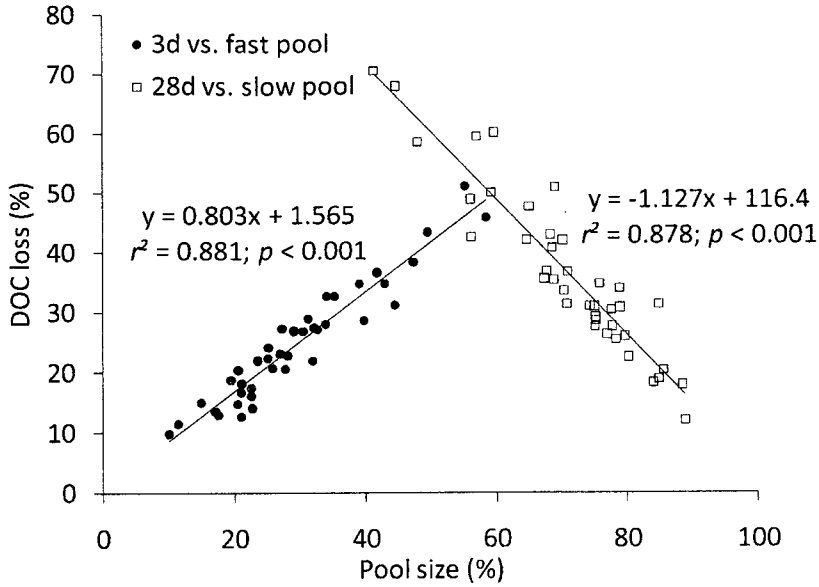


Figure 5.3. Relationship between the SUVA (L mg DOC^{-1} m^{-1}) of the initial water extract and after an incubation of 28 d.

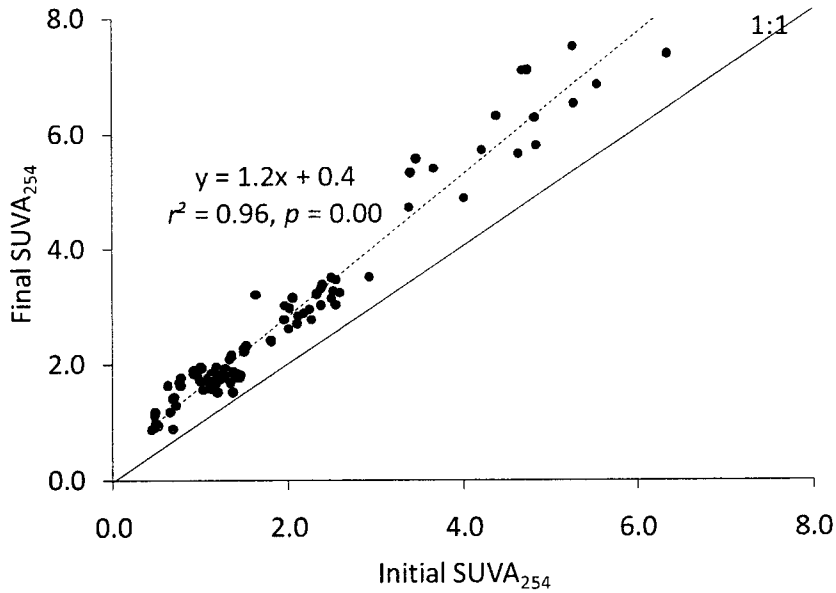


Figure 5.4. Scatterplots of the significant Spearman correlation between initial SUVA and (a) slow pool size, (b) fast pool size, (c) mean residence time 2 (MRT_2), (d) k_2, (e) loss of DOC during the first 3 d and (f) loss of DOC during the 28 d of the incubation.

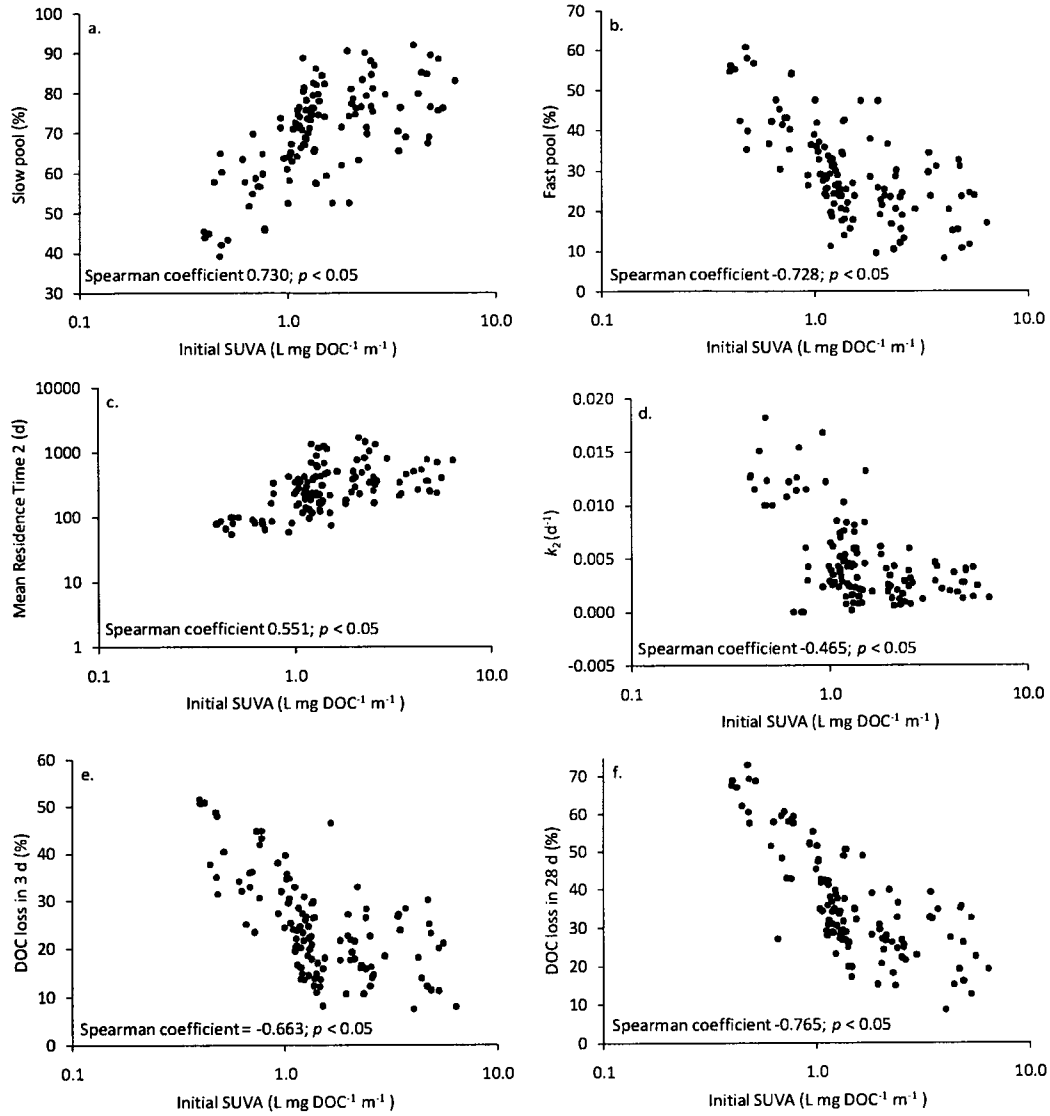


Figure 5.5. Relationship between DOC loss per d in the water extracts and CO_2-C emission per d after days 3 and 7 of the incubation.

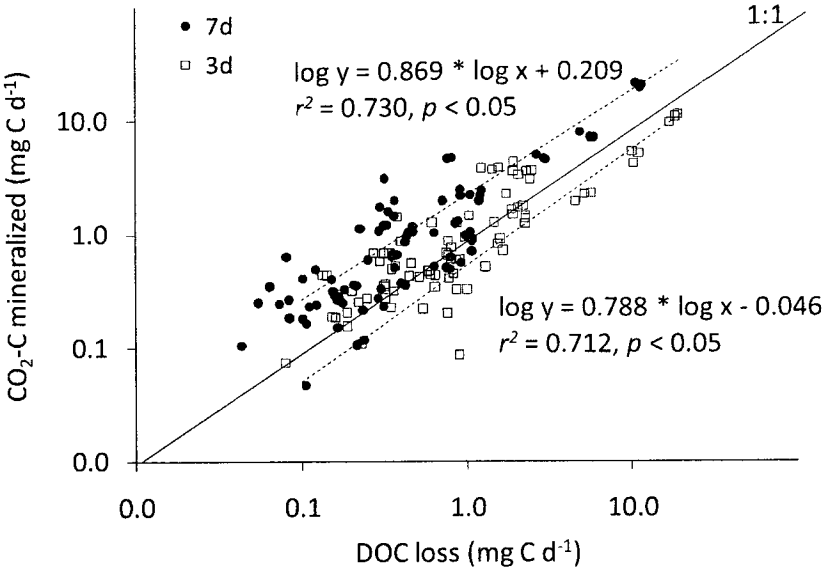
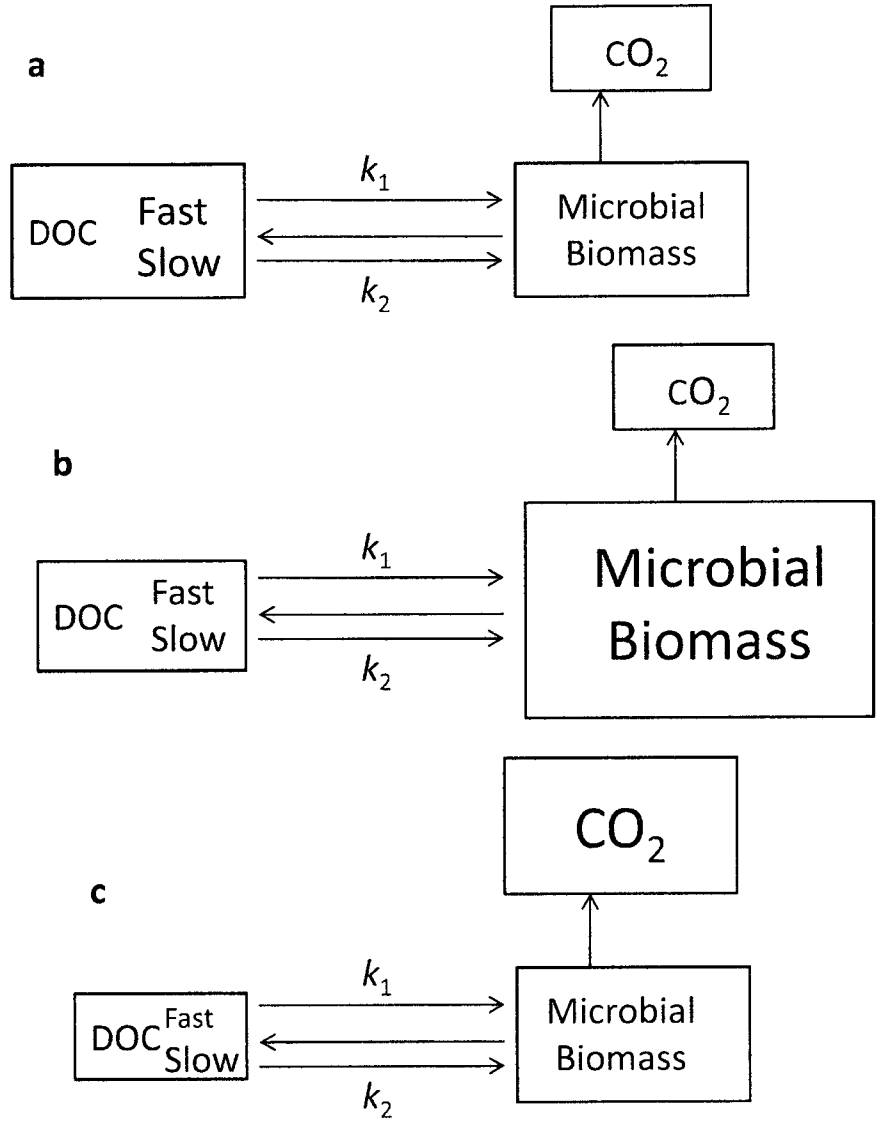


Figure 5.6. Conceptual model representing the dynamic of DOC degradation and CO_2 production during the incubation. a) start of the incubation: a large amount of DOC comprising fast and slow pools each with a characteristic rate constant, a small microbial biomass and small CO_2 production b) Early stage of incubation (~ day 3), smaller amount of DOC, a larger microbial and a small CO_2 production, c) later stages of incubations (~ day 28): less DOC with fast (which has decreased in size) and slow pools, a smaller microbial biomass and larger CO_2 production. Adapted from Gregorich et al. (2000).



Chapter 6 – Transformation of dissolved nitrogen in water-extracts from Canadian forest floors (manuscript #4)

A brief overview – Context within the thesis

Finally, after determining production rates and the interaction of dissolved C, N and P, and evaluating the potential for biodegradation of dissolved organic C, it was logical to look at the biodegradation of DON and associated transformations of NO_3-N and NH_4-N during the bioassay. In Chapter 6, the data from the bioassay are used to evaluate the dynamic and complicated transformations between organic and inorganic forms of nitrogen. Very few studies from the literature have look at these transformations simultaneously with DOC biodegradation, and most of those studies focused solely on DON.

During the course of the bioassay, a rapid initial loss of TDN and DON was observed. These results support the observations from the previous manuscript indicating that during the bioassay, the microbial biomass initially increased due to the availability / lability of C and N. Following this initial change, an increase of dissolved inorganic N was observed, further supporting the shift between the initial increases of microbial biomass to a more steady state incubation, hence the production of waste production of the decomposition of DON. Even though there were no stoichiometric links between the biodegradation of DOC and DON, we observed strong links between DOC and TDN concentrations lost during the incubation, emphasizing the retention of TDN (internally mineralized between DON and NO_3-N, NH_4-N) while DOC was mineralized into CO_2, resulting in a net lost from the bioassay.

6.1. Introduction

Soil dissolved carbon (C) and nitrogen (N) are transformed and transported in surface water in a multitude of ways. These processes help link the soil to the atmosphere (i.e. greenhouse gases emission, denitrification), to the biosphere (i.e. plant assimilation, microbial immobilization) and to the hydrosphere (i.e. leaching). Furthermore, the dissolved C and N takes part in major ecosystem and soil processes such as soil formation, mineral weathering, and soil and water acidification (Likens et al., 1981 Stevenson and Cole, 1999; Kaiser et al., 2001; Kalbitz et al., 2000). The characteristics of dissolved elements, such as chemical composition, reactivity and availability can trigger or inhibit these processes. The role of the dissolved phase in the C and N global cycles is still poorly understood (McDowell, 2003).

The biodegradation of a compound is defined as the process whereby organic matter is transformed into simpler compounds by biological activity. The process of biodegradation in soil has been studied, and literature stressed its important role in nutrient cycling, C sequestration and greenhouse gas emission (Boyer and Groffman, 1996; Gregorich et al., 2003; McDowell et al., 2006). Our understanding of the biodegradability of dissolved organic nitrogen (DON) and associated transformations of N forms lags far behind that of dissolved organic carbon (DOC). Biodegradable DON (BDON) is believed to behave similarly to BDOC with a slow decomposing pool and a fast decomposing pool (e.g. Qualls and Haines, 1992; Gregorich et al., 2003), but mineral forms of dissolved N are believed to be far more dynamic and complex in terms of transformations. For this reason, we need a better understanding of the different processes that affect the amount and form of dissolved N in soils as well as their interactions.

6.1.1. Nitrogen transformations

Nitrogen is a key element, ubiquitous to most systems, but often in quantity that limits the growth of plants and micro-organisms in forest ecosystems (Stevenson and Cole, 1999; Lovett et al., 2004; Gradowski and Thomas, 2006). Unlike the transformations that can occur during the biodegradation of DOC (mineralization into carbon dioxide, microbial biomass storage or exudation of DOC compounds, see Turgeon

et al., Chapter 5), the transformations that affect dissolved nitrogen (N) can be rather complex. In soils, dissolved N is expected to be found in three major forms: 1) nitrate (NO_3-N), 2) ammonium (NH_4-N) and 3) dissolved organic N (DON). In the water-extracts, the dissolved N originates from the solubilization of the organic N that compose the sample and the accumulation of both organic and inorganic N at the surface of the sample. In natural environments, the major source of N in soils is either from atmospheric depositions (dry or wet) and N fixation performed by either the micro-organisms, plants or symbiotic associations between plants and bacteria. According to Stevenson and Cole (1999), the bacteria capable of N fixation (i.e. cyanobacteria and free-living bacteria) are present in both soils and aquatic ecosystems. The N found in the liquid phase of soils (dissolved) can undergo different processes that transform the form (organic vs. inorganic) in which they can be measured. Mineralization is the process by which the organic N (DON) is transformed into inorganic N, mainly NH_4-N, a process called ammonification. The NH_4-N can further be transformed into NO_3-N by a process called nitrification. Dissolved N can be lost from a system through plant uptake (called assimilation), microbial immobilization, denitrification (NO_3-N into gaseous N_2 compounds back to the atmosphere) and leaching through running and surface water systems. Note that the different ways that N is lost from soil are the major means of transport / exchange between soils and the atmosphere, biosphere and hydrosphere.

6.1.2. Stoichiometry

One of the major current understanding of nutrients cycling in soils is the constant interactions occurring between the essential elements (i.e. C, N and phosphorus (P)). However, despite our acknowledgement of these interactions, we know very little about the actual behaviour of those elements, and about the factors that controls the different pathways of transformation. In ecology, stoichiometry (the study of the balance of elements among organisms and their environment [Sterner and Elser, 2002; Hessen et al., 2004]) is used to study these interactions. In soil science, stoichiometry is mainly used to infer soil (or organic matter/litter) quality and decomposability (i.e. C:N quotient), because of its role on the different microbially mediated processes occurring in soils (i.e. decomposition, mineralization, nitrification, biodegradation) (Dodds et al., 2004; Wang et al., 2004, McGroddy et al., 2004).

6.1.3. Objectives

In this study, we had the following 3 objectives:

1. To determine the rates of DON biodegradation and the changes of TDN, NO_3-N and NH_4-N in water-extracts of organic matter from forest floors across Canada;
2. To establish the controls on the rates of biodegradation and transformation DON, such as stand type and degree of decomposition of the original organic matter; and
3. To determine the stoichiometric links between the biodegradation of DOC, DON and TDN.

6.2. Materials and methods

6.2.1. Samples

To characterize the variability of Canadian forests, we chose 10 forests across Canada. The forests sampled were: 1) Douglas fir (*Pseudotsuga mensiesii*), 2) black spruce (*Picea mariana*), 3) jack pine (*Pinus banksiana*), 4) aspen (*Populus tremuloïdes*) with hazel (*Corylus spp.*) understory , 5) boreal mixed woods (aspen (*P. tremuloïdes*), black spruce (*P. mariana*), white spruce (*Picea glauca*), white birch (*Betula papyrifera*) and balsam fir (*Abies balsamea*)), 6) white pine (*Pinus strobes*), 7) black spruce (*P. mariana*) and jack pine (*P. strobus*) 8), balsam fir (*A. balsamea*), 9) sugar maple (*Acer saccharum*) and 10) sugar maple (*A. saccharum*) and american beech (*F. grandifolia*). More details about the study sites are provided by Turgeon et al. (Chapter 3).

A total of 40 samples of OM were gathered. At each site, two soil pits were dug, horizon designation performed (Agriculture Canada Expert Committee on Soil Survey, 1987), followed by sampling of the L, F and H horizons. The two samples were then mixed to produce a composite sample for the site. When available, we collected needles from the trees and fresh litter, old needles and plants were directly collected from the ground. At the aspen site, litterfall was collected in the fall and separated into aspen and hazel leaves (hereafter called aspen and hazel litterfall). Field moisture of the samples was preserved and the samples were kept at 4°C until analysis. The samples were grouped into stand type (coniferous, deciduous and others) and degree of OM

decomposition (Fresh, L, F and H horizons) for statistical analysis. The complete list of samples in every group is listed in Turgeon et al. (Chapter 3).

6.2.2. Water extraction procedure

The water extraction of the 40 samples of organic material was done using a soil: solution mixture ratio of 1:100 (dry weight), that was gently shaken on a rotating shaker for 30 min. The bottles containing the soil:solution mixture were centrifuged at 4500 rpm for 20 min, the supernatant was filtered through a 0.45 um filter paper (Macherey-Nagel 85/90 BF) and frozen until analyses and incubation.

6.2.3. Incubation

The incubation was modified from Gregorich et al. (2003). The frozen water-extracts were allowed to thaw at 4°C three days before the beginning of the incubation. The inoculum was prepared using a mixture of all 40 OM samples (4 g of the mixture) mixed with 45ml of deionised water, shaken vigorously by hand and allowed to incubate during 24 hrs at 20°C. Minutes before the inoculation, the inoculum was passed through fibre glass wool to avoid any particulate matter to enter the solution during inoculation. Samples were incubated in triplicates: 200 ml of water-extract, diluted to between 10 and 20 mg DOC L^{-1} to avoid excessive growth of microbial biomass (e.g. Kalbitz et al., 2003, McDowell et al. 2006) was gently mixed with 2 ml of inoculum (representing 1% v/v inoculum/solution) and 2 filter papers were added (cut in half; (Macherey-Nagel 85/90 BF)) to allow a medium for the growth of microbes in a glass Erlenmeyer flask. Blanks were run to verify for contamination during the incubations. We did not adjust the nutrient content of the water extracts (McDowell et al. 2006) so that we could measure both the biodegradability (mineralization / immobilization) of DOC and dissolved N simultaneously. The flask were kept in the dark at 20°C during the incubation, and shaken gently every other day. Aliquots of 30 ml of the incubated solution were taken from the flask at days 1, 3, 7, 14 and 28, and filtered though a 0.45 um filter-paper (Macherey-Nagel 85/90 BF) and analysed for DOC and TDN on a Shimadzu VSN TOC/TN analyzer. A 10 ml sub-sample of the aliquot was analysed for NO_3-N and NH_4-N by colorimetric method with a Flow Injection Analyser (FIA, Lachat). Dissolved organic N was calculated by subtracting DIN from TDN.

6.2.4. Statistical analyses

First, the different variables of the dataset were tested for normality with a Shapiro-Wilk test. The variables that did not fit the normal distribution were log-transformed. When the log-transformation did not result with normality, non-parametric tests were used for the statistical analysis of the results (i.e. Kruskall-wallis, Spearman correlations, etc). Otherwise, parametric tests were used on raw or log-transformed data (i.e. ANOVA, *t*-test, etc). The normality of the residuals of the regression models were tested with the Shapiro-Wilk test. The data of DON disappearance were analysed with a double exponential decay model (% DON remaining = $[(100-b)e^{-K1t}] + [\ be^{-K2t}]$, where 10-$b$ is the size of the fast pool, k_1 is the decomposing rate constant of the fast pool, b is the size of the slow pool and k_2 is the decomposing rate constant of the slow pool).

6.3. Results

6.3.1. Dissolved N concentrations

The concentration of TDN in the water extracts varied from 0.8 (spruce/pine H) to 29.5 mg L^{-1} (hazel litterfall), with an average of 8 ± 6 mg L^{-1} (Table 6.1). These concentrations were significantly correlated (Spearman coefficient 0.50, $p < 0.05$) with the TDN concentration measured in the leaching experiment described in Turgeon et al. (Chapter 4), using the same organic samples. The H horizon TDN concentrations are statistically ($p < 0.05$) smaller (4 ± 3 mg L^{-1}) than the fresh material (9 ± 7 mg L^{-1}), the L horizon (10 ± 4 mg L^{-1}) and the F horizon concentrations (10 ± 8 mg L^{-1}). Furthermore, coniferous TDN concentrations are statistically ($p < 0.001$) larger (12 ± 7 mg L^{-1}) than those from deciduous samples (5 ± 4 mg L^{-1}).

The concentration of NO_3-N in the water-extracts ranged from 0.1 (spruce/pine H) to 25.6 mg L^{-1} (aspen F) (Table 6.1), with an average of 3 ± 5 mg L^{-1}. The F horizon showed statistically larger NO_3-N concentrations (6.1 ± 8.4 L^{-1}, $p < 0.05$) than the fresh material (1.4 ± 1.9 -N L^{-1}), the L horizon (1.6 ± 1.0 L^{-1}) and the H horizon (1.6 ± 1.8 L^{-1}). The NO_3-N concentrations in the coniferous group (5.2 ± 6.7 L^{-1}) were significantly larger ($p < 0.05$) than those from the deciduous group (1.0 ± 1.3 L^{-1}) and the other vegetation group (0.8 ± 0.6 L^{-1}).

The concentrations of NH_4-N ranged between 0.2 (spruce/pine H) to 11.3 mg L^{-1} (white pine L) (Table 6.1), with an average of 3.3 ± 2.6 mg L^{-1}. The fresh material NH_4-N

(3.5 ± 2.2 mg L^{-1}) was not significantly different from the L horizon (4.9 ± 2.6 mg L^{-1}), but both were significantly larger ($p < 0.05$) than the F horizon (3.1 ± 2.8 L^{-1}) and the H horizon samples (1.6 ± 1.2 mg L^{-1}), which were not significantly different from one another ($p > 0.05$). No significant differences were observed according to stand type ($p > 0.05$).

The concentration of DON, estimated as the difference between TDN and DIN, varied between 0 (mixed woods F and aspen F and H) and 14.8 mg L^{-1} (hazel litterfall) (Table 6.1), with an average of 2.2 ± 2.6 mg L^{-1}. The DON concentration of the fresh material (3.5 ± 3.5 mg L^{-1}) was not significantly different from the L horizon (3.3 ± 2.3 mg L^{-1}). The F horizon samples (0.9 ± 0.6 mg L^{-1}) were not significantly different from the H horizon samples (0.6 ± 0.4 mg L^{-1}), but both are significantly smaller ($p < 0.05$) than the fresh material and L horizon samples. Concentration of DON in the coniferous group (3.0 ± 4.0 mg L^{-1}) was statistically larger ($p < 0.05$) than the deciduous group (1.6 ± 1.1 mg L^{-1}). The initial DON concentration of the water-extracts was not correlated ($p > 0.05$) with any of the biodegradation parameters presented in this study.

6.3.2. Changes of total N and inorganic N during the incubation

The percentage of original TDN remaining in the solution fell sharply during the first 3 or 7 days of the incubation, followed by a general increase. An example of the trend is shown in Figure 6.1 with the data from the Douglas fir samples.

The percentage of original NO_3-N and NH_4-N remaining in the solution behaved in a more irregular pattern than TDN, in some cases increasing, in others decreasing. Expressed as dissolved inorganic N (DIN), the combination of NO_3-N and NH_4-N, there was a general increase during the incubation, illustrated by the samples from the white pine site (Figure 6.2).

Although the incubation lasted 28-d the gain or loss was calculated on a 14-d basis because some data from day 28 were missing; where the dataset was complete, most of the change in concentration occurred by day 14. The largest loss of TDN was measured for black spruce F (-36%) while 3 of our samples showed gain of total N: mixed woods F (+9%), mixed woods H (+4%) and aspen F (+7%). The average percentage loss of TDN was -11 ± 10%. The largest loss of NO_3-N was measured for aspen and hazel litterfall, and aspen L horizon (94-96%) and the largest gain was measured for *Dicranum* (+847%)

and white pine L horizon (+285%). The average change of NO_3-N was +15 ± 168%. The H horizon of black spruce forest presented the largest loss of NH_4-N (-83%) while both F and H horizon from spruce/ pine forest gave the largest gain of NH_4-N (+79 and 57% respectively), with an average change of +2 ± 35%.

When compared by degree of decomposition of the OM, the fresh material group had a significantly larger loss of TDN, while the loss or gain of NO_3-N was not significantly different between degrees of OM decomposition (Table 6.2). Ammonium show some variability between the different groups, with fresh material NH_4-N loss being significantly larger than from F horizon, but both were not significant different from L and H horizons. The coniferous samples generally lost less TDN than deciduous samples, but show no significant differences for NO_3-N and NH_4-N.

6.3.3. Biodegradation of DON

The change in DON during the incubation follows, for most of the samples, the double-exponential decay model. Figure 6.3 shows the example of the black spruce site. Some cases had to be excluded from the model because of missing values or no fit with the decay model (i.e. an increase in concentration). We tested both single and double exponential models and the latter always gave better r^2 values (r^2 ranging between 0.54 and 0.99; $p < 0.05$).

The double-exponential decay model parameters for DON are presented in table 6.3. Neither the degree of OM decomposition nor stand type was significantly different for any of the decay parameters ($p > 0.05$). None of the parameters presented in Table 6.3 seems to show specific trends between soils horizons (L, F and H horizon). The largest fast pool size is observed for the H horizon of the sugar maple sites (77%). The H horizon of the maple/beech site, the L horizon of the white pine and the jack pine fresh needles also presented fast pool size above the 70% threshold (73, 76 and 73 respectively). The smallest fast pool was measured for the sugar maple fresh litter (15%). Values of k_1 above 6 d^{-1} were observed for *Sphagnum* and the L horizons of spruce/pine, sugar maple and maple/beech sites. The largest k_2 was calculated for maple/beech F horizon (0.194 d^{-1}), and the smallest for jack pine old needles, balsam fir L horizon and maple F horizon (below 0.001 d^{-1}).

When looking at the loss/gain of DON during the first 14 days of the incubation, 4 samples resulted in a gain of DON: jack pine H horizon, aspen and hazel litterfall and mixed woods H horizon. On the other hand, 3 samples resulted in a total disappearance of DON of 90% or more: *Dicranum*, white pine L horizon and maple / beech F horizon. As observed for the double exponential decay parameters, the percentage lost of DON did not present consistent trend between the soil horizons (L, F and H horizon) within a site. When divided into groups, no differences were observed between degrees of OM decomposition, but the coniferous samples lost significantly less than deciduous (Table 6.2).

6.3.4. Comparison with DOC results

We measured the disappearance of DOC and dissolved N simultaneously during the incubations, the former being reported in Turgeon et al. (Chapter 5). The initial concentrations of DOC and DON of the water-extracts were statistically correlated (Figure 6.4), but their losses during the first 14 d were not correlated ($p > 0.05$)5, with a wide distribution around the 1:1 line (Figure 6.5). Furthermore, none of the biodegradation parameters (k, % and MRT, for DON, in Table 6.3 and for DOC in Turgeon et al., Chapter 5) or both the slow and fast pools in DOC and DON are statistically related (Spearman $p > 0.05$). The initial concentration of DOC is correlated with the initial concentration of TDN (Figure 6.6) and their respective loss are correlated to each other (Figure 6.7, Spearman coefficient 0.617, $p = 0.00$), although more DOC was lost than TDN.

6.3.5. Stoichiometry between carbon and nitrogen

The initial DOC:TDN quotient of the water-extracts is statistically correlated with the final solution DOC:TDN quotient (Figure 6.8). Even though significantly related, the initial and final DOC:DON quotient show a weaker correlation compared to the DOC:TDN quotient. Furthermore, while the initial vs. final DOC:TDN quotient is always below the 1 to 1 line (Figure 6.8), the initial vs. final DOC:DON quotient is spread both above and below the 1 to 1 line.

Figure 6.9 shows the relationships between initial C:N quotient of the solution and the lost of DOC and TDN. The initial DOC:TDN quotient is statistically related ($p < 0.05$) to

the lost of DOC (Spearman coefficient = -0.543), the lost of TDN (Spearman coefficient = -0.636), the lost of NH_4-N (Spearman coefficient = -0.301) but not correlated to either the lost of DON or NO_3-N ($p > 0.05$). The initial DOC:DON quotient is statistically correlated ($p < 0.05$) to the lost of DOC (spearman coefficient -0.212) and the lost of NH_4-N (spearman coefficient -0.490) but not correlated to the lost of TDN, DON or NO_3-N. Consequently, smaller initial DOC:TDN quotients lose smaller percentage of DOC and TDN, and *vice versa*, the larger initial DOC:TDN quotient lose larger percentage of both DOC and TDN during the first 14 days. However, the organic dissolved quotient (DOC:DON) do not have a great predicting power over the lost of dissolved N and C.

6.4. Discussion

6.4.1. Dissolved nitrogen

Few studies have examined the biodegradability of dissolved N in soil, and of those that have, the focus was on DON. Hence, there are few data with which to compare our results of biodegradability/mineralization/immobilization of dissolved N (e.g. Qualls and Haines, 1992; Gregorich et al. 2003; Cleveland et al. 2004). The trends observed for TDN, showing an initial sharp decrease in the first 3 days followed by an increasing trend, were similar to those observed by Cleveland et al. (2004). Taken together, these results support our findings reported in Turgeon et al. (Chapter 5) on the biodegradability of DOC. In that study, a discrepancy between results of DOC disappearance and carbon dioxide (CO_2-C) mineralization at the beginning of the incubation, suggested that during the first 3 days of the incubation, microbial biomass was growing, resulting in a larger loss of DOC compared to the amount respired as CO_2. The sharp decrease of TDN also suggests immobilization of N by a growing microbial biomass, since microbes need both C and N for their growth and maintenance (Figure 6.10b). Hence the disappearance of N was related to the fast uptake of C. Following, the increasing TDN concentration trend is probably the result of steady microbial biomass releasing N as a waste product, and transforming organic and inorganic forms (e.g. Cleveland et al. 2004) (Figure 6.10c).

At day 14 of the incubation, 3 samples resulted in an increase if TDN: aspen F horizon (+7% ±1), mixed woods F horizon (+9% ±4) and H horizon (+4% ± 4). These

increasing trends were replicated in each triplicates. The temporal trends of these 3 samples showed very little change in TDN concentration through the incubation.

Both NO_3-N and NH_4-N show irregular patterns during the incubation. However, when looked at as the sum of inorganic nitrogen (DIN), we can conclude there was net mineralization during the incubation. We believe, supported by the results of DOC, TDN and DON, that there is a net immobilization at the beginning of the incubation, followed in decomposition and possibly ammonification / nitrification (e.g. Gregorich et al., 2003), releasing dissolved inorganic N into the solution. *Dicranum* and the L horizon of the white pine site resulted in large gain of NO_3-N. Simultaneously, these two samples showed very large lost of DON, resulting in an average TDN lost around -15% and -5% respectively for *Dicranum* and white pine L horizon. In general, the fresh material had a larger TDN lost, probably due to the availability and lability of its initial material, resulting in a large utilisation by the microbial biomass.

6.4.2. Dissolved organic nitrogen

In general, the double exponential model for the biodegradation of DON resulted in large fast pool, sometimes larger than 70%. Some samples even lost up to 90% of their initial concentration during the first 14 days of the incubation. Also, most of the DON was lost during the first 3 days of the incubation (between 4 to 94%). Gregorich et al. (2003) mentioned that this rapid disappearance of DON during the incubation (reporting a lost between 63 to 73% in the first 3 days) is the result of DON containing readily decomposable compounds, also supporting the hypothesis presented in figure 10 of increasing microbial biomass at the beginning of the incubation. Unlike observed in Kiikkila et al. (2005; 2006) and Qualls and Haines (1992), we did not found significant differences between BDON parameters and the different type of samples (degree of OM decomposition and stand type).

6.4.3. Stoichiometry

Unlike observed by Wiegner and Seitzinger (2004), our lost of DON was not always larger than the lost in DOC. Which means that DON does not decay faster than DOC as stated in Gregorich et al., (2003) and that DON is as refractory as DOC (e.g. Qualls and Haines 1992). Our DOC:TDN quotients varies between 2 to 36, well within the range of

what other studies observed: 12 to 21 (Gregorich et al., 2003) and 30 to 58 (Qualls and Haines 1992). While the DOC:TDN quotients consistently decreased during the incubations, as observed by Qualls and Haines (1992) and to the contrary of what Gregorich et al. (2003) observed, The DOC:DON quotient showed both increasing and decreasing trends during the incubation. The DOC:TDN quotient trend however suggest that nitrogen is retained in the solution while the DOC is mineralized. Taken together, these results support that the mineralization of dissolved N is linked to the mineralization of DOC as suggested by Qualls and Haines (1992). The smaller initial quotients resulting in smaller lost of both DOC and TDN, suggesting a link between the C:N quotient of the solution and the biodegradation as suggested in Fellman et al. (2008). Ours results clearly show stoichiometric relationships of dissolved C and N transformations, emphasizing the importance of linkages between nutrients and substrate during soil biological processes.

6.4.4. Conclusions

Taken together, our data of biodegradation of DOC and DON and mineralization/ immobilization of dissolved total and inorganic N supported interpretation of the dynamics during the bioassay presented in the first part of this study focussing on BDOC. At the beginning of the incubation, the sharp decrease of both TDN and DON supported the fast uptake by an increasing microbial biomass. Following this rapid increase of microbial biomass, probably due to the readily available DOC and dissolved N, the net increase of TDN, and general increased in inorganic N are the result of decomposition of DON along with DOC, and the net mineralization of DIN as waste-products of biodegradation. The general decrease of the DOC:TDN quotient being the result of a general lost of DOC, mainly as CO_2 mineralization, and the retention of N in solution, emphasizing a strong link between processes that transforms C and N during solution incubation.

We were unable to find strong relationships between the biodegradation of DON and either of the degree of OM decomposition or stand type. Furthermore, the comparison between the lost of DOC and DON during the incubation showed that there is not a general trend showing that C is more or less refractory than N. Hence, the distribution of their respective lost was almost evenly represented above and below the 1:1 line of the graph. Even if the biodegradation parameters between DOC and DON did not correlated,

our study strongly showed a clear link in the simultaneous transformations of C and N during the bioassay, stressing the importance of both C and N, necessary for microbial biomass growth and maintenance. Obviously, the microbial biomass plays a key role in the biodegradation and difference transformation of dissolved N in soils.

Table 6.1. Initial concentrations of nitrate (NO_3-N), ammonium (NH_4-N) and dissolved organic nitrogen (DON) and the percentage of original concentration lost during the 14-d incubation period for total dissolved nitrogen (TDN), NO_3-N, NH_4-N, DON and dissolved organic carbon (DOC). [a, b, c] Different letters indicate significant differences between samples for a site (Kruskall-Wallis, $p < 0.05$). *n/a* are indicated for samples that had no or negative calculated DON in the initial solution, unabeling the calculation of lost.
(*see next page*)

	NO_3-N	NH_4-N	DON	TDN	NO_3-N	NH_4-N	DON	DOC
	Initial concentration (mg L^{-1})				Loss over 14 d (% of initial concentration)			
Douglas fir, Campbell River								
Douglas fir fresh needles	0.3	0.7	1.4	-31^{a} ±4	-7^{a} ±6	-9^{a} ±7	-59^{a} ±18	-65^{a} ± 4
Douglas fir L	2.5	2.2	2.0	-12^{b} ±1	-66^{b} ±5	$+34^{b}$ ±17	-55^{a} ±5	-29^{b} ± 1
Douglas fir F	0.2	1.3	0.7	-15^{b} ±2	-48^{c} ±2	$+18^{ab}$ ±6	-59^{a} ±16	-37^{c} ± 1
Douglas fir H	0.6	0.4	0.5	-12^{b} ±4	$+6^{d}$ ±1	$+38^{b}$ ±13	-76^{a} ±11	-35^{c} ± 2
Black spruce, Waskesiu								
black spruce fresh needles	1.6	0.3	2.9	-24^{a} ±7	$+9^{a}$ ±5	-52^{a} ±9	-43^{a} ±7	-49^{a} ± 4
feather moss	0.7	7.8	3.7	-10^{b} ±1	$+5^{a}$ ±1	$+6^{b}$ ±7	-43^{a} ±7	-19^{b} ± 0.4
Sphagnum	1.8	3.5	2.8	-16^{ab} ±2	$+9^{a}$ ±5	$+10^{bc}$ ±5	-53^{ab} ±3	-35^{c} ± 3
black spruce L	0.4	4.8	2.0	-15^{ab} ±2	$+82^{b}$ ±29	-14^{ab} ±12	-67^{b} ±5	-27^{d} ±0.3
black spruce F	0.1	0.3	0.8	-36^{c} ±5	-88^{c} ±4	$+51^{c}$ ±37	-49^{a} ±3	-41^{c} ± 1
black spruce H	0.5	0.3	0.6	-7^{d} ±3	$+132^{b}$ ±39	-83^{a} ±5	-45^{a} ±6	-28^{d} ± 3
Jack pine, Waskesiu								
jack pine fresh needles	0.2	0.8	1.2	-30^{a} ±5	$+16^{a}$ ±7	$+33^{a}$ ±26	-77^{a} ±2	-62^{a} ± 1
jack pine old needles	1.7	1.8	2.5	-15^{b} ±3	$+2^{b}$ ±2	$+32^{a}$ ±25	-58^{a} ±10	-47^{b} ± 1
jack pine H	0.6	2.8	1.1	-3^{c} ±2	-92^{c} ±5	-6^{b} ±6	$+54^{b}$ ±21	-25^{c} ± 5
Aspen, Waskesiu								
aspen litterfall	1.5	3.1	3.5	-20^{a} ±6	-96^{a} ±2	-30^{a} ±10	$+21^{a}$ ±9	-53^{ab} ± 1
hazel litterfall	7.4	4.0	14.4	-25^{a} ±14	-95^{a} ±1	-77^{b} ±5	$+34^{a}$ ±16	-43^{a} ± 5
aspen L	2.6	2.8	9.2	-25^{a} ±6	-94^{a} ±1	-19^{ac} ±6	-17^{b} ±1	-56^{b} ± 1
aspen F	25.6	2.1	-	$+7^{b}$ ±1	$+2^{b}$ ±1	-12^{cd} ±5	*n/a*	-27^{c} ± 2
aspen H	5.8	3.0	-	-2^{b} ±0	-1^{b} ±1	$+3^{d}$ ±4	*n/a*	-22^{c} ± 8
Boreal mixed wood, Groundhog River								
mixed woods L	1.6	6.1	3.8	-10^{a} ±4	-95^{a} ±1	$+2^{a}$ ±4	-17^{a} ±3	-38^{a} ± 5
mixed woods F	14.4	1.4	-	$+9^{b}$ ±4	-1^{b} ±1	-22^{b} ±5	*n/a*	-34^{a} ± 1
mixed woods H	2.4	0.4	0.1	$+4^{b}$ ±4	-1^{b} ±2	-5^{c} ±3	$+39^{b}$ ±25	-37^{a} ± 10
White pine, Turkey Point								
white pine fresh needles	0.6	5.2	2.6	-11^{ab} ±2	-5^{a} ±3	$+8^{a}$ ±11	-52^{ab} ±26	-49^{a} ± 2
Dicranum	0.6	4.9	3.1	-15^{a} ±6	$+847^{b}$ ±31	-60^{b} ±5	-97^{a} ±1	-44^{a} ± 5
white pine L	3.0	11.0	3.6	-5^{bc} ±1	$+235^{c}$ ±7	-37^{c} ±2	-90^{a} ±0.3	-25^{b} ±0.3
white pine F	5.1	7.5	0.5	-2^{c} ±1	$+22^{a}$ ±16	-8^{a} ±6	-36^{b} ±23	-24^{b} ±3
Black spruce and jack pine, Tirasse Lake								
spruce / pine L	0.6	5.5	1.8	-9^{a} ±2	$+349^{a}$ ±25	-28^{a} ±4	-75^{a} ±13	-31^{a} ±2
spruce / pine F	0.6	0.9	2.0	-16^{a} ±5	-93^{b} ±1	$+79^{b}$ ±27	-37^{b} ±18	-32^{a} ±5
spruce / pine H	0.1	0.2	0.5	-8^{a} ±3	-91^{b} ±1	$+57^{b}$ ±37	-14^{b} ±7	-22^{b} ±1
Balsam fir, Laflamme Lake								
balsam fir fresh litter	1.3	5.4	4.3	-11^{a} ±3	-89^{a} ±4	$+22^{a}$ ±2	-31^{a} ±9	-20^{ab} ±2
balsam fir L	0.9	2.8	0.8	-2^{a} ±1	$+16^{b}$ ±14	$+11^{a}$ ±7	-48^{a} ±11	-13^{b} ±6
balsam fir F	0.5	3.4	1.4	-10^{a} ±3	$+8^{b}$ ±3	-1^{b} ±1	-49^{a} ±9	-28^{a} ±3
balsam fir H	0.3	1.7	0.9	-11^{a} ±7	-85^{a} ±1	$+13^{a}$ ±5	-32^{a} ±10	-25^{a} ±2
Sugar maple, Hermine - Saint-Hippolyte								
maple fresh litter	0.6	3.4	1.6	-6^{ab} ±2	-92^{a} ±0.4	$+10^{a}$ ±3	-11^{a} ±5	-26^{a} ±3
maple L	0.6	5.1	2.5	-12^{ab} ±1	-88^{a} ±1	$+6^{a}$ ±4	-32^{a} ±10	-35^{a} ±2
maple F	0.3	2.4	1.0	-7^{ab} ±6	-88^{a} ±0	$+28^{b}$ ±4	-19^{a} ±5	-30^{a} ±7
maple H	1.5	2.7	1.3	-14^{a} ±6	$+6^{b}$ ±3	$+8^{a}$ ±5	-80^{b} ±8	-34^{a} ±5
Roots	0.3	2.5	0.9	-3^{b} ±1	$+41^{c}$ ±17	$+18^{ab}$ ±6	-60^{b} ±10	-26^{a} ±3
Sugar maple and american beech, Mont Saint-Hilaire								
maple / beech L	1.8	6.6	5.1	-6^{a} ±7	$+25^{a}$ ±6	$+23^{a}$ ±4	-53^{a} ±10	-17^{a} ±5
maple / beech F	8.8	8.4	1.4	-5^{a} ±2	$+8^{b}$ ±1	$+8^{b}$ ±2	-94^{b} ±4	-12^{a} ±2
maple / beech H	2.5	2.4	0.5	-3^{a} ±6	$+9^{b}$ ±1	-3^{c} ±2	-64^{a} ±16	-39^{b} ±9

Table 6.2. The loss of N forms (total dissolved nitrogen (TDN), nitrate (NO_3-N), ammonium (NH_4-N), dissolved organic nitrogen (DON)) and dissolved organic carbon (DOC) during the first 14 days of the incubation, expressed as a percentage (mean ± standard deviation) of the original concentration; positive values indicate a gain. [a, b] Different letters represent significantly different values (Kruskal-Wallis, $p < 0.05$) between groups based on degree of organic matter decomposition and stand type.

Sample	TDN (%)	NO_3-N (%)	NH_4-N (%)	DON (%)	DOC (%)
			Degree of organic matter decomposition		
Fresh	a -17 ± 10	a +45 ± 50	a -10 ± 36	a -39 ± 38	a -41 ± 16
L horizon	b -11 ± 7	a +44 ± 166	ab +1 ± 26	a -51 ± 24	b -32 ± 13
F horizon	b -8 ± 13	a -31 ± 46	b +16 ± 34	a -49 ± 25	b -29 ± 9
H horizon	b -6 ± 7	a -14 ± 70	ab +3 ± 40	a -26 ± 50	b -29 ± 8
			Stand type		
Coniferous	a -8 ± 11	a -41 ± 50	a -5 ± 25	a -23 ± 43	a -33 ± 13
Deciduous	b -14 ± 10	a +13 ± 126	a +8 ± 40	b -47 ± 31	a -34 ± 14
Others	ab -11 ± 6	b+225 ± 336	a -6 ± 33	b -63 ± 22	a -31 ± 10

Table 6.3. Parameters of the double-exponential decay model used to fit the loss of DON (%) for the different organic matter samples. % DON remaining = $[(100-b)e^{-K1t}] + [\ be^{-K2t}]$. [a] equivalent to $(100 - b)$, [b] MRT = mean residence time = $(1\ /K)$, [c] equivalent to b. *Italicized* values of *0.001* represent a k_2 values smaller than 0.001

	Fast pool			Slow pool		
	Size[a] (%)	k_1 (day^{-1})	MRT[b] (days)	Size[c] (%)	k_2 (day^{-1})	MRT[b] (days)
Douglas fir, Campbell River						
Douglas fir fresh needles	54	0.60	1.8	46	0.019	52
Douglas fir L	39	0.61	2.1	61	0.017	65
Douglas fir F	33	2.45	1.1	67	0.032	31
Douglas fir H	67	1.12	0.9	33	0.019	53
Black spruce, Waskesiu						
black spruce fresh needles	40	2.11	1.2	60	0.008	124
feather moss	36	1.09	1.1	64	0.006	369
Sphagnum	29	6.01	0.2	71	0.015	77
black spruce L	36	4.66	0.2	63	0.045	24
black spruce F	37	1.99	0.8	63	0.039	27
black spruce H	37	2.52	0.5	58	0.019	75
Jack pine, Waskesiu						
jack pine fresh needles	73	2.52	0.4	27	0.013	76
jack pine old needles	54	0.83	1.7	46	*0.001*	>1000
Boreal mixed wood, Groundhog River						
mixed woods L	28	3.84	0.2	72	0.014	51
White pine, Turkey Point						
white pine fresh needles	54	5.10	0.2	46	0.035	32
Dicranum	43	3.30	0.2	57	0.131	8
white pine L	76	2.71	1.2	24	0.027	38
white pine F	44	2.27	1.1	56	0.042	25
Black spruce and jack pine, Tirasse Lake						
spruce / pine L	57	6.75	0.1	33	0.008	303
spruce / pine F	38	4.79	0.2	62	0.008	120
spruce / pine H	29	2.97	0.2	71	0.011	107
Balsam fir, Laflamme Lake						
balsam fir fresh litter	34	3.69	0.3	66	0.002	749
balsam fir L	49	0.25	4.5	53	*0.001*	>1000
balsam fir F	59	0.70	1.5	41	0.004	334
balsam fir H	39	5.03	0.2	61	0.013	79
Sugar maple, Hermine - Saint-Hippolyte						
maple fresh litter	15	0.50	2.0	85	0.007	150
maple L	43	6.02	0.2	57	0.007	143
maple F	33	5.30	0.2	67	0.001	688
maple H	77	1.02	1.2	23	0.015	86
Roots	43	0.46	2.5	57	0.029	34
Sugar maple and american beech, Mont Saint-Hilaire						
maple / beech L	22	6.30	0.2	78	0.030	33
maple / beech F	52	0.49	2.4	48	0.194	6
maple / beech H	73	0.49	2.1	27	0.038	27

Figure 6.1. Temporal evolution of the total dissolved nitrogen (TDN) remaining (%) in the solution during the 28 days incubation for the Douglas fir site. Each point is a mean of three replicate samples.

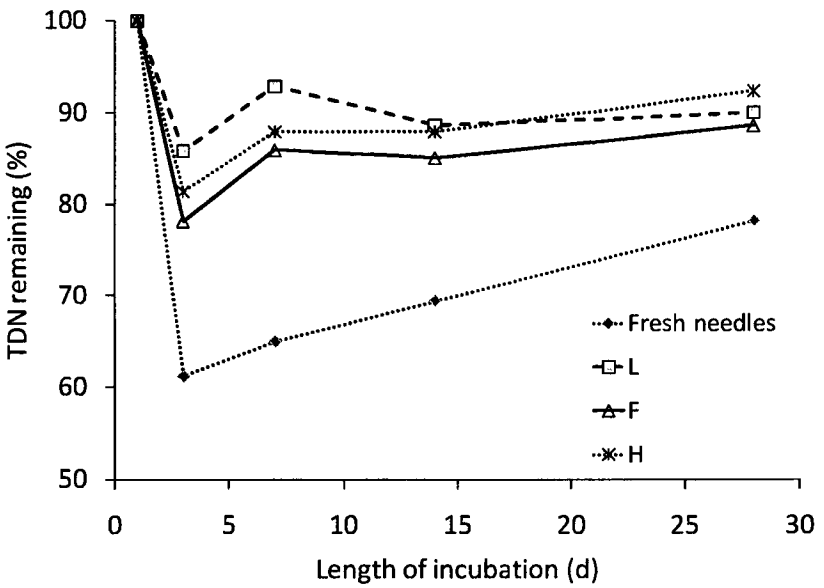


Figure 6.2. Changes in the dissolved inorganic nitrogen (DIN) remaining (%) in the solution during the 28 day incubation for the white pine site. Each point is the mean of triplicate samples.

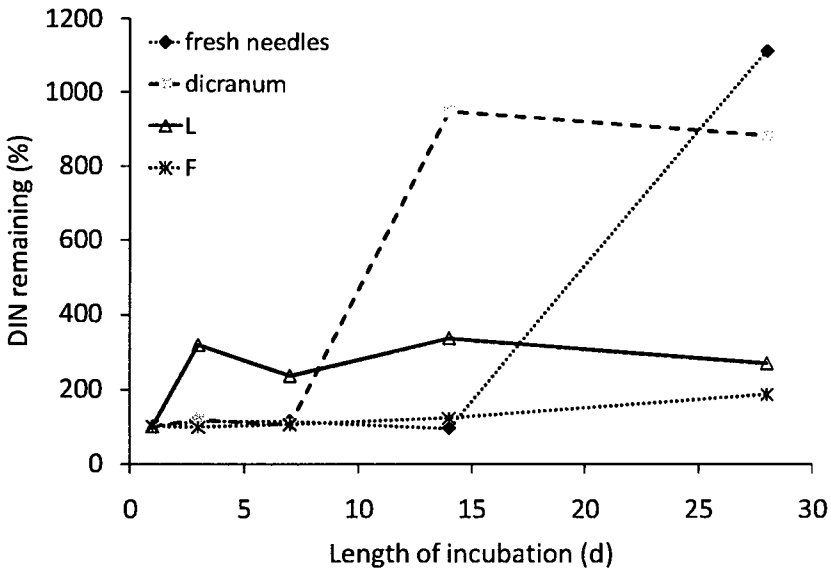


Figure 6.3. Changes in the dissolved organic nitrogen (DON) remaining (%) in the solution during the 28 day incubation for the black spruce site. Each point is the mean of triplicate samples.

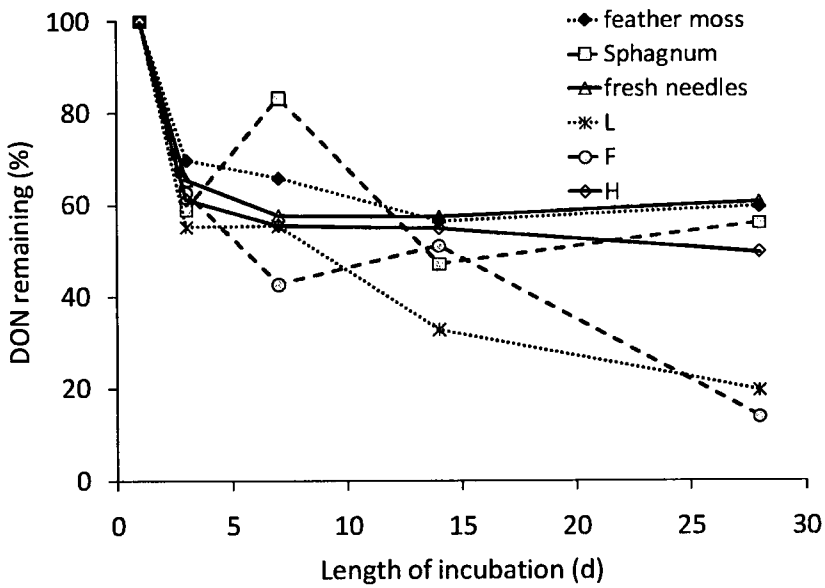


Figure 6.4. Scatterplot between the concentrations of dissolved organic carbon (DOC) and dissolved organic nitrogen (DON) from the water extracts (initial) and the solution at the end of the incubation (final).

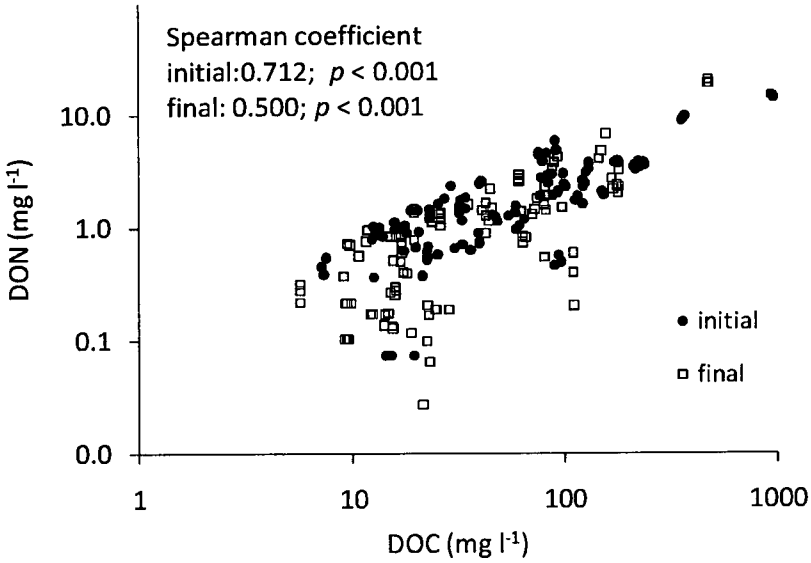


Figure 6.5.Comparison between the proportions of dissolved organic carbon (DOC) and dissolved organic nitrogen (DON) loss during the first 14 days of the incubation.

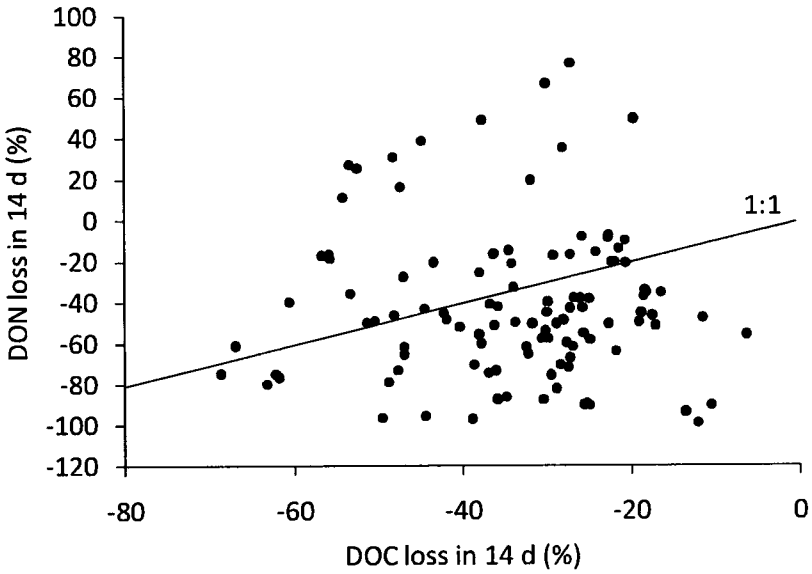


Figure 6.6. Scatterplot between the concentrations of dissolved organic carbon (DOC) and total dissolved nitrogen (TDN) in the water extracts at the start (initial) and end of the incubation (final).

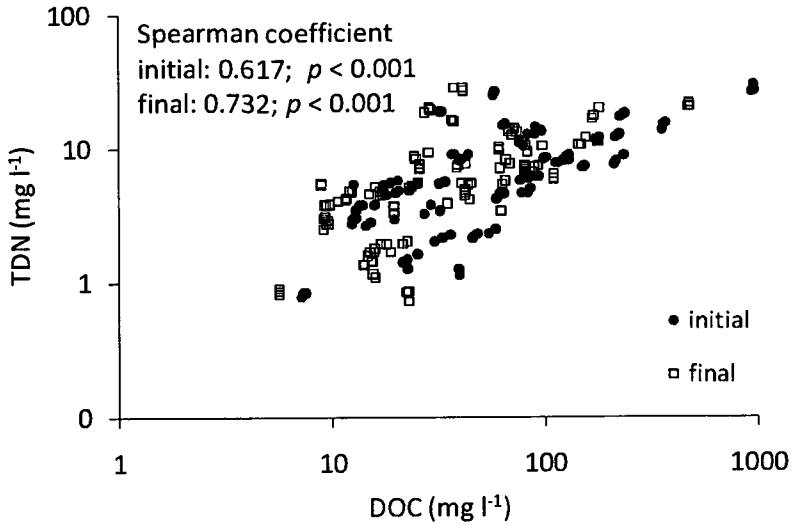


Figure 6.7. Relationship between the proportions of dissolved organic carbon (DOC) and total dissolved nitrogen (TDN) lost during the first 14 days of the incubation.

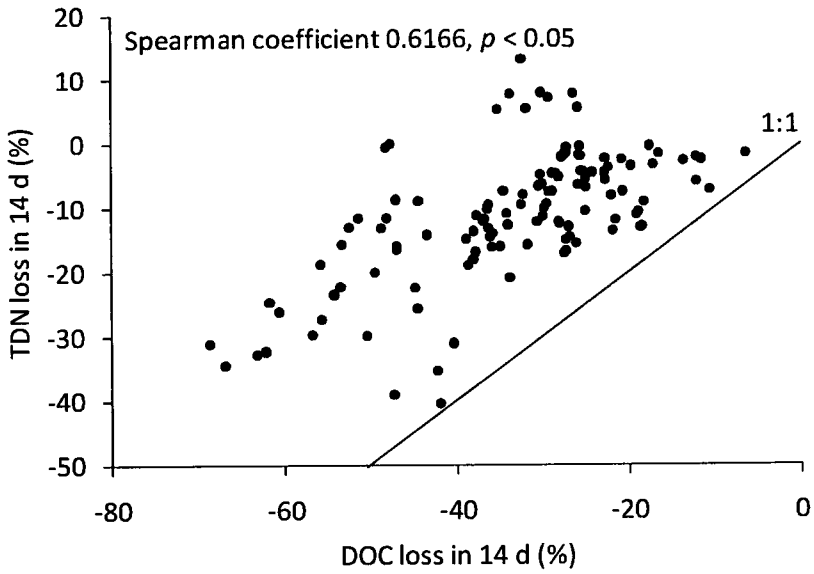


Figure 6.8. Scatterplot between the initial DOC:TDN ratio of the water-extracts and DOC:TDN quotient of the incubated solution on day 28 (final).

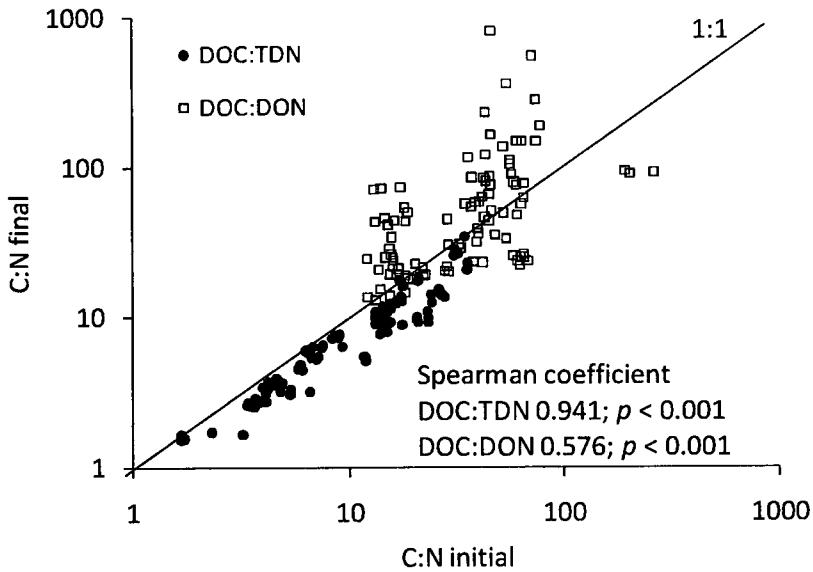


Figure 6.9. Percentage of DOC or TDN lost during the incubation as a function of the DOC:TDN ratio of the water extracts at the start of the incubation.

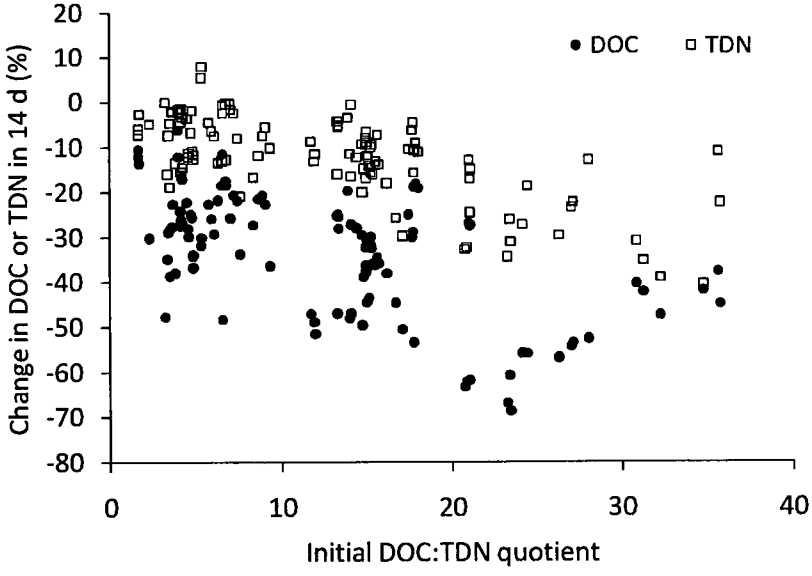
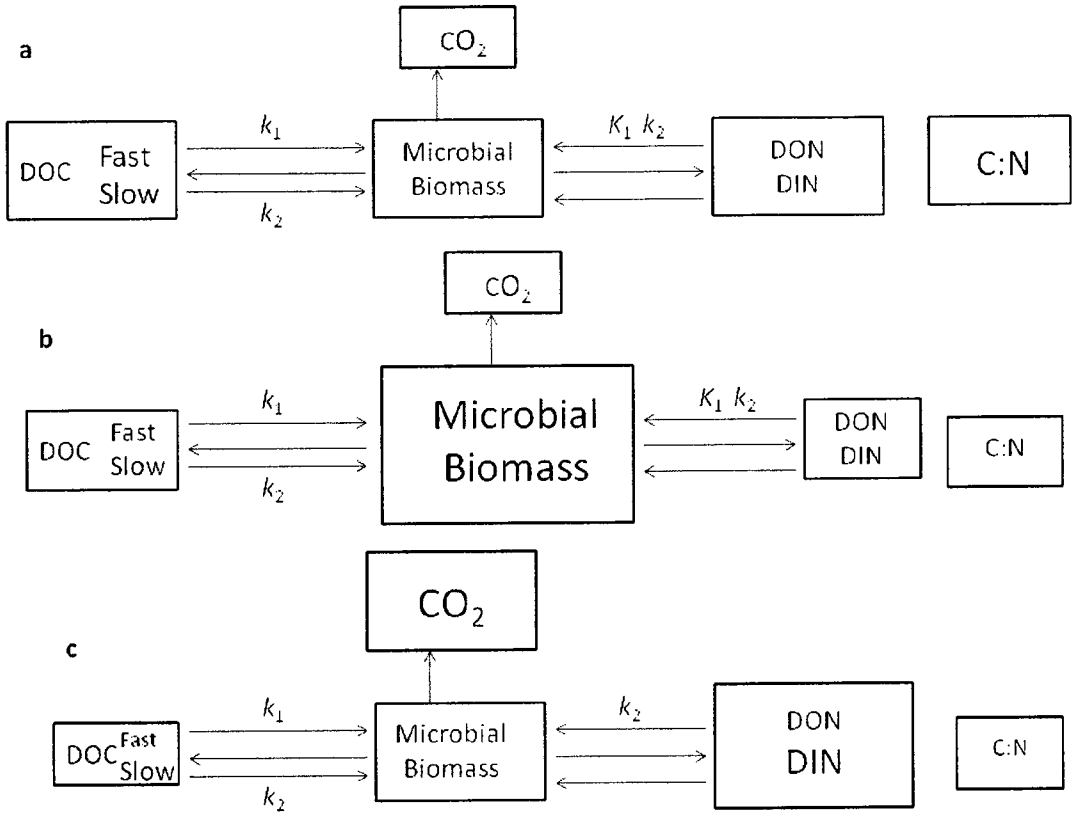


Figure 6.10. Hypothesized changes in the size of DOC and DON/DIN pools during deceomposition of organic matter in soil (indicated by the size of the boxes and font size). a) start of the decomposition: a relatively large DOC pool with fast and slow pools, a small microbial biomass and small amount of CO_2 production and a relatively large pool of DON and DIN, with high C:N quotient b) Early stage of decomposition (~ day 3), relatively small DOC pool with fast and slow pools, a larger microbial biomass which has grown and a small amount of CO_2 production and smaller amount of DOC and DIN, and a decrease of C:N quotient c) later stages of incubation (~ day 28): smaller DOC pool with fast (which has decrease in size) and slow pools, a smaller microbial biomass by larger amount of CO_2 production, smaller DON pool and larger DIN pool. Adapted from Gregorich et al. (2000).



Chapter 7 – Summary and Conclusions

Even though past research demonstrates the overall importance of DOC in biotic processes such as plant and microbial uptake, chemical processes such as pedogenesis and weathering, as well as soil and water acidification, knowledge of the dynamics of DOC in forest soils is still incomplete. After several decades of research, studies still debate about the major source of production of DOM (fresh material *versus* humified material), whereas the understanding of dissolved N and P lags behind that of DOC.

In chapter 3, I showed that the production of DOC and CO_2-C decreased as the degree of OM decomposition increased within 42 samples from forest floors across Canada. Fresh material produced statistically more DOC and CO_2-C than the L > F > H horizons. The coniferous and deciduous samples did not show a statistical difference in their rate of production, but the live tissues, such as *Sphagnum* and *Dicranum* mosses, lichen and roots, produced significantly larger amounts of DOC than the other organic matter samples, contributing large amount of DOC to the entire profile. The relationships between the initial C content or C:N quotient of the initial material are not sufficiently strong to predict the amount of DOC and CO_2-C produced in spite of significant regressions. Gross DOC production was difficult, if not impossible, to calculate from DOC and CO_2-C production, because of microbial biomass C similarity between pre- and post-incubation samples.

In chapter 4, the production of TDP followed the degree of OM decomposition pathway, with fresh material producing more TDP than humified material, but the results are not as strong as observed for DOC. The production of TDN and its species were larger in the F horizon than the L and H horizons. The large production of TDN and species in the F horizon occurs in response to the decomposition mid-point and processes occurring during both the initial and late stage of decomposition. Fresh material release soluble compounds stimulating microbial biomass and causing mineralization and immobilization within the more humified material. Strong stoichiometric relationships were observed between the initial material, the dissolved phase, and the microbial biomass quotients, which indicates that the release of C, N and P are strongly interactive and depend on the initial material and microbial quotients.

In Chapter 5, my results showed that neither of the biodegradation of DOC parameters obtained from the double exponential decay model varied according to the degree of OM decomposition or stand type. A discrepancy was observed between the DOC disappearance and CO_2-C evolved at days 3 and 7, supporting the hypothesis that microbial biomass increases rapidly during the initial stage of the bioassay, consuming DOC for microbial storage, with a smaller amount of CO_2-C produced by microbial respiration. Following this initial stage, microbial biomass reaches equilibrium, resulting in a reduction in the difference between the DOC and CO_2-C produced due to reduced biomass storage. At this later stage, most of the DOC consumed is respired. SUVA measurements of the water-extracts are a good predictor of several of the DOC biodegradation parameters measured with the double-exponential decay model and the disappearance after 3 and 28 days.

In chapter 6, I showed that the biodegradation of DON also followed the double-exponential decay model, but no links were found between the parameters of biodegradable DOC and DON. Dissolved organic C and DON seem to be similarly refractory. In contrast to the literature, the behaviour of DON does not mimic DOC, but has a complex dynamic of its own that necessitates further research. Strong relationships were observed between the changes of DOC and TDN, suggesting a dynamic disappearance of DOC and retention of TDN. Stoichiometric relationships occur during assimilation and transformation of dissolved C and N as biomass needs both nutrients and substrates (present in a variety of C:N quotients of the leachate) to meet their required C:N quotient for growth, maintenance and reproduction. Dissolved N dynamics displayed an initial sharp decrease in the first few days of the incubation, followed by a general increase, observed for both DIN and TDN. These findings support the initial increase in microbial biomass observed with DOC results.

The first objective of the thesis is to determine the potential rates of production and biodegradation of dissolved C, N and P compounds. The second objective of the thesis is to compare production and biodegradation rates of the OM found across Canadian forests. These data, on both production and biodegradation are, one of the most significant contributions of this thesis: a database of 42 organic matter samples, collected across Canada, representing varying degree of organic matter decomposition and stand

type. It is the only collection of dissolved C, N and P production and biodegradation data, obtained from an identical method, allowing comparisons among samples by eliminating the differences between treatments and methods found in the literature. It highlights the importance of the degree of organic matter decomposition on the production of DOC, resolving one of the major debate of the scientific community. Under the same conditions, fresh organic matter produced more DOC than humified material. However, the absence of significant differences between stand type for both production and biodegradation of DOC emphasize the large variability between samples, which must be taken into account when one wants to generalize. Both production and biodegradation data suggest that, in field conditions, the DOC from the upper soils horizon (i.e. fresh material) contributes to the stimulation of the microbial activity within the humified horizon (i.e. H horizon), resulting in further decomposition and leaching. This being said, research needs to be done to verify if it can truly result in larger contribution from humified material because of the possible quasi-total consumption by micro-organisms of the DOC from the fresh horizons. The challenge with laboratory results is the transferability to the field processes. In the laboratory, many variables are controlled (i.e. temperature) and other variables are excluded (i.e. plants, roots, throughfall, DOC cascading between one horizon to another). Here, my results can be used to better understand DOC production and biodegradation processes and create new hypotheses, but field studies are needed to get an even better understanding of soil DOC cycling.

The third objective of my thesis was to determine various predictors of production and biodegradation of the dissolved component. In chapters 3, 4 and 5, previously established relationships were both confirmed and contradicted. Production and biodegradation is the result of processes controlled by a multitude of variables, but some simple relationships were found, which allows an easy evaluation of production and biodegradation of dissolved C, N and P. On the one hand, SUVA (specific UV absorbance) was found to be a very good predictor of some calculated parameters of biodegradability of DOC. This specific result has the potential to save substantial resources (i.e. money and time) in estimating the potential biodegradability, using a fast measurement of SUVA absorbance rather than a bioassay, lasting several days to months. This estimation can help evaluate what proportion of the DOC will be mineralized as it is

transported through the soil and contributes to the CO_2-C emission to the atmosphere. On the other hand, the C, N and P content and microbial biomass C and N of the initial material were not strong predictors of production of dissolved C, N and P, in spite their significant regressions. Even though not perfect, these relationships could help estimate a possible range of production and biodegradation parameters without long bioassays. Other organic matter quality indices (i.e. ^{13}C-NMR, proximate analysis, etc) may provide better relationships with C, N and P release, however, the right C compounds need to be identified, as previous studies were deceptive in the predictive value of such analyses.

The thesis contributes to the knowledge by tracing, for the first time, the stoichiometric relationships in soils and the dissolved and microbial phases, as masses and as quotients (objective 4). I showed in chapters 4 and 6 the applicability of stoichiometric relationships for the estimation of the production and biodegradation of dissolved C, N and P. Nutrients and substrate are continuously interacting, resulting in variable production and biodegradation of dissolved components. Because these relationships were stronger than the ones observed with single elements (previous paragraph), future research should always consider the multi-element interaction controlling the production / biodegradation of the soil dissolved phase. These stoichiometric relationships can help understand variability across sites, time and substrate.

To better understand dissolved compounds dynamics in soils, future research could include a laboratory incubation involving cascading of samples between forest floor horizons (i.e. L, F and H), with simultaneous measurement of DOC and CO_2-C. This experiment would allow for the measurement of the priming effect amplitude on those production rates, a step closer to field conditions. For a better prediction of production rates, measurements of C quality (i.e. proximate analysis, ^{13}C-NMR) could help the understanding of the factors that control DOC and CO_2-C production. But as previously mentioned, these methods have been used, and were not always successful. We need research to identify specifically which fraction / compound of C contributes to the DOC production. A multidisciplinary collaboration between field, laboratory and model results would aid in the understanding of soil cycling dynamics and create database and tools for a better prediction. Existing models as well as new models should consider the

stoichiometry between C and N. Further investigation and research are needed if one wants to specifically quantify gross DOC production. Experiments looking at microbial biomass dynamics and characterization could help quantify which proportion of dissolved element cycling is specifically controlled by biotic processes. In this thesis, the contribution of fine roots decomposition to the dissolved pool of elements was shown to be quite significant. Further understanding of the role of root exudates and release during decomposition are needed.

Given the usual focus on CO_2 as major factor in climate change, the importance of DOC in the global C cycle needs to be thoroughly considered as I have shown its substantial contribution to CO_2-C emission. Furthermore, the results of my study demonstrated the importance of site specific characteristics (i.e. vegetation type, degree of organic matter decomposition, sample quality, etc.) in the amount of dissolved C, N and P production and biodegradation. The production data paired with biodegradation implies an even larger role of DOC within the global C cycle than previously thought, as it could stimulate decomposition and C release through a priming effect during the cascading of leachates between horizons. Recent studies also showed the overall importance of DOC as major control on CO_2-C production (Bengtson and Bengtsson, 2008).

The contemporary concerns regarding climate change, affecting both temperature and precipitation, are believed to have the potential to change the cycling of dissolved elements in soils. Both the rates of production and biodegradation of dissolved C, N and P may be greatly affected under changing climate and possibly exacerbate the predicted consequences, such as CO_2 emissions. The sensitivity of DOC production to temperature have been studied, and suggested that decomposition was temperature sensitive whereas other factors might have a larger control on DOC production. A possible change in the patterns and amount of precipitation could greatly influence the total release of DOC from soils. The contribution of my thesis, with a large database and novel stoichiometric relationships helps our understanding of the complex dynamics of dissolved C, N and P. The degree of organic matter decomposition and the quotients of the initial material are, as per this thesis, the best predictors of dissolved compounds production. Future research needs to be done to evaluate the role of other factors, but fresh litter / plants components of the ecosystem should definitely be the focus. Furthermore, the results I presented in

this thesis, particularly the stoichiometry and biodegradation data, allowed hypothesizing on the limitation of decomposition / mineralization: Are biotic processes limited by nutrient quantity (i.e. N or P) or by the bioavailability / biodegradability of the substrate (i.e. BDOC)?

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