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# SOLUBLE ORGANIC MATTER, ITS BIODEGRADATION, DYNAMICS AND ABIOTIC PRODUCTION

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By:

Ehsan Razavy Toosi

University of Canterbury

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*O Holy spirit! Thy blessing the guide of my path, make;*

*For, to my goal, long is the path; new to journeying am I*

*(Hafez)*

## Abstract

Soluble organic matter represent less than 1% of total soil organic matter (SOM) - but it contributes to many terrestrial ecosystem processes, due to its high mobility and reactivity in soil. Although it has been suggested that soluble organic matter (OM) may serve as an early indicator of soil quality changes as a result of shifts in land-use and management practices, only a few studies have addressed the dynamics of soluble OM in relation to land-use and specifically soil depth.

This study focuses on two aspects of soluble OM. In the first part, I hypothesized that extractable OM obtained by aqueous solutions is a continuum of substances that depending on the extraction method can be separated into two operationally different fractions. The size and properties of these fractions may consistently differ among different land uses and at different soil depths. The objective of this part of the study was then to assess dynamics (size and properties, biodegradability and seasonality) of water extractable organic matter (WEOM) and salt extractable organic matter (SEOM) in a sequence of human dominated land-uses at topsoil and subsoil.

At the second part of the study, I tested the regulatory gate hypothesis –abiotic solubilization of OM- as a primary controlling factor in soluble OM production. The objective of this study was to evaluate the impact of the microbial activity on the net production of dissolved organic matter (DOM) from the native SOM in the presence of added DOM and plant residue.

For the first part of the experiment, the soil samples were collected from four land-uses under bog pine (*Halocarpus bidwillii*) woodland, tussock grassland (*Festuca novae-zelandiae* and *Heiracium pilosella*), cropland (*Medicago sativa*) and plantation forest (*Pinus nigra*). The selected land uses were located in the Mackenzie Basin, Canterbury, New Zealand and occurring on the same soils, topography and experienced similar climates. Soil samples were obtained from topsoil (0-20 cm) and subsoil (60-80 cm) at the end of each season (November, February, May and August) during 2007-2008. The sampled soils were adjusted to the same water status prior to extraction. While WEOM was obtained during a mild extraction procedure and using 0.01 M

CaCl<sub>2</sub>, SEOM was extracted with 0.5M K<sub>2</sub>SO<sub>4</sub> at high temperature (75°C for 90 min). Both extracts were filtered through a 0.45 µm filter size.

In the first part of the study, I assessed the biodegradation dynamics of WEOM and SEOM (spring samples), using a double-exponential decay model. The WEOM and SEOM were inoculated and incubated at 22°C for 90d under aerobic conditions. Subsamples were removed on days 1, 3, 7, 12, 16, 30, 42, 60, 75, and 90, filtered (0.22 µm), and analyzed for organic C and N content, UV absorption, and <sup>13</sup>C natural abundance (δ<sup>13</sup>C).

The results of the biodegradation experiment indicated a similar pattern for both C and N of SEOM and WEOM as that of previously shown for soil DOM. However, C and N mineralization rate were considerably larger in the WEOM than SEOM. The parameters of the double-exponential model suggested that regardless of the land-use and soil depth, both the WEOM and SEOM can be modeled in two biological pools, with a largely similar “fast decomposable” but different “slowly decomposable” pools. However, since the extraction was not sequentially followed, a very small portion of the SEOM was comprised of the WEOM and given the greater observed biodegradability of the WEOM, the overall biodegradable portion of the SEOM would be lower than the observed. Despite a greater biodegradability of the organic N than C of both WEOM and SEOM; mainly due to a longer HL of the slowly biodegradable pool of C; the C/N ratio of the samples did not change very much during the biodegradation. This led us to conclude that the biodegradation of soluble OM may occur as a function of N availability.

Parallel to C and N loss, a considerable increase in SUVA<sub>254</sub> of SEOM, and particularly WEOM occurred during the incubation period. The greater increase in the proportion of aromatic compounds (assessed by SUVA) in the WEOM than SEOM, implied consumption of simple compounds (*vs.* very humified) during decomposition and further supported the observed faster biodegradation rate of the WEOM. The data indicated a relatively strong correlation (R<sup>2</sup>=0.66 and 0.74 for the WEOM and SEOM, respectively) between the amount of biodegraded C and the increase in SUVA<sub>254</sub>. This

suggested that SUVA<sub>254</sub> can be used as a simple, low-cost but reliable approach for describing the biodegradability of soluble OM, as previously suggested by others.

At the end of the bioassay, the <sup>13</sup>C natural abundance of the WEOM was significantly depleted, and showed a clear relationship with the proportion of the biodegraded C. This confirmed the previously suggested preferential biodegradation of simple organic constituents (<sup>13</sup>C enriched), resulting in the accumulation of more depleted <sup>13</sup>C compounds (often recalcitrant compounds). Moreover, the results of the δ<sup>13</sup>C technique revealed that the relatively greater <sup>13</sup>C enrichment of the WEOM obtained from subsoil, seems to be due to the presence of root exudates (often highly <sup>13</sup>C enriched). In contrast, a proportionally greater <sup>13</sup>C depletion observed in the SEOM particularly at subsoil samples, suggests that there is a close relationship between the SEOM and the typically <sup>13</sup>C depleted humified SOM.

The results of the biodegradation model (half-life of both C and N), in addition to dynamics of SUVA<sub>254</sub> and δ<sup>13</sup>C of the WEOM and SEOM were very comparable between top and subsoil samples. This implied that the potential biodegradability of soluble OM under laboratory conditions does not necessarily reflect the reported lower in situ biodegradability at soil depth, in agreement with recent evidence suggested by others. Instead, this may be largely due to the lack of optimum conditions (oxygen, nutrients, and moisture) for the decomposer community at soil depth.

Although there was a tendency for a generally greater biodegradability of the samples from the soils under the crop land (both WEOC and SEOC), along with relatively greater increase in SUVA, there was not a consistent trend of the effect of land use on the biodegradation of either WEOM or SEOM. The lower C/N ratio of the soils under the crop land seemed to be related with the observed proportionally greater biodegradability of these soils.

During the second part of the study, I assessed seasonal variations of the size and properties of the previously defined WEOM and SEOM, collected from top-and subsoil from the land-uses. I observed that 10-year after conversion of the degraded tussock grassland to cropland or plantation, the total C stock of topsoil (0-20 cm) when above- and below-ground plant biomass is excluded; has remained unchanged. This was attributed to the limited biomass production of the region, more likely as a result of low

productivity of the soil, but also harsh climatic conditions. Not only soil depth, but land-use affected both C concentration and C/N ratio of soil organic matter (SOM), with the greatest C concentration of soils under grassland and plantation in topsoil and subsoil, respectively. Despite the WEOM, the size of SEOM was largely unaffected by land-use and soil depth; instead, the properties of SEOM was more consistent with the effect of soil depth. Given the observed large temporal and spatial variability of the WEOM, the study suggests that the SEOM more consistently reflects the influence of land use and soil depth. No consistent effect of seasonality was observed in terms of size or properties of the SOM and the WEOM and SEOM. Overall comparison of the size and properties of the WEOM and SEOM indicated that OM extraction efficiency may vary largely, depending on extraction conditions. Using more concentrated salt solutions consistently yielded greater amount of OM (N, and especially C) release from soil with properties resembling more those of total soil OM (more humified) compared to the WEOM. The SEOM was also less variable by time and space.

The last part of the study was aimed to assess biotic *vs.* non-biotic solubilization of OM in the presence of added plant residue. Given the need to recognize the source of the solubilized OM during the experiment, I used enriched ( $^{13}\text{C}$ ) plant residue as the source of fresh OM. The above-ground part of ryegrass was added to soil either as plant residue or residue extract (extracted with  $\text{CaCl}_2$  followed by  $0.45\mu\text{m}$  filtration) -termed DOM. These two forms of added OM (residue/DOM) were conceived to represent two levels of bioavailability for the decomposer community for further assessing possible biotic solubilization of OM. Two soils similar in their OM content and other properties, but different in mineralogy were selected for the experiment. Soils were incubated for 90d under sterilized *vs.* non-sterile conditions and leached regularly with a dilute aqueous solution ( $0.05\text{M}$   $\text{CaCl}_2$ ). Plant residue was added to soil (1:100, residue: soil, w/w) prior to the start of the incubation, but DOM was frequently applied to the soils along with each leaching experiment.

The greater C and N concentration in the leachates of both sterilized residue-amended and DOM-amended soils compared to that of living soils, indicated a high microbial activity, as determined by  $\text{CO}_2$  loss, in the living soils. However, the proportion of the solubilized C (determined by  $^{13}\text{C}$ ) from sterilized soils was largely

comparable to that of living soils. This supports the recently suggested “regulatory gate” hypothesis, stating that solubilisation of OM largely occurs independent of the size or community structure of microorganisms. In addition, I observed that even with the presence of adequate amount of added fresh OM (ryegrass residue), about 70% of the solubilized C consistently originated from the humified soil OM, highlighting the role of native soil OM as the source of soluble OM in soil. In addition, in the DOM-amended soils, there was strong evidence, indicating that in the sterilized soils, the added DOM was exchanged with the humified soil OM as observed by an increase in SUVA, and humification index (HI) of the leached OM. Although the results of the study did not show a considerable difference in the solubilisation rate of added OM as a function of biological activity (either in the residue- or DOM-amended soils), there was clear evidence that the presence of microbial activity has resulted in further decomposition of the solubilised OM through biological transformations.

Together, the results suggested that the proposed fractionation method can be used to separate two operationally defined pools of soluble OM with consistent differences in their size (C and N), properties ( $\delta^{13}\text{C}$ ,  $\text{SUVA}_{254}$ , and C/N ratio) and biodegradability across the land-uses and soil depth. The second part of the study supported the primary role of abiotic factors on the production of soluble OM from native soil OM. Although the abiotic mechanisms involved in the solubilization remain to be addressed by future studies. Cons and pros of the methods with some suggestions for further works have been mentioned in the last chapter.



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## List of acronyms

ANOVA	Analysis of variance
CP	Cross polarization
Da	Dalton
DOC	Dissolved organic Carbon
DOM	Dissolved organic matter
DON	Dissolved organic Nitrogen
DOP	Dissolved organic Phosphorus
EC	Electrical conductivity
EOM	Extractable organic matter
eq	Equivalent
g	gram
HPLC	High performance Liquid chromatography
HI	Humification index
Ka	Acid dissociation constant
M	Mole
n	Number
NMR	Nuclear magnetic resonance
OM	Organic matter
p	-log
PCR	Polymerase chain reaction
pH	-log H <sup>+</sup>
PLFA	Phospholipid fatty Acids
R <sup>2</sup>	Coefficient of determination
SD	Standard deviation
SE	Standard error
SEOM	Salt extractable organic matter
SOM	Soil organic matter
SUVA <sub>254</sub>	Specific ultraviolet absorption at 254 nm
WEOM	water extractable organic matter

## Chapter One

# INTRODUCTION

### 1.1. DOM/WEOM in soil, importance and functions

Dissolved organic matter (DOM) and water extractable organic matter (WEOM) comprise a fraction of soil organic matter (SOM) which is present in the soil solution and is considered as the soluble form ( $<0.45\mu\text{m}$ ) of organic matter. Although in many studies of soluble OM, the terms DOM and WEOM have been used interchangeably; recent studies suggest that these forms may each represent different pools of soluble OM (Burton et al., 2007; Ros et al., 2009) which can be characterized by their solubility, mobility and lability (Zsolnay., 1996; McDowell et al., 2006). DOM/WEOM comprises only a small portion of the total SOM but it contributes to many important processes in soil (Kalbitz and Kaizer, 2003). Apart from its role in soil genesis, specifically at soil depth (e.g. Buscot, 2005), DOM/WEOM acts as a substrate for the soil microbial community (Zsolnay, 1996), the engine of the soil system. From an environmental point of view, it controls the mobility and bioavailability of heavy metals (e.g. Fotovat and Naidu, 1998) and is involved in binding and co-transportation of a variety of organic pollutants (e.g. Muller et al., 2007). DOM/WEOM also contributes to  $\text{CO}_2$  efflux from the soil (Glatzel et al, 2003) and denitrification (McCarty and Bremner, 1993), both significant processes in global warming issue. Recently, Haynes (2000) suggested that this fraction may be utilized as a potential indicator for soil quality assessment.

The small amount of DOM/WEOM in the soil along with its high biodegradability and reactivity to the total pool of OM in soil make it difficult for hydrologists, soil and environmental scientists and ecologists to obtain a genuine and representative pool of the soluble OM. While DOM is more often obtained *in situ* using suction cups, or zero-tension lysimeters, WEOM is obtained by extraction procedures mainly using different solutions (Herbert and Bertsch, 1995). Due to the difficulties associated with the maintenance of suction cups or lysimeters in managed lands, WEOM is the preferred

form of soluble OM studied in agricultural soils (Chantigny, 2003). Different procedures that have been practiced for obtaining DOM/EOM (extractable organic matter), make it difficult to compare the data reported for DOM and WEOM in the literature. Thus, developing standardized extraction methods would help to reduce the uncertainty when comparing results from different studies.

## **1.2. Aspects of DOM/WEOM studies in soil and ecosystem**

The main focus on DOM/WEOM in soil related literature has been on its production, its dynamics in terrestrial ecosystems and its fate. Despite it now being well established that the above- and below-ground plant litter, microbial activities, and decomposition of the added/residual OM are the sources of DOM/WEOM in soil (Kalbitz et al., 2000b), very little is known about the kinetics of the release of DOM from these sources and how it is affected by biotic and abiotic interactions. For example, Kemmitt et al (2008) recently challenged the commonly accepted Winogradsky's theory (Winogradsky 1924) by presenting their "regulatory gate hypothesis" which indicates the unseen role of abiotic factors in the control of solubilization of OM as a source of soluble OM.

Due to its highly mobile nature, DOM/WEOM can easily move both vertically and horizontally through the soil profile. This process is, however, limited by its physical immobilization in soil as a result of its sorption onto mineral particles, mainly clay, or from being trapped within stable aggregates. These processes have been suggested to delay the biodegradation of soluble OM up to a few decades (Guggenberger and Kaiser, 2003). Nevertheless, microbial consumption, known as biodegradation is the final step through which DOM/WEOM is partly released as CO<sub>2</sub> and partly assimilated in to the microbial biomass to provide energy and nutrients needed for soil microbial communities. This complex procedure is carried out by a variety of microorganisms in different ecosystems and at different soil depths.

Edaphic conditions, biological activities, environmental factors, management practices and land use are the key parameters affecting DOM/WEOM in soil and the

combination of these factors determines the quality and quantity of DOM/WEOM. Land use and management are the key parameters controlling soil DOM properties (Kalbitz et al, 2000). While management practices have generally short duration impacts on DOM, long-term effects are more related to vegetation type and to the amount of above- and below-ground litter (Chantigny, 2003). Most of the research on DOM has been carried out on forest soils and fewer studies have considered the DOM properties in agricultural soils. Agricultural activities (ploughing, liming, fertilization, irrigation, crop rotation, etc) alter the amount and properties of DOM/WEOM (Chantigny, 2003). Thus, given the suggested key roles of DOM/WEOM in soil systems, the evaluation of the impact of human activities on DOM/WEOM properties under different agricultural management systems is of great interest in sustainable land management.

While the properties of DOM/WEOM in the topsoil is thought to affect the microbial dynamics and thus reflects the quality functions of soil, DOM fluxes and properties in subsoil can be of importance in C sequestration, de-nitrification, and co-transportation of pollutants. In addition to the changes in the quality and quantity of soil OM as a source of DOM, the biological processes and activities as the other main source of DOM are also affected by soil depth. Consequently, the properties of DOM/WEOM at soil depth are expected to be different with those of the topsoil. However, most related studies have addressed DOM/WEOM dynamics in the topsoil with less emphasis on the DOM/WEOM processes at depth in the soil profile. Therefore, the comparison of DOM/WEOM obtained from top- and subsoil may help to broaden our understanding of the links and dynamics of DOM/WEOM within the soil profile.

### **1.3. Outlines of the study**

In this dissertation, I have addressed some aspects of soluble OM that are related to its production, land use and soil depth, and potential decomposition (Fig. 1.1). As a result of my review of the literature (chapter 2) and evaluation of gaps in our knowledge, I became interested in developing a fractionation method for obtaining extractable organic matter (EOM). The lack of such a procedure has led to some contradictions when

comparing results reported in different studies. I tested the liability of the fractionation by i) a relatively long-term biodegradation assay and ii) seasonal changes of the obtained fractions of EOM. Given the importance of the impact of land use and soil depth on the properties of DOM/EOM, I evaluated the impact of land use and soil depth on the properties of each of the fractions of EOM. I collected the soil samples from a sequence of land uses and two soil depths (0-20 cm and 60-80 cm) within each land use. This helped me to assess how strongly the amount and properties of the fractions of EOM are affected by land use and soil depth.

I selected the land uses based upon the need to ensure the similar climatic conditions and edaphic properties. The land uses, located in the Mackenzie Basin, Canterbury, New Zealand, were comprised of plantation forest (*Pinus nigra*), cropland (*Medicago sativa*), degraded rangeland (*Festuca novaezelandia* and *Heiracium pilosella*) and bog pine woodland (*Halocarpus bidwilli*). Soil samples were collected at different seasons during one year from topsoil (0-20 cm) and subsoil (60-80 cm). I defined a fractionation procedure to extract the water and salt extractable fractions of EOM. A biodegradation experiment (chapter 3 and 4) was then set up using the prepared solutions from each of the two fractions of the EOM obtained from soil sampled. Changes in DOC, DON, C/N ratio (Chapter 3), UV Abs., and  $^{13}\text{C}$  (Chapter 4) were monitored during a 90 day incubation period. Collecting data on changes in C and N during the biodegradation assay enabled me to develop a model to explain the biodegradation dynamics of the fractions of EOM and to test for the previously suggested slow and fast biodegradable pools of DOM/EOM (Qualls and Haynes, 1992; McDowell et al., 2006) as a function of land use and soil depth. The combination of results obtained from the chemical (Chapter 3) and spectroscopic/isotopic (Chapter 4) methods aid in interpreting the results and support the determination of the efficiency of the fractionation of EOM and the biodegradation dynamics of EOM.

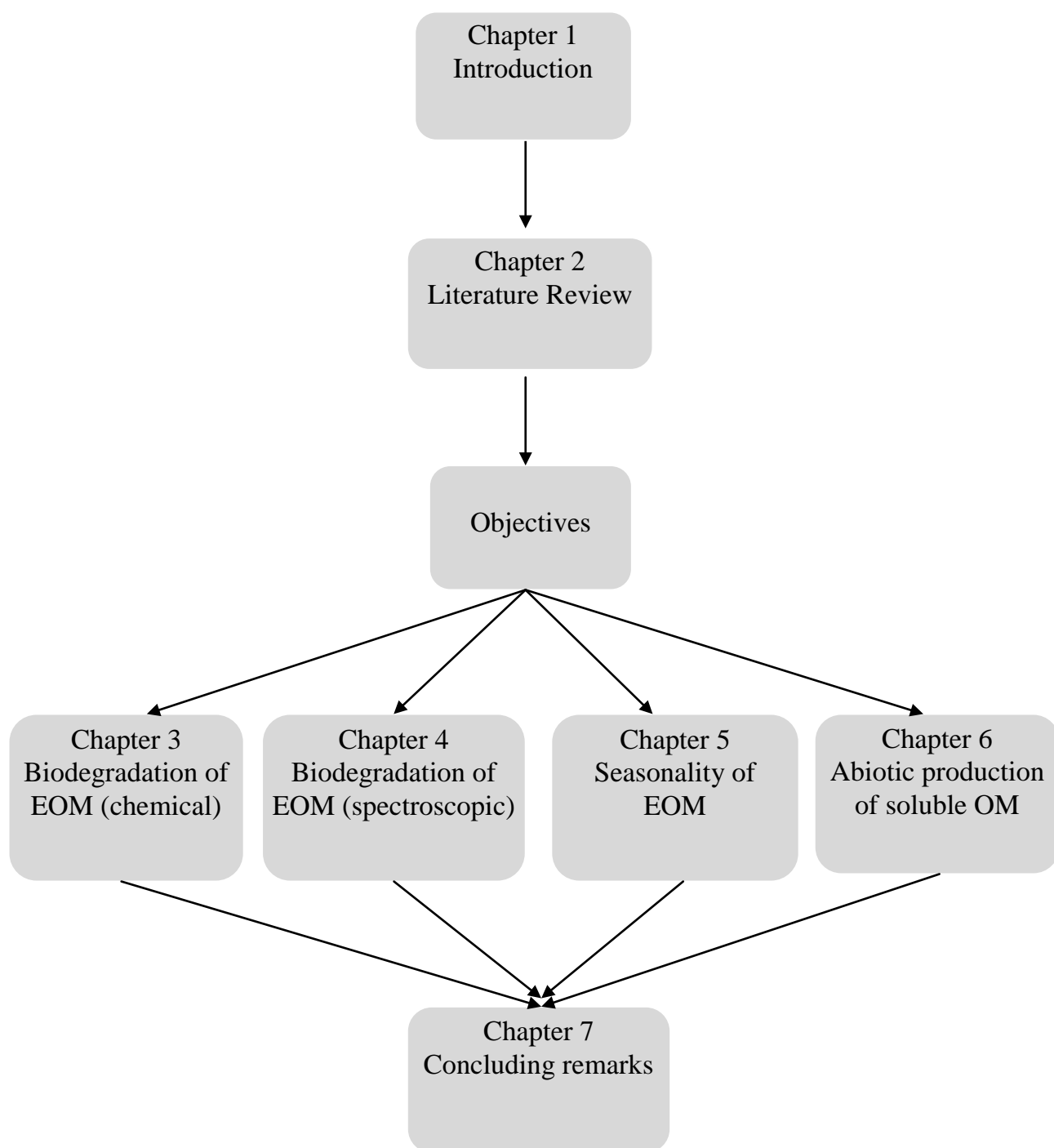
In the second part of the study (Chapter 5), I evaluated the seasonal variability of EOM. The findings of this chapter can support the reliability of the fractionation procedure that I developed. Given the high temporal variability of DOM/EOM (e.g. Don

and Schulze, 2008), the results of this chapter also extend our knowledge about the impact of landuse and soil depth on the properties of EOM over a reasonable time scale. I used the same fractionation procedure for obtaining the fractions of EOM from the soil samples collected during different seasons. DOC, DON and UV absorption were analysed in the water and salt extractable OM fractions.

In recent times, more emphasize has been given to the role of abiotic factors on the OM dynamics in soil (e.g. Kemmitt et al., 2008). In the third part of the study (Chapter 6), I investigated the impact of presence/absence of biological activity on the solubilization of OM (DOM production) from two freshly OM amended soils. Given the need for artificially  $^{13}\text{C}$  enriched OM, and highly experienced staff, this experiment was carried out at University of California, Davis. Two soils with different mineralogical properties were collected from south and central California.  $^{13}\text{C}$  OM (once) and  $^{13}\text{C}$  DOM (repeatedly) were added to the soils during a 90 day incubation assay. The leachate obtained from the sterilized and un-sterilized subsamples were collected and analysed for  $^{13}\text{C}$  DOC, DON, UV abs., and fluorescence of the obtained DOM.



**Figure. 1.1** Schematic diagram representing the structure of the thesis



## **Chapter Two**

# **LITERATURE REVIEW**

### **2.1. Definitions of soil organic matter and its soluble pools**

#### **2.1.1. Soil organic matter**

Soil Organic Matter (SOM) is a continuum; generally composed of the plant and microbial residues and their transformation products/by-products (Guggenberger, 2006). The presence of OM distinguishes soil from a mass of fine mineral particles and recognises its role as a complex living system (Schnitzer, 2004). In addition to the pedogenic functions, OM makes a significant contribution to different properties of soil (e.g. colour, water holding capacity, source of nutrients and elements, formation of complexes, etc.) (Morris, 2004). The OM content of soil is largely affected by the pedogenic factors and vegetation (Brady and Weil, 2007) and ranges from less than 1% to near to 100% in organic soils. A typical agricultural soil usually contains between 1-5% OM in the topsoil (Schnitzer, 2004).

The complexity of the soil system and the nature of the SOM make any attempts to categorize soil organic components “at best, imperfect” (Swift, 1996). Despite the heterogeneity and complexity of SOM, attempts have been made to improve our understanding of this continuum (SOM) by its conceptual separation into different pools. Different extraction procedures have been developed to distinguish the meaningful SOM pools that can be related to C and N dynamics and to management impacts in agricultural systems and at a global scale (Olk and Gregorich, 2006). In this regard, a large number of methods have been employed for separation of different components of SOM into entities that vary in terms of source, composition and turn-over (Guggenberger, 2006). Evidences suggest that the classical chemical fractionation of SOM according to its solubility characteristics in strong acid and base solutions (chemical extraction) is not useful in this respect (Stevenson and Elliott, 1989). More recently, researchers have tried to distinguish the meaningful fractions of OM that may reflect i) the impacts of different management

systems or ii) serve as the input components of OM models. (Wander, 2004). Although chemical characterization of SOM was dominant for long time, more recently physical fractionation (Golchin et al., 1994), analysis of the DOM obtained from soil solution (Guggenberger et al., 1994), biological characterization of SOM extracts (Gregorich et al., 2003), and evaluation of microbial biomass (Magdoff and Weil, 2004) have been suggested as potential promising approaches to OM fractionation. However, definitions based on physical fractionations are widely preferred since physical separation is thought to be related to the role that OM plays in soil structure and soil functions. Ideally such fractionation methods should partition SOM into components that vary in their turnover, chemistry, and origin (microbial vs. plant) (Kogel-Knabner et al., 2006)

### **2.1.2. Dissolved and extractable soil organic matter**

A small portion of the total soil OM is present in soluble form. Despite its low proportion, the soluble form of OM has been of increasing interest due to its solubility, dynamicity and lability in soil and water systems (Baldock, 2002, Hayes, 2005). Dissolved organic matter (DOM) is probably the most commonly used term for recognition of soil soluble OM in the literature. DOM has been defined as the OM dissolved in soil solution which passes a 0.45  $\mu\text{m}$  filter (Thurman, 1985). Because of difficulties in collecting soil DOM, water extractable OM (WEOM) has been used as a surrogate for soil solution DOM (Herbert and Bertsch, 1995). Consequently, some researchers have used these two terms interchangeably when refer to soluble OM. This has led to misinterpretation of results reported in the literature (Zsolnay, 2003, Ros et al., 2009). WEOM is defined as “the DOM obtained by extracting a given mass or volume of soil with an aqueous solution” (Zsolnay, 1996). This definition of WEOM can be improved as “the soluble OM (0.45 $\mu\text{m}$ ) that can be dissolved in and extracted with pure water or diluted salt solutions under a mild extraction procedure”. Water has been used as a common extractant agent of soluble OM. However, it should be noted that the properties of the OM obtained through the extraction procedure using “water” may vary widely. This could be due to the variability of the ionic strength of soil solution extracted

with “water” that leads to the release of OM not only from soil solution but also as a result of destruction of soil structure (Rennert et al., 2007). On the other hand, H<sub>2</sub>O solubilises hydrophobic compounds of OM that are not dissolved in the natural electrolyte concentration of soil solution (Rennert et al., 2007). Thus, the amount and properties of the extracted OM using water may substantially change. In an effort to standardize the chemical characteristics of “water”, low molarity, diluted salt solutions have been commonly used for the extraction of WEOM. 10 mM CaCl<sub>2</sub> seems to be the predominantly used solution for obtaining WEOM. Such a dilute salt solution is believed to resemble the natural electrolyte properties of the soil solution and thus may better reflect the properties of soil solution *in situ* (Zsolnay, 2003).

While DOM is typically collected by centrifugation of a field-moist soil in laboratory conditions or using suction cups or zero tension lysimeters *in situ* (Herbert and Bertsch, 1995), WEOM is obtained in laboratory conditions and its application is more common in cultivated land studies where soils are more disturbed due to management activities (Chantigny, 2003). The processes of obtaining WEOM can be considerably affected by experimental conditions including temperature, ionic strength, the ratio of soil to extractant, etc. (Herbert and Bertsch, 1995; Zsolnay, 1996). More recently, Ros et al., (2009) suggested that the term “extractable organic matter” (EOM) can be used for all forms of OM obtained in laboratory conditions. Regarding the considerable impact of methodology on the size and properties of fractions of OM in dissolved form (<0.45 µm), it has been suggested that the interchangeable use of DOM and extractable (aqueous or salt) OM should be avoided whenever possible (Ros et al, 2008; Zsolnay, 1996).

Some researchers (Zsolnay, 1996; Tipping, 1998) have tried to partition the total pool of DOM into mobile and immobile fractions based on the distribution of this substance within different soil aggregate classes. Using this concept, DOM may be separated into three components based on its association with micropores (<0.2 µm; DOM I), mesopores (0.2-0.6 µm; DOM II), and macropores (>0.6 µm; DOM III). WEOM was then suggested as comprising the DOM in mesopores and macropores (DOM II and

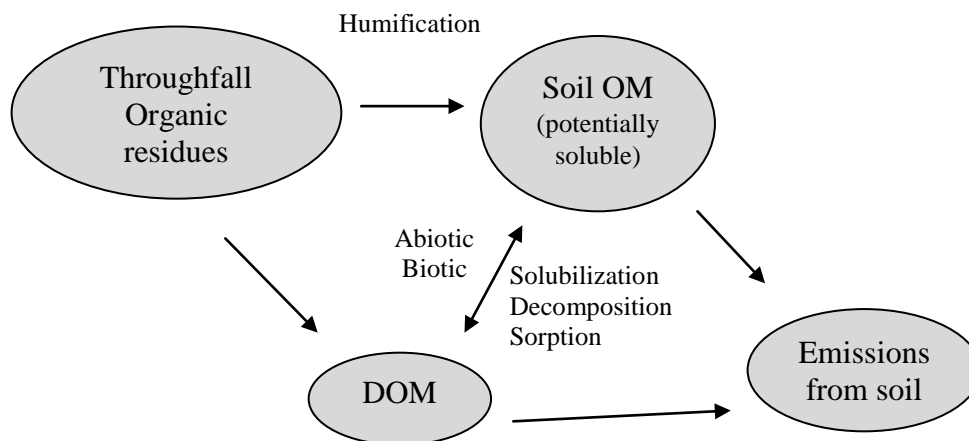
DOM III). This classification has not been widely adopted by soil scientists, possibly because of some ambiguities in the methodology used.

In the research described in this dissertation the terms DOM and WEOM will be used based on the nomenclature in the mentioned references. However, since it is not possible to define a common term for DOM and WEOM, the acronym DOM/WEOM will be used whenever I refer to soluble forms of OM. Regarding the extractants that I used for obtaining soluble OM in this research (diluted and concentrated salt solutions), I will use the term extractable organic matter (EOM) when referring the results obtained from my experiment.

## **2.2. Nature and properties of DOM/WEOM**

Regarding different sources and production processes of DOM/WEOM in soil, most of what has been termed DOM/WEOM in soil is a heterogeneous mixture of macromolecules, mainly humic substances and a variety of simple compounds. Thus, suggesting a general chemical definition for DOM/WEOM is problematic (Kalbitz, et al, 2000). At the upper boundary of its cutoff ( $0.45\mu\text{m}$ ), DOM/WEOM may exist in any colloidal or particulate organic materials with high molecular weight. A large portion of the DOM/WEOM in soil solution is present as humic substances with an average molecular weight (MW) of approximately 1000 Dalton (Da) (Thurman, 1985). At the smaller end of its size scale, DOM/WEOM consists of individual molecules such as amino sugars, amino acids, carbohydrates, monomeric acids, and simple aliphatic acids, phenols, phenolic acids (Stevenson, 1994; Baldock, 2002). DOM has been suggested as being composed of about 50% humic substances, particularly fulvic acids, 30% macromolecular hydrophilic acids, and about 20% identifiable organic compounds (carbohydrates, carboxylic acids, amino acids, and amino sugars) (Thurman, 1985; McDowell and Likens, 1988; Qualls and Haines, 1991). The variety of chemical compounds in DOM/WEOM reflects the various degradation stages of this fraction of OM depending on its the sources (Stevenson, 1994). Figure 2.1 shows a simplified model representing different organic sources of DOM that through biotic and

abiotic processes determine DOM dynamics in soil. Because of the complex nature of DOM/WEOM different characterization methods have been developed and employed by soil and water scientists to improve our understanding of DOM/WEOM properties.



**Figure 2.1.** A simplified model of the DOM interactions as a pool of soil OM. Organic forms including throughfall, above- and below-ground litter, root exudates and intact OM are the sources of DOM in soil. These sources control the amount and properties of DOM in soil through biotic and abiotic processes (e.g. solubilization, diffusion, desorption). Although adsorption to colloids surfaces is a sink of DOM in mid to long-term periods, biodegradation and release of C from DOM to the atmosphere is the final pathway of the DOM cycle in soil (modified from Kalbitz et al., 2000b).

## 2.2.1. DOM/WEOM characterization

### 2.2.1.1. Elemental characterization

#### 2.2.1.1.1. Carbon

DOM/WEOM can be characterized based on its constituent elements, mainly C and N. Since C is the structural and measurable component of all organics, in DOM/WEOM studies similar to all other organic compounds, the amount of C is determined and reported as the indicator of the total organic content after correction for C content factor (e.i. 1.72). DOC is the C component of DOM and an accurate quantification of DOM pools and fluxes. The concentration of dissolved organic Carbon (DOC) or water extractable organic Carbon (WEOC) in soil solution can vary greatly and

substantially depends on the soil-to-solution ratio and specific conditions under which the solutions are recovered (e.g. temperature, pH, etc.). However, it should be noted that soil type, landuse, management history, etc. have a large impact on the C content of the DOM/WEOM. The total amount of DOC/WEOM in field-moist soil usually ranges 2-30 mg C kg<sup>-1</sup> soil (Herbert and Bertsch, 1995) which represents between 0.05-0.4% and 0.25-2.0% of soil organic C in agricultural and forest soils, respectively (Haynes, 2005). Because of its structural role in organic compounds, carbon has been the center of focus in all DOM/WEOM studies. However, dissolved organic Nitrogen (DON), and more recently dissolved organic phosphorus (DOP) have been of interest for their environmental and ecological magnitude.

#### **2.2.1.1.2. Nitrogen**

Dissolved organic Nitrogen (DON) represents a significant pool of soluble N in most ecosystems (Christou et al, 2006). Despite its low proportion to the total N in soil (0.15-0.19% in arable and 0.15-0.61% in pasture lands) (Haynes, 2005), recent studies indicate that (DON) may play an important regulatory role in the soil–plant N cycle (Murphy et al., 2000; Neff et al., 2003; Chen and Xu, 2006). DON has been suggested to have an important role in N supply for plants in N-limited environments (Jones, et al, 2005). By analysing a relatively broad range of soils, Ghani et al., (2007) showed that there is a significant and relatively strong correlation ( $r^2=0.71$ ) between EON (extracted by 0.5 M K<sub>2</sub>SO<sub>4</sub>) and EOC in pastoral soils.

From the environmental point of view, organic N has been shown to be the dominant form of N in water bodies adjacent to forest watersheds. It has therefore, been suggested that terrestrial sourced DON may contribute to the pollution of waterways via leaching and run off (Sollins and McCorinson, 1981; Hedin et al., 1995). There has been increasing attention regarding the possible roles of DON in terrestrial ecosystems.

DON is likely to originate from microbial transformations of soil OM and accumulates in soil as microbial by-products (McDowell, 2003, Ogawa et al, 2001). It is composed of many compounds that enter the soil from a range of sources including dry

and wet depositions, throughfall, litterfall, root and microbial exudates, turnover products of roots and organisms, urine and feces, and organic fertilizer additions to soil (Kalbitz et al, 2000). Primary DON is composed of many individual components ranging from low molecular weight compounds such as amino acids, amino sugars, urea and purines to high molecular weight compounds such as proteins, chlorophyll and DNA (Antia et al., 1991). Vegetation, despite its great influence on the proportion of pool of DON, has been observed to be of less importance in terms of its contribution to the forms of amino acids and peptides in DON. Secondary DON can be produced as a result of abiotic synthesis of chemicals in soil, resulting in the production of a range of high molecular weight polyphenolic materials (Stevenson, 1994). Considering the major biological inputs into soil and their unknown chemical composition, some researchers (Jones et al., 2005; Christou, et al., 2006) have hypothesized that the dominant DON compounds entering soil are free and polymeric amino acids (proteins and peptides). In an attempt to characterize DON using chromatographic approaches, Khalid et al., (2007) showed that DON comprised of free amino acids accounted for only 3% of the total soil DON, with approximately 95% of this substance remaining chemically unidentified. Despite the growing information about the contribution of DON in soil ecosystem, few attempts have been made to identify DON dynamics, its components and how its size relates to other soil chemical, physical and biological factors. This has been partly related to the analytical limitations (e.g. lack of available techniques, very low concentrations, poor clean-up procedures) and difficulties in the isolation of DON constituents (Christou et al., 2006).

Plants have been shown to take up organic Nitrogen compounds as a source of Nitrogen although this is limited only to low molecular weight DON (e.g. urea, amino acids, polyamines, small polypeptides (DiTomaso et al., 1992, Yu et al., 2002; Jones et al., 2005). However, the major part of the soil's DON appears to be not directly bioavailable for plants (Jones et al., 2005).



### **2.2.1.1.3. Phosphorus**

In contrast to DOM and DON, few attempts have been made to address the functions and properties of dissolved organic phosphorus (DOP) in soil. However, it has been suggested that this is one of the priorities for better understanding of DOM/WEOM dynamics in soil (McDowell, 2003). Although P has been of environmental concern particularly as a result of the transportation of P-rich soil particles to water bodies through topsoil erosion, little is known about the soluble organic forms of P (DOP/WEOP) in soil and its possible functions. DOP has been suggested as largely prevalent in the hydrophilic fraction of DOM, resulting in its loss during temporal flushes from top-to subsoil (Qualls and Haines, 1991; Kaiser, 2001). In addition, P sorption can be easily influenced by the presence of C-rich compounds, resulting in the release of the adsorbed P compounds to the soil solution phase (Bhatti et al., 1998). In a batch DOP sorption-desorption experiments, Gjettermann et al., (2007) observed that DOP sorption decreases sharply with increase in soil pH from acidic to neutral. Therefore, application of alkaline amendments (e.g. gypsum) may result in the solubilization of P compounds specifically in the soils treated with P-rich organic amendments (e.g. manure and poultry litter). Overall, there seems to be potential for future DOP studies to focus on i) the downward DOP losses through the soil profile and, ii) the role of DOP in the contamination of water bodies.

### **2.2.2. Chromatographic characterization**

The chemical nature of DOM/WEOM has conventionally been characterized by “sorption chromatography” based on its adsorption to non-ionic and ion exchange resins that separate acids, bases and neutral substances with hydrophobic and hydrophilic groups (Leenheer, 1981; Aiken and Leenheer, 1993; Qualls et al, 1991). This technique was believed to fractionate DOM/WEOM based on properties that regulate DOM/WEOM interactions colloids surfaces in soil (Qualls and Haines, 1991).

Using hydrophobic and hydrophilic resins in the sorption chromatography approach, DOM/WEOM is typically regarded as being dominated by hydrophobic and hydrophilic

acid fractions including fatty acids, humic materials and polyphenoles (Herbert and Bertsch, 1995). The hydrophobic materials, primarily hydrophobic acids, are the main component of the DOM/WEOM, comprising 26-60% of its volume. Weak hydrophobic acids are mainly phenols and account for 10-20% of DOM/WEOM. Hydrophilic acids account for 20-30% and consist of humic and non-humic substances with low molecular weight compounds and a high content of carboxylic acids (Cook and Allan, 1992; Smolander et al, 2001; Souminen et al., 2003). Hydrophilic neutrals consist of free carbohydrates and alcohols and make up 3-20% of DOC/WEOM (Qualls et al, 1991; Smolander et al, 2001). Although the hydrophobic acid fraction comprises the major part of DOC, DON and specially DOP and DOS are concentrated predominantly in the hydrophilic acid fraction (Kaiser, 2001; Qualls, and Haines, 1991). Hydrophobic acids are composed of plant-derived compounds that may become water-soluble by oxidative biodegradation (Guggenberger and Zech, 1994). Hydrophilic acids are oxidation products of hydrophobic acids (Guggenberger and Zech, 1994) and may migrate more effectively in the soil profile, whereas hydrophobic acids are likely to be adsorbed in the humus layer (Thurman, 1985). The relative proportion of hydrophobic acids decreases with depth in the soil profile. This indicates that the hydrophilic fraction can be preferentially transported through the soil (Herbert and Bertsch, 1995).

Despite the long-term usage and commonly established sorption chromatography method for DOM characterization, the method is been less popular in recent studies. This appears to be due to the lack of relevance of the suggested hydrophobic/hydrophilic fractions in the possible functions of DOM in terrestrial ecosystems (personal communications, Greenfield, L; Sollins, P.). In contrast, DOM characterization through quantification of identifiable compounds may be better suited when the compounds are directly related to particular biogeochemical processes of interest. These compounds can act as molecular markers which aid in identifying specific soil microbial and other biochemical processes (Herbert and Bertsch, 1995).

### 2.2.3. Spectroscopic characterization

Although characterization of DOM/WEOM through the quantification of specific organic classes has been hampered because of the complexity of DOM/WEOM structure (Qualls and Haines, 1991), and the low concentrations of many of its constituents (Jones et al., 2005a), more focus has recently been paid to identifying and comparing DOM/WEOM compounds through the spectroscopic and chromatographic approaches. Rapid and simple optical measurements, namely UV absorption and fluorescence emission spectroscopy, have been used as indicators of DOM properties, mainly because of their strong association to DOM/WEOM biodegradability and its reactions with heavy metals (Her et al., 2003; Akagi et al., 2007; Hunt and Ohno, 2007). Comparing the hydrophobic/hydrophilic fractionation method with spectroscopic approaches, Kalbitz (2001) suggested that the traditional “sorption chromatography” is not sensitive enough to reflect the chemical composition of DOM in relation to the impact of landuse and depth. Instead, he suggested that UV and fluorescence spectroscopy appears to be more sensitive to detect small shifts in DOM composition.

Studies have demonstrated that aromatic Carbon content and the absorbance of UV light are important indicators of DOM reactivity in a number of environmental processes (Weishaar, et al., 2003; Choudhry, 1984). It is well known that the specific ultraviolet absorbance (SUVA) of DOM/WEOM measured in the vicinity of 250 nm (e.g., 254, 272, 280 nm) is correlated with the aromatic Carbon content of DOM (Chantigny et al., 2008). In addition, studies have shown a negative correlation between DOM/WEOM bioavailability and its content of aromatic structures measured by UV absorbance that has been attributed to their recalcitrance against degradation (Gilbert, 1988; Kalbitz et al., 2003b; Embacher et al 2007). Although UV absorption has been shown to be a good predictor of general chemical characteristics of DOM/WEOM, it does not provide information about reactivity of DOM/WEOM derived from different types of source materials (Weishaar, et al., 2003). UV absorption also does not reveal any direct information about and the chemical structure and properties of DOM/WEOM. However it seems that the simplicity of this method together with its reliability for certain purposes

(e.g. biodegradability experiments) are the main reasons behind its widespread application.

Fluorescence spectroscopy analysis has been suggested as a tool that can highlight important but subtle differences in DOM/WEOM properties (Corvasce et al., 2006). Along with UV absorption technique, researchers have used this method as a tool to estimate bioavailability of DOM/WEOM. This is based on the assumption that a decrease in bioavailability of DOM/WEOM usually occurs with an increase in the conjugation and/or condensation of aromatic structures (Zsolnay et al, 1999; Glatzel et al, 2003; Kalbitz et al, 2003a). Thus, this method has been used to characterize the bioavailability and aromaticity of DOM/WEOM from different land uses and at different depths (Corvasce et al, 2006, Kalbitz, 2001, Kalbitz, 2003b; Zsolnay, 2003). The fluorescence property of DOM/WEOM is represented as a humification index (HI). HI is considered to be associated with an increase in the C/H ratio of DOM, and with a resulting shift to higher fluorescence emission wavelengths (Stevenson, 1994; Zsolnay et al., 1999).

Nuclear magnetic resonance spectroscopy (NMR) is probably the most applicable spectroscopic method for the determination of the chemical structure of the DOM/WEOM. Combining pyrolysis field ionization (PFI) and NMR, Leinweber et al., (1995) and Landgraf et al., (2006) detected the presence of carbohydrates, phenols, lignin monomers, and N-containing compounds in WEOM obtained from arable and forest soils, respectively. Using  $^{13}\text{C}$  cross polarization mass spectroscopy (CP-MS) Novak and Bertsch (1991) suggested that WEOM has a greater proportion of O-Alkyl Carbon reflecting the presence of polysaccharides and aliphatic acids and lower portion of aromatic Carbon than fulvic and humic acid. This finding supports the idea that WEOM is mainly comprised of the non-humic compounds with a relatively short turn over time. In a similar study but using high temperature extraction, comparison of the NMR spectra of the SOM and WEOM showed that the existence of large alkyl Carbon peak in SOM spectra is related to the higher contents of lipids, cutins, and suberins in SOM, (Balaría et al., 2009). In contrast, the weak peaks of these compounds in WEOM NMR spectra was suggested to relate their low solubility in aqueous solutions even after extraction under

high temperature conditions. Furthermore, Balaria et al. (2009) showed that while the bulk SOM spectra represents high aromatic and greater lignin content, hot WEOM spectra are dominated by the O-alkyl peaks, indicating the abundance of carbohydrates in their structure.

By using pyrolysis mass techniques, Guggenberger and Zech (1994) and Huang et al., (1998) showed that in comparison with bulk SOM, DOM has a larger accumulation of more oxidized lignin and aromatic compounds. This implies that DOM could be the highly oxidized form/s of organic matter. They also suggested that polysaccharide compounds in DOM could be the modified form of polysaccharides in the bulk SOM but with higher furan structures. These results suggest that although DOM and WEOM are terms that are commonly used interchangeably in the literature, they appear to reflect different fractions of SOM with different proportion of constituent compounds.

#### **2.2.4. Other chemical based approaches**

Some attempts have been made to fractionate DOM/WEOM based on the molecular sizes or weights that are related to its properties. Using such fractionation procedures DOM/WEOM can be classified into components with different size classes generally as small (<10 kDa), medium (10-100 kDa) and large (>100 kDa) classes of constituents. Based on such a physico-chemical approach, the most abundant molecular size group can be classified as medium range size (10-100 kDa) (Suominen et al., 2003). On the other hand, Her et al (2003) demonstrated that the medium molecular weight (500-1000 gr/M) has the greatest aromaticity whilst the lower molecular weight is thought to be rich in protein-like and aliphatic compounds. The major drawback of the size/weight fractionation methods seems to be the lack of information that are related to the transformation pathways or chemical properties of DOM/WEOM (Souminen et al., 2003).

Molecular size distribution of DOM/WEOM has been suggested to be largely unaffected by plant species and depth (Smolander et al., 2001; Souminen et al., 2003). Since WEOM has a greater proportion of large molecular weight components compared

with DOM (Delprat et al., 1997), it seems that the extraction methodology (lysimeter/suction cups *vs.* water/salt solution extraction) affects the molecular weight distribution property. Boddy et al., (2007) suggested that regarding the quick turnover rate (up to 4000 times/yr) of the low molecular weight (LMW) components of DOM (e.g. amino sugars, carbohydrates, amino acids), DOM may play a substantial role in microbiological dynamics. The turnover rate of the fraction of DOM that is comprised of low molecular weight components has however been indicated to be substantially variable with seasonal temperature fluctuations (Boddy et al., 2008).

DOM/WEOM in forest soils may have a considerable amount of phenolic compounds and tannins. These are secondary compounds originating from plant decomposition that accumulate in forest soils (Souminen et al., 2003; Pizzeghello et al., 2003). Polyphenolic compounds may compose up to 23% of the total WEOM obtained from the litter layer (Pohlman and McColl, 1988). In the WEOM obtained from forest soils, however, the total concentration of phenolic acids (Pizzeghello et al., 2006) has been reported to be 79-187  $\mu\text{M}$  depending to the stand and age of the tree. Regarding their recalcitrant nature, phenolics and tannins have been of certain interest in terms of their contribution in Carbon and Nitrogen cycles in forest ecosystems (Northup et al., 1995; Fierer et al., 2001).

Titrateable acidity ( $\text{pH} < 7$ ) is another parameter that has been used for characterization of DOM/WEOM. Although it reflects the presence of free organic acids in organic matter (Tan, 1996), it does not any reveal information about the chemical structure of the related acidic functional groups. Herbert and Bertsch, (1995) reported that titrateable acidity for a range of soil derived DOM and WEOM samples varied between 6.5-11.5 and 9.6-14.9 eq/kg for DOM and WEOM (Herbert et al., 1993), respectively. A few researchers have attempted to link a relationship between  $\text{pK}$  and chemical components of WEOM. For example, while the  $-\text{COOH}$  groups with a  $\text{pK}$  3.5-3.8 has been suggested to be related to the presence of the low weight acids (formic, malic, glycolic, lactic), the  $\text{pK}$  between 4.6-4.9 characterize hydrooxalate and dihydrocitrate anions and fumaric and butyric acids components of WEOM (Shamrikova et al., 2006).

### **2.2.5. Biological characterization**

Some researchers have tried to characterize the biodegradability of DOM/WEOM from a biological-based characterization perspective. This approach may improve our understanding of the biological related properties Of DOM/WEOM obtained from different sources (Kalbitz et al., 2003b), obtaining methods (DOM vs. WEOM) (McDowell, et al., 2006), extraction temperature (Gregorich et al., 2003). The biodegradation aspects of DOM/WEOM will be discussed in details in the section “fate of DOM/WEOM” (part 2.5.1.) and Chapter 4 of this thesis.

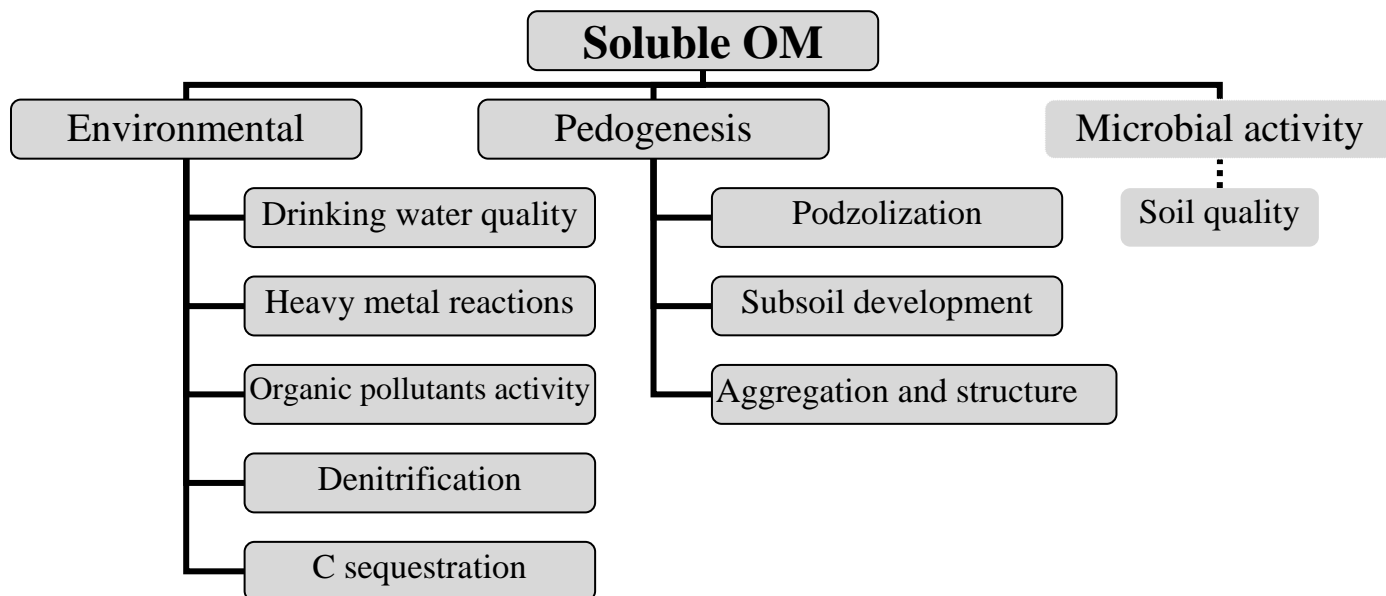
## **2.3. Key Functions of DOM/WEOM in soil**

DOM/WEOM is an important constituent of OM in both terrestrial and aquatic ecosystems. In fact, it has been suggested that it acts as the bottleneck between the terrestrial and marine ecosystems (Zsolnay, 2003). Due to its dynamics and reactivity, DOM/WEOM may play a key role in many important ecosystem functions. Its role in environmental transport and transformations, pedogenesis processes, nutrient supply, and the maintenance of soil quality are briefly discussed in this section (Fig. 2.2).

### **2.3.1. Environmental importance**

DOM/WEOM is known to interact with metal ions in soils (Stevenson, 1994). Metal ions, which are bound to the soil matrix, become mobile not only as a result of changes in the chemistry of the soil solution, but also through interaction with DOM (Fotovat and Naidu, 1996). When there is no significant change in solution conditions, DOM is most likely the chief controlling factor of the fate of metal ions in soils (Martinez and Motto, 2000; Fest et al., 2008). DOM can mobilize metal ions through interacting with them directly (*e.g.*, Cu) (Gondar and Bernal, 2009) or indirectly by competing with them for binding sites on the soil matrix (*e.g.*, Cs) (Staunton et al., 2002). Organo-metal complexes of DOM with heavy metals control the mobility, toxicity and bioavailability of metals (Brümmer et al., 1986; Ma et al., 1999). The degree of interaction and

**Figure 2.2.** Schematic diagram representing the ecological roles of DOM (dotted lines show the roles yet to be proved).



change in both the toxicity and availability of a metal depends on the quality of DOM (Inaba and Takenaka, 2005). The increase in concentration of DOM in surface and ground waters can lead to deterioration of water quality by potentially bringing pollutants such as heavy metals into the hydrosphere. DOM also influences water quality by changing its colour which causes UV (sourced from radiation) absorption (Engelhaupt et al., 2003; Houser et al., 2003). For drinking water purposes, this needs to be removed at considerable expense to achieve the public health standards (Oulehle and Hruska, 2009). Moreover, in water treatment facilities, DOM can react with chlorine and light during chlorination and produce carcinogen chloroform and other toxic halogenated organics (Rook, 1976).

It is well known that DOM significantly affects the sorption and mobility of pesticides in the soil ecosystem (Muller et al., 2007; Jiang et al., 2008). It has been reported that DOM is of some importance in the toxicity and availability of polyaromatic hydrocarbons (PAH) (Steinberg et al., 2000; Akkanen et al., 2001). The association of PAH with DOM is known to considerably increase the solubility of PAH and results in



their facilitated transport (Chiou et al., 1986; Fang et al., 1998; Sabbah et al., 2004). This association with DOM is probably due to a partition-like interaction of the solute with the microscopic intra-molecular DOM, which may consist of micelle like structures (Chien et al., 1997; Ragle et al., 1997). The degree of DOM interaction with PAH is not only dependent on the quality of DOM, but also the concentration of DOM (Persson et al., 2003). Since DOM is an aggregate of both high and low molecular weight OM, the changes in the toxicity and bioavailability of PAH that results from DOM sorption are difficult to observe. Furthermore, the solution conditions such as pH, metal ion concentration and ionic strength of the medium are known to affect DOM-pollutant interactions (Carter and Suffet, 1982; Lee and Farmer, 1989; Döring and Marschner, 1998).

DOM may participate in denitrification process through depletion of available Oxygen in the soil and also through providing the electrons needed for denitrification (McCarty and Bremner, 1993). Yeomans et al (1992) reported that there is a strong relationship between denitrification potential (DNP) and C availability even in deep soil layers down three meters, suggesting that DOM can be an important source of C for the denitrifier community, particularly in C-limited environments. However, contradictory results (strong and weak correlations) have been reported regarding DOM/WEOM concentration with soil DNP in the literature (Burford and Bremner, 1975; Christensen, 1991; Elliott and Jong, 1993; Siemens et al., 2003). This contradiction has been suggested to be as a result of i) different methodological approaches adopted for obtaining DOM/WEOM, ii) different DNP assessment methods, and iii) variability of DOM quality (Zsolnay, 1996).

### **2.3.2. Pedogenic functions**

In general, DOM plays a significant role in the transportation of different elements specifically metal ions which at the soil profile scale can increase the depth that nutrients are available for plant roots and microorganisms. It can also act as the source of energy and C needed for microbial communities active at soil depth and results in sustaining of

the biological activity of the soil down to subsoil (Buscot, 2005; Gorbushina and Krumbien, 2005). DOM in particular in forest ecosystems, translocates large amounts of organic compounds down to subsoil and thus is important in the development of the downward distribution of OM during ecosystem development (Sollins et al, 1983). Aside from its influence on soil microbial community activities at soil depth, DOM enhances soil conditions for biological activities through the improvement of soil aggregation and structure in subsoil (see part 2.3.3.2). Thus, DOM has both direct and indirect impacts on the soil chemical weathering processes with depth (Chotte, 2005; Gorbushina and Krumbien, 2005).

In cold and high altitude regions, the interactions of metals with soil solution (metal-DOM complexes) play an important role in soil weathering processes, the so called podzolization (Brümmer et al., 1986; Hongve et al., 2000). DOM is therefore, considered to take part particularly in the unique development process in Spodosols. This is known to be related to its role in the mobility of Al, Fe, along with organic compounds (Jansen et al., 2005). Further researches have elucidated that the role of DOM in podzolization is strongly determined by the amount and composition of the low molecular weight organic acids constituents of DOM (Lundström, 1993; Jansen et al., 2004, 2005).

### **2.3.3. Soil quality indicator**

#### **2.3.3.1. Biochemical contribution**

Although soil organic matter is considered to be probably one of the most common indicators of soil quality, DOM/WEOM appears to be a better indicator reflecting the ability of soil to deliver key soil functions and is more sensitive to short- to medium-term changes in the environment than SOM as a whole. Different management practices e.g. addition of crop residues (Graham et al, 2002); cultivation-fallow to continuous cultivation (Campbell et al., 1999a and 1999b), shifting from traditional to organic cropping (Lundquist et al., 1999), stock camping by grazing animals (Haynes and Williams, 1999) have been shown to affect DOM/WEOM markedly and faster than

SOM. Therefore, determination of DOM properties may serve as a suitable method for monitoring the adverse impacts of management on soil quality (Silveria, 2005). Zsolnay (1996) suggested that regarding the relatively high biodegradability of DOM, its concentration (particularly the concentration of its labile portion), is supposed to be minimum in optimum soil condition (in aerobic conditions). Thus, DOM/WEOM has the potential to be used as an indicator of soil conditions (Zsolnay, 1996). At a larger scale, DOM is also considered to be the bottleneck that links terrestrial ecosystems and water bodies. Therefore, it may act as early indicator of shifts in ecological processes (Zsolnay, 2003).

Similar to other suggested soil quality indicators, attempts have been made to quantify DOM/WEOM properties as an indicator of soil quality. Strong correlations (>80%) between water soluble C and cropping history in arable and pasturelands was reported by Haynes (2000). He showed that there is a strong relationship between water soluble C, microbial biomass and light fraction of SOM; two other suggested soil quality indicators; proposing that they are interrelating properties of SOM. Using Hot water extractable Carbon (HWE) from a broad range of land uses, Ghani et al., (2003) demonstrated that this attribute of DOM strongly differentiates between the impact of different long-term grazing intensities within a pastoral ecosystem. Ghani et al., (2003) suggested that HWE is a sensitive indicator and its depletion could give an early indication of the deterioration or decline of soil organic C. Their results also revealed that among the range of soil biochemical and biological measurements, HWE was the most consistent measurable factor to distinguish between treatments within and across their studied ecosystems.

Despite the suggested potential of DOM/WEOM as a soil quality indicator, the limited size of this in soil and its highly labile nature (Baldock and Nelson, 2000), in addition to its low concentration soil solution (specifically its labile portion) under normal field conditions suggest that the flux of DOM is more relevant than its size when used as a soil quality indicator (Haynes (2005). However, constantly monitoring the flux of DOM in soil under field conditions involves practical difficulties (e.g. expenses, its

low concentrations, rapid biodegradation, etc.) that should be considered for long-term surveys. In addition, the large temporal variability of DOM/WEOM due to its sensitivity to many environmental factors (e.g. moisture, temperature) and management activities (cultivation) may also constrain the use of DOM/WEOM as a spoil quality indicator. However, HWEOM and salt extractable organic matter (SEOM) appear to be more stable and thus, reliable for long-term monitoring soil quality indicator than the DOM/WEOM.

#### **2.3.3.2. Physical contribution**

The indirect impact of DOM/WEOM on sustaining soil physical properties is another feature of DOM/WEOM relation to soil quality studies. As a labile pool of OM, DOM/WEOM acts as the fuel that drives the soil food web via microbial community (Weil et al., 2003). The microbial products and by-products are well known for their beneficial impacts on soil physical properties (e.g. water stable aggregates, soil aggregation) (Tisdall and Oades, 1982; Gunapala and Scow, 1998). Thus, DOM/WEOM plays an indirect but large role in improving and sustaining soil physical properties. For example, addition of the DOM extracted from compost was shown to enhance the percentage of water stable aggregates in soils with poor physical conditions (Kohler et al., 2008). This was related to DOM fostering of the activity of the microbial community and was supported by significant increases in de-hydrogenase and urease activities. However, given the low initial OM content of the soils, the improvement was observed to diminish shortly after suspending the addition of the OM source (DOM extract) to soils.

Although evidence suggests that DOM/WEOM may act as an aggregate stabilizer agent (Cheshire et al., 1983, 1984; Chaney and Swift, 1984, 1986; Piccolo and Mbagwu, 1989), adverse impacts of DOM/WEOM on aggregation have also been reported by some authors (Gupta et al., 1984; Visser and Caillier, 1988). On the whole, it appears that DOM/WEOM acts as a dispersing agent when it is bound with monovalent cations (*e.g.*, Na, K), while being bound with polyvalent cations (*e.g.*, Al, Fe, Ca, and Mg, Gu and Doner, 1993), DOM/WEOM stabilizes soil aggregates. In addition, presence of humic acids (Chaney and Swift, 1984; Mbagwu and Piccolo, 1989), polysaccharides (Cheshire

et al., 1983, 1984), or readily metabolizable chemicals (Tisdall and Oades, 1982), in DOM/WEOM has been reported that improves soil physical properties through aggregation. Thus DOM/WEOM may contribute to land remediation and rehabilitation measures through the enhancement of soil physical properties.

#### **2.3.3.3. Soluble OM, the substrate for microbial activity**

Little is known about the chemical composition of DOM (see part 2.2.) but it is well documented that a portion of DOM/WEOM is comprised of labile compounds mainly of simple carbohydrate monomers, low molecular organic acids, amino acids, amino sugars, and peptides (see parts 2.2.2 and 2.2.3.) which are water soluble and biodegradable (Stevenson, 1994 ). The increase in CO<sub>2</sub> production (microbial activity) after addition of fresh DOM/WEOM to soil or moistening dry OM-amended soils, strongly suggest the importance of DOM in microbial metabolism (Zsolnay, 1996, Haynes, 2005). Soil microorganisms are totally dependent on water content of the soil and all microbial uptake mechanisms require an aqueous environment (Metting, 1993). Since DOM/WEOM exists in soil solution phase, it is, therefore, the gate through which microbial uptakes and exudations occur. On the other hand, microbial consumption of DOM/WEOM can strongly influence the redox conditions of soils. Anoxic pockets or micro-sites can be created, which in turn may result in the production and release of environmentally important gases such as nitrous oxide and methane (Zsolnay, 1996; Siemens et al., 2003). Under anaerobic conditions (lack of sufficient oxygen), DOM may act as the electron donor for microorganisms when required. The microbially processed Carbon from DOM may become partially decomposed during several pathways when it is finally transformed to methane and poorly biodegradable compounds, accumulating in soil (Zsolnay, 1996). There is a wide gap in our knowledge about the sequences through which organic compounds are assimilated by different groups of microorganisms, in particular in anaerobic conditions. Proteomics (Solaiman et al., 2007), polymerase chain reaction (Solaiman and Marschner, 2007) and PLFA (Marschner, 2007) have been suggested as the promising approaches for revealing a part of such ambiguities.

The amount of DOM which is biodegradable by soil microbes is not constant and varies with soil depth, land use, and soil conditions (Boyer and Groffman, 1996; Lundquist et al., 1999). DOM quality, and as a result its suitability as a substrate, may also be influenced by environmental stress such as by dry-wet and freeze-thaw cycles. In addition to its chemical composition, the accessibility of DOM for soil microorganisms depends on its location in the soil matrix (Zsolnay, 2003) and its interactions with soil aggregates (e.g. DOM I, II, and III, see part 2.1.2.). Consequently, only a part of freshly introduced DOM to the soil (e.g. throughfall, root exudates, litter leachate) may become accessible for microorganisms existing in soil aggregates. A part of this freshly introduced DOM may leach through the soil profile and serves as a microbial nutrient/energy source at soil depth or is lost through leaching to deeper layers. Given the fast turnover rate of the DOM/WEOM (vanHees et al., 2005; Boddy et al., 2008), it appears that not only the amount of this pool but also its flux is of crucial importance when microbial interactions of DOM/WEOM is studied. This may, however, be strongly affected by temporal variability of the flux of DOM in soil.

#### **2.4. Sources of DOM/WEOM**

Despite the ecological importance of DOM/WEOM, little is known about the sources of DOM (McDowell, 2003; Uselman, et al., 2007). It is now well established that DOM/WEOM originates from both above- and below-ground litter (throughfall, leaf litter, root exudates, decaying fine roots), microbial activities (biomass and exudates) and transformation of fresh and residual organic matter in soil (Kalbitz et al., 2000b; Haynes, 2005). Thus, both biological and non-biological factors are involved in the production of the DOM in soil (Fig. 2.1). While the flux of DOM/WEOM (the net result of the processes that release DOM; Kalbitz et al., 2000b) under different vegetation and climatic conditions are relatively well studied (e.g. Qualls, et al, 2000; Fujii et al., 2009), far less is known about the production mechanisms of DOM/WEOM in soil.

There is a considerable uncertainty and contradictory results in the literature regarding the importance of the fresh biological (microorganisms/plants) *vs.* relatively

stable OM as sources of DOM/WEOM in soil. McDowel and Likens (1988) postulated that leaching and microbial degradation of OM control DOM production in forest floor.  $^{14}\text{C}$  radioactive results demonstrated that fresh litter ( $\text{O}_i$ ) has no significant contribution to DOC flux in the mineral soil layer (below 15 cm) (Froberg et al., 2007). Therefore, it appears that the primary source of DOC in forest soils is either the underlying soil organic horizons ( $\text{O}_e$  or  $\text{O}_a$ ) or the residual SOM. In addition, the high ratio of humus to fresh litter has also been suggested as a convincing reason that residual SOM mainly controls DOM production (Zsolnay, 1996). On the other hand, Qualls and Haynes (1991) observed that rainfall largely affects the hydrophilic neutral fraction, a part of DOM that is influenced by fresh litter and mainly consists of simple compounds. Similarly, the majority of DON collected from pine forest soils was observed to originate from freshly fallen litter and partially decomposed litter layer (Casals et al., 1995), supporting the importance of fresh OM as the main source of DOM.

The rhizosphere is commonly associated with large C flux due to root decay and exudation and has been reported to be of particular important source of DOM production in forest ecosystems (Kalbitz et al., 2000b). However, due to accelerated decomposition and turnover of OM in rhizosphere, it usually serves as a source of energy and C for microbial community, resulting in high  $\text{CO}_2$  efflux, than a direct source of DOM to soil (Paterson et al., 2007)

The soil microbial biomass pool has also been suggested as a potentially important source of DOM/WEOM (Williams and Edwards, 1993). Hexose/pentose biomarker tracing of DOM revealed that DOM obtained from forest and grassland soils can largely form during microbial transformations of fresh and intact OM (Guggenberger et al., 1994; Huang et al., 1998). Among soil microflora, fungi were suggested to be more important in the formation of DOM in labile C-limited environments due to their predominant activity (Moller et al., 1999). Combining three different biomarker approaches (pentose/hexose ratio, GluN/GalN ratio, and the neutral sugar/uronic acid ratio), Fischer et al., (2007) suggested that the vegetation (*vs.* microbial community) is the determining source of the low molecular weight organic compounds (LMWOC) in

the soil solution. Their results suggested that compared to plants, microorganisms play only a minor role in the production of LMWOC with a larger contribution for bacteria than fungi.

Despite the proposed large contribution of vegetation in the production of DOM/WEOM in forest ecosystems, tree species has been observed that do not significantly affect the main properties of WEOM (e.g. molecular size distribution, chemical composition) (Smolander et al., 2002). However, it has been suggested that applying advanced and sophisticated analytical procedures (e.g. determination of the individual compounds of DOM/WEOM) may reveal more details about the biochemical contribution of different plant species to DOM/WEOM properties (Howard et al., 1998; Fischer et al., 2007).

## **2.5. Sinks of DOM/WEOM**

As mentioned before, despite representing only a small proportion of the total pool of SOM, DOM/WEOM is one of the most labile and active pools of OM in soil. Thus, the released DOM/WEOM from different sources is unstable and exposed to various fates. Regarding the roles of DOM/WEOM in the soil, the sink pathways of DOM/WEOM have been suggested to be important in terms of the fate of the organic and inorganic pollutants in soil (Martinez and Motto, 2000; Muller et al., 2007), depletion of C and N during soil solution passage through the soil profile (Fujji et al., 2009; Ruark et al., 2010) and from watersheds to streams (Fellman et al., 2009), denitrification at soil depth (Burford and Bremner, 1975), Carbon efflux from the soil to the atmosphere (Glatzel et al., 2003, Boddy et al., 2007), and cycling of elements (e.g. C and N) in terrestrial ecosystems (Zsolnay, 2003). Although a very small portion of the simple compounds existing in the soluble OM can be absorbed by plant roots (Jones et al., 2005a.) or mineralized during abiotic processes (Van Hees et al., 2005), microbial degradation and adsorption onto the soil colloids are the two main final processes controlling the fate of soluble OM in soil (Fig. 1) (Kalbitz et al., 2000; Guggenberger and Kaiser, 2003). The following section discusses the biodegradation and adsorption of DOM/WEOM.



### 2.5.1. Biodegradation

Soluble OM and specifically its simple components have been shown to be quickly metabolizable/mineralizable by the soil microbial community (van Hees et al., 2005; Boddy et al., 2007). A part of the microbially decomposed DOM/WEOM is released into the atmosphere as CO<sub>2</sub>, but a part of the metabolized DOM is transformed into microbial biomass and by-products and remains within the soil. Unlike the major part of the microbial biomass, the microbial by-products part of DOM is believed to be composed of more humified and refractory compounds than the original assimilated DOM (Don and Kalbitz, 2005; Ogawa et al., 2001). This part of DOM may remain in the soil longer than the original DOM, not only as a result of its recalcitrance nature but also due to its stronger affinity to the SOM than to the soil matrix (Kalbitz et al., 2003b). As a result, the biodegraded DOM may become immobilized due to adsorption to the bulk SOM. Assimilation of DOM to microbial biomass during DOM biodegradation, however, cannot be considered as DOM immobilization because of the relatively fast turn-over of microbial biomass in soil (Jones and Kielland, 2002).

DOM/WEOM is generally considered a labile substrate for the soil microbial community. Laboratory experiments have shown that there is a strong relationship between CO<sub>2</sub> efflux and the amount of soluble organic C in soil (Kalbitz et al., 2003b; Haynes, 2005), supporting its strong correlation with microbial activity. Soil solution incubations have revealed that while considerable amounts of DOM/WEOM may be readily degradable, the major portion of this substance is not readily available for the decomposer community in the soil. (Boyer and Groffman, 1996; Qualls and Haynes, 1992; Wagai and Sollins, 2002). This pool may comprise up to 76 and 94% of DOM/WEOM obtained from top- or subsoil, respectively (Schwesig et al., 2003; Fellman et al., 2008).

In an order to elucidate the dynamics of DOM/WEOM biodegradation, a number of laboratory experiments have shown that DOM/WEOM may be comprised of the “fast” and “slowly” biodegradable pools. The results of these experiments suggest that the turnover of the fast and slowly biodegradable pools may vary 0.22-2.5 day<sup>-1</sup> and 0.0022-

0.0122 day<sup>-1</sup>, with the labile (fast biodegradable) pool comprising 11-44.4% of the total DOM/WEOM (Qualls and Haynes, 1992; Kalbitz et al., 2003b; McDowell et al., 2006). The relatively widely reported range for the turnover time and proportion of the fast and slowly biodegradable pools appears to be related to the sources of DOM, procedure used for obtaining DOM/WEOM (DOM vs. WEOM), time of sampling, landuse, incubation period, experimental design, and analytical measures (CO<sub>2</sub> vs. DOC). To explain some of the contradictory results reported by authors and to try and standardize incubation experiments, McDowell et al., (2006) suggested that 7 and 42 days are the appropriate incubation periods for the evaluation of the fast and slowly biodegradable pools of DOM/WEOM. Their finding also revealed that CO<sub>2</sub> measurement (vs. DOC analysis) is the preferred method for biodegradability assessment experiments along with addition of nutrients when the potential biodegradable pool of DOM/WEOM is desired. The biodegradable pool of DOM has been suggested to be considerably less than WEOM (13-16% vs. 18-27%) (Wagai and Sollins, 2002). However, findings of experiment carried out by McDowell et al., (2006) suggested that the results of the biodegradation experiments performed either with DOC or WEOC are comparable.

Given the role of the laboratory biodegradation assessments in improvement of our understanding of the dynamics of DOM/WEOM, it is important to bear in mind that the biodegradability of DOM/WEOM in nature is substantially affected by the environmental conditions (e.g. temperature, moisture) and the intrinsic properties of the soil solution (e.g. pH, ionic strength, nutrients availability, toxic elements, etc.) (Marschner and Kalbitz, 2003). Thus, the results provided by batch experiments may not necessarily reflect the DOM biodegradation potentials and dynamics of DOM/WEOM in soil under natural conditions. The biodegradability of DOM/WEOM in nature can be recognized by its temporal variability, not only in terms of the impact of temperature on the plant and microorganisms activity but also by providing moisture needed for microbial activities, specifically during dry seasons. Such variable biodegradability of DOM has been suggested to affect the i) biogeochemical cycle of nutrients and ii)

ecology of microorganisms in the water streams affected by runoff containing DOM which is sourced from uplands (Fellman et al., 2009).

Despite the growing body of information about biodegradation rate of DOM/WEOM and its related pools, little is known about the long-term impact of land use and management on the biodegradability of either of the slowly or fast decomposable pools of DOM/WEOM and their proportion. In addition, most studies have focused only on the biodegradation dynamics in the topsoil while knowledge of the biodegradation dynamics at soil depth is largely lacking. Such studies may be essential when addressing issues such as C sequestration and nutrient losses in ecosystems.

From a biochemical perspective, the readily biodegradable portion of DOM/WEOM is a determinant of soil microbial activity (Haynes, 2005) and has been suggested to be largely comprised of the low molecular size/weight class compounds (carbohydrate, amino acids and amino sugars) (Qualls, and Haynes, 1992; Jones, 1999; van Hees et al., 2003; Jones et al., 2005b; Boddy et al., 2007). Poor biodegradability of DOM is believed to be related mainly to the presence of larger molecular size/weight classes (e.g. humic substances) (Haynes, 2005), and the presence of aromatic compounds (vs. aliphatic), tannins, phenols, polyphenols and terpenoids (Gianfreda, 1995; Williams and Gray, 1974). Given the larger contribution of bacteria over fungi in the degradation of labile DOM/WEOM (Møller et al., 1999), bacteria appear to make a considerable contribution during the initial stages of the biodegradation while fungi may play a more significant role in the later stages of biodegradation, mainly through decomposition of more humified/aromatic constituents of DOM/WEOM. By comparing different properties that are involved in the biodegradation, Marschner and Kalbitz (2003) suggested that molecular and structural characteristics of DOM/WEOM act as the primary intrinsic factors controlling biodegradation of DOM/WEOM. Given the chemical complexity of DOM/WEOM (see part 2.2.) and the broad range of microorganisms involving in its decomposition, future works needs to address the contribution of different groups of microorganisms to the biodegradation of different classes or compounds and how

individual compounds are cross-assimilated by different microorganisms (Jones, 1999; Jones et al., 2005b; Kiikkila et al., 2006).

During biodegradation CO<sub>2</sub> is released into the atmosphere as a part of microbial transformation of OM. However, given that only a minor part of DOM/WEOM is biodegradable in a short time period (as discussed above), and the high adsorption capacity of the soil for long-term retention of DOM/WEOM (Kaiser and Guggenberger, 2000; Kalbitz et al., 2005), it seems that the sorption of DOM/WEOM on soil colloids also acts as the significant controlling factor of the fate of DOM/WEOM in terrestrial ecosystems.

### **2.5.2. Sorption**

DOM adsorption especially in the subsurface layers of mineral soils has been suggested as having a significant role in C stabilization within terrestrial ecosystems and may be of importance in C sequestration (Zsolnay, 1996; Sanderman and Amandson, 2007). Sorption is defined as the transfer of the solute (sorbate) from solution to an existing solid phase (sorbent) (Sposito, 1984). Thus, the dissolved state is a prerequisite for the sorption process. Estimates show that about 10–25% of total C input to the forest floor through litter fall is leached from the organic surface layers (McDowell and Likens, 1988; Goggenberger, 1992). Michalzik et al., (2001) reported that although 10–40 g DOC m<sup>-2</sup> yr<sup>-1</sup> is translocated from the organic surface layer into the mineral soil horizons, the flux of DOC decreases to about 1–10 g C m<sup>-2</sup> yr<sup>-1</sup> in deep mineral forest soil. Similarly, sorption of large amounts of WEOM at soil surface (0–10 cm) was reported after manure application (equivalent to 150 kg N ha<sup>-1</sup>) to medium-heavy texture soils (Angers et al., 2006). Such a pronounced decline in the concentration and flux of DOM/WEOM beneath topsoil is assumed to be due to the adsorption of DOM onto soil minerals (Kalbitz et al., 2000). DOM flux at a soil depth of 90–100 cm is assumed to represent the DOM exported by leaching (Guggenberger and Kaiser, 2003). Sanderman and Amandson (2007) reported that DOC which was transported and subsequently absorbed in the clay-rich subsoil had a mean residence time of 90–150 years. Their results also revealed that

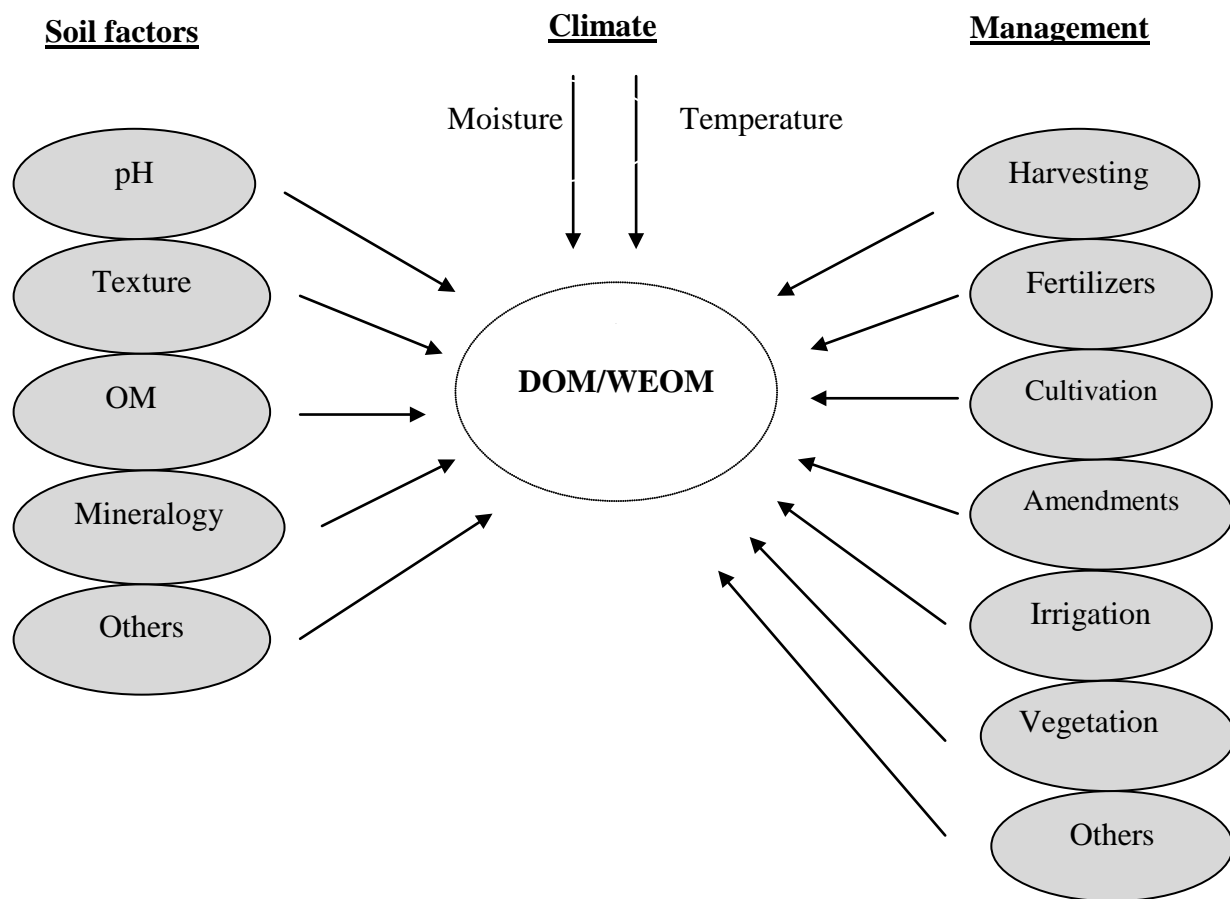
retention of DOC could be responsible for up to 20% of the total mineral soil C stock to 1 m depth in a forest soil and 9% in a grassland prairie soil.

DOM retention involves the formation of stable complexes between surface cationic metal and polar or acidic functional groups of the organic molecules. The predominant chemi-sorption (Guggenberger and Kaizer, 2003) process suggests that DOM sorption is sensitive to both the chemical structure of DOM and the surface properties of the minerals (Kaiser and Guggenberger, 2000). *Ligand exchange* reactions involving i) carboxyl and hydroxyl groups of natural organic matter and i) surface hydroxyl groups of solid components have been reported to be the predominant mechanisms for DOM sorption onto oxy-hydroxide minerals (Gu et al., 1994; Chorover and Amistadi, 2001). The high coating efficiency of DOM ( $0.18 \text{ m}^2 \text{ mg}^{-1} \text{ C}$ ) onto goethite was related to the high *polyfunctionality* of DOM as a result of its being a mixture of a variety of compounds (Modl et al., 2007). In addition to chemisorptions, physic-sorption also plays an important role in the adsorption of DOM via Van der Waals forces onto hydroxy-oxide minerals (Stevenson, 1994). Silicate clay minerals, have been suggested to have an even larger capacity for retention of DOC than oxides (Kahle et al., 2003). Apart from the significant impact of the sorbent properties (silicates vs. oxy-hydroxy minerals), the distribution of the compounds in the soluble OM appears to be important in its absorption. For example, the aromaticity of the DOM/WEOM down soil profile suggests that heavier molecular weight fractions are preferentially adsorbed by soil particles leading to the simple components either becoming biodegraded in topsoil or leached to subsoil due to their hydrophilic properties (Chorover & Amistadi, 2001; Hur and Schlautman, 2004; Omoike & Chorover, 2005).

## **2.6. DOM/WEOM in relation to land use and depth**

Land use changes and management activities such as plantation, clear-cutting, liming and fertilization, cultivation, ploughing, shifting cultivation has been widely shown to influence the properties of DOM/WEOM. The impacts of land use and management on DOM/WEOM contribute through changing both the quantity and quality

of OM input to the soil (Kalbitz, et al, 2000). This may change directly DOM/WEOM sources through the OM input (e.g. manure or fresh plant residues, throughfall) or through the rate and extent of the microbial pathways related to DOM synthesis and degradation (Cronan et al., 1992). Fig. 2.3 illustrates different management factors that affect DOM properties in soil.



**Figure 2.3.** Schematic of the impact of edaphic, environmental and management factors influencing the properties of DOM/WEOM in soil

Using molar ratio of C:N:P in DOM extracted from different land uses, Mattsson, et al., (2009) suggested that in addition to the impact of land use on the flux of DOM, the

changes in the quality of DOM has a substantial environmental importance when the release of DOM in to water catchments is of concern.

In general, DOM/WEOM concentration varies in the order of forest soil>grassland soil>arable soil, mainly due to the influence of different vegetation types (Zsolnay, 1996; Chantigny, 2003). The greater fungal biomass (Alexander, 1977), larger contents of the total OM (as the source of DOM) (Zsolnay, 1996; Klbitz et al., 2000), and greater proportion of lignin and other recalcitrant compounds (i.e. tannins and phenolic acids) (Chantigny, 2003) have been related to the higher content of DOM/WEOM in forest soils than agricultural lands.

Vegetation type, fertilization and liming are important factors related to the impact of land use and management on DOM/WEOM. While the impact of vegetation on the properties of DOM/WEOM has been addressed in the literature, the underlying mechanisms by which how different plant species affect the properties of DOM/WEOM are poorly understood. In terms of DOM constituents, tree species has been shown to have a stronger impact on DON than DOC in natural forests (Smolander and Kitonen, 2002) which may be due to the different composition of phenol (tannins and flavonoids) and amino acid/peptide compounds of the root exudates of different tree species (Qualls and Haynes, 1991). greater DOM concentrations in coniferous than in deciduous forest soils may be as a result of a greater accumulation of decomposing litter and the lower decomposition rate of DOM originated from coniferous trees (Kuiters, 1993; Currie et al., 1997). Furthermore, the greater DOM release into soil from coniferous roots especially during the winter period when no photosynthesis occurs in deciduous stands, may be another possible reason for the large DOM content in coniferous than in deciduous forest soils (Chantigny, 2003).

Far less information is available about the impact of vegetation type on the DOM/WEOM properties in agricultural soils. Higher concentration of WEOM in arable topsoil than grassland has been attributed to the different root exudation patterns, and amounts, and solubility of legume root exudates compared with those from grass (gramineae) roots in the soils (Chantigny et al., 1997). It is well-known that application

of organic wastes in agricultural lands increases the OM content of soil and thus DOM/WEOM. However, the impact of inorganic fertilizers on DOM/WEOM is more complex and less consistent than organic fertilizers (Chantigny, 2003).

Comparing laboratory and field results, McDowell et al., (1998) suggested that in laboratory conditions N fertilization stimulates microbial activities leading to the consumption of DOM/WEOM. However, under field conditions, the DOM/WEOM is replaced at the same rate through the decomposition of fresh litter, root exudation, and microbial by-products, partly due to higher microbial activities induced by added OM. The impact of N fertilization on the microbial activities in the forest soils and subsequently accelerated biodegradation of DOM may lead to the preferential use of easily decomposable compounds (e.g. low-molecular-weight acids), leaving a higher proportion of the slowly decomposable pool of DOM (Cronan et al., 1992). This may affect the quality of the OM content in the long-term and shift the microbial community composition through favouring fungi over bacteria. In contrast, repeated inorganic N applications in long-term experiments was reported to have no impact on the amount of DOM in forest soils (Gundersen et al., 1998; Yano et al., 2000). In agricultural soils a significant (Campbell et al., 1999 and 1999a) or not significant (Zsolany and Grlitz, 1994) increase in WEOM content of the soil was observed in plots that had undergone long-term treatment with N fertilizers. The significant increase in WEOM was related to a greater crop residue input in the fertilized soils. Overall, inorganic N fertilization could stimulate both DOM/WEOM production and consumption processes at the same time. The net effect has been suggested to be difficult to draw under field conditions and may vary from case to case (Chantigny, 2003).

Contradictory results have been reported for the impact of liming on DOM/WEOM properties. Increase in the i) organic matter solubility (Murayama and Ikono, 1975; Erich and Trusty, 1997), ii) microbial activity (Guggenberger et al., 1994), and iii) displacement of the previously adsorbed DOM by other mobilized anions (Kalbitz et al., 2000b) have been attributed to the release of DOM/WEOM following lime application. However, mechanisms such as i) consumption of DOM/WEOM as a result of



improving microbial activity (Anderson et al., 1994) and DOM flocculation and ii) adsorption by cation bridging due to high  $\text{Ca}^{2+}$  concentration (Romsdens and Dolfing, 1998) may result in a decrease in DOM/WEOM concentration. Apart from the concentration, liming can affect the composition of DOM/WEOM by precipitating large-molecular-weight DOM with  $\text{Ca}^{2+}$  (Romsdens and Dolfing, 1998), resulting in a greater proportion of smaller molecules in DOM/WEOM (Erich and Trusty, 1997).

Despite a relatively large number of studies addressing the impact of land use on DOM/WEOM, far less information is available on changes in DOM/WEOM pool size and properties with soil depth in soils under different vegetations. A decrease in the concentration of DOM/WEOM from the topsoil down to subsoil has been addressed frequently. For example, Michalzik and Matzner (1999) observed a substantial decrease in the average flux of the DON and DOC in forest floor from 5.9 and 146.6 Kg/ha/yr to <0.2 and 58.8 at 20 cm soil depth and “very little” and 16.5 kg/ha/yr at 90 cm soil depth, respectively. Their results suggested that the “low correlation” of DOC and DON is even weaker at soil depth. The WEOM concentration in deeper horizons (2B, 2Bt2, 2B/C) has been proposed to be strongly influenced by soil clay content, whereas WEOM behaviour is more independent of the textural characteristics in topsoil (Corvasce et al, 2006). The well-documented decrease of DOM/WEOM down the soil profile is widely thought to be due to the adsorption of DOM/WEOM onto soil particles or fast biodegradation of the labile pool of this substrate during the passage through the soil profile (Angers et al., 2006, Gu et al., 1994; Guggenberger and Kaiser, 2003).

The effects of land use (and management) on DOM should be identifiable down to the subsoil as a result of the translocation of considerable amounts of DOM from the topsoil to deeper soil horizons (Qualls et al., 1991). However, the results reported from comparison of the properties of DOM/WEOM obtained/extracted from different depths do not necessarily support this idea. Analysing DOM extracted from different sites, Kalbitz (2001) reported that although land use had a significant effect on DOM composition in the soil solution down to 95 cm depth, the DOC concentration was not affected by land use in soil depth. He then concluded that the depth-dependent change/s

in DOM composition could conceal effects of land use on DOM in deeper soil horizons. Angers et al., (2006) observed that despite a considerable impact on WEOM concentration, application of liquid or solid manure had little effect on WEOM content in 10-30 cm depth in both loamy and clay soils, possibly due to high adsorption capacity of the humic like materials. Aromaticity and humification indices of WEOM extracted from an agricultural soil showed a consistent decrease of molecular size and structural complexity of WEOM from topsoil ( $AP_1$ ) down to soil depth horizons ( $2B_{t2}$  and  $2B/C$ ) (Corvacse et al., 2006). Regarding the gentle extraction procedure for obtaining WEOM (OM extracted from soil solution) in the experiment, the results suggest the presence of a preferential flow of structurally simple molecules in WEOM towards deeper soil horizons.

## 2.7. Objectives of the study:

Based on my understanding from the literature review, it is clear that improving our knowledge of the dynamics of DOM/WEOM in soil systems is essential for a better understanding of ecological functions of DOM/WEOM. Despite using a number of extractants for obtaining EOM, the lack of a unified approach has led to contradictory results in the literature and hampered our understanding of the dynamics of this important pool of OM. As a basis for my research I, therefore, hypothesized that *although EOM is a continuum of substances, depending to the extraction method, it can be separated into two operationally different fractions. The size and properties of these fractions may consistently differ among different land uses and at different soil depths.* Identification of different fractions may improve our understanding of EOM dynamics in terrestrial ecosystems.

This research will focus on:

- 1- Defining a fractionation procedure for obtaining EOM and testing the reliability of the method by the assessment of i) the biodegradation of the fractions and ii) the size and properties of the fractions.
- 2- Evaluate the chemical and spectroscopic properties of the EOM during biodegradation.
- 3- Evaluate the impact of land use and soil depth on the rate of biodegradation of the fractions of EOM.
- 4- Evaluate the impact of land use and soil depth on the size and properties of the fractions of EOM for different seasons.
- 5- Evaluate the net DOM production through the solubilization of the SOM in the presence vs. absence of microorganisms.

## Chapter Three

# AMOUNT AND BIODEGRADABILITY OF WATER AND SALT EXTRACTABLE ORGANIC MATTER FRACTIONS AS AFFECTED BY LAND USE AND SOIL DEPTH

### 3.1. Introduction

Soluble organic matter (OM) comprises only a very small portion of total soil OM but it has been suggested to be a key contributor to many important ecological and biogeochemical processes in soils (Neff and Asner, 2001; Zsolnay, 1996). The movement of soluble OM through the soil profile is not only an important mechanism involved in soil formation (e.g. McDowell and Wood, 1984), but also contributes to the distribution and stabilization of soil carbon (Sollins et al., 1996). Soluble OM serves as one of the most labile OM pools and thus strongly influences the activity of microorganisms within the soil profile (Cleveland et al., 2004; Kalbitz et al., 2003a). The size and quality of the soluble OM pool may play an important role in soil nutrient fluxes, specifically N, P and S (Kalbitz et al., 2000b).

The biodegradability of soluble OM is a key factor in the stabilization and de-stabilization of soil organic matter (Marschner and Kalbitz, 2003). Incubation studies have shown that depending on the quality of soluble OM, usually 10-40% of the soluble OM obtained from topsoil is biodegradable under experimental conditions (Kalbitz et al., 2000b). Although information is limited, soluble OM obtained from subsoil appears to be much more resistant to biodegradation (<5% degradable) (Schwesig et al., 2003). Despite a number of studies, the long-term impact of land-use systems on the properties and biodegradability of soluble OM, especially in subsoil is poorly understood. Such studies can improve our understanding of biogeochemical cycles in terrestrial ecosystems.

While several studies have focused on the total and biodegradable C content of soluble OM, little is known about the composition of N-rich components of soluble OM

and their ecological importance (Jones et al., 2004; McDowell, 2003). Soluble organic nitrogen has been suggested as a part of recalcitrant pool of N, resulting from extensive microbial degradation of SOM (Ogawa et al., 2001) that may comprise a considerable portion of plant N uptake in N-limited environments (Jones et al., 2005a). Depending on edaphic conditions, this pool may act as a retained N pool within the soil profile due to adsorption on mineral surfaces (Kaiser and Zech, 2000) or comprise a significant portion of the N lost from agroecosystems (van Kessel et al., 2009). Only a few studies (e.g. Gregorich et al., 2003; Qualls and Haines 1992) have addressed the dynamics of both C and N during biodegradation of soluble OM. It may well be that both the quality and quantity of N have a significant role on the decomposability of soluble OM given the close relationship of C and N in OM degradation (Tate, 2000).

Biodegradation studies have been undertaken under a wide range of laboratory conditions, using soluble OM obtained by different procedures. This may be one of the reasons for the often contradictory results reported for the kinetics of soluble OM decomposition in the literature (e.g. Qualls and Haines, 1992; Gregorich et al., 2003; Kalbitz et al., 2003a; Schwesig et al., 2003). Dissolved and extractable OM are both soluble forms of OM. While dissolved OM (DOM) is typically defined as soluble organic matter in the soil solution and is often collected *in situ* using lysimeters or suction cups (Herbert and Berstch, 1995), extractable OM (EOM) is the soluble organic matter obtained under controlled laboratory conditions using different extractants. Among different extractants, distilled water, 0.01 M  $\text{CaCl}_2$  and 0.5 M  $\text{K}_2\text{SO}_4$  are the most commonly-used solutions for obtaining EOM (Rennert et al., 2007; Zsolnay, 1996). The properties of EOM are known to be influenced by the extractant itself. For example, dilute salt solutions have been used to simulate the ionic strength of the soil solution and thereby recover the soluble OM that occurs freely in soil solution (Reemtsma et al., 1999). In contrast, concentrated salt solutions (e.g. 0.5 M  $\text{K}_2\text{SO}_4$ ) have been proposed as a means of extracting OM held in the exchangeable phase (Jones and Willet, 2006). Because EOM is more likely to consist of a continuum of substances, we hypothesized that the frequently -used soil extractants 0.01M  $\text{CaCl}_2$  and 0.5M  $\text{K}_2\text{SO}_4$  would extract

two fractions of EOM with different biochemical characteristics. This experiment was designed to i) compare the amount and biodegradability of C and N of water extractable OM (WEOM) and salt extractable OM (SEOM) and, ii) evaluate the impact of land-use and soil depth on the size and activity of biodegradable pools in both fractions of EOM.

### **3.2. Materials and methods**

#### **3.2.1. Sites and sample collection**

Four land-uses typical of those found in the Mackenzie Basin, inland Canterbury, New Zealand (44°S, 170°E), were selected for the study (Table 1). Site selection was based on the need to ensure sites experienced a similar rainfall (700-800 mm yr<sup>-1</sup>), occurred at a similar elevation (600-700 m) and had similar soil types. At all sites, the current vegetation cover had been on the site for at least 14 years. The bog pine (*Halocarpus bidwillii*) woodland is a remnant of the pre-human vegetation that is likely to have occurred more widely across the Mackenzie Basin, while the degraded tussock grassland (*Festuca novae-zelandiae* and *Heiracium pilosella*) is typical of unimproved vegetation in the area induced by historic burning of the woodland and subsequent grazing. The two other vegetation types, cropland (*Medicago sativa*) and plantation forest (*Pinus nigra*) represent two very different types of land improvement, with the cropland involving cultivation, sowing and fertilizer application, while the plantation is dominated by fast growing exotic tree species planted in the degraded grass land. Soils are categorized as Orthic Brown (Andic Ustochrept) Pukaki soil based on New Zealand soil classification system. Soil samples were collected in December 2007 from topsoil (0-20 cm) and subsoil (60-80 cm) at three separate locations under each land-use using a 10 cm diameter stainless steel auger. Samples were held in an icebox and transferred to the laboratory within 24 h. The sampled materials were mixed well after sieving (2 mm) and removal of visible roots and litter. To minimize the effects of antecedent soil moisture on the amount and/or quality EOM obtained from different soil depths, all samples were adjusted to the same water status (60% of water holding capacity) prior to extraction. (For details see Appendices A and B)

### 3.2.2. Extractable fractions of organic matter

Water extractable organic matter (WEOM) was obtained by shaking (110 rpm, 15 min) the soil with 0.01 M  $\text{CaCl}_2$  at room temperature with a soil-to-extractant ratio of 1:2 (w/v). The extracts were then centrifuged (10 min, 1500 rpm) followed by filtration through pre-washed (with 10 ml DW) 0.45  $\mu\text{m}$  cellulose nitrate syringe filters. This method is considered a mild extraction procedure which reflects the *in situ* OM content of the soil solution (Zsolnay, 2003). Salt extractable organic matter (SEOM) was obtained by extracting the soil with 0.5M  $\text{K}_2\text{SO}_4$  at 1:1 soil-to-extractant ratio (w/v). Briefly, 20 g soil was transferred to a 50 ml plastic centrifuge tube. This leaves a small space in the bottle during the intense subsequent shaking procedure, avoiding break down of soil aggregate. The tubes were then placed in a water bath at 75°C for 90 min followed by 60 min shaking at room temperature. The tubes were centrifuged at 4800 rpm and the supernatant was filtered as above. Soil extraction using concentrated salt solutions (e.g. 0.5M  $\text{K}_2\text{SO}_4$ ) is more likely to release OM bound (exchangeable OM) with minerals (Jones and Willet, 2006; Murphy et al, 2000), which is potentially soluble. It should be noted that the SEOM obtained using this method includes the WEOM fraction. Both extracts were held at 4°C prior to further analysis (<4 d). For further impact of temperature and soil:extractant ratio on the release of organic C and N and microbial biomass see Appendices C and D, respectively.

### 3.2.3. Biodegradation assay

The biodegradability of the WEOM and SEOM was measured as the change in both organic C and N concentration of filtered solutions during an aerobic incubation. Under these experimental conditions, biodegradable OM included both mineralized and microbially assimilated C and N (Gregorich et al., 2003; Qualls and Haines, 1992). The bioassay for each sample was conducted by placing 150 ml of each extract solution into a 250 ml wide-mouth container. Each extract solution was incubated in duplicate without addition of supplementary nutrients. The SEOM solutions were diluted 10 times with

deionized water to reduce the osmotic pressure and to lower the dissolved C concentration of the extract solution.

A microbial inoculum was prepared and added to each bioassay extract at the beginning and after 42 d of the incubation. The inoculum solution was prepared by adding 100 ml distilled water to 10 g of a composite soil composed of equal parts of each soil sample (Gregorich et al., 2003). The suspension was shaken (10 min, 90 rpm) and then incubated for 48 h at room temperature. Before inoculation, the suspension was shaken and allowed to stand for 2 h. 120  $\mu$ l of the supernatant was added to each bioassay extract solution as the microbial inoculum. The blank was prepared by adding the same amount of inoculum to deionized ultra pure water ( $<18.2 \text{ M}\Omega \text{ cm}^{-1}$ , MilliQ, Millipore). The amount of C and N in the inoculated blank was not detectable. An inoculated glucose solution ( $20 \text{ mg C l}^{-1}$ ) was used to check the activity of microorganisms (Kalbitz et al., 2003a). Two pieces of shredded glass fibre filter were added to the solutions to provide physical support for microbial growth (Qualls and Haynes, 1992). Inoculated containers were sealed and placed in the dark at room temperature ( $22^\circ\text{C}$ ) during the experiment. To ensure aerobic conditions, all containers were agitated manually and opened daily in the first two weeks and less regularly for the remainder of the experiment. The incubation experiment ran for 90 days and subsamples were removed on days 1, 3, 7, 12, 16, 30, 42, 60, 75, and 90, filtered through  $0.22 \mu\text{m}$  filters to remove colloids and microbial colonies and analyzed for their C and N content as described below.

#### 3.2.4. Analytical methods

Total soil C and N were determined using a LECO CNS-200 analyzer (LECO Corp, St. Joseph, MI). The soil texture was determined using the hydrometer method (Kroetsch and Wang, 2008). Soil pH was measured in saturated paste with a standard glass electrode. Extracts obtained from the saturated pastes were used for determining the electrical conductivity (EC) of soils.

The extractable OM solutions were analysed for organic C and total N (TN) in duplicate with a TOC-TN analyzer (Apollo 9000 TOC/TN, Hewlett Packard, USA).



Organic C was measured by quantifying the CO<sub>2</sub> (after acidification), using the high catalytic oxidation (680°C) method. Mineral N forms (NO<sub>3</sub><sup>-</sup> and NH<sub>4</sub><sup>+</sup>) were determined colorimetrically by a flow injection analyzer (Flow Solution, TM 3000, ALPKEM, USA). Organic N was calculated as the difference between the concentration of the total N and the mineral N forms.

## 2.5. Assumptions and analysis of data

Previous studies have shown that soluble OM is composed of at least two distinct biodegradable pools that are best described using a double exponential model (Gregorich et al., 2003; Kalbitz et al., 2003a; Qualls and Haines, 1992). We used the same approach to describe the biodegradation rate of organic C and N in the WEOM and SEOM. The equation parameters and its components are defined as:

$$\text{Mineralized C or N} = (100-a) (1-e^{-k_1 t}) + a (1-e^{-k_2 t})$$

Where “100-a” (%) and “a” (%) represent the fast and slow decomposable components of EOM respectively.  $k_1$  and  $k_2$  are mineralization rate constants (day<sup>-1</sup>) of the fast and slow decomposable pools of EOM; and “t” the biodegradation period (day). The *half-life* (day) of the fast and slow decomposable pools of EOM was estimated as  $\ln 2/k_1$  and  $\ln 2/k_2$ , respectively. Decomposition curves were fitted to the model using the Gauss-Newton method with SAS software. To ensure the suitability of the applied model, the data were also run through the one-pool model (see Appendix E). The effects of land-use (within the same soil depth) on the i) concentration of C and N, and C:N ratio of EOM and ii) proportion of the slow and fast pools and their turnover rate were evaluated by analysis of variance (ANOVA), using Tukey’s test to compare differences between mean values. The impact of soil depth (within the same land-use) on these parameters was also analysed by t Test using SAS software (SAS Inc.).

## 3.3. Results

### 3.3.1. Carbon and nitrogen contents of the fractions

As expected, the topsoil samples from the four land-uses generally had higher concentrations of total organic C, total N and soluble salts than subsoil samples (Table 1). The soil texture classes were similar across the sites for both soil depths.

While only 0.02-0.09% of the total soil organic C was recovered as water extractable organic carbon (WEOC), the amount of salt extractable organic carbon (SEOC) ranged from 0.4% to 1.2% of the soil organic carbon (Table 2). As a proportion of total soil N, the difference between the water extractable organic nitrogen (WEON, 0.04-0.17%) and salt extractable organic nitrogen (SEON, 0.24-0.55%) was less than that for carbon. The proportion of SEON to the TN and particularly SEOC to TOC increased with soil depth at all sites, but this difference was not evident for WEOC and WEON. Both the proportions of C and N of the WEOM to those of SOM were significantly affected by land-use at both soil depths (Table 2), with plantation and cropland representing the highest proportion of WEOC/TOC and WEON/TN, respectively. In contrast, only the proportion of SEON to TN at topsoil was significantly affected by land-use.

Although the topsoil under plantation forest had the highest concentration of WEOC, the topsoil from the cropland site had the highest concentration of N in both WEOM and SEOM (Table 3). Despite having the lowest concentration of WEOC, the bog pine site had the highest concentration of SEOC in both topsoil and subsoil. Extractable organic N (EON), however, did not follow the same trends as those described for extractable organic C (EOC). The C content of the SEOM was much greater than WEOM, ranging from 5 to nearly 70 times (Table 3). The same pattern was observed for EON (1.7-8.8 times), but there was less difference in the amount of EON obtained by the two different extraction methods.

Compared with WEOM, the C and N content of the SEOM was highly comparable among the land-uses and between the soil depths (Table 3). The C/N ratios of the SEOM were considerably greater than those of the WEOM (Table 3), particularly for the subsoil samples. While the WEOM had a greater C/N in the topsoil than subsoil samples, the C/N ratio of the SEOM increased with soil depth at all sites.

### 3.3.2. Biodegradability of the fractions

The pattern of biodegradation of both WEOM and SEOM can be characterized by a fast initial phase of decomposition followed by a much slower, more or less linear phase of decomposition for both C (Fig. 1a and 1b) and N (Fig. 2a and 2b). With a few exceptions, 50% or more of C and N mineralization occurred within the first 16 days of the incubation. In comparison with C, there was slightly greater net N mineralization from both EOM fractions (Fig. 1 and 2). By the end of the incubation period, 39-70% of C (Fig. 1a) and 49-75% of N (Fig. 2a) was mineralized in the WEOM solutions. In contrast, the proportion of biodegradable C (15-30%) and N (22-44%) was much lower for SEOM (Fig. 1b and 2b). In most cases, the ratio of WEOC-to-SEOC and WEON-to-SEON declined significantly during the incubation experiment (Table 4).

The proportion of biodegradable of C (Fig. 1a and 1b) and N (Fig. 2a and 2b) in each of the fractions varied among soils with different land management histories and between the two soil depths. Although extracts of subsoils from the cropland site had the largest proportion of the total biodegradable WEOC (70%) and SEOC (30%), there were no consistent differences among land-uses and between soil depths in either of fractions. There was a general tendency for the WEOM and SEOM from cropland and plantation forest soils at both depths to have a larger percentage of total biodegradable C than extracts from the bog pine and degraded tussock soils. This was also the case for biodegradable N in the SEOM, but not for the WEOM where soils from bog pine sites had both the lowest (topsoils) and the highest (subsoils) percentage of biodegradable C compared to other land-uses. In most cases, there was little or no increase in the C/N ratio of the WEOM and SEOM remaining during the bioassay (Fig. 3a and 3b).

Results of the biodegradation assays provided strong evidence for the existence of two operationally different pools of biodegradable OM in both WEOM and SEOM. These are referred to here as the fast and slow pools. The fast pool of biodegradable C made up a larger proportion of the total biodegradable OM in the WEOM (13-22%) than SEOM (8-13%, Table 5) obtained from topsoils. However, there were relatively few

differences between land-uses in the percentage of total biodegradable C that was associated with the fast and slow pools of WEOM and SEOM from topsoils. By comparison, the pool size of the fast biodegradable C made up a much greater percentage of the total biodegradable WEOM in subsoils (28-50%) than topsoils (13–22 %), but this pool was highly comparable in the SEOM obtained from topsoil (8-13%) and subsoil (8-13%). There were also greater differences among land-uses in the relative pool size of fast and slow biodegradable C in the WEOM than SEOM.

Overall, the half-life of the fast pool of biodegradable C was similar in both WEOM (2.6-6.2 days) and SEOM (2.4-12.4 days) across land-uses and both soil depths (Table 5) and was significantly affected by the impact of land-use (Table 5). In contrast, the half-life of the slow pool of biodegradable C was much greater for SEOM (249-767 days) than for WEOM (112-171 days), irrespective of land-use or sample depth. While there were no or very few significant effects of soil depth on the half-life of fast or slow biodegradable SEOC, the half-life of the fast pool of biodegradable WEOC was higher in subsoils than topsoils under all land-uses except the cropland soils.

The effects of land-use and soil depth on the relative size and half-life of fast and slow pools of biodegradable N in WEOM and SEOM were different from those of biodegradable C (Table 5). Our results showed that the fast pool of biodegradable N generally made up a larger proportion of the total biodegradable N in the WEOM (21-38 %) than the SEOM (11-13%, Table 6) from topsoils in all land-uses except the plantation forest soils where the fast pool of SEOM made up a much larger proportion of the total biodegradable N (36%) than the fast pool in WEOM (11%). While in topsoil, the degraded tussock site had the highest proportion of biodegradable N in the fast pool of WEOM (38%), the highest proportion of biodegradable N in the fast pool of SEOM was observed in plantation forest (36%, Table 6). Similar to biodegradable C, the effects of soil depth on the proportion of the fast pool of biodegradable N were more obvious for WEOM than SEOM. The fast pool of biodegradable N made up a greater proportion of the total biodegradable N in subsoils of only the plantation forest and bog pine sites compared to the topsoils. In the SEOM, the only significant effect of soil depth on the

size of the fast biodegradable N was observed at the cropland sites where subsoils had a higher proportion of fast biodegradable N than topsoils (Table 6).

In general, the half-life of the fast pools of biodegradable N was in a similar range for both WEOM and SEOM across all land-uses and both sample depths (Table 6). As for biodegradable C, the half-life of the slow pool of biodegradable N was much greater for SEOM (178-585 d) than for WEOM (62-138 d), irrespective of land-use or sample depth. There was a stronger effect of land-use than soil depth on the half-life of fast and slow pools of biodegradable N from WEOM and SEOM. Significant effects of land-use on the half-life of biodegradable N in fast and slow pools of both fractions were observed in topsoils but less evident in subsoils. In comparison with the topsoil samples, there were fewer significant differences in the half-life of the fast and slow pools of biodegradable C and N in subsoil samples of both WEOM and SEOM. Comparison of the mineralization rate constant of the fast ( $K_1$ ) and slow ( $K_2$ ) decomposable pools of C (Fig. 4a) and N (Fig. 4b) showed that although the WEOM and SEOM have a similar range of mineralization rate constant of the fast pool of biodegradable C and N, the range of mineralization rate constant of the slow pool is significantly ( $P < 0.0001$ ) larger in the WEOM than SEOM. This resulted in a substantially longer half-life of the slow biodegradable pool of C and N of the SEOM than WEOM as mentioned above.

### **3.4. Discussion**

#### **3.4.1. Fractionation of EOM**

The amount of C and N recovered in the WEOM and SEOM in this study (Table 3) was in accordance with the literature (e.g. Burton et al., 2007; Haynes, 2005). Few studies have compared the impact of different extractants on properties of EOM (e.g. Burton et al., 2007; Rees and Parker, 2005). We observed that the C and N content and the C/N ratio of the EOM were strongly influenced by the extraction method regardless of the land-use and soil depth (Table 3). Similar to Burton et al (2007), we found that the C and N content of the SEOM is less affected by land-use and soil depth compared with the WEOM (Table 3).

The considerable differences in the concentration of C and N in the WEOM and SEOM as well as differences in their C/N ratios (Table 3) and biodegradation rates (Table 4 and Figs 1 and 2) suggest that the two fractions are chemically different and their dynamics are controlled by different mechanisms. While WEOM is thought to be enriched with low molecular weight organic compounds (e.g. free carbohydrates, amino acids) (Kalbitz et al., 2003b; Owen and Jones, 2001; Volk et al., 1997), the higher C/N ratio of the SEOM, particularly that obtained from subsoil implies that only a small portion of this fraction is comprised of microbial residues and perhaps fresh organic matter. Comparison of C/N ratio suggests that unlike the SEOM, WEOM shows a greater richness of N compounds than the total SOM (Tables 1 and 3). These results emphasize the necessity to ensure the comparability of results when pools of soluble OM are studied.

Although only a small portion ( $\leq 1.2\%$ ) of the total soil organic C was recovered as the SEOC (Table 2), significant amounts of the SEOC may have been removed during the microfiltration ( $< 0.45 \mu\text{m}$ ) (Rees and Parker, 2005). The size of the pool of OM extracted by concentrated salt solutions (2M KCl or 0.5M  $\text{K}_2\text{SO}_4$ ) has been suggested to be largely comparable (Burton et al., 2007) and reflects the amount of the OM held in exchangeable phase (Jones and Willett, 2005). However, because of the interference of  $\text{Cl}^-$  (derived from KCl extractant) with the reactions involved in the persulfate oxidation method (Aiken, 1992; McKenna and Doering, 1995), 0.5M  $\text{K}_2\text{SO}_4$  seems to be the appropriate extractant when persulfate method is used for C and/or N determination. Additionally, extraction of the soil with 0.5M  $\text{K}_2\text{SO}_4$  minimizes the disruption of soil aggregates (Haney et al., 1999) and thus, the release of the occluded OM during the extraction procedure.

Since we did not apply a sequential extraction, the SEOM was also comprised of WEOM. However, comparison of the C and N concentrations in the two fractions indicates that organic matter in soil solution (WEOM) made up only a small portion of the SEOM (Table 3). Instead, the major portion of SEOM is likely to be comprised of the organic matter which is bound to soil colloids (potentially soluble OM).

Given the higher temperature and higher ionic strength of the solution used to extract the SEOM, it is likely that in addition to exchangeable forms of SOM, The SEOM also comprised some microbial biomass (Sparling et al., 1998). In our experiment, the increase of extraction temperature from 25°C to 75°C, resulted to 13.8-21.5% increase of the released C from soils, equivalent with 8-13% of the microbial biomass C of the soils (data not shown). It seems that only a part of the temperature-induced C release can be comprised of the lysed microbial biomass C with the rest sourced from the exchangeable and perhaps thermally solubilized OM. On the other hand, the high C/N ratio of the SEOM (table 3) supports the results reported by Chantigny et al (2010), suggesting that even the high temperature extraction (80°C, 16h) does not necessarily result in the release of N-rich microbial tissues ( $C/N \leq 6$ ). Furthermore, the low initial moisture content of the soils (7.8-11.9% w/w) at the time of sampling may have reduced the impact of temperature on the release of microbial biomass C due to the low amount of living biomass in the soils.

#### 3.4.2 Biodegradation of C and N

The initial decomposition of C and N of both fractions of EOM was very rapid. The decomposition of the fast biodegradable pool of C and N of both fractions occurred within the initial 12 and 16 days, respectively (Fig. 1 and 2). This is a slightly longer than the suggested 7-day incubation period for the assessment of the labile pool of EOM (McDowell et al., 2006) and may be related to the lack of nutrients addition in this experiment. Addition of nutrients increases the activity of the decomposing microorganisms (McDowell et al., 2006) and thus, enhances the biodegradation rate. The observed trend of biodegradation of C and N for both fractions of EOM supports the suggestion of using a 42-day incubation period to evaluate the slow biodegradable pool (McDowell et al., 2006). However, the longer period of incubation (90 days) in our experiment was intended to improve the approximation of the half-life of the slow decomposable pool by providing more observations for the model.

We did not find any relationship between the initial amounts of C and N in either of the EOM fractions and their decomposability. Contradictory results have been reported elsewhere for the relationship between the quantities of soluble OM and the rate of C mineralization, with studies showing either a positive correlation or no correlation (Cook and Allan 1992; Petrone et al., 2009; Quall and Haines, 1992; Smolander and Kitunen, 2002).

The pattern of biodegradation of C and N was similar in both fractions of EOM (Fig. 1 and 2). This is in accordance with earlier studies (e.g. Kalbitz et al., 2003a; McDowell et al., 2006), indicating that soluble OM consists of two kinetically distinct pools. However, the proportion of the biodegraded C and N was substantially different between the two fractions. In the other hand, we observed a significant loss in the proportion of C and N of the WEOM compared to those of the SEOM (Table 4). The low biodegradability of C and N of the SEOM (Figs. 1b and 2b) appears to be related to its higher C/N ratio (Table 3). The strong relationship between C/N ratio and OM biodegradation has been well documented (e.g. Fellman et al., 2008; Wiegner and Seitzinger, 2004).

The range of half-lives of the fast and slow biodegradable pools in our experiment were very comparable with those reported by McDowell et al. (2006) for both  $K_1$  (0.18-2.5d<sup>-1</sup>) and  $K_2$  (0.003-0.012 d<sup>-1</sup>). There is a relatively broad range of half-lives reported for the fast and particularly for the slow biodegradable pool -varying up to 10 times- of soluble organic C (e.g. Kalbitz et al., 2003a; Qualls and Haines, 1992; Schwesig et al., 2003). This contradiction seems to be associated with extraction procedure (EOM vs. DOM), length of biodegradation period (Kalbitz et al., 2003a), biodegradation assessment method (DOC loss vs. CO<sub>2</sub> production; McDowell et al., 2006), time and depth of soil sampling (Qualls and Haines, 1992), and soil vegetation. The estimated K values of the slow biodegradable C pool (Fig. 4a) ranged from 0.001 to 0.003 d<sup>-1</sup> in the WEOM and from 0.004 to 0.006 d<sup>-1</sup> in the SEOM, comparable with that reported for lignin (0.003 d<sup>-1</sup>, Paul and Clark, 1996). This support Kalbitz et al (2003a) in saying that depending on the



extraction procedure, a portion of soluble OM contains labile OM and the rest is comprised of partially to highly stable organic compounds.

Despite the higher proportion of the fast biodegradable pool (both C and N) in the WEOM than SEOM, the half-life of this pool is rather comparable between the two fractions (Table 5 and 6). On the contrary, comparison of the biodegradation rate constants of C (Fig. 4a) and N (Fig. 4b) of the WEOM and SEOM indicates that different biodegradability of the fractions is primarily due to the biodegradation rate constant of the slow biodegradable pool ( $K_2$ ). This implies that the extraction procedure had a significant influence on the half-life of the slow biodegradable pool ( $HL_2$ ) as measured by both C and N. Given that the extraction procedure was not carried out sequentially, a small proportion of the SEOM is likely to have comprised WEOM. Thus, the half-life of the SEOM may have been underestimated in our experiment. However, the comparability of our results with the literature (e.g. McDowell et al, 2006), in addition to the extended period of the incubation (90 days) imply a negligible influence of the WEOM included in the SEOM.

Despite a similar pattern of C and N biodegradation, a greater proportion of N was biodegraded in both fractions (Fig. 1 and 2). Comparison of the half-life of the fast and slow biodegradable pools of C and N indicated a longer half-life of C than N in the slow biodegradable pool in both WEOM (Mean: 118 *vs.* 83 days;  $P < 0.001$ ) and SEOM (Mean: 458 *vs.* 355 days;  $P = 0.06$ ). Accordingly, the observed reduced biodegradation of C than N is largely contributed to the slow biodegradable pool of EOM. The greater biodegradability of soluble N than C reported in the literature (e.g. Petrone et al., 2009) has been attributed to the N-based biodegradation of OM (Gregorich et al., 2003; Wiegner et al., 2004). In addition, the larger proportion of biodegraded N than C suggests that the metabolized OM was relatively rich in readily decomposable N compounds. The proportion of the metabolized organic N has been reported (Petrone et al., 2009) to be highly correlated with the N content of the hydrophilic (*vs.* hydrophobic) fraction which is mainly comprised of N-rich compounds (Aiken et al, 1992).

We observed similar results to Qualls and Haines (1992) indicating that in most cases (specifically SEOM), the C/N ratio regardless of its initial value did not substantially change during the incubation period (Figs. 3a and 3b). The C/N ratio change during biodegradation reflects the degree to which the microbial community metabolizes the N-rich components (Qualls and Haines, 1992). The fast initial increase in the C/N ratio which was observed during biodegradation of the SEOM, appears to be due to the quick consumption of N-rich compounds (Petrone et al., 2009). These compounds however, comprise only a small portion of soluble OM (Jones et al., 2004; Jones et al., 2005b).

### 3.4.3 Soil depth and land-use

The greater biodegradability of the WEOC obtained from the subsoil than topsoil (Fig. 1a) may be due to the i) lower C/N ratio of the WEOM (Table 3) and ii) significantly higher proportion of the fast biodegradable pool in the subsoil WEOM (Table 5). This could be as a result of leaching of hydrophilic (N-rich) soluble OM (Petrone et al., 2009) and higher proportion of labile root exudates at soil depth at the time of sampling (spring). The larger proportion of biodegradable pool of soluble OM during spring has been reported in both cultivated and forest soils (Embacher et al., 2007; Lundquist et al., 1999). In addition, retention of more humified compounds of soluble OM during its movement through the soil profile (Kalbitz, 2001; Qualls and Haines, 1992), and thus the higher proportion of labile soluble OM within the subsoil may further explain the greater biodegradability of WEOM in the subsoil samples.

The turnover of the slow biodegradable pool of soluble OM obtained from subsoil has been suggested to be longer than that of topsoil (Schwesig et al., 2003). We did not find any evidence (Tables 5 and 6) indicating that the turnover of either the fast or slow biodegradable pool of EOM obtained from the subsoil is longer than topsoil. Fontaine et al., (2007) compared the structure and turnover rate of the SOM in topsoil (0-20cm) and subsoil (60-80 cm) using spectroscopic and isotopic ( $^{13}\text{C}$  and  $^{14}\text{C}$ ) methods. Their results indicated that despite similar  $^{13}\text{C}$  CPMAS-NMR spectra of different soil depths, the

estimated mean residence time of the OM in the subsoil was considerably longer than the topsoil (2560 vs. 320 yr). The similar potential biodegradability of both fractions of EOM obtained from the topsoil and subsoil in our experiment agrees with these findings, implying that the suggested longer half-life of the soluble OM in the subsoil can be largely due to inappropriate conditions for the decomposer community at soil depth in field conditions. Additionally, our findings proposed that soil depth did not have a strong influence on either of the proportion or half-life of the fast and slow biodegradable pools of C and N (Tables 5 and 6).

Similar to other studies, the soluble organic C obtained from cropland soil was more biodegradable than that forest and woodland soils (Kalbitz et al., 2003a; Macdonald et al., 2007). However, there was not a consistent effect of land-use in relation to C and specifically N biodegradation. The higher biodegradability of soluble OM of cropland has been attributed to the lower C/N ratio of crop litter and proportionally higher abundance of recalcitrant compounds in the soil solution obtained from forest soils (Boyer and Groffman, 1996, Kalbitz et al., 2003a). The SEOM particularly in bog pine and degraded tussock grassland soils represented a substantially N-depleted pool of OM and accordingly less biodegradable than that of cropland and plantation soils. This agrees with previous studies that have shown that a large proportion of soluble OM obtained from soils under woodland (Macdonald et al., 2007) and forest soils (Smolander et al., 2001) may be comprised of highly C-rich compounds (C/N ratio: 20-40).

Only a few studies have addressed the impact of land-use on the biodegradation dynamics of soluble OM. It has been suggested that afforestation of arable lands i) has no influence on either of the pools size and turnover rate of the fast and slow decomposable pools of EOM (Zhao et al., 2008) or, ii) affects only the size (but not the turnover rate) of these pools of EOM (Llorente and Turrion, 2010). The latter is in accordance with our observation that land-use has a significant impact on the proportion of the fast and slow biodegradable pools of EOM (both C and N). However, we did not find a consistent effect of land-use on the proportion and half-life of the fast or slow biodegradable pools. In addition, land-use had a strong influence on the turnover rate (HL) of the fast

biodegradable pool (both C and N) of the WEOM and SEOM, but the turnover rate of the slow biodegradable pool was largely unaffected by land-use. These results suggest that despite the influence of land-use on the quality and quantity of OM input to the soil, it has a limited influence, on the turnover rate of the slow biodegradable pool of soluble OM. By seasonal monitoring of soluble OM obtained from different land-uses Kawahigashi et al. (2003) noticed that land-use significantly affected the concentration of compounds known to comprise the fast biodegradable pool of soluble OM (e.g. pentoses and hexoses), but it did not influence the concentration of slow biodegradable compounds (e.g. phenolic acids). There is an increasing body of evidence indicating that a high proportion of the soluble OM may source from the humified OM (e.g. Sanderman et al., 2008). The lack of considerable effect of land-use on the turnover of the slow biodegradable pool of soluble OM may be associated with the SOM-derived pool of soluble OM. Comparison of the impact of land-use and soil depth on the components of biodegradation model suggests that the effect of land-use on the composition of EOM is stronger than that dependent on soil depth, In agreement with Kalbitz (2001).

## **Conclusion**

The failure of different studies to use a standard extraction method may explain some of contradictory results reported for the kinetics of EOM. Extractable OM ( $OM < 0.45 \mu m$ ) is a continuum comprised of different pools but it can be operationally partitioned into two fractions (WEOM and SEOM) with different C and N contents and biodegradability constants. Using a unified fractionation approach (e.g. dilute followed by a concentrated salt solution) for obtaining EOM may help to reduce the contradictory results. The major part of EOM (SEOM) reflected the properties of the potentially soluble OM, the OM pool which is mainly bound with soil colloids (Jones and willet, 2006). This fraction is comprised of relatively humified substances with limited C biodegradability. Unlike the SEOM, the OM obtained from soil solution, known as the WEOM, is largely affected by land-use and soil depth. The lower C/N ratio of this fraction seems to be related to its greater biodegradability. Extractable OM is commonly known as a highly

labile fraction of the SOM. However, our data showed only a small portion of the EOM (WEOM) can be characterized by a greater degree of biodegradability. The identified fractions of EOM in this study have potential for further studies, focused on roles of EOM in soil quality and ecosystem function. Both fractions of EOM were shown to be comprised of the fast and slow biodegradable pools. While the fast decomposable pool is thought to act as a substrate for microbial activity, the slow decomposable pool is of interest for its contribution to the preservation of OM. Although no relationship was found between the size of biodegradable C of EOM and its initial C content, the rate of C biodegradation appeared to be related with the C/N ratio.

**Table 3.1.** Selected characteristics of soils under different land uses

Land use	Depth (cm)	Texture	pH	EC (m $\mu$ cm <sup>-1</sup> )	Total C (g kg <sup>-1</sup> )	Total N (g kg <sup>-1</sup> )	C/N
Plantation	0-20	Silty Clay	4.9	204	33.3 $\pm$ 0.4	2.48 $\pm$ 0.08	13.4 $\pm$ 0.6
Cropland			5.3	128	31.4 $\pm$ 0.2	2.23 $\pm$ 0.01	14.0 $\pm$ 0.6
Grassland		Loam	5.5	109	35.6 $\pm$ 0.9	2.66 $\pm$ 0.07	13.4 $\pm$ 0.5
Bog pine			4.9	65	36.6 $\pm$ 0.8	1.97 $\pm$ 0.17	18.5 $\pm$ 0.8
Plantation	60-80	Silt Loam	5.2	62	18.2 $\pm$ 0.2	1.38 $\pm$ 0.04	12.8 $\pm$ 0.9
Cropland			5.2	163	15.0 $\pm$ 0.2	1.05 $\pm$ 0.03	14.3 $\pm$ 1.1
Grassland			5.5	28	11.8 $\pm$ 0.6	1.01 $\pm$ 0.12	11.7 $\pm$ 0.8
Bog Pine			5.0	56	19.2 $\pm$ 0.5	1.16 $\pm$ 0.38	16.5 $\pm$ 0.8

Values are given as mean  $\pm$  S.D

**Table 3.2.**

The C and N content of WEOM and SEOM as a percentage of total soil organic C (TOC) and total N (TN) and their probability value (*P*) as affected by land-use.

Land-use	Depth (cm)	WEOM		SEOM	
		WEOC/TOC	WEON/TN	SEOC/TOC	SEON/TN
Plantation	0-20	0.09 <sup>a</sup>	0.09 <sup>b</sup>	0.53 <sup>a</sup>	0.39 <sup>b</sup>
Cropland		0.07 <sup>a</sup>	0.28 <sup>a</sup>	0.41 <sup>b</sup>	0.47 <sup>a</sup>
Degradedland		0.04 <sup>b</sup>	0.04 <sup>c</sup>	0.49 <sup>ab</sup>	0.24 <sup>c</sup>
Bog pine		0.02 <sup>b</sup>	0.05 <sup>c</sup>	0.49 <sup>ab</sup>	0.29 <sup>c</sup>
<i>P</i>		<0.01	<0.01	0.06	<0.01
Plantation	60-80	0.05 <sup>a</sup>	0.11 <sup>b</sup>	0.86	0.46
Cropland		0.05 <sup>a</sup>	0.21 <sup>a</sup>	0.85	0.55
Degradedland		0.03 <sup>b</sup>	0.06 <sup>c</sup>	1.22	0.46
Bog pine		0.02 <sup>b</sup>	0.05 <sup>c</sup>	1.02	0.42
<i>P</i>		0.02	<0.01	0.21	0.44

**Table 3.3.** The effect of land use and depth on the concentration of Carbon and Nitrogen ( $\text{mg kg}^{-1}$ ) and C/N ratio in the water and salt extractable OM

Fraction	Element	Depth	Land use				P	F
			Plantation	Cropland	Grassland	Bog pine		
WEOM	C	Topsoil	29.9±2.7 <sup>A</sup>	22.5±1.2 <sup>A</sup>	12.4±0.7 <sup>B</sup>	5.6±0.4 <sup>C</sup>	<0.001	35.1
		Subsoil	9.7±1.0 <sup>A</sup>	7.0±0.5 <sup>B</sup>	3.8±0.5 <sup>C</sup>	2.7±0.2 <sup>C</sup>	<0.001	18.7
		Depth impact	**	**	**	**		
	N	Topsoil	2.2±0.2 <sup>B</sup>	3.9±0.5 <sup>A</sup>	1.2±0.2 <sup>C</sup>	1.0±0.12 <sup>C</sup>	<0.001	70.3
		Subsoil	1.5±0.2 <sup>A</sup>	1.2±0.2 <sup>A</sup>	0.6±0.06 <sup>B</sup>	0.5±0.0 <sup>B</sup>	<0.001	58.6
		Depth impact	*	**	NS	*		
	C/N	Topsoil	14.0±1.2 <sup>A</sup>	5.7±0.2 <sup>B</sup>	11.3±1.6 <sup>A</sup>	5.8±0.7 <sup>B</sup>	0.008	8.2
		Subsoil	6.7±0.7 <sup>A</sup>	5.8±0.5 <sup>A</sup>	6.6±0.6 <sup>A</sup>	5.3±0.5 <sup>A</sup>	0.45	0.9
		Depth impact	*	NS	NS	NS		
SEOM	C	Topsoil	176±15 <sup>A</sup>	129±11 <sup>B</sup>	173±16 <sup>A</sup>	180±8 <sup>A</sup>	0.005	9.7
		Subsoil	154±5 <sup>B</sup>	126±6 <sup>C</sup>	142±13 <sup>B</sup>	183±11 <sup>A</sup>	<0.001	18.4
		Depth impact	NS	NS	NS	NS		
	N	Topsoil	9.9±0.9 <sup>A</sup>	10.6±0.8 <sup>A</sup>	6.5±0.6 <sup>B</sup>	5.6±0.4 <sup>B</sup>	<0.001	36.4
		Subsoil	6.3±0.2 <sup>A</sup>	5.8±0.3 <sup>A</sup>	4.5±0.4 <sup>B</sup>	4.5±0.3 <sup>B</sup>	<0.001	19.4
		Depth impact	**	**	*	*		
	C/N	Topsoil	17.9±0.0 <sup>B</sup>	12.2±0.9 <sup>C</sup>	27.2±4.9 <sup>A</sup>	32.3±2.4 <sup>A</sup>	<0.001	30.9
		Subsoil	24.7±1.7 <sup>C</sup>	21.8±0.8 <sup>C</sup>	31.6±2.2 <sup>B</sup>	40.4±1.1 <sup>A</sup>	<0.001	80.5
		Depth impact	*	**	NS	**		

Capital letters: Significant difference among the land uses in a same soil depth with related P and F

\* and \*\* Significant difference between topsoil and subsoil in the same land use at P=0.05 and P=0.01 probability levels, respectively

Values are given as mean ± S.D.

NS: Not significant



**Table 3.4.** Comparison of the changes in the ratio of C and N in the WEOM to the SEOM before and after biodegradation

Land use	Depth (cm)	$C_W/C_S$		$N_W/N_S$	
		Before	After	Before	After
Plantation	0-20	0.172±0.043	0.127±0.033	0.221±0.006	0.189±0.014 *
Cropland		0.174±0.012	0.116±0.015 **	0.600±0.027	0.308±0.046 **
Grassland		0.072±0.002	0.053±0.003	0.184±0.081	0.093±0.052
Bog pine		0.031±0.003	0.025±0.003	0.171±0.014	0.120±0.017 *
Plantation	60-80	0.062±0.011	0.03±0.007 *	0.231±0.032	0.136±0.022 *
Cropland		0.056±0.005	0.024±0.002 **	0.382±0.023	0.199±0.007 **
Grassland		0.027±0.005	0.015±0.004 *	0.128±0.002	0.063±0.001 **
Bog pine		0.015±0.003	0.007±0.002 *	0.113±0.030	0.035±0.028 **

\* and \*\* in rows: Significant difference of the ratio between before and after biodegradation and at 0.05 and 0.01 probability levels, respectively

$C_W/C_S$  : The ratio of C in the WEOM to C in the SEOM fraction

$N_W/N_S$ : The ratio of N in the WEOM to N in the SEOM fraction

Values are given as mean ± S.D.

**Table 3.5.** Parameters of the decomposition model for the water and salt extractable organic Carbon

Pool	Elements of the equation	Depth	Land use				P	F
			Plantation	Cropland	Grassland	Bog pine		
WEOM								
Fast (100-a)	Proportion (%)	Topsoil	21.6±2.0 <sup>A</sup>	20.8±2.4 <sup>A</sup>	15.9±0.7 <sup>B</sup>	13.3±1.9 <sup>B</sup>	0.038	4.5
		Subsoil	27.9±1.9 <sup>B</sup>	50.6±3.6 <sup>A</sup>	28.1±4.1 <sup>B</sup>	41±3.8 <sup>A</sup>	0.004	9.9
		Depth effect	NS	**	*	**		
	HL <sub>1</sub> (day)	Topsoil	4.7±0.68 <sup>A</sup>	5.9±0.28 <sup>A</sup>	2.6±0.37 <sup>B</sup>	3.2±0.53 <sup>B</sup>	0.006	8.9
		Subsoil	5.6±0.62 <sup>A</sup>	3.5±0.36 <sup>B</sup>	4.9±0.0 <sup>A</sup>	6.2±0.18 <sup>A</sup>	0.005	9.7
		Depth effect	NS	**	**	**		
Slow (a)	Proportion (%)	Topsoil	78.4±2.0 <sup>A</sup>	79.2±2.4 <sup>A</sup>	84.1±0.7 <sup>A</sup>	86.7±1.9 <sup>A</sup>	0.035	4.7
		Subsoil	72.1±1.9 <sup>A</sup>	49.4±3.6 <sup>B</sup>	71.9±4.1 <sup>A</sup>	59.0±3.8 <sup>B</sup>	0.004	10.0
		Depth effect	NS	**	*	**		
	HL <sub>2</sub> (day)	Topsoil	167±17 <sup>A</sup>	141±2.0 <sup>A</sup>	171±22 <sup>A</sup>	160±10 <sup>A</sup>	0.53	0.78
		Subsoil	112±5.5 <sup>A</sup>	126±34 <sup>A</sup>	136±24 <sup>A</sup>	123±12 <sup>A</sup>	0.77	0.37
		Depth effect	*	NS	NS	NS		
SEOM								
Fast (100-a)	Proportion (%)	Topsoil	13.0±0.2 <sup>A</sup>	7.9±1.1 <sup>B</sup>	8.4±1.4 <sup>B</sup>	12.0±1.5 <sup>A</sup>	0.059	3.7
		Subsoil	13.0±0.3 <sup>A</sup>	10.8±0.9 <sup>A</sup>	7.6±1.2 <sup>B</sup>	8.1±0.7 <sup>B</sup>	0.005	9.4
		Depth effect	NS	NS	NS	NS		
	HL <sub>1</sub> (day)	Topsoil	2.4±0.1 <sup>C</sup>	11.3±2.3 <sup>A</sup>	5.4±0.69 <sup>B</sup>	7.4±0.46 <sup>B</sup>	0.006	9.2
		Subsoil	6.3±1.0 <sup>B</sup>	12.4±0.3 <sup>A</sup>	4.5±0.38 <sup>B</sup>	6.9±0.75 <sup>B</sup>	0.0002	25.7
		Depth effect	*	NS	NS	NS		
Slow (a)	Proportion (%)	Topsoil	87.0±0.2 <sup>A</sup>	92.1±1.1 <sup>A</sup>	91.6±1.4 <sup>A</sup>	88.0±1.5 <sup>A</sup>	0.056	4.6
		Subsoil	86.8±0.3 <sup>B</sup>	89.2±0.9 <sup>A</sup>	92.4±1.2 <sup>A</sup>	91.9±0.7 <sup>A</sup>	0.005	9.7
		Depth effect	NS	NS	NS	NS		
	HL <sub>2</sub> (day)	Topsoil	426±60 <sup>A</sup>	337±68 <sup>A</sup>	449±28 <sup>A</sup>	419±110 <sup>A</sup>	0.72	0.45
		Subsoil	330±37 <sup>A</sup>	249±38 <sup>B</sup>	693±129 <sup>A</sup>	767±123 <sup>A</sup>	0.026	5.3
		Depth effect	NS	NS	NS	NS		

Capital letters: Significant difference among the land uses in a same soil depth

\* and \*\* Significant difference between topsoil and subsoil in the same land use at 0.05 and 0.01 probability level, respectively.

NS: No significant difference at <0.05; HL<sub>1</sub> & <sub>2</sub>: half life of fast and slow decomposable pools

P and F: ANOVA for the effect of land use; Values are given as mean ± S.E.

**Table 3.6.** Parameters of the decomposition model for the water and salt extractable fractions of organic Nitrogen

Pool	Elements of the equation	Depth	Land use				P	F
			Plantation	Cropland	Grassland	Bog pine		
WEOM								
Fast (100-a)	Proportion (%)	Topsoil	10.7±1.4 <sup>C</sup>	30.9±2.2 <sup>A</sup>	38.2±3.9 <sup>A</sup>	20.8±0.7 <sup>B</sup>	0.0001	27.6
		Subsoil	27.6±3.7 <sup>B</sup>	30.5±1.7 <sup>B</sup>	29.5±1.5 <sup>B</sup>	40.3±2.7 <sup>A</sup>	0.029	5.1
		Depth effect	*	NS	NS	**		
	HL <sub>1</sub> (day)	Topsoil	16.8±1.4 <sup>A</sup>	9.9±1.3 <sup>B</sup>	5.9±0.8 <sup>C</sup>	5.8±0.8 <sup>C</sup>	0.0002	26.3
		Subsoil	5.9±0.5 <sup>A</sup>	5.6±0.7 <sup>A</sup>	5.6±0.9 <sup>A</sup>	8.3±0.9 <sup>A</sup>	0.128	2.6
		Depth effect	**	*	NS	NS		
Slow (a)	Proportion (%)	Topsoil	89.3±1.4 <sup>A</sup>	69.1±2.2 <sup>C</sup>	61.8±3.9 <sup>C</sup>	79.2±0.7 <sup>B</sup>	0.0001	29.5
		Subsoil	72.4±3.7 <sup>A</sup>	69.5±1.7 <sup>A</sup>	70.5±1.5 <sup>A</sup>	59.7±2.7 <sup>B</sup>	0.029	5.1
		Depth effect	*	NS	NS	**		
	HL <sub>2</sub> (day)	Topsoil	116±8.8 <sup>A</sup>	106±9.5 <sup>B</sup>	82±6.6 <sup>B</sup>	138±2.9 <sup>A</sup>	0.065	3.6
		Subsoil	95±5.3 <sup>A</sup>	95±3.9 <sup>A</sup>	86±1.4 <sup>A</sup>	62±7.0 <sup>B</sup>	0.004	10.3
		Depth effect	NS	NS	NS	*		
SEOM								
Fast (100-a)	Proportion (%)	Topsoil	35.6±3.8 <sup>A</sup>	10.9±1.1 <sup>B</sup>	12.5±4.9 <sup>B</sup>	12.8±1.7 <sup>B</sup>	0.001	13.6
		Subsoil	25.3±5.6 <sup>A</sup>	19.2±2.5 <sup>A</sup>	13.6±1.9 <sup>B</sup>	12.6±0.8 <sup>B</sup>	0.081	3.2
		Depth effect	NS	*	NS	NS		
	HL <sub>1</sub> (day)	Topsoil	4.8±0.7 <sup>B</sup>	3.1±0.1 <sup>B</sup>	4.4±0.3 <sup>B</sup>	7.8±1.0 <sup>A</sup>	0.004	10.5
		Subsoil	3.7±0.1 <sup>B</sup>	4.1±0.6 <sup>B</sup>	6.9±0.9 <sup>A</sup>	6.2±0.5 <sup>A</sup>	0.013	6.9
		Depth effect	NS	NS	NS	NS		
Slow (a)	Proportion (%)	Topsoil	64.4±3.8 <sup>B</sup>	89.1±1.1 <sup>A</sup>	87.5±4.9 <sup>A</sup>	87.2±1.7 <sup>A</sup>	0.001	13.7
		Subsoil	74.7±5.6 <sup>A</sup>	80.8±2.5 <sup>A</sup>	86.4±1.9 <sup>A</sup>	87.4±0.8 <sup>A</sup>	0.080	3.2
		Depth effect	NS	*	NS	NS		
	HL <sub>2</sub> (day)	Topsoil	419±22 <sup>A</sup>	231±17 <sup>C</sup>	178±9.7 <sup>C</sup>	348±47 <sup>B</sup>	0.001	15.3
		Subsoil	284±26 <sup>A</sup>	287±34 <sup>A</sup>	508±130 <sup>A</sup>	585±138 <sup>A</sup>	0.132	2.5
		Depth effect	**	NS	NS	NS		

Capital letters: Significant difference among the land uses in a same soil depth

\* and \*\* Significant difference between topsoil and subsoil in the same land use at 0.05 and 0.01 probability level, respectively.

NS: No significant difference at <0.05; HL<sub>1</sub> & 2: half life of fast and slow decomposable pools

P and F: ANOVA for the effect of land use; Values are given as mean ± S.E.

**Figure 3.1.** The proportion of the biodegraded C in the WEOM (a) and SEOM (b) fractions

Fig. 3.1a

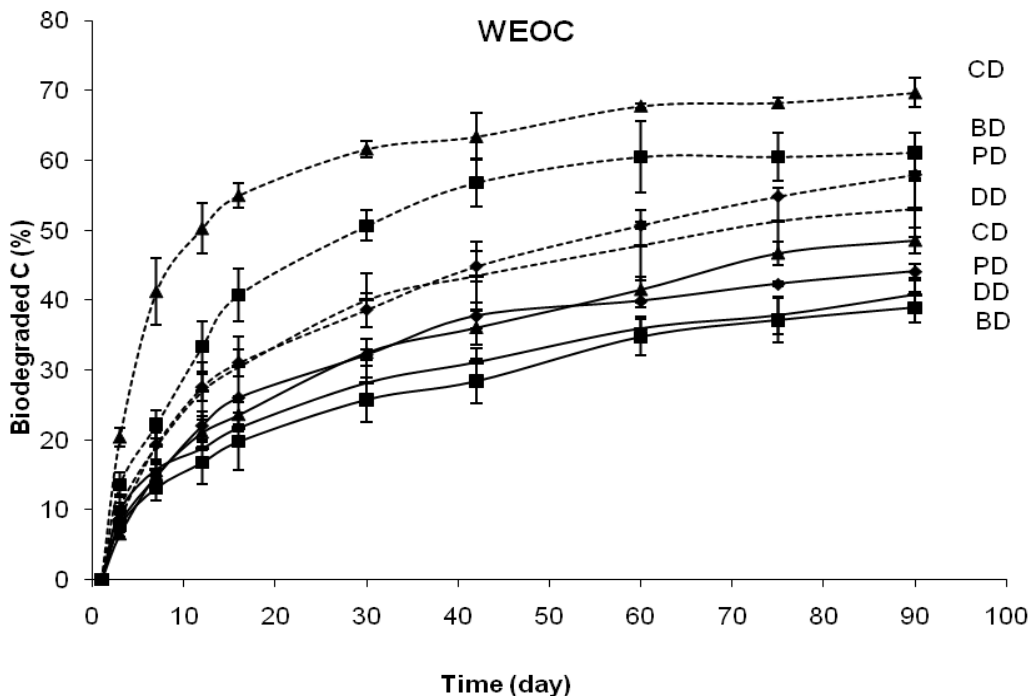
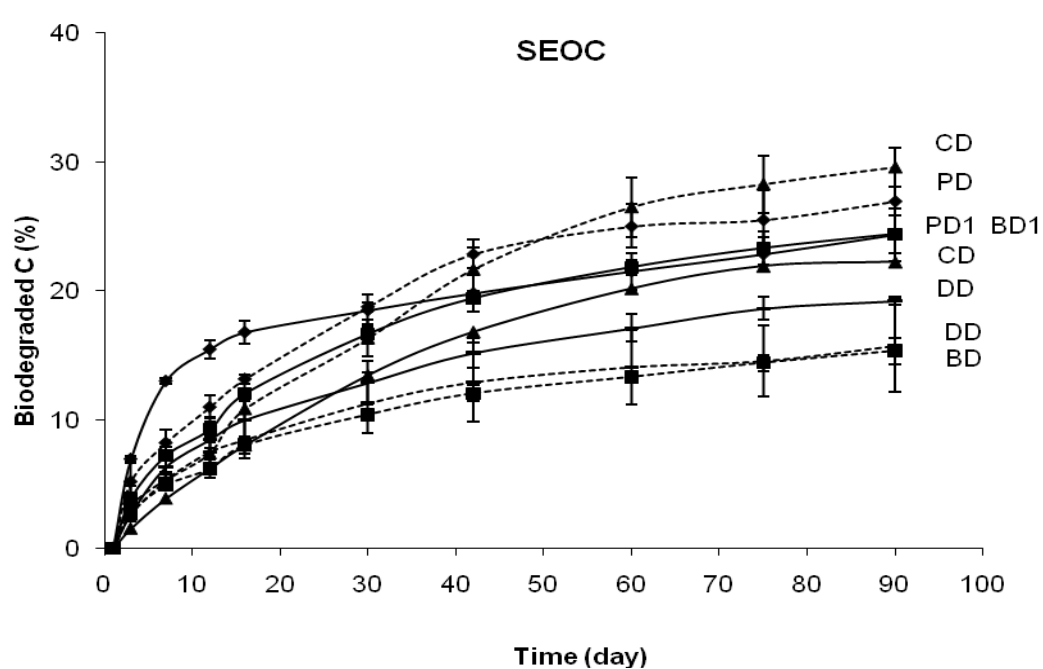


Fig. 3.1b



B: Bog pine; C: Cropland; G: Grass land; P: Plantation

D<sub>1</sub>: Depth 0-20 cm; D<sub>2</sub>: Depth 60-80 cm

Points represent the mean of three replicates. Values are given as mean  $\pm$  S.E.

**Figure 3.2.** The proportion of the biodegraded N in the WEOM (a) and SEOM (b) fractions

Fig. 3.2a

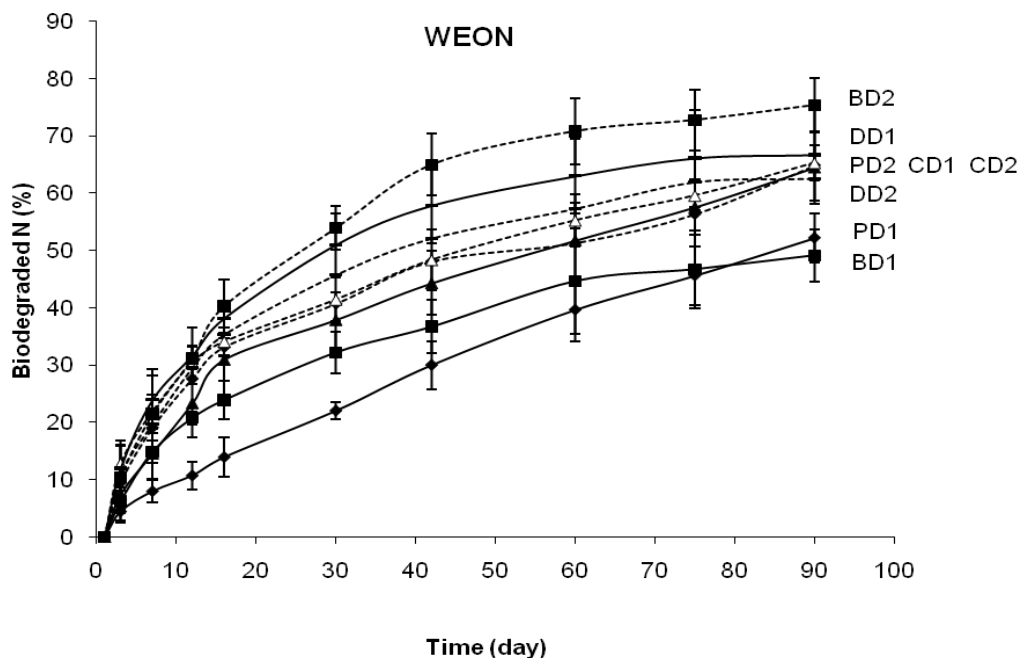
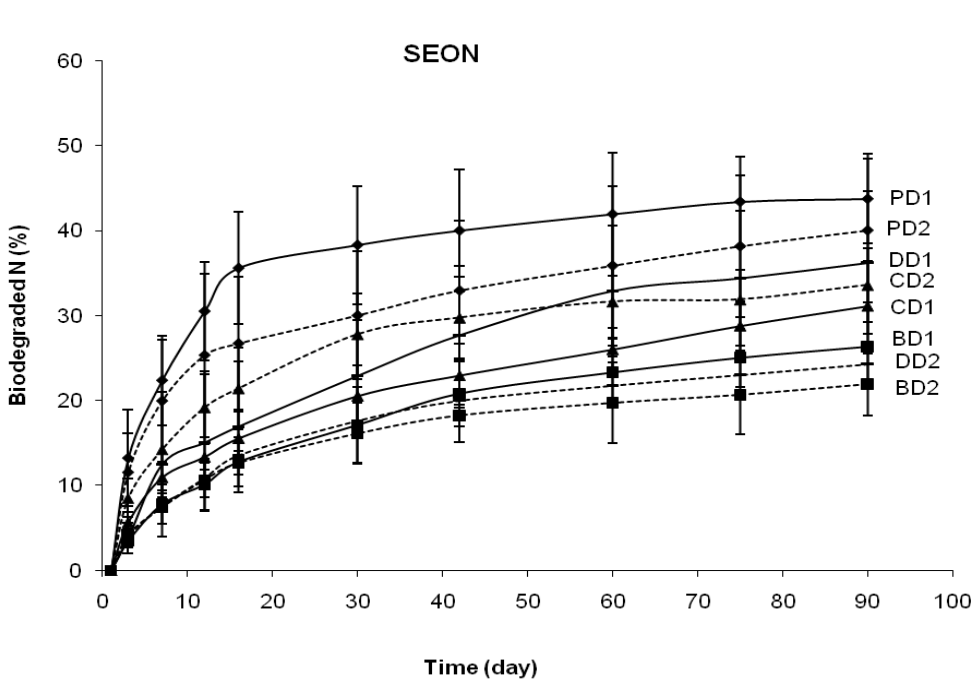


Fig. 3.2b



B: Bog pine; C: Cropland; G: Grass land; P: Plantation

D<sub>1</sub>: Depth 0-20 cm; D<sub>2</sub>: Depth 60-80 cm

Points represent the mean of three replicates. Values are given as mean  $\pm$  S.E.

**Figure 3.3.** Changes in C/N ratio of the WEOM (a) and SEOM (b) during biodegradation  
Fig. 3.3a

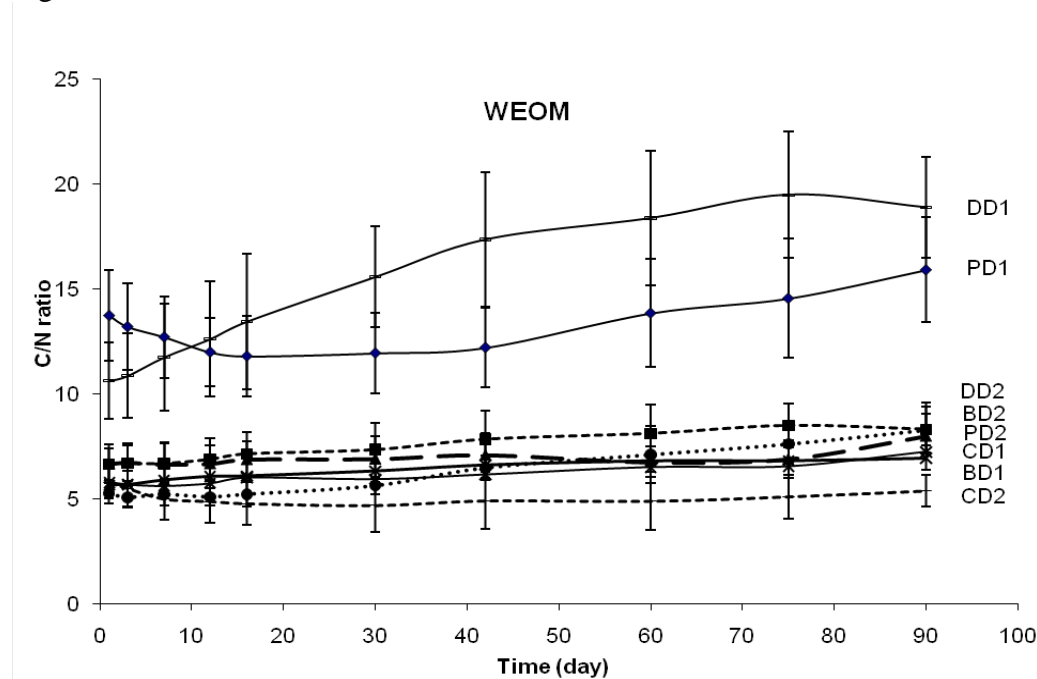
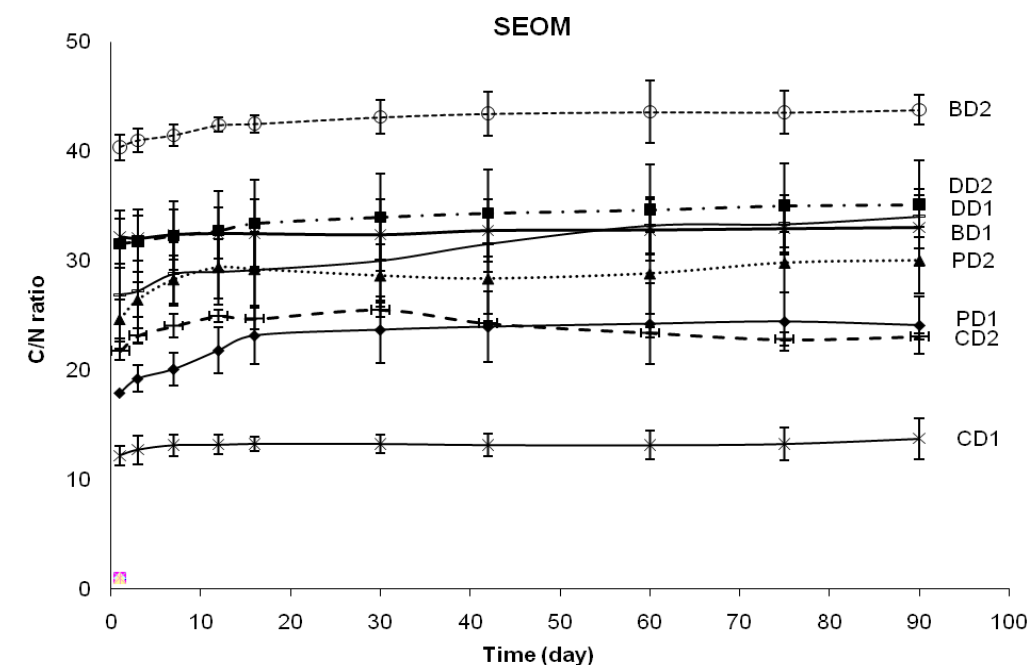


Fig. 3.3b



B: Bog pine; C: Cropland; G: Grass land; P: Plantation

D<sub>1</sub>: Depth 0-20 cm; D<sub>2</sub>: Depth 60-80 cm

Points represent the mean of three replicates. Values are given as mean  $\pm$  S.E.

**Figure 3.4:**  $K_2$  vs.  $K_1$  in the water and salt extractable organic Carbon (a) and Nitrogen (b)

Fig. 3.4a

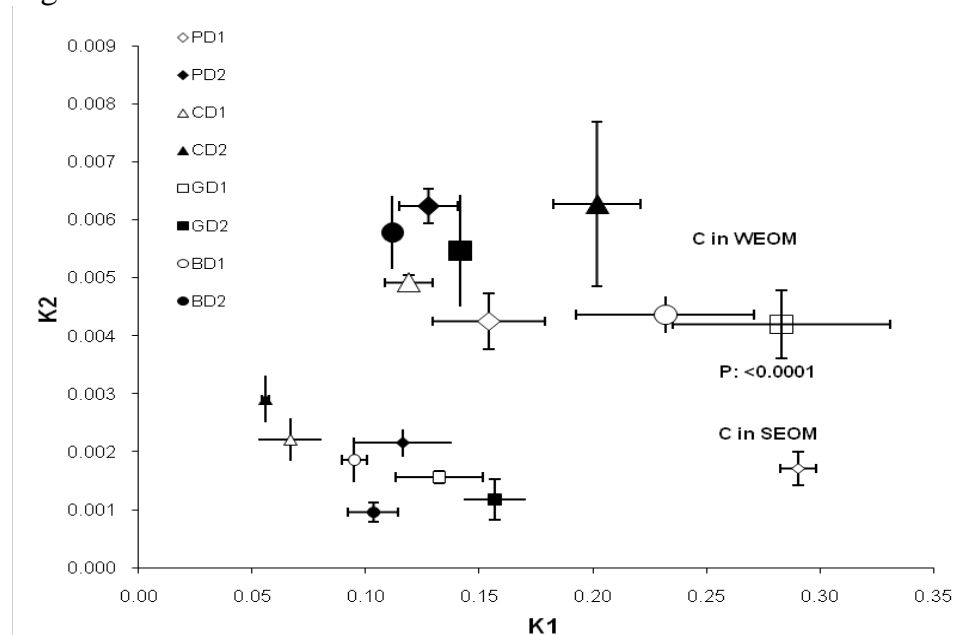
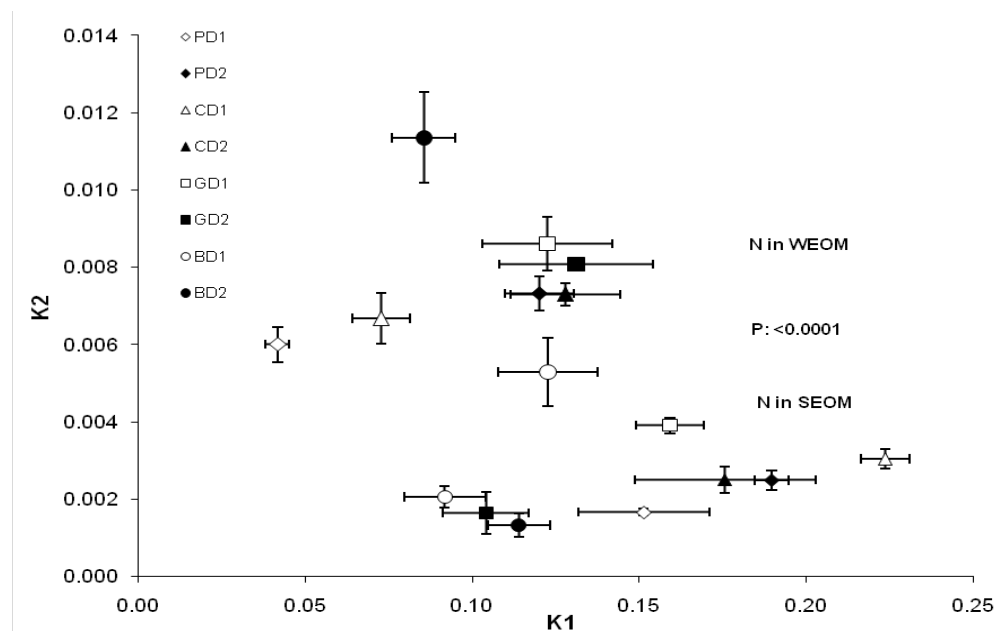


Fig. 3.4b



B: Bog pine; C: Cropland; G: Grass land; P: Plantation

D<sub>1</sub>: Depth 0-20 cm; D<sub>2</sub>: Depth 60-80 cm

Large and small legends represent the soluble and exchangeable fractions, respectively.

Values are given as mean  $\pm$  S.E.

## Chapter Four

# CHANGES IN THE UV ABSORPTION AND $\delta^{13}\text{C}$ OF THE WATER AND SALT EXTRACTABLE ORGANIC MATTER DURING BIODEGRADATION

### 4.1. Introduction

Soluble organic matter (OM) is often defined as the pool of organic matter that can pass through 0.45  $\mu\text{m}$  filter (Herbert and Bertsch, 1995). Although it represents only a small portion of the total SOM (generally  $< 0.5\%$ ), the pool of OM which exists in soluble form has been of interest in many soil and environmental studies due to its high mobility, reactivity, and the fact that it contains large amounts of labile compounds that may serve as source of energy and nutrients for microorganisms (Zsolnay, 1996; Kalbitz and Kaizer, 2003). In addition to its significance in transportation of organic and inorganic pollutants (e.g. Brumer et al., 1986; Muller et al., 2007), soluble OM appears to considerably contribute to the annual C inputs in subsoil leading to C accumulation at soil depth (Kalbitz and Kaiser, 2008).

Dissolved organic matter (DOM) and extractable organic matter (EOM) are both the soluble forms of OM in soil. While DOM is typically collected by suction cups or zero tension lysimeters *in situ* (Herbert and Bertsch, 1995), EOM is obtained in laboratory conditions and its application is more common in studies carried out in cultivated lands where soils are frequently disturbed due to management activities (Chantigny, 2003). The amount and properties of EOM can be considerably influenced by experimental conditions e.g. extractant, the ratio of soil to extractant, temperature, etc. (Herbert and Bertsch, 1995; Zsolnay, 1996). Accordingly, obtaining EOM in a number of laboratory conditions has led to inconsistency in the reported results for the amounts and properties of the EOM. Recently, Jones and Willet (2006) suggested the application of dilute (0.05 M  $\text{CaCl}_2$ ) and concentrated (0.5 M  $\text{K}_2\text{SO}_4$ ) aqueous extractants to reflect the properties of the OM existing in the soil solution and exchangeable sites, respectively. It



appears that such a fractionation procedure for obtaining EOM can help to address some of the conflicting results reported for the dynamics and properties of EOM.

Biodegradation of DOM/EOM is the process which yields the release of nutrients and energy for soil microbial community (Metting, 1993). Regarding the roles of DOM/EOM in pollutant transformation/reactions, the biodegradation process also affects dynamics of the fate of pollutants within vadose zone (Mullet et al., 2007). Carbon is the main constituent of DOM/EOM and its dynamics have been frequently assessed during biodegradation experiments. Such studies have revealed that although DOM/EOM is a continuum of a variety of organic substances, its biodegradation is carried out in a fast followed by a slow decomposition step (e.g. Qualls and Haynes, 1992; McDowell et al., 2006). Such experiments however, do not reveal information about dynamics of the constituent components of DOM/EOM during biodegradation. Spectroscopic approaches have been suggested to improve our understanding of the biodegradation processes by indirect characterization of organic constituents (Klabitz et al., 2003). These methods do not reveal information about the structure or amount of each of the single components of organic matter; yet, there is a strong relationship between i) certain area/s of the spectrum (e.g. in NMR or FT-IR) or ii) specific wavelengths (e.g. in UV or fluorescence spectroscopy) with particular functional groups related to specific organic compounds (Stevenson, 1994).

UV absorption is a fast, simple, inexpensive, and more importantly, a sensitive method for evaluation of some of the DOM/EOM properties (Abbt-Braun et al., 2004). Comparison of the results obtained by  $^{13}\text{C}$ NMR and UV absorption has indicated that there is a strong correlation ( $R^2=0.97$ ) between specific UV absorbance (normalized by DOC concentration) and the content of aromatic compounds of DOM/EOM (e.g. Weishaar et al., 2003; Leenheer and Croue, 2003). Based on this property, UV absorption have been extensively used for characterization of DOM/EOM to reflect the properties of DOM/EOM that are related to its fingerprint (Hur et al., 2006), metal bindings (Van Schaik et al., 2010), and biodegradation (Klabitz et al., 2003).

Isotope ratio mass spectroscopy (IR-MS) has been widely employed to address the turnover and the land use related dynamics of SOM (Glaser, 2005). This technique is based on the higher kinetic energy of lighter isotopes (e.g.  $^{12}\text{C}$  vs.  $^{13}\text{C}$ ), and the different values of  $\delta^{13}\text{C}$  among different classes of compounds during biochemical processes. Thus, analysis of  $^{13}\text{C}$  natural abundance may not only reveal some of structural changes during DOM/EOM biodegradation (Lichtfouse, 2000), but also provides an approximation of the degree of biodegradation of OM (Balesdent and Mariotti, 1996). Such information, when combined with spectroscopic findings, can expand our knowledge of the process of microbial transformation of DOM/EOM.

In this study we assumed that developing a fractionation procedure may help to separate two fractions of EOM with different  $\delta^{13}\text{C}$  and aromaticity. Therefore, the objectives of this chapter are to combine UV absorbance and  $\delta^{13}\text{C}$  approaches to evaluate i) the differences of aromaticity and  $\delta^{13}\text{C}$  in the water and salt EOM obtained from different land uses and soil depths and, ii) changes of these properties during the biodegradation of EOM.

## **4.2. Materials and methods**

### **4.2.1. Site and soil sampling**

Four land uses were selected from the Mackenzie Basin, Canterbury, New Zealand (44°S, 170°E). At all sites, the current vegetation cover had been on the site for at least 10 years. Cropland (*Medicago sativa*) and plantation forest (*Pinus nigra*) represent two different types of land improvement. While the plantation site is dominated by fast-growing exotic tree species planted in the degraded grass land, the cropland involves conventional agricultural management activities in the same site. The Bog pine (*Halocarpus bidwilli*) woodland is a remnant of the pre-human vegetation that is assumed to have occurred widely across the Mackenzie Basin. In turn, the degraded tussock grassland (*Festuca novaezelandia* and *Heiracium pilosella*) is typical of unimproved vegetation in the area induced by historic burning of the woodland followed by intense grazing. Soils are categorized as Orthic Brown (Andic Ustochrept) Pukaki fine sandy

loam based on New Zealand soil classification system. Soil samples were collected in December 2007.

Samples were taken from the topsoil (0-20 cm) and subsoil (60-80 cm) at three separate locations at each site using 10 cm diameter stainless steel auger. Samples were transferred in an icebox to the laboratory within 24 hours. The soil samples were mixed well followed by sieving (2 mm) and removal of visible roots and litter. All the soil samples were adjusted to 60% of water holding capacity before obtaining EOM. This helps to avoid the differences in the quality and quantity of EOM obtained from field-moist samples with different initial amounts of moisture content (Zsolnay, 2003). For details on sampling and sample preparation see appendices A and B.

#### 4.2.2. Sample preparation

We defined a weak and harsh extraction procedure (modified from Zsolnay, 2003; Jones and Willet, 2006) as follows for obtaining the water and salt extractable fractions of OM, respectively.

The WEOM was obtained by shaking the soil with 10 mM  $\text{CaCl}_2$  in horizontal shaker (110 rpm, 15 min) at room temperature with a soil-to-extractant ratio of 1:2 (w/v). The extracts were then centrifuged (15 min, 4500 rpm) followed by microfiltration (0.45  $\mu\text{m}$ ).

The SEOM was collected by extracting the soil with 0.5 M  $\text{K}_2\text{SO}_4$  at 1:1 soil-to-extractant ratio (w:v) in 50 ml centrifuge tubes. The tubes were then placed in a water bath at 75°C for two hours followed by shaking (360 rpm) for 60 minutes at room temperature. The samples were then centrifuged at 4800 rpm (20 min) to accelerate subsequent filtration through pre-washed 0.45  $\mu\text{m}$  cellulose nitrate filters. The samples were stored at 4°C before analysis (<5d).

#### 4.2.3. Biodegradation assay

The biodegradable experiment was carried out in a 90-day incubation experiment in aerobic conditions. Details of the experiment are given in Toosi et al., (2011). Briefly,

150 ml of each solution was transferred into 250 ml wide-mouth containers. The solution containing SEOM was diluted 50 times with de-ionized water to reduce the high osmotic pressure and C concentration of the extract solution to more closely match with that of the WEOM solutions. To make sure of the optimum size of decomposer community a microbial inoculum (modified by Gregorich et al., 2003) was added to the samples at the beginning and after 42 days of the experiment. The blank was prepared by adding the same amount of inoculum to ultra pure water. The amount of C and N in the inoculated blank was not detectable. An inoculated glucose solution (20 mg C l<sup>-1</sup>) was used to check the activity of microorganisms (Kalbitz et al., 2003). Inoculated containers were sealed and placed in dark at room temperature during the experiment. All containers were shaken manually and opened daily in the first two weeks and less regular for the remainder of the experiment. The subsamples were removed at 1, 7, 42, and 90 days, filtered (0.22 µm), and analyzed for dissolved C, UV absorption and <sup>13</sup>C natural abundance as follows.

#### 4.2.4. Analysis

The extractable OM solutions were analysed for organic C and total N (TN) in duplicate with a TOC-TN analyzer (Apollo 9000 TOC/TN, Hewlett Packard, USA). Organic C was measured by quantifying the CO<sub>2</sub> (after acidification) using the high catalytic oxidation (680°C) method.

*Specific UV absorption (SUVA).* The filtered (0.22µm) EOM subsamples were analyzed for UV absorbance at 254 nm (room temperature) using UV spectrophotometer (Varian Co., Cary 50 Probe, Au). Samples with UV absorbance ≥0.3 cm<sup>-1</sup> were diluted to ensure the comparability of the results (Embacher et al., 2007). Specific UV absorption (SUVA) was then calculated after normalizing as follow:

$$\text{SUVA} = 100 * \text{UV}_{\text{abs}} / \text{DOC}$$

Where

SUVA<sub>(L mg<sup>-1</sup> C m<sup>-1</sup>)</sub> = Specific Ultra Violet Absorption

UV<sub>abs (cm<sup>-1</sup>)</sub> = UV absorption at 254 nm

DOC<sub>(mg L<sup>-1</sup>)</sub> = Carbon concentration in EOM solutions

The calculated SUVA<sub>254</sub> has been shown to be strongly correlated with the presence of aromatic compounds in soluble OM (Weishaar et al., 2003).

*<sup>13</sup>C natural abundance.* The ground freeze-dried subsamples of EOM were weighed into tin capsules and loaded into a PDZ Europa GSL elemental analyzer (Cheshire, UK). The CO<sub>2</sub> produced was separated by a GC column linked to a PDZ Europa 20-20 isotope ratio mass spectrometer (Sercon Ltd., Cheshire, UK) to determine <sup>13</sup>C/<sup>12</sup>C ratio. The samples were analysed in duplicate with analytical errors less than 0.1‰ between replicates. Each run of samples included working DOC standards prepared with EDTA of known enrichment in order to ensure accuracy within and between runs. The EDTA had been normalized to V-PDB against the international standard IAEA-CH-6. <sup>13</sup>C natural abundance (δ<sup>13</sup>C) value (‰) expresses the enrichment of <sup>13</sup>C in the samples relative to the <sup>13</sup>C content of CO<sub>2</sub> prepared from a calcareous belemnite of the cretaceous Peedee formation, South Carolina.

#### 4.2.5. Statistical analysis

To evaluate the impact of soil depth on δ<sup>13</sup>C in the water and salt extractable OM, and δ<sup>13</sup>C changes during biodegradation of EOM, the data were analysed by analysis of variance (ANOVA) and student test, respectively, using SAS (9.2) statistical software (SAS Inc.,). The relationship between the biodegraded C and N (%) and increase in SUVA, or <sup>13</sup>C depletion was also estimated by regression coefficient.

### 4.3. Results

#### 4.3.1. SUVA

The initial SUVA value in the WEOM samples varied from 0.55 to 2.6 L/mg<sup>-1</sup>C m<sup>-1</sup> (mean 1.39±0.63 L mg<sup>-1</sup>C m<sup>-1</sup>) (Table 4.1). SUVA increased substantially during the biodegradation period in the WEOM (mean 2.6±0.71 times) (Fig. 4.1.a). During the biodegradation period, SUVA value increased initially but this was followed by with a relatively stable trend for the rest of the period. SUVA value increased from 54%

(plantation, topsoil) to 273% (bog pine (subsoil) in the WEOM (Fig. 4.1.a). At the end of the experiment, the smallest and greatest amount of aromaticity ( $0.85$  and  $5.5 \text{ L mg}^{-1}\text{C m}^{-1}$ , respectively) was observed in the WEOM obtained from plantation (topsoil) and cropland (topsoil), respectively.

SUVA value of the SEOM was similar to that of the WEOM (Fig. 4.1.b). Although the initial SUVA value of SEOM ( $1.3\text{-}3.0$ ; mean  $1.71 \pm 0.56 \text{ L mg}^{-1}\text{C m}^{-1}$ ) was larger than WEOM ( $0.55\text{-}2.6 \text{ L mg}^{-1}\text{C m}^{-1}$ ) (Table 4.1), SUVA value of SEOM after 90d of incubation ( $1.7\text{-}4.3$ ; mean  $2.89 \pm 0.86 \text{ L mg}^{-1}\text{C m}^{-1}$ ) was less than that of WEOM solutions ( $0.85\text{-}5.5$ ; mean  $3.7 \pm 1.7 \text{ L mg}^{-1}\text{C m}^{-1}$ ). Therefore, the proportion of SUVA increase in the SEOM (Fig. 4.2.b) was considerably less than that in the WEOM solutions (mean  $72\% \pm 29$  vs.  $167\% \pm 72$ ) (Fig. 4.1.a and b). The sequence of the proportion of SUVA value increase in the WEOM ( $\text{BD}_2 > \text{CD}_2 > \text{GD}_2 > \text{PD}_2 > \text{GD}_1 > \text{CD}_1 > \text{BD}_1 > \text{PD}_1$ ) was different with that in the SEOM ( $\text{PD}_2 > \text{CD}_2 > \text{BD}_1 > \text{PD}_1 > \text{GD}_1 > \text{GD}_2 > \text{CD}_1 > \text{BD}_2$ ) (Fig. 4.1.a and b). Thus, changes in the aromaticity of the samples did not follow a similar pattern between the fractions either in terms of land use or soil depth.

We observed a relatively strong relationship between the proportion of the biodegraded C (%) and SUVA increase (%) in the WEOM ( $R^2=0.66$ ) and specifically SEOM ( $R^2=0.74$ ) fractions (Figs. 4.2a and b, respectively). Furthermore, there was a strong relationship between the proportion of the total biodegraded N (%) with the increase in SUVA (%) in WEOM ( $R^2=0.74$ ) (Fig. 4.3.a). This relationship was however, far less for SEOM ( $R^2=0.38$ ) (Fig. 4.3.b).

#### 4.3.2. $^{13}\text{C}$ natural abundance

The  $\delta^{13}\text{C}$  of the samples varied from  $-24.15$  to  $-26.05\text{‰}$  and  $-27.74$  to  $-32.03\text{‰}$  in WEOM and SEOM, respectively (Fig. 4.4). Comparison of the  $\delta^{13}\text{C}$  in WEOM and SEOM showed that  $\delta^{13}\text{C}$  value in SEOM is significantly ( $P > t$ :  $<0.001$ ) less than that in WEOM (Fig. 4.4). Although  $\delta^{13}\text{C}$  value in the SEOM samples obtained from subsoil was significantly less than those sampled from topsoil, this was observed only in the cropland and grassland in WEOM (Table 4.2).

$\delta^{13}\text{C}$  values of EOM decreased during the biodegradation period (Fig. 4.5.) but this was significant in only some of the samples. The larger proportion of decrease in  $\delta^{13}\text{C}$  was observed in WEOM than SEOM (Fig. 4.5). In comparison with WEOM, the significant depletion of  $\delta^{13}\text{C}$  was observed in only SEOM sampled from plantation site (Fig. 4.5). This seems to be related with the less biodegradable C content of SEOM than WEOM (Fig. 4.6). Comparison of the biodegraded C (Fig. 4.6) and  $\delta^{13}\text{C}$  depletion (Fig. 4.5) suggests that the larger amounts of C loss during biodegradation in the samples (WEOM obtained from subsoil) was consistent with the significant decrease in  $\delta^{13}\text{C}$ . Figure 4.7 indicates that there is a relatively good correlation ( $R^2=0.41$ ) between the decrease in  $\delta^{13}\text{C}$  of EOM and Carbon loss of the samples during the biodegradation.

#### 4.4. Discussion

##### 4.4.1. UV absorption

The range of initial SUVA value was similar in the WEOM ( $0.55\text{--}2.6 \text{ L mg}^{-1}\text{C m}^{-1}$ ) and SEOM ( $1.4\text{--}3.0 \text{ L mg}^{-1}\text{C m}^{-1}$ ) and in accordance with the range reported for SUVA value in the literature (e.g. Kalbitz et al., 2003; Michel et al., 2006). Based on the model suggested by Weishaar et al (2003), the range in SUVA value corresponds to the presence of 7-20% and 11-26% aromatic compounds in the WEOM and SEOM, respectively. SUVA value has been shown to be strongly correlated with the aromatic content of DOM (Linheer et al., 2003) and the degree of the oxidation of lignin-derived compounds (Michel et al., 2006). Thus, the larger initial SUVA of SEOM (mean  $1.7\pm0.3 \text{ L mg}^{-1}\text{C m}^{-1}$ ) than WEOM ( $1.4\pm0.6, \text{ L mg}^{-1}\text{C m}^{-1}$ ) appears to be related with a greater content of humic-like constituents. This can also be supported by the relatively wide C/N ratio of the SEOM (Table 4.3). The SUVA value seems to be affected by the obtaining procedure (DOM *vs.* WEOM) (Michel et al., 2006) and possibly the source of DOM/EOM (aquatic *vs.* soil sourced DOM).

The pattern of change in SUVA value during biodegradation assay was similar in both fractions of EOM (Fig. 4.1.a and b) and similar to the observed trend for C biodegradation (Fig. 4.1.a and b). Similar to the proportion of the biodegraded C in

WEOM and SEOM, the proportion of SUVA value increase was also larger in WEOM than SEOM ( $72\% \pm 29$  vs.  $167\% \pm 72$ ). This suggests that the increase in SUVA value is well correlated with the proportion of biodegradable OM in each of the fractions of EOM.

Despite the relatively strong relationship reported between initial SUVA value and biodegradability of DOM/WEOM (e.g. Fellman et al., 2008), we did not observe such a relationship ( $R^2 < 0.01$ ). Instead, our results indicated an overall strong correlation between the amount of the biodegraded OM of both WEOM and SEOM (Figs. 4.2 and 4.3 a and b) and SUVA value increase. This implies that the microbial decomposition of organic substances existing in EOM occurs along with an increase in the aromaticity of WEOM and SEOM despite their relatively constant C/N ratio during biodegradation period (Fig. 4.3.a. and b).

The proportion of SUVA value increase in SEOM did not show a consistent trend with either land use or soil depth. However, the larger increase of SUVA in WEOM in subsoil (Fig. 4.1 a) was in line with the larger biodegradability of WEOC in samples obtained from subsoil (Fig. 4.6).

SUVA values in soluble OM have been reported to decrease (Hagedorn et al., 2000; Hassouna et al., 2010) or remain stable (Kalbitz, 2001; Corvasce et al., 2006) from topsoil down to subsoil. This has been attributed to the capacity of soil mineral particles for the adsorption of the aromatic components of soluble OM (Kalbitz, 2001). Our results suggest that SUVA values may increase or decrease from topsoil down to subsoil, depending on land use (Table 4.1). Regarding the similar soil properties of all the land uses (see chapter 3), this seems to be related with the properties of the EOM obtained from different land use and the presence/absence of the in-situ produced simple, low aromatic compounds (amino acids, amino sugars and carbohydrates, root exudates; Jones et al., 2005) at soil depth.

Although SUVA value, specifically at 254 or 280 nm has been commonly used as a relatively simple way to distinguish different DOM properties (e.g. Michel et al., 2006; Fellman et al., 2008), other UV absorption approaches, mainly the ratio of absorbance at



two different wavelengths) have also been used in DOM characterization studies. For instance, i) the absorbance ratio of 465–665 nm (i.e., the E4/E6 ratio) as an inverse indicator of DOM size (You et al., 1999) and the degree of condensation of DOM aromatic groups (i.e., humification) (Chin et al., 1994; Stevenson, 1994); ii) the ratio of  $\text{Abs}_{254}/\text{Abs}_{436}$  to estimate the relative composition of autochthonous versus terrestrial DOM (Battin, 1998; Jaffe et al., 2004) and, iii) the ratio of UV absorbance in the range 251–256 to that in the range 202–205 nm as an indicator of the relative proportion of unsaturated to saturated (or aromatic to aliphatic) moieties present in DOM (Korshin et al., 1997). Using a number of UV absorption points and ratios, Hur et al. (2006) suggested that  $\text{SUVA}_{254}$  could be considered to be the most reliable zero-order UV–visible absorption index to discriminate DOM composition. However, one major advantage of using ratios of UV absorbance (e.g.  $\text{E}_{254}/\text{E}_{204}$ ) over calculated SUVA (normalized by DOC) is the fact that UV ratio approach does not require an independent measurement of the DOC concentration. This advantage could possibly override the benefit of having more precision with the SUVA index, in particular when trying to make real-time, in-situ field applications (Hur et al., 2006).

#### 4.4.2. $^{13}\text{C}$ natural abundance

The  $\delta^{13}\text{C}$  values of the EOM samples varied from -23.7‰ to -32.0‰, a  $^{13}\text{C}$  signature that represents organic material derived from C3 plants with signatures ranging from -40 to -20‰ (Staddon, 2004). The considerable differences between  $\delta^{13}\text{C}$  of WEOM and particularly SEOM obtained from topsoil and subsoil in most samples (Table 4.2) suggest that the dynamics of these two fractions in topsoil and subsoil are controlled by different mechanisms.  $\delta^{13}\text{C}$  of DOM has been observed to increase (Kaiser et al., 2001) or decrease (Ludwig et al., 2000; Schiff et al., 1990) with soil depth. This has been attributed to the i) preferential decomposition of labile compounds and/or ii) preferential sorption of naturally depleted compounds (e.g. lignin) (Ludwig et al., 2000; Kaiser et al., 2001). Potentially labile compounds such as carbohydrates (e.g. cellulose, hemicelluloses) and amino acids are enriched in  $^{13}\text{C}$  compared with the bulk organic

matter (Macko and Estep, 1984; Benner et al., 1987; Schleser et al., 1999). Thus, their microbial decomposition leads to a relative enrichment of  $^{13}\text{C}$ -depleted compounds in the organic matter (Agren et al., 1996). On the other hand, lignin-derived aromatic acid and phenol constituents of soil solution are preferentially absorbed onto mineral surfaces (McKnight et al. 1992; Kaiser et al. 1997). Lignin and lipids are depleted in  $^{13}\text{C}$ , whereas carboxyl groups are strongly enriched in  $^{13}\text{C}$  (Macko and Estep, 1984; Benner et al. 1987). Accordingly,  $\delta^{13}\text{C}$  of the DOM/EOM may increase or decrease during its passage through soil profile, depending on its compositional change of DOM/WEOM due to the sorption and biodegradation (Schiff et al. 1990; Trumbore et al. 1992; Amelung et al. 1999).

Given the low moisture content of the soil at the time of sampling (7.8-12.9% of WHC), the downward movement of the freshly decomposed soluble organic compounds (rich in  $^{13}\text{C}$ ) does not appear to be a major source of enriched  $\delta^{13}\text{C}$  WEOM at soil depth. Instead, we assumed that the increase (depletion) in  $\delta^{13}\text{C}$  of WEOM in cropland and grassland in topsoil compared with that in subsoil may be due to the presence of labile root exudates (rich in carbohydrates and amino acids) and related microbial products. The significantly higher  $\delta^{13}\text{C}$  in the WEOM obtained from subsoil (cropland and grass land) than that in topsoil along with the considerable decrease in C content of WEOM (Table 4.3.) suggest that microbial decomposition has particularly contributed to the loss of dissolved organic C (through release of  $\text{CO}_2$ ) with increasing depth because aerobic metabolic processes preserve  $^{13}\text{C}$  (in biomass) and release  $^{12}\text{C}$  via the evolution of  $\text{CO}_2$  (Blair et al., 1985; Kaiser et al., 2001), resulting in  $^{13}\text{C}$  enrichment in the microbial products.

The highly  $^{13}\text{C}$  depleted SEOM compared with WEOM suggests that this fractions originates from the native soil OM instead of fresh biomass input (plant/microbial products). This can be supported by its higher C/N (Table 4.3.) ratio and SUVA value (Table 4.1) in comparison with WEOM. On the other hand, the similar concentration of SEOC in topsoil and subsoil (Table 4.3) in addition to the considerably larger  $\delta^{13}\text{C}$  values of SEOM obtained from subsoil than that of topsoil in all land uses (Table 4.2.) along

with the reported higher  $^{13}\text{C}$  depletion of SOM at soil depth (e.g. Weimeier et al., 2009), support the hypothesis that WEOM and SEOM originate from different sources of OM in soil.

The significant depletion of  $^{13}\text{C}$  in the WEOM obtained from subsoil (Fig. 4.5) was in accordance with the relatively larger C loss of these samples (Fig. 4.6) during the biodegradation period. The relatively larger biodegradability of SEOM obtained from the plantation site at both soil depths (Fig. 4.6) was along with their significant  $^{13}\text{C}$  depletion (Fig. 4.5). The correlation ( $R^2=0.41$ ) between the C loss and  $^{13}\text{C}$  depletion (Fig. 4.7) supports the previous findings (e.g. Raymond and Bauer, 2001) indicating that the preferential microbial utilization of more biodegradable compounds (e.g. amino acids, carbohydrates, ...) can be safely traced by isotope signature changes during biodegradation period. Using spectroscopic approaches ( $^1\text{H}$  NMR and FT-IR), Kalbitz et al. (2003) concluded that the increase in  $\delta^{13}\text{C}$  during the biodegradation of a relatively wide range of DOM/WEOM obtained from forest and agricultural soils is in line with an increase in lignin and a decrease in carbohydrate compounds. The observed decrease in  $\delta^{13}\text{C}$  in SEOM extracted from Bog pine soil (subsoil) can be attributed to the possible larger amount of microbial biomass and their by-products (rich in  $^{13}\text{C}$ ) which compensated the enrichment of lignin degradation products and thus  $^{13}\text{C}$  depletion (Kalbitz et al., 2003)

#### 4.5. Conclusion

Spectroscopic approaches have been used as an appropriate approach assisting researchers to interpret the findings of fractionation and biodegradation experiments. In our study, we observed that application of UV absorbance and  $^{13}\text{C}$  natural abundance supported our hypothesis that EOM can be operationally separated into two fractions. These fractions have relatively different content of aromatic constituents (as shown by SUVA value) and more likely originate from different pools (e.g. fresh vs. native) of OM in soil (as shown by  $\delta^{13}\text{C}$ ). Comparison of the  $\delta^{13}\text{C}$  values in topsoil and subsoil suggested that the two fractions of EOM; specifically SEOM; are controlled by different mechanisms in the topsoil and subsoil. Along with the findings of the previous studies,

we observed a larger proportion of aromatic compounds (shown by SUVA value) and the depletion of  $^{13}\text{C}$  during the biodegradation of EOM. Both approaches, specifically SUVA values showed a relatively strong correlation with the proportion of C and N loss during the biodegradation assay. However, given that SUVA determination is rather easier, faster and far less expensive than isotopic approach, the UV approach is probably the preferred method as a common laboratory method when assessing DOM biodegradation.

**Table 4.1.** The initial value of  $\text{SUVA}_{254}$  ( $\text{L mg}^{-1}\text{C m}^{-1}$ ) in the water and salt extractable OM

EOM fractions	Land use							
	Plantation		Cropland		Grassland		Bog pine	
	topsoil	subsoil	topsoil	subsoil	topsoil	subsoil	topsoil	subsoil
WEOM	0.55	0.83	2.59	1.50	1.03	1.64	1.66	1.30
SEOM	1.43	1.67	2.97	1.30	1.90	1.13	1.63	1.63

**Table 4.2.** The probability ( $\text{Pr}>\text{F}$ ) of the impact of soil depth (0-20 *vs.* 60-80 cm) on the  $^{13}\text{C}$  abundance of the WEOM and SEOM in different land uses

fraction	Land use			
	plantation	Cropland	Grassland	Bog pine
WEOM	0.260	<0.001	0.025	0.943
SEOM	0.004	<0.001	<0.001	<0.001

**Table 4.3.** The effect of land use and depth on the concentration of Carbon and Nitrogen ( $\text{mg kg}^{-1}$ ) and C/N ratio of the water and salt extractable OM

Fraction	Element	Depth	Land use				P	F
			Plantation	Cropland	Grassland	Bog pine		
WEOM	C	Topsoil	29.9±2.7 <sup>A</sup>	22.5±1.2 <sup>A</sup>	12.4±0.7 <sup>B</sup>	5.6±0.4 <sup>C</sup>	<0.001	35.1
		Subsoil	9.7±1.0 <sup>A</sup>	7.0±0.5 <sup>B</sup>	3.8±0.5 <sup>C</sup>	2.7±0.2 <sup>C</sup>	<0.001	18.7
		Depth impact	**	**	**	**		
	N	Topsoil	2.2±0.2 <sup>B</sup>	3.9±0.5 <sup>A</sup>	1.2±0.2 <sup>C</sup>	1.0±0.12 <sup>C</sup>	<0.001	70.3
		Subsoil	1.5±0.2 <sup>A</sup>	1.2±0.2 <sup>A</sup>	0.6±0.06 <sup>B</sup>	0.5±0.0 <sup>B</sup>	<0.001	58.6
		Depth impact	*	**	NS	*		
	C/N	Topsoil	14.0±1.2 <sup>A</sup>	5.7±0.2 <sup>B</sup>	11.3±1.6 <sup>A</sup>	5.8±0.7 <sup>B</sup>	0.008	8.2
		Subsoil	6.7±0.7 <sup>A</sup>	5.8±0.5 <sup>A</sup>	6.6±0.6 <sup>A</sup>	5.3±0.5 <sup>A</sup>	0.45	0.9
		Depth impact	*	NS	NS	NS		
SEOM	C	Topsoil	176±15 <sup>A</sup>	129±11 <sup>B</sup>	173±16 <sup>A</sup>	180±8 <sup>A</sup>	0.005	9.7
		Subsoil	154±5 <sup>B</sup>	126±6 <sup>C</sup>	142±13 <sup>B</sup>	183±11 <sup>A</sup>	<0.001	18.4
		Depth impact	NS	NS	NS	NS		
	N	Topsoil	9.9±0.9 <sup>A</sup>	10.6±0.8 <sup>A</sup>	6.5±0.6 <sup>B</sup>	5.6±0.4 <sup>B</sup>	<0.001	36.4
		Subsoil	6.3±0.2 <sup>A</sup>	5.8±0.3 <sup>A</sup>	4.5±0.4 <sup>B</sup>	4.5±0.3 <sup>B</sup>	<0.001	19.4
		Depth impact	**	**	*	*		
	C/N	Topsoil	17.9±0.0 <sup>B</sup>	12.2±0.9 <sup>C</sup>	27.2±4.9 <sup>A</sup>	32.3±2.4 <sup>A</sup>	<0.001	30.9
		Subsoil	24.7±1.7 <sup>C</sup>	21.8±0.8 <sup>C</sup>	31.6±2.2 <sup>B</sup>	40.4±1.1 <sup>A</sup>	<0.001	80.5
		Depth impact	*	**	NS	**		

Capital letters: Significant difference among the land uses in topsoil or subsoil with their related P and F

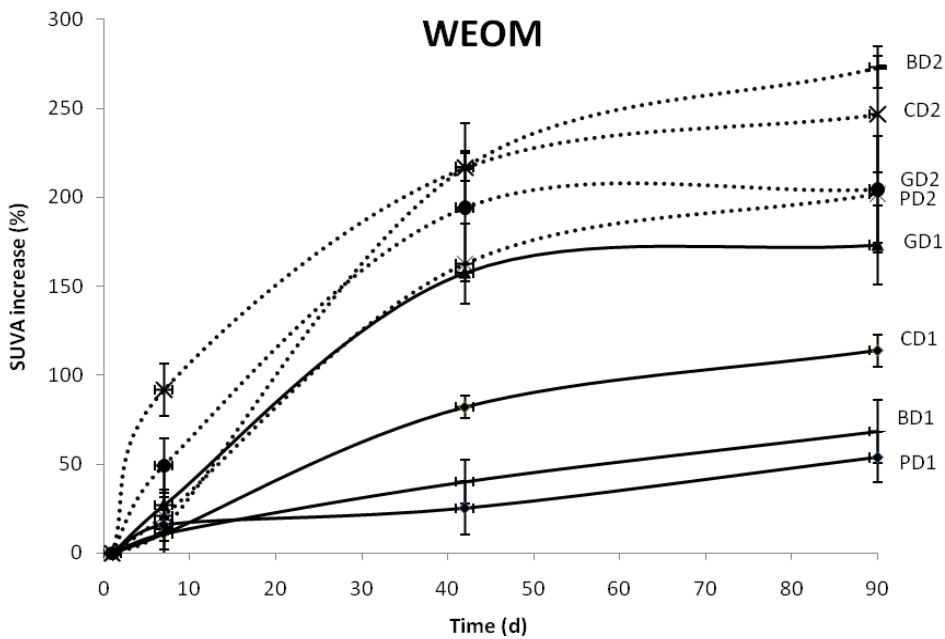
\* and \*\* Significant difference between topsoil and subsoil in the same land use at P=0.05 and P=0.01 probability levels, respectively

Values are given as mean ± S.D.

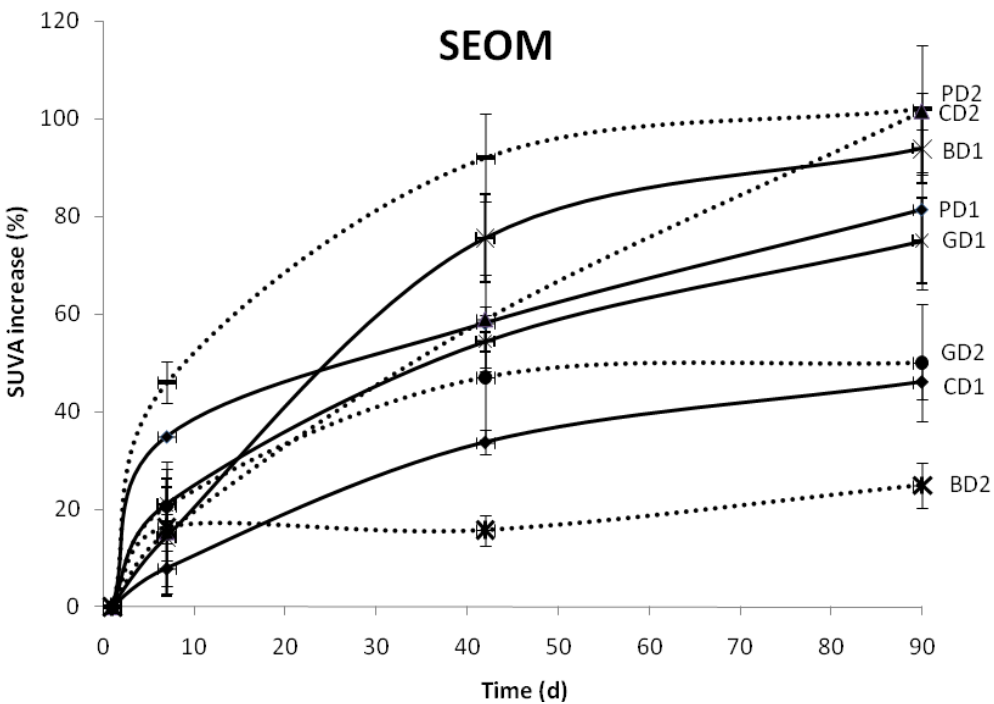
NS: Not significant

**Figure 4.1.** The proportion of SUVA increase (% of initial value) during biodegradation of WEOM (5.1.a) and SEOM (5.1.b).

**Figure 4.1.a**



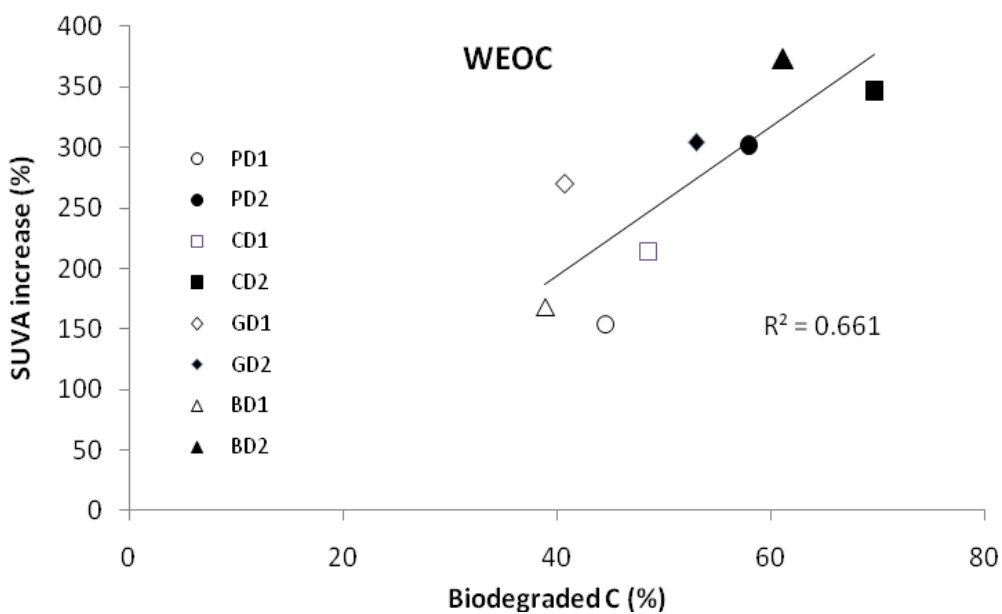
**Figure 4.1.b**



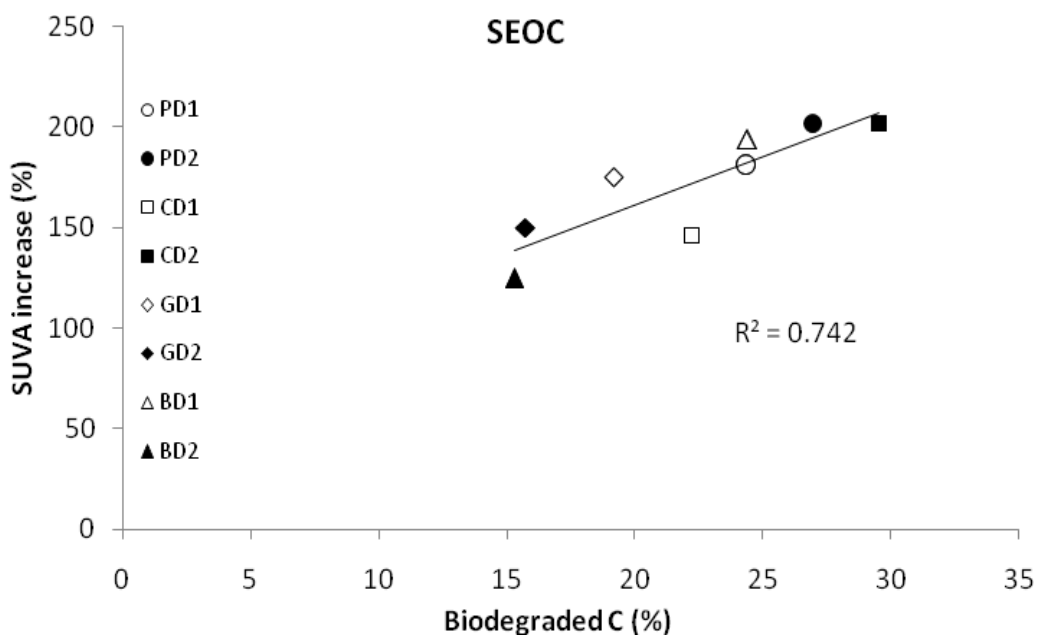
P: Plantation; C: Cropland; G: Degraded grassland; B: Bog pine  
D<sub>1</sub>: Depth 0-20 cm; D<sub>2</sub>: Depth 60-80 cm  
Error bars represent SD (n=3)

**Figure 4.2** The relationship between the proportion of the biodegraded C and  $SUVA_{254}$  increase (% of initial value) in WEOM (5.2.a) and SEOM (5.2b)

**Figure 4.2.a**



**Figure 4.2.b**

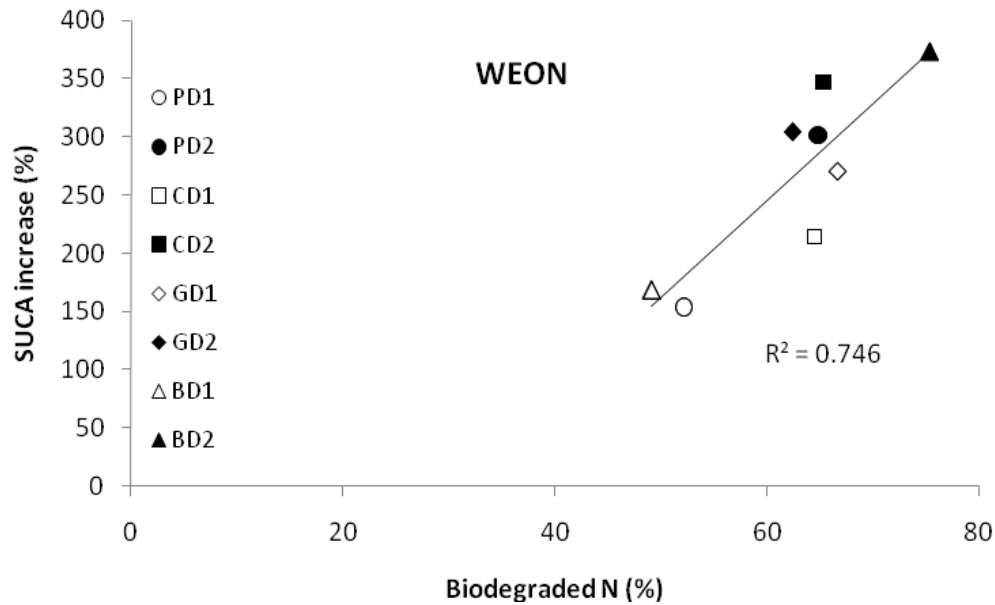


P: Plantation; C: Cropland; G: Degraded grassland; B: Bog pine  
D<sub>1</sub>: Depth 0-20 cm; D<sub>2</sub>: Depth 60-80 cm;

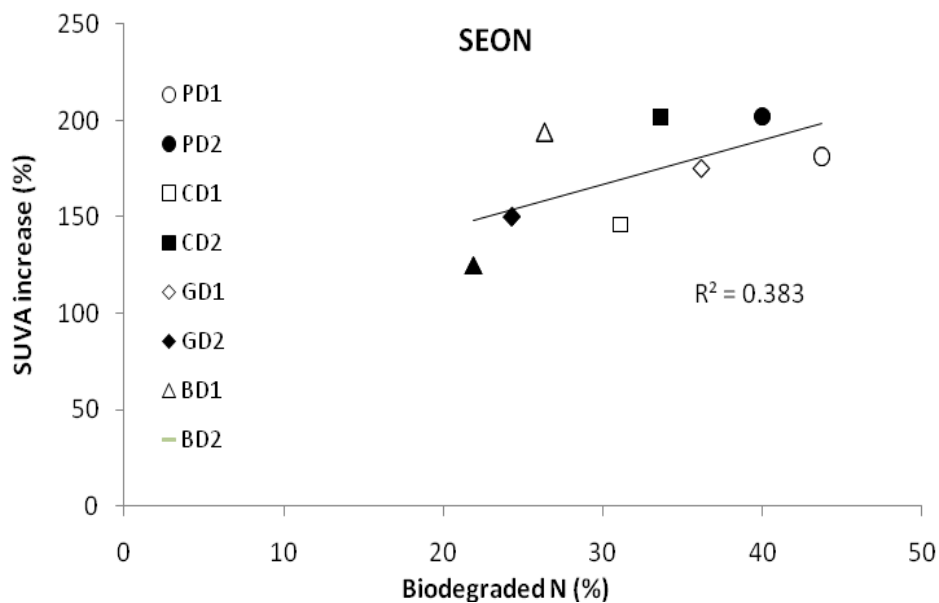


**Figure 4.3.** The relationship between the proportion of the biodegraded N and  $\text{SUVA}_{254}$  increase (% of initial value) in WEOM (5.3.a) and SEOM (5.3.b)

**Figure 4.3.a**

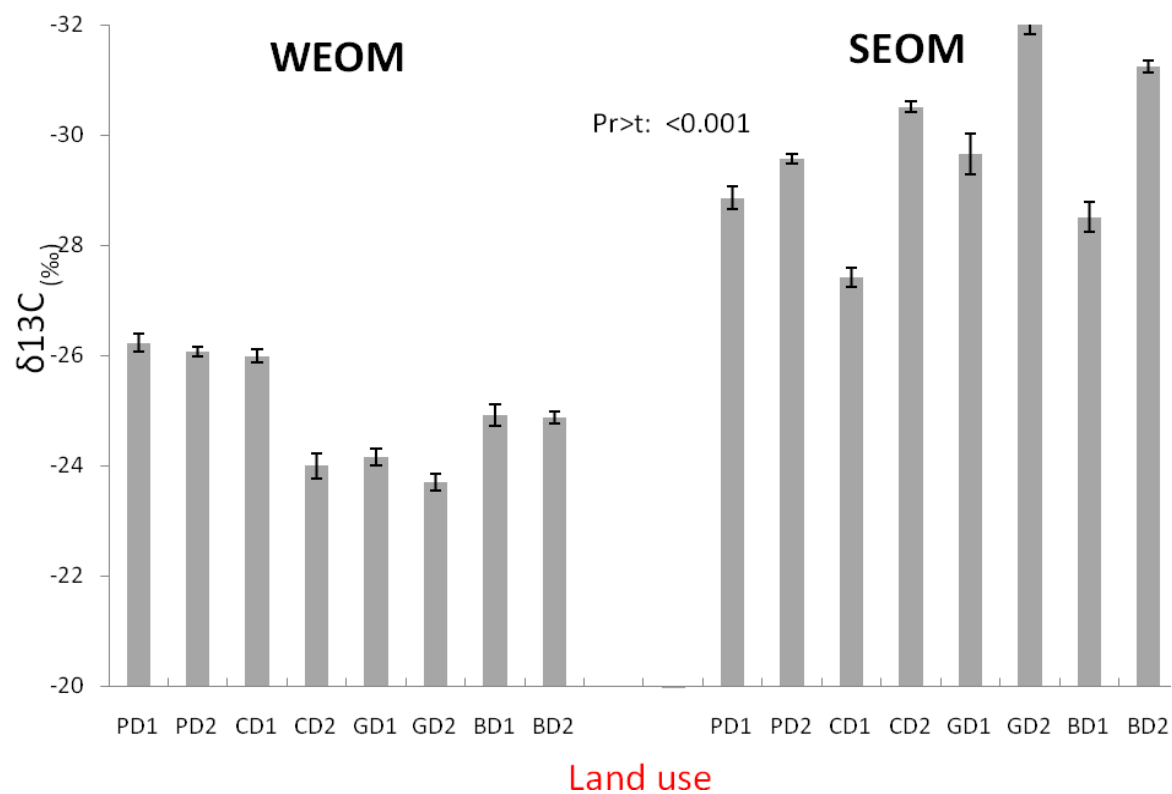


**Figure 4.3.b**



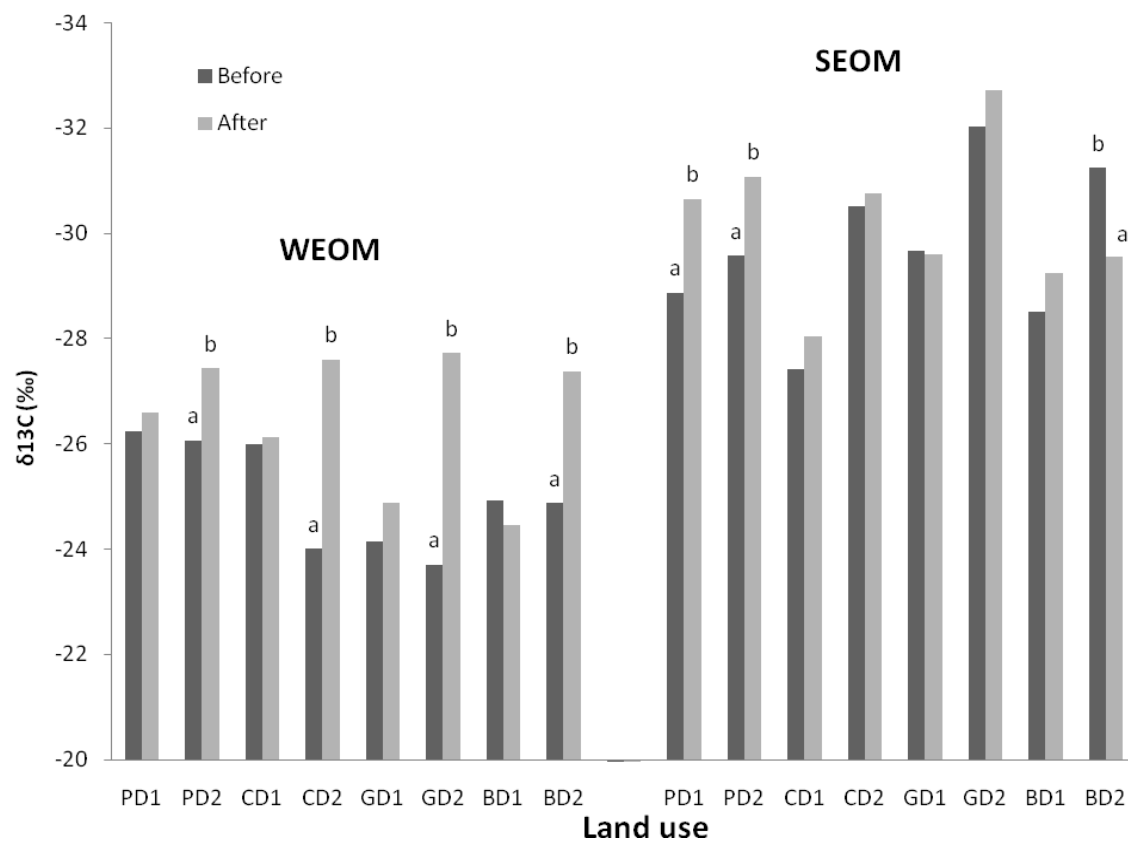
P: Plantation; C: Cropland; G: Degraded grassland; B: Bog pine  
D<sub>1</sub>: Depth 0-20 cm; D<sub>2</sub>: Depth 60-80 cm

**Figure 4.4.** The initial natural abundance of  $^{13}\text{C}$  of the two fractions of extractable OM



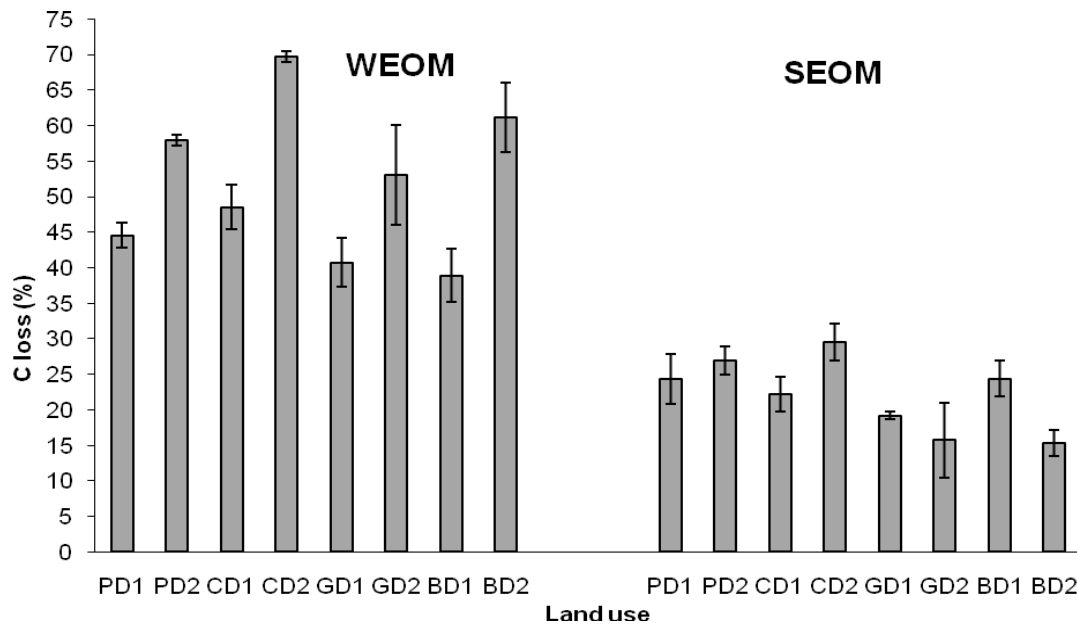
P: Plantation; C: Cropland; G: Degraded grassland; B: Bog pine  
D<sub>1</sub>: Depth 0-20 cm; D<sub>2</sub>: Depth 60-80 cm  
Error bars represent SD (n=3)

**Figure 4.5.** Changes in  $\delta^{13}\text{C}$  in the water and salt extractable OM during the biodegradation assay

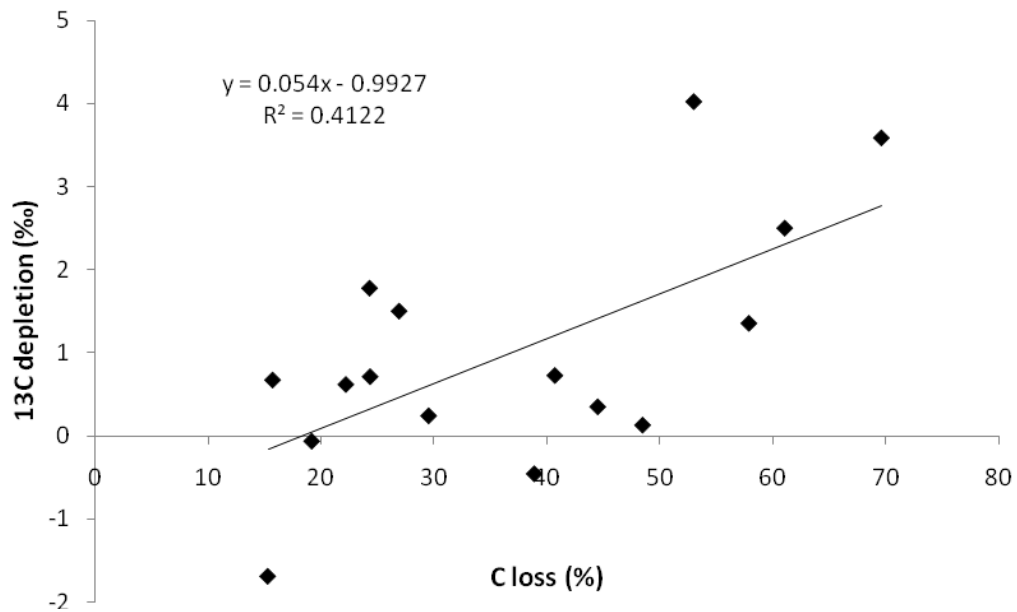


P: Plantation; C: Cropland; G: Degraded grassland; B: Bog pine  
D<sub>1</sub>: Depth 0-20 cm; D<sub>2</sub>: Depth 60-80 cm

**Figure 4.6.** The proportion of C loss in the water and salt extractable OM  
Error bars represent SD (n=3)



**Figure 4.7** The relationship between the proportion of the biodegraded C and depleted  $^{13}\text{C}$  in the EOM samples



P: Plantation; C: Cropland; G: Degraded grassland; B: Bog pine  
D<sub>1</sub>: Depth 0-20 cm; D<sub>2</sub>: Depth 60-80 cm

## **Chapter Five**

# **DYNAMICS OF THE WATER AND SALT EXTRACTABLE ORGANIC MATTER AS AFFECTED BY LAND USE AND SOIL DEPTH**

### **5.1. Introduction**

The amount and quality of soil organic matter (SOM) substantially affect a large number of important soil properties. Dissolved organic matter (DOM) and extractable organic matter (EOM) are the soluble forms of OM that have been shown to be linked with many processes involved in soil dynamics. Soluble OM is considered a highly dynamic pool of SOM which is closely associated with microbial activity and thus CO<sub>2</sub> efflux from the soil (Patrick et al., 2005). It has been suggested that this pool of organic matter acts as a bottleneck between soil and water bodies (Zsolnay, 1996) and controls the nutrient dynamics, especially C and N turnover during OM transformation in soil (Qualls et al., 1991; Jones et al., 2004). In addition to its important role as a substrate for microbial metabolism (Murphy et al., 2000; Cookson et al., 2005), soluble OM facilitates transportation of metals and organic compounds to depth in the soil, a process that plays a key role in soil formation (Hongve et al., 2000; Gorbashina and Krumbein, 2005). Recent evidence shows that soluble OM may contribute significantly to the annual inputs of C at soil depth (Kalbitz and Kaiser, 2008), indicating the magnitude of this pool of OM in C retention by soil.

Despite the growing body of the literature related to the mechanisms that control the dynamics of soluble OM in soil, the impacts of factors controlling interactions between these mechanisms on the production and properties of soluble OM is not well-understood. Among the factors that affect soluble OM, land use has been shown to contribute substantially to the amount and properties of this pool of SOM since it controls vegetation which is the primary source of the OM and thereby of soluble OM (Chantigny, 2003). The impact of land use on soluble OM is of some interest, especially with regard

to its role in i) the export of soluble OM into streams through watersheds (Qualls, and Richardson. 2003; Mattsson et al., 2009); ii) cycling and losses of N (Van Kessel et al., 2009; Neff et al., 2002); iii) the potential significance of land use on C retention by soil (Kalbitz and Kaiser, 2008; Sanderman and Amundson, 2009); iv) on the labile fractions of OM (Haynes, 2000).

The literature on soluble OM dynamics in soil is derived mainly from studies carried out in temperate or cold forest ecosystems where a major part of the released soluble OM originates from the litter layer and organic horizons (Chantigny, 2003). However, only limited information is available about the impact of land use on the properties of soluble OM in agrosystems and grasslands that lack surface litter and organic layer/s, generally receive lower OM input to soil, and are under strong influence of human activities.

While it has been commonly accepted that the amount of the soluble OM substantially decreases during its passage through the soil profile, the literature on the impact of land use on the properties of the soluble OM at soil depth is largely lacking. Although it has been suggested that land use may affect the properties of soluble OM down to the subsoil (95 cm) (Kalbitz, 2001), recent evidence suggests that a large proportion of soluble OM at soil depth is produced *in situ* from the intact OM (Froberg et al., 2007; Sanderman et al., 2009), thus, it may less be affected by land use. The impact of land use on the flux and properties of the soluble OM at soil depth becomes more profound under rainfall flush conditions when the soluble OM derived from litter and/or surface layers directly reaches the subsoil (Don and Schulze, 2008). Therefore, the temporal pattern of the water flux into soil appears to interact with the impact of land use on the distribution and properties of the soluble OM down to the soil profile.

To avoid the practical difficulties associated with obtaining soluble OM, this pool of OM is commonly obtained by soil extraction using aqueous solutions (Haynes, 2005). In addition to the OM that exists in the soil solution, OM obtained by aqueous extracts may also be derived from the pool of potentially soluble OM, depending on the extraction procedure (Zsolnay, 1996). The lack of consensus in the methodology for obtaining

soluble OM has resulted in a range of data in the literature while the possible relationships between the measures are still poorly known.  $\text{CaCl}_2$  (0.05M) and  $\text{K}_2\text{SO}_4$  (0.5 M) are probably the most common extractants used for obtaining soluble OM and have been suggested to obtain the “OM in soil solution” (Reemtsma et al., 1999) and “the potentially soluble OM” (Jones and Willet, 2006), respectively.

Although soluble OM is a continuum of organic compounds smaller than  $0.45\ \mu\text{m}$  in size, in this experiment, we hypothesized that by using different extraction procedures, the two extractants, 0.05M  $\text{CaCl}_2$  and 0.5 M  $\text{K}_2\text{SO}_4$ , may help to separate two fractions of soluble OM from the soil. The objective of the study was therefore to evaluate the impact of land use on the amount and properties of the two fractions of the soluble OM in topsoil and subsoil. Given the large temporal variability of the soluble OM in soil (Kaiser et al., 2002; Fellman et al., 2009), we measured seasonal fluctuations of EOM to better address the impact of land uses and soil depth on EOM and its properties.

## **5.2. Materials and methods**

### **5.2.1. Site description**

The study was undertaken in the Mackenzie Basin, upper Waitaki catchment, South Island, New Zealand ( $44^\circ\text{S}$ ,  $170^\circ\text{E}$ ). The moderately leached Pukaki series soil in the area has developed from a 40-50 cm greywacke loess layer over alluvium. Soils are categorized as Orthic Brown Pukaki fine sandy loams (Andic Ustochrept) (Webb, 1992). The dominant land-cover is degraded tussock grassland, with scattered tussocks of *Festuca novae-zelandiae* in a matrix dominated by the invasive herb *Hieracium pilosella* (Ledgard, 1997). We selected sites based on similar rainfall (MAP:  $646\ \text{mm yr}^{-1}$ ), temperature (MAT:  $9.1^\circ\text{C}$ ), elevation (600-700 m) and soil type. All the sites have been under the same vegetation for at least ten years. While the bog pine (*Halocarpus bidwilli*) shrubland represents the pre-human undisturbed vegetation of the area, the degraded tussock grassland is the dominant vegetation of the area after ca. 750 years of human induced-burning and subsequent intensive livestock grazing. Both sites had not been cultivated, fertilized or burnt since 1897 (Hunt et al., 2004). Plantation forest (*Pinus*

*nigra*) and cropland (*Medicago sativa*) represent two alternative rehabilitation management systems for the area. The plantation site was established through planting fast-growing exotic trees directly into the depleted grassland with no further management inputs, the cropland soil has been under intensive agricultural activities (cultivation, fertilization, mechanical harvesting) for number of years.

#### 5.2.2. Soil sampling and sample preparation

Soil samples were collected using 10 cm diameter stainless steel augers from 0-20 cm (after removal of the litter layer) and 60-80 cm at three separate locations at each site. To assess seasonal variation of soluble OM, soil sampling was carried out at the end of each season (November, February, May and August) during 2007-2008. Soil samples were transferred in an icebox to the laboratory within 24 hours of collection. The soils were mixed well after sieving (2 mm) and visible impurities (litter, organisms, etc) were removed before storage at 4°C until the extraction procedure. To avoid differences in the quality and quantity of the extractable OM obtained from field-moist samples collected during different seasons and from different depths, all soil samples were adjusted to the same water status (60% of water holding capacity) prior to extraction. Extractable OM was obtained during the first week after the sample collection. The following extraction procedures were conducted to obtain two fractions of the soluble OM. For details of sampling and sample preparation see Appendices A and B

#### 5.2.3. Extractable OM

Water extractable OM was obtained by shaking the soils with 10 mM CaCl<sub>2</sub> at 110 rpm (15 min) at room temperature with a soil:extractant ratio of 1:2 (w/v). This mild extraction procedure is assumed to approximate the properties of soil solution *in situ* (Zsolnay, 1996; Embacher et al, 2007). The extracts were then centrifuged at 4500 rpm (15 min) followed by filtration through 0.45 µm cellulose nitrate syringe filters.

Salt extractable OM was collected by extracting the soils with 0.5 M K<sub>2</sub>SO<sub>4</sub> at 1:5 soil:extractant ratio (w:v). Briefly, 7 g soil was transferred to 50 ml plastic centrifuge



bottles. The bottles were shaken for 60 min at 360 rpm at room temperature. The samples were then centrifuged at 4800 rpm (20 min) to accelerate the subsequent microfiltration (0.45  $\mu\text{m}$ ). This procedure is assumed to reflect the OM bound with minerals (exchangeable OM) although it is comprised of the OM in the soil solution (WEOM).

#### 5.2.4. Soil analysis

Total Soil C and N were determined using a LECO CNS-200 analyzer (LECO Corp, St. Joseph, MI). The soluble OM samples were analysed for organic C and total N (TN) in duplicate with a TOC-TN analyzer (Apollo 9000 TOC/TN, Hewlett Packard, USA). Organic C was measured by quantifying the  $\text{CO}_2$  (after acidification) using the high temperature (680°C) catalytic oxidation followed by NDIR detection. Mineral N forms ( $\text{NO}_3^-$  and  $\text{NH}_4^+$ ) were determined colorimetrically by a flow injection analyzer (Flow Solution, TM 3000, ALPKEM, USA). Organic N was then calculated as the difference between the concentrations of total N and the total mineral N. The soil texture was determined using the hydrometer method. Soil pH was measured in saturated paste with a standard glass electrode. Saturated extracts were then used for determining the electrical conductivity of soils. Bulk density in each site was measured as three replicates using cylindrical soil cores (5.2 cm inner diameter; 5 cm depth) by weighing undisturbed soil and oven-drying at 105°C for 24 h (Blake, 1965).

UV Absorbance was measured at 254 nm at room temperature using UV spectrophotometer (Varian Co., Cary 50 Probe, Au). All the solutions with UV absorbance that is more than 0.3 were diluted to ensure the comparability of the results. Specific UV absorption (SUVA,  $\text{l mg C}^{-1} \text{ m}^{-1}$ ) was then calculated after normalizing the UV absorption data using related DOC concentrations. SUVA at 254 nm ( $\text{SUVA}_{254}$ ) has been shown to strongly reflect the proportion of aromatic compounds in soluble OM (Weishaar et al., 2003).

#### 5.2.5. Statistical analysis

All data analyse was conducted using SAS (9.2) statistical software (SAS Inc.,). We used analysis of variance (ANOVA) to evaluate the impact of the land use on the C

and N content of the soils. Three way ANOVA was also used to assess the impact of factors of seasonal variation, land use, and soil depth and their interactions on the concentration of C and N of the pools of the total SOM and the water and salt extractable OM, and the impact of the factors on the properties of the SOM (C/N ratio) and water and salt extractable OM (C/N ratio and SUVA). The results represent the mean of and standard error of three replicates.

### **5.3. Results**

#### **5.3. 1. Temperature and rainfall**

Figure 5.1 shows that the rainfall is almost evenly distributed in the Mackenzie area throughout the year with an average rainfall of 50 mm/month and a maximum 30% seasonal variation. The maximum (62 mm) and minimum (35 mm) rainfall occurred in August and February, respectively. In contrast to the rainfall, the ambient temperature of the area is highly variable during the year with the maximum temperature (17.5°C) in January and minimum (2.1°C) in June (Fig.5.1). While the rainfall of the area is minimum from late spring to late summer (November to February), the temperature reaches its maximum during this period, limiting the vegetation growth. In addition, the relatively higher rainfall during the winter (June-August) is along with a decline in the temperature to its lowest point (Fig. 5.1).

#### **5.3.2. General soil properties**

The soil texture was medium to slightly heavy in all of the sites with a similar textural class at a similar depth (Table 5.1). However, the amount of clay decreases slightly from topsoil down to subsoil. Soil pH varied from 4.9 to 5.5, representing the slightly acidic range for all the land uses and without any marked change with soil depth (Table 5.1). The electrical conductivity of the soils varied considerably among the land uses in both the topsoil (65-204  $\mu\text{m cm}^{-1}$ ) and subsoil (28-62  $\mu\text{m cm}^{-1}$ ) with the least amount of total soluble salts in the soil under bog pine. With the exception of the cropland, the electrical conductivity of the soils decreased with soil depth. The soils

under the plantation had the lowest bulk density in both the topsoil and subsoil while bulk density was similar for the other sites (Table 5.1).

### 5.3.3. Total soil C and N

Soil C concentration was comparable in all the land uses at the same soil depth. However, land use had a significant impact of the C concentration of soil at a same depth (Table 5.2). While total C varied from 3.1% (cropland) to 4.6% (grass land) in topsoil, it ranged from 0.61% (bog pine) to 1.8% (plantation) in subsoil (Fig. 5.2). This suggests that the concentration of C was more variable in the subsoil than topsoil. Soil C was significantly ( $P < 0.01$ ) less in the subsoil than topsoil (Fig. 5.2 and Table 5.2). In spite of a general increase in C concentration of the topsoils from spring to winter (Fig. 5.2), the seasonal changes in soil C concentration were not statistically significant (Table 5.2). The annual variation of the concentration of C varied 40% and 100% from the mean of C concentration in topsoil and subsoil, respectively.

Total soil C mass was similar among the topsoils ( $P > 0.05$ ) but bog pine had significantly less total C mass than the other land uses in the subsoil (Table 5.1).

The concentration of total soil N ranged from 0.19% (bog pine) to 0.31% (grassland) in the topsoil and from 0.05% (bog pine) to 0.14% (grassland) in the subsoil (Fig. 5.3). Although soil N content was minimum in both the topsoil and subsoil under bog pine (Fig. 5.3), the differences in N content of the soils was not statistically significant among land uses (Table 5.2). In parallel to total C, total N slightly increased from spring to winter with a maximum N content in winter (Fig. 5.3). This however, was not statistically significant (Table 5.2). Soil N content decreased significantly from topsoil down to subsoil in all the land uses (Table 5.2). Similar to C, the annual variation of N concentration in the topsoil was less than that in the subsoil (60% vs. 91% of the mean).

Total N mass of the topsoil was significantly greater in the soils under the cropland than other soils (Table 5.1). However, in the subsoil the impact of cropland on the total N mass was not statistically significantly different with soils under plantation or

grassland. Soils under Bog pine have a statistically significant lower N content in the subsoil (Table 5.1).

The average C/N ratio of the topsoil was greater than that in the subsoil (14.6 vs. 12.5). While the highest C/N ratio was observed in the topsoil beneath bog pine (mean  $17.0 \pm 0.89$ ), the C/N ratio was lowest in samples obtained from subsoil beneath the grassland site (mean  $11.5 \pm 1.0$ ) (Fig. 5.4). Both land use and soil depth significantly affected the C/N ratio of both the topsoil and subsoil (Table 5.2). The C/N ratio of the soils was stable ( $P > 0.05$ ) during the course of sampling programme (Table 5.2).

#### 5.3.4. Extractable organic carbon (EOC)

The concentration of C in the WEOM fraction of the soils ranged from 5.3 to 50 mg C kg<sup>-1</sup> in the topsoil and from 2.4 to 24.4 mg C kg<sup>-1</sup> in the subsoil (Fig. 5.5). The concentration of WEOC of the soils was significantly affected by soil depth and specifically by land use (Table 5.2) with a relatively larger amount of WEOC in the topsoil than subsoil ( $P > 0.01$ ). There was a significantly more WEOC in soils at the plantation site than under other land uses (Fig. 5.5.). In spite of the relatively large seasonal variability of WEOC (Fig. 5.5), the ANOVA model did not show a significant impact of season on the C concentration of the WEOM but the interaction of the 3 factors of season, land use and depth was statistically significant on the C content of the WEOM (Table 5.2).

The concentration of C in the SEOM fraction of the soils ranged from 128 to 251 mg C kg<sup>-1</sup> (Fig. 5.6). The size of C pool of the SEOM was greater than that of the WEOM at both topsoil (mean 11.9 times) and subsoil (mean 16.9 times). Despite WEOC, the amount of salt extractable organic C (SEOC) was highly similar across the land uses and between the topsoil and subsoil with no statistically significant change (Table 5.2). The seasonal fluctuation in the amount of SEOC was less in the topsoil (up to 18.2%) than that in the subsoil (up to 28%). However, SEOC did not alter significantly during the year period of the experiment for both factors of soil depth and land use (Table 5.2).

#### 5.3.5. Extractable organic Nitrogen (EON)

The amount of water extractable organic N (WEON) was greater in the topsoil than subsoil, ranging from 0.4 to 3.9 mg N kg<sup>-1</sup> in the topsoil and from 0.3 to 2.9 mg N kg<sup>-1</sup> in the subsoil (Fig. 5.7), but these differences were not significant (Table 5.2). This suggests that the variation range of WEON was similar to that of the WEOC and in a similar range between the two soil depths (10 times). On average, grassland and Bog pine soils had, the largest and smallest amounts of N, respectively in the WEOM at both soil depths (Fig. 5.7). Despite the relative differences in the N content of WEOM in the topsoils (average 1.5 mg N kg<sup>-1</sup>) and subsoils (1.1 mg N kg<sup>-1</sup>) and in particular its seasonal fluctuations (Fig. 5.7), the ANOVA model did not reveal any statistical significant impact of the soil depth and seasonality on the size of WEON of the soils (Table 5.2).

Similar to C, the N pool of the SEOM was considerably greater than that of the WEOM at both topsoil (mean 6.8 times) and subsoil (mean 6.4 times). The amount of the salt extractable organic nitrogen (SEON) varied from 6.0 to 14.0 (mean 10.2±1.5) mg N kg<sup>-1</sup> in the topsoil and from 4.8 to 9.2 (mean 7.1±0.7) mg N kg<sup>-1</sup> in the subsoil samples (Fig. 5.8). The N concentration of the SEOM obtained from topsoil was significantly larger than that of the subsoil (Table 2). In comparison with the WEON, the amount of SEON was less variable among the land uses at a similar soil depth (Fig. 5.8). The SEON was not significantly affected by either of the land use or season factors (Table 5.2).

#### 5.3.6. Properties of EOM (C/N ratio and SUVA)

The C/N ratio of the WEOM varied greatly among the land uses and at different sampling times from 5.7 to 19.6 in topsoil and from 5.3 to 27.3 in subsoil (Fig. 5.9). The differences of the C/N ratio of the WEOM were however, not statistically significant (Table 5.2). The variation in the C/N ratio of the WEOM was larger for the subsoils than topsoils. Comparison of the C/N ratio of the WEOM suggested that the C/N ratio was greatest during summer in almost all of the sites and at both soil depths (Fig. 5.9) although this was not statistically significant (Table 5.2). In three of the four sites (i. g.

cropland, grass land and bog pine) the C/N ratio of the WEOM was less than that in the total SOM (fig. 5.4 and Fig. 5.9). However, the C/N ratio of WEOM was larger than that in the total SOM in summer sampled soils. Given the large variations in the C/N ratio of the WEOM, land use, soil depth and season were not shown to consistently affect the C/N ratio and thus, did not have a significant impact on the C/N ratio of WEOM (Table 5.2).

The C/N ratio of SEOM ranged from 11.3 to 33.7 in the topsoil and from 17.1 to 41.7 in the subsoil (Fig. 5.10), and there was a significant difference in C/N ratio with soil depth (Table 5.2). The C/N ratio of the SEOM was larger and more constant than that of the WEOM (Fig. 5.10). Neither land use nor season significantly influenced the C/N ratio of the SEOM (Table 5.2). At both the grass land and bog pine sites the C/N ratio of the SEOM (topsoil and subsoil) decreased during the sampling year with the minimum C/N of SEOM in winter (Fig. 5.10) but these differences were not significant. In contrast with WEOM, in almost all of the soils the C/N ratio of the SEOM was larger than that in total SOM (Fig. 5.4 and 5.10),

The value of the specific UV absorption (SUVA) of the WEOM ranged from 0.21 to 0.82 l mg C<sup>-1</sup> m<sup>-1</sup> in the topsoil and from 0.35 to 0.91 l mg C<sup>-1</sup> m<sup>-1</sup> in the subsoil (Fig. 5.11). The SUVA value of the WEOM showed a considerable variation during the year of the study. Although it was consistently greater in the WEOM obtained from the topsoil in autumn (Fig. 5.10), there was no statistically significant seasonal change/s. The average SUVA value of the WEOM was similar in the topsoil (0.51±0.1 l mg C<sup>-1</sup> m<sup>-1</sup>) and subsoil (0.50±0.1 l mg C<sup>-1</sup> m<sup>-1</sup>) with no significant impact of soil depth on this property (Table 5.2).

Comparison of the SUVA value of the fractions suggested that the SUVA value of the SEOM (Fig. 5.12) was considerably greater than that of the WEOM in both the topsoil (0.68±0.06 vs. 0.51±0.1 l mg C<sup>-1</sup> m<sup>-1</sup>) and subsoil (0.73±0.07 vs. 0.50±0.1 l mg C<sup>-1</sup> m<sup>-1</sup>). The SUVA value of the SEOM was also far more constant among the land uses and during different seasons (Fig. 5.11). The ANOVA results indicate that the SUVA value of

the SEOM obtained from the subsoil ( $0.73 \pm 0.07 \text{ l mg C}^{-1} \text{ m}^{-1}$ ) was significantly larger than that of the topsoil ( $0.68 \pm 0.06 \text{ l mg C}^{-1} \text{ m}^{-1}$ ) (Table 5.2).

## 5.4. Discussion

### 5.4.1. Pools of C and N in the water and salt extractable OM

The size of C and N in each of the fractions of EOM is consistent with the range reported in the literature (e.g. Embacher et al., 2007; Rennert et al., 2007; Burton et al., 2007). We observed that while the amount of C and N of the WEOM varied largely (21 and 13 times, respectively) among soils under different land-uses and across seasons, the amount of C and N of the SEOM was less variable (2 and 3 times). Similarly, Burton et al (2007) indicated that in comparison with the SEOM (obtained by 0.5 M  $\text{K}_2\text{SO}_4$ ), the amount of C and N of the WEOM is far more influenced by land use and soil depth. Our data also show a greater variability in the C and N content of the WEOM than of the SEOM among the sample replicates. The large temporal and spatial variability of the components (C and N) of the WEOM appears to be due to the influence of a variety of mechanisms controlling the dynamics of this very labile and mobile portion of the soluble OM (Kalbitz et al., 2000b; von Lutzow et al., 2007). In contrast, the SEOM acts as a potentially soluble OM (Jones and Willet, 2006), mainly bound with colloids (Matlou and Haynes, 2006) and given to its relatively wide C/N ratio (Fig. 5.10), is less affected by biotic and abiotic factors. When corrected for the bulk density (Table 5.1), the existing soluble C pool (WEOM) represents 11-114 (mean 40) and 5.7-60 (mean 24)  $\text{kg C ha}^{-1}$  in topsoil and subsoil, respectively. The size of the potentially soluble C pool (SEOC) represents far more C, ranging from 372 to 575 (mean 478)  $\text{kg C ha}^{-1}$  in the topsoil and from 290 to 552 (mean 407)  $\text{kg C ha}^{-1}$  in the subsoil. Both WEOM and SEOM comprise very small portion of the total C of the soil (78-89 and 21-37  $\text{Mg-C ha}^{-1}$  in topsoil and subsoil, respectively).

Regardless of the land use and soil depth, the amount of N and particularly C were substantially larger in the SEOM. For example, the amount of C present in the SEOM, averaged across land use and seasons, was 11.9 (topsoil) and 16.9 (subsoil) times larger

than that of the WEOM. The larger proportion of the SEOC than WEOC in subsoil than topsoil appears to be related to the substantial decrease in the amount of WEOC at soil depth whilst the amount of SEOC was less affected by soil depth (Table 5.2). On the other hand, the difference between the amount of C of the WEOM and SEOM is larger in topsoil (mean 191 mg C kg<sup>-1</sup>) than subsoil (mean 167 mg C kg<sup>-1</sup>). Similar to C, the difference between the N content of the WEOM and SEOM was also more pronounced in the topsoil than subsoil (mean 8.7 vs. 6.0 mg N kg<sup>-1</sup>). Murphy et al. (2001) suggested that the difference between the amount of the EOM obtained from dilute and concentrated extractants increases with the increase in clay component of the soil. This appears to be due to the large capacity of clay particles retain OM in the exchangeable form that can be substituted during extraction with concentrated salt solutions (e.g. 0.5 M K<sub>2</sub>SO<sub>4</sub>). Thus, the greater clay content of the topsoil than subsoil (Silty Clay Loam vs. Silt Loam, respectively) may contribute to the proportionally larger difference in the pool of exchangeable C and N (difference between SEOM and WEOM) in the topsoil. The difference between the amount of C of the two fractions (WEOM and SEOM) may reflect the size of the adsorbed OM (substituted by K<sub>2</sub>SO<sub>4</sub>).

Dilute CaCl<sub>2</sub>, 2M KCl and 0.5 M K<sub>2</sub>SO<sub>4</sub> are probably the most commonly used aqueous solutions among a number of extractants used for obtaining EOM. Dilute aqueous solutions (e.g. 0.05 M CaCl<sub>2</sub>) may reflect *in situ* properties of the soil solution at the time (Zsolnay, 2003). In contrast, the concentrated salt solutions (e. g. 2 M KCl and 0.5 M K<sub>2</sub>SO<sub>4</sub>) approximate the OM held in the exchangeable sites (Murphy et al., 2000; Jones and Willet, 2006). K<sub>2</sub>SO<sub>4</sub> and KCl are extensively used in routine soil studies for determination of the microbial biomass (Vance et al., 1987) and soil available N (Mulvaney, 1996), respectively. Thus, it seems logical to use samples of the same soil extract for the extractable C and N measurements. The pool size of the OM released by 2 M KCl and 0.5 M K<sub>2</sub>SO<sub>4</sub> has been suggested to be comparable (Chang and Preston, 1998; Jones and Willet, 2006) with the concentration of C and N strongly correlated between the extracts obtained by 2 M KCl and 0.5 M K<sub>2</sub>SO<sub>4</sub> solutions ( $R^2=0.80\%$  for C and  $R^2=0.73\%$  for N; Burton et al., 2007). However, Cl<sup>-</sup> ions derived from KCl



extractant may interfere with DOC in the wet oxidation (persulfate) method which is used in DOC determination (Aiken, 1992; McKenna and Doering, 1995). In addition, the concentration of N in the KCl extracts may vary depending to the N determination method (e. g. Kjeldahl digestion *vs.*  $\text{K}_2\text{S}_2\text{O}_8$  oxidation) (Cabrera and Beare, 1993). Apart from that, when the exchangeable pool of OM is desired, the extraction of the soil with 0.5 M  $\text{K}_2\text{SO}_4$  avoids the disruption of soil colloids (Haney et al., 1999), minimizing the partial release of the occluded OM during harsh extraction procedure. Therefore, the application of 0.5 M  $\text{K}_2\text{SO}_4$  as the suitable extractant for approximating the exchangeable OM is preferred.

Different extractants and extraction procedures not only affect the size, but also the quality of the obtained pool of OM (Haney et al., 1999; Reemtsma et al., 1999; Zsolnay, 2003). We used the C/N ratio and SUVA of the fractions of EOM as indicators that respectively, reflect the degree of microbial alteration (Baisden et al., 2002) and the proportion of the aromatic compounds of OM (Weishaar et al., 2003). Our data showed an overall larger variability of the C/N ratio of WEOM at both topsoil and subsoil (4.3 and 5.2 times, respectively) than that of the SEOM (3.0 and 2.4 times, respectively). As discussed before, this could be related to the more dynamic nature of the WEOM than SEOM. In addition to its variation, the overall C/N ratio of the SEOM in both topsoil and subsoil (mean  $22.1 \pm 3.6$  and  $26.2 \pm 3.8$ , respectively) was wider than that of the WEOM (mean  $13.8 \pm 3.8$  and  $10.4 \pm 3.6$ , respectively). This suggests that the proportion of the N-rich organic compounds is greater in the OM existing in the soil solution (WEOM) than in the potentially soluble (adsorbed) OM (SEOM). The greater proportion of N content of the OM (low C/N) has been proposed to correlate well with the biodegradability of OM (e.g. Vossbrinck et al., 1979). Comparing the biodegradability of the WEOM and SEOM, Razavy et al. (Chapter 5.3) observed that the greater proportion of N in the WEOM contributes to its considerably greater biodegradability than that of SEOM. The more significant P value of the impact of the land-uses and season on the C/N ratio of the WEOM than SEOM (Table 5.2) may indicate that the dynamics of WEOM are closely associated with the biological activity.

Although there is a lot of temporal variability, the average SUVA value of the WEOM was similar ( $0.5 \pm 0.1$ ) in the topsoil and subsoil, respectively. According to the linear model developed by Weishaar et al. (2003) the range of SUVA values of the WEOM may correspond to approximately 5–9% aromatic C compounds. The larger average SUVA values of the SEOM ( $0.68 \pm 0.06$  and  $0.73 \pm 0.07$  in the topsoil and subsoil, respectively) than that of the WEOM appears to be related with the larger proportion of aromatic components released during harsh extraction procedure (0.5 M  $K_2SO_4$ ). The overall lower SUVA values of WEOM is in accordance with the previous findings (e.g. Reemtsma et al., 1999) that suggest that a considerable portion (up to 51%) of the WEOM obtained by dilute  $CaCl_2$  solution may be comprised of the low molecular weight organic compounds.

#### 5.4.2. The impact of land use on the pools of OM

Land use may affect the quality and quantity of the SOM through changes in the input of the above- and below-ground OM to the soil (Chantigny, 2003). Although in a similar range, the C concentration was statistically different among the land uses (Fig. 5.2 and Table 5.2). The lower concentration of both C and N in subsoil and topsoil of bog pine soil is related to the slow growth rate of the native vegetation of the area (Wardle, 1979) and thus, its low above- and below-ground biomass production and turnover. Conversion of grasslands to coniferous plantation may reduce the OM content of the soil at least within the initial few years of afforestation (Guo and Gifford, 2002; Paul et al., 2002). For example, Guo et al. (2008) reported that C and N content of a soil (down to 1m depth) under native pasture has decreased 20% and 15% sixteen years after pine plantation. However, covariance analysis of the data collected over 10 year period showed that in Balmoral site neither of C and N concentrations of the soil were significantly affected by the afforestation of the degraded grassland (Davis et al., 2007). This has been contributed to the low productivity of soils in this area (Davis et al., 2007; Webb, 1992).

Abiotic solubilization of SOM, biodegradation of OM, and the OM input from above- and below-ground litter determine the amount and quality of the OM existing in the soluble form (Kalbitz et al., 2000b, Stutter et al., 2007). Land use not only directly affects the amount and properties of the soluble OM through the quality and size of the OM input, but also indirectly through the rate and extent of biological pathways and changes in the microbial community of the soil (e.g. Cronan et al., 1992; Smolander and Kitunen, 2001; Cookson, et al., 2005). In our study the amount of WEOM varied as plantation>cropland>degraded grassland>bog pine at both soil depths. The proportionally greater C concentration of the WEOM obtained from the plantation site is in agreement with the literature suggesting that the size of the soluble C decreases from forest to degraded lands or soils under unimproved vegetation (Zsolnay, 1996). The presence of the litter layer and the abundance of the root exudates in forest soils have been suggested to maintain the proportionally larger amount of soluble C and N at topsoil and subsoil in forest soils (Zsolnay, 1996; Chantigny, 2003). In addition, the relatively low biodegradability of the coniferous litter (Kuiters, 1993 and Currie et al., 1997) and its derived leachate (Ganjegunte et al., 2006) may result in the longer half-life of the WEOM obtained from soils under the plantation. The cultivation practices (irrigation, fertilization etc.) improve the plant growth at the cropland site and thus, the production of the WEOM due to the enhanced plant-microorganism activities. The proportionally larger amount of the N in the WEOM obtained from the cropland (alfalfa) soil appears to be related to the fast turnover of its roots (Guo et al., 2006) and its considerable effect on the production of the soluble organic N (Yano et al., 2005), the greater N input in soils under legumes (Macdonald et al., 2007), and the presence of the N rich root exudates (van Hees et al., 2005). In contrast, the low-productivity (Webb, 1992) and therefore, the low OM input (Hunt et al., 2004) and its fast decomposition in the soils under degraded grassland (Hunt et al., 2002; Hunt et al., 2004) and bog pine result in the low WEOM content of these soils.

In comparison with the WEOM, the amount of C and N in the SEOM were far more similar among the land uses (Fig. 5.6 and 5.7; Table 5.2) with a reasonable

correlation between the amount of C and N in the SEOM with those in the total SOM ( $R^2 = 0.39$  and  $0.44$  for C and N, respectively). These data are in accordance with the literature that differences in the extractable organic C and N among contrasting land uses are far less pronounced when concentrated salts rather than water/dilute solutions are used (Matlou and Hynes, 2005; Burton et al., 2007). Given the similar textural class and parent materials of the soils, the lack of a considerable difference in the amount of the SEOM in the soils under different land uses may suggest that the OM extractable with concentrated salt is more affected by the edaphic (CEC, pH, etc.) conditions than the vegetation. This is somehow in agreement with findings of Don and Schulze (2008) who suggested that physicochemical properties of the soil (e.g. adsorption capacity) affect the dynamics of DOC more strongly than land use.

#### 5.4.3. The impact of soil depth on the pools OM

Our results indicated that soil depth has a significant impact on the concentration of the total C and N of the soils (table 5.2). This is in agreement with the literature that the concentration of soil OM decreases considerably at soil depth due to OM input being limited through the turnover of the below-ground OM and the OM leached from the topsoil. Land use not only affects the size and quality of the OM at soil depth but also the depth that the OM enters and/or accumulates in the soil (Lorenz and Lal, 2005). Our data (Table 5.1) indicate that depending on the land use, the amount of C and N is respectively, 2-4 and 2-3 times larger in the topsoil (0-20 cm) than that in the subsoil (60-80). Using meta analysis of a large number of publications ( $n=74$ ), Guo and Gifford (2002) concluded that conversion of arable land to pasture may result in a considerable increase in C stock at soil depth (below 100 cm). In contrast, changes in land use from forest to pasture or arable land did not alter the SOM content of subsoil deeper than 100 and 60 cm, respectively. Our results (Table 5.1) are similar to those reported by Davis et al. (2007) that plantation or cultivation of the degraded grass land did not change the C stock of the topsoil. The alteration of land use also did not change the C content of the subsoils. However, we observed that a change in the native vegetation (bog pine) to other

land uses has resulted in a significant C and N accumulation at soil depth (Table 5.1). To date the conversion of this degraded grass land to either productive cropland or exotic forest has not resulted in further increases in soil C and N. However, since the existing OM in the litter layer has not been considered in our experiment, the total amount of either of C and N under the 14 year old trees is far more than that reported here. Kirschbaum et al. (2008) indicated that despite a significant depletion of soil C and N after pine forestation of a degraded pasture, including the biomass of the pine stand and the above-ground litter result to respectively, an exceeding of 88 and 6.1 t C ha<sup>-1</sup> and 0.39 and 0.11 t N ha<sup>-1</sup> than those of pasture.

In addition to the size of the SOM, the C/N ratio of the soils was also significantly affected by soil depth (Table 5.2). The observed decline in the C/N ratio with soil depth is in accordance with the literature (e.g. Stevenson, 1994; Corvasce et al., 2006) and is due to the leaching of organic (VanKessel et al., 2009) and inorganic (Stevenson, 1994) N forms, the presence of N-rich root exudates (van Hees et al., 2005), the turnover rate of roots, and microbial residues and metabolites as an important source of organic N at soil depth (Yano et al., 2005).

Along with the data in the literature (e.g. Hassouna, et al., 2010) we observed that the size of both C and N of the WEOM declined at soil depth, although this effect was only significant for C (Table 5.2). This may be due to the large variability in N concentrations of WEOM during the sampling period. However, the more pronounced decrease in C than N of the WEOM may also be due to the preferential absorption of the more humified components of the soluble OM in the topsoil, leading to the proportional abundance of the N-rich compounds (Kaiser et al., 2002). Alternatively the *in situ* release of N-rich WEOM through root activities (Van Hees et al., 2007) may explain the change in the C/N ratio of the WEOM at soil depth. The pronounced decline in the amount of soluble OM content at soil depth has been attributed to its i) limited *in situ* production at soil depth, ii) the fast biodegradation of its labile pool, and ii) its absorption by soil mineral particles (Kaiser et al., 2002; Guggenburger and Kaiser, 2003; Don and Schulze, 2008; ).

Our results are in accordance with Burton et al. (2007) that in contrast with the WEOC, the SEOC is far less affected by soil depth. The weak impact of land use and depth on the SEOC implies that the impact of land use or soil depth on the size of the C in the EOM may be considerably affected by the extraction method. This amplifies the need for unified approach to selecting an extraction procedure when the impact of soil depth or land use on the EOM is considered. However, we observed a significant decrease in the N content of the SEOM at the soil depth. This along with the impact of the soil depth on the WEOC (Table 5.2), suggest that the soil depth may differently affect the dynamics of N and C pools of the EOM.

A growing body of the recent literature suggests that the impact of land use on the WEOM/DOM is mainly limited to the topsoil with the subsoil WEOM/DOM originating *in situ*, partly from the turnover of the biomass but largely from transformations of the native OM and thus, less affected by land use (e.g. Chen et al., 2004; Sanderman et al., 2008; Hassouna et al., 2010). However, our results suggest a significant interaction of land use-soil depth for the size of the C in the WEOM (Table 2). Given the pronounced role of the fresh OM input in the biotic solubilization of the intact SOM at soil depth (Fontaine et al., 2007), land use appears to partly control the rate limiting step in the production of the soluble OM at soil depth due to its impact on the fresh OM input in soil depth. This is specifically of importance during the rainy season with a flush of the fresh soluble OM sourced from throughfall or litter layer to the subsoil (Kaiser and Guggenberger, 2005; Don and Schulze, 2008).

The C/N ratio of the SEOM obtained from the subsoil was significantly ( $P>0.05$ ) greater than that of the topsoil in all land uses (Table 5.2) (mean  $26.2\pm3.8$  vs.  $22.0\pm3.6$ , respectively). This in addition to a significant increase of the SUVA value of the SEOM (Table 2) from topsoil to subsoil ( $0.67\pm0.06$  vs.  $0.73\pm0.07$ , respectively) suggest respectively, an increase in the microbial alteration (Baisden et al., 2002) and proportion of aromatic compounds (Weishaar et al., 2003) of the SEOM at soil depth. In contrast, neither the C/N ratio nor SUVA value of the WEOM showed a consistent increase or decline with increasing soil depth (Table 5.2). This may be due to the large variation in

the properties of WEOM across the land uses and between different seasons. The C/N ratio and SUVA value of the WEOM has been shown elsewhere to decrease with increasing soil depth (e.g. Corvasce et al., 2006; Strahm et al., 2009; Hassouna et al., 2010) as a result of the selective elimination of WEOM constituents during its passage down the soil profile (Mcknight et al., 1992; Kaiser et al., 2002). As suggested, the different impact of soil depth on the properties of the two fractions of the EOM suggests that the dynamics of these fractions are controlled by different mechanisms.

#### 5.4.4. Seasonal variation of the EOM

We did not observe a significant impact of season on the C and N content of the total SOM in either of the topsoil and subsoil although there was a consistent but slight increase in the size of the total C and N of topsoil from spring to winter (Table 5.2, Fig. 5.2 and 5.3). Similar to the size of C and N pools, the C/N ratio of the SOM was not significantly affected by the season factor. The lack of significant impact of the seasons on the components of the SOM appears to be partly due to the reported long-time root-zone water deficit along with the low but evenly distributed rainfall in Mackenzie area (Hunt et al., 20002; Hunt et al., 2004). Although the temperature of the area varies substantially ( $<0$  to  $>30^{\circ}\text{C}$ ; average  $9.1^{\circ}\text{C}$ ), the area has 154 days of the year ground frost (Webb, 1992). These in addition to the shortage of essential nutrients (Hunt et al., 2004), may result to a lack of substantial changes in the above- and below-ground biological activities and thus, OM input to the soils. Therefore, the proportion of OM input to the soil during different seasons and its transformations within the soil profile may not be enough to affect the size and properties of SOM.

Despite the considerably greater seasonal fluctuations of the size (C and N) and properties (C/N ratio and SUVA) of the WEOM than those of the SEOM, the impact of season was not significant either on the components (C and N) or properties (C/N ratio and SUVA) of both fractions (Table 5.2). The large seasonality of the soluble forms of OM and its biochemical components has been attributed to the seasonal changes in i) biological transformations of the SOM and ii) the quality and size of the above- and

below-ground input to the soil (e. g. Kawahigashi et al., 2003; Clarke et al., 2007). The large variability in the size of the WEOM and its properties and thus the lack of a constant trend in their seasonal pattern can be explained by considering the highly variable nature of WEOM in each aspect of its production, dynamics and degradation (Haynes, 2005). Some researchers have shown the time-dependent dynamics of the soluble OM (e. g. Embacher et al., 2007), and the significance of rainfall and topsoil moisture on the amount of WEOM (Hassouna et al., 2010). However, our results are in agreement with Boyer and Groffman (1996) and Clarke et al. (2007), suggesting the lack of a consistent temporal fluctuations of the soluble OM and its properties. The poor vegetation cover and the slow plant growth in the area have been suggested to hamper the microbial activity of the soils due to low supply of readily mineralizable substrates (Hunt et al., 2004). However, our results suggest that despite the suggested low productivity of the site (Webb, 1992; Hunt et al., 2002), there were still large fluctuations in the amount and properties of the SEOM and in particular, WEOM, implying the substantial changes in the biological activity in the soil.

## **Conclusion**

EOM has been suggested to act as a sensitive indicator of shifts in soil systems (Haynes, 2000; Ghani et al., 2003). However, it is a continuum of soluble organic compounds of which composition reflects the extraction procedure employed. The use of different extractants under different experimental conditions in addition to the extraction-dependent properties of the OM obtained, have led to some contradictory results in the literature. In this chapter, the comparison of the EOM collected in different seasons and obtained by the two commonly used extraction procedures indicated that WEOM (obtained by 0.05M CaCl<sub>2</sub>) and SEOM (obtained by 0.5 M K<sub>2</sub>SO<sub>4</sub>) are largely different in their size and properties. This supports our previous findings (Chapters 3 and 4), suggesting that the WEOM and SEOM may represent two fractions of EOM differentiated/characterized by their inherent biodegradability and isotopic properties.



We observed that after 10 yr, the conversion of the degraded grassland to either the productive cropland or pine plantation has not resulted in changes in the soil C and N in topsoil which is in agreement with Davis, et al., (2007) and also in the subsoil. Whether or not, changes in OM pool/s occur needs to be the subject of future research. In addition, the factors of land use and soil depth significantly affected the size (C) and quality (C/N ratio) of the total soil OM. The size and properties of the two fractions of the EOM were found to be considerably different in terms of their response to the impact of the land use and soil depth. While land use and soil depth influenced the size of the WEOM, they did not affect the size of the SEOM. However, soil depth significantly affected the properties (SUVA and C/N ratio) of the SEOM. Unlike land use and soil depth, season had no statistically significant impact on either of the size or properties of the SOM and EOM.

Comparison of the C/N ratio of the fractions of the EOM with that of the SOM indicated that while the C/N ratio of the WEOM varied in a range similar or less than that of the SOM (in subsoil), the C/N ratio of the SEOM was generally greater than that of the SOM. This suggests that the potentially soluble OM (OM bound with colloids) may have undergone substantial microbial alteration. Unlike the case with SEOM, the C/N ratio of the SOM and WEOM was greater in topsoil than subsoil. The overall amount of both C and N of the SOM and EOM were greater in topsoil than subsoil. In contrast to the SOM and SEOM, the WEOM showed large variation in terms of land use, soil depth and season. Such variability in the size and properties of WEOM may constrain its applicability as a useful indicator of the functional pool of C in soil (von Lutzow et al., 2007) and as a sensitive indicator of soil quality in comparative studies. Our results suggest that the impact of land use and soil depth on the size and properties of the EOM may vary largely depending on the extraction procedure. Therefore, a unifying methodology may be necessary when the impact of these factors on the soluble pool of OM is studied.

**Table 5.1.** Selected properties of soils

Land use	Depth (cm)	Texture	pH	EC (m $\mu$ cm <sup>-1</sup> )	Bulk density	C* (Mg C ha <sup>-1</sup> )	N* (Mg C ha <sup>-1</sup> )
Plantation	0-20	Silty Clay Loam	4.9	204	1.04	78 $\pm$ 3.1 <sup>A</sup>	5.4 $\pm$ 0.05 <sup>B</sup>
Cropland			5.3	128	1.20	84 $\pm$ 1.7 <sup>A</sup>	6.7 $\pm$ 0.07 <sup>A</sup>
Grassland			5.5	109	1.13	89 $\pm$ 8.0 <sup>A</sup>	5.6 $\pm$ 0.1 <sup>B</sup>
Bog pine			4.9	65	1.16	81 $\pm$ 14.6 <sup>A</sup>	5.0 $\pm$ 0.9 <sup>B</sup>
Plantation	60-80	Silt Loam	5.2	62	1.23	37 $\pm$ 9.6 <sup>a</sup>	2.7 $\pm$ 0.04 <sup>a</sup>
Cropland			5.2	163	1.34	34 $\pm$ 5.8 <sup>a</sup>	2.7 $\pm$ 0.04 <sup>a</sup>
Grassland			5.5	28	1.32	34 $\pm$ 3.4 <sup>a</sup>	2.9 $\pm$ 0.04 <sup>a</sup>
Bog Pine			5.0	56	1.32	21 $\pm$ 5.2 <sup>b</sup>	1.8 $\pm$ 0.04 <sup>b</sup>

\*The amount of C and N in topsoil (0-20 cm) and subsoil (60-80 cm)

Capital and small letters represent the statistical difference among the land uses at topsoil or subsoil, respectively

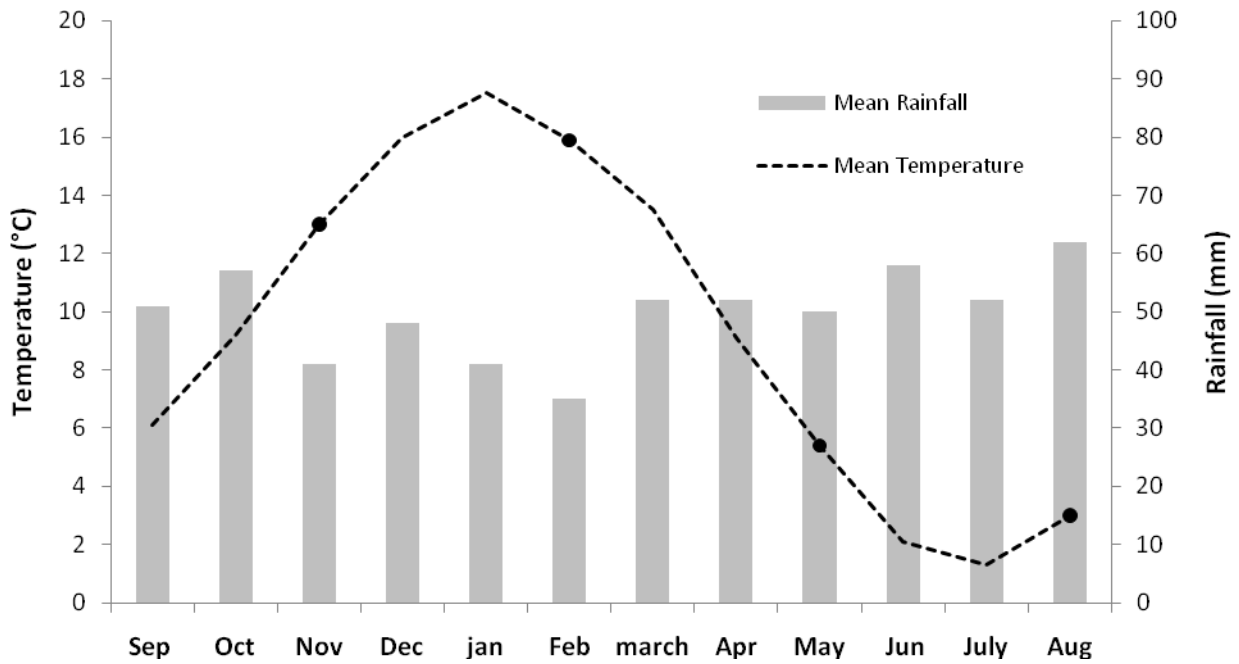
\* Data are mean $\pm$ SD (n=3)

**Table 5.2.** Probability values (three way ANOVA) for the impact of landuse, soil depth, and seasonality and their interactions on the properties of the SOM and EOM fractions

Factor	WEOM				SEOM				Total OM		
	C	N	C/N	SUVA	C	N	C/N	SUVA	C	N	C/N
landuse	<0.01	0.66	0.09	0.08	0.33	0.60	0.88	0.25	0.01	0.96	0.01
Depth	0.05	0.17	0.60	0.50	0.57	0.04	0.04	0.02	<0.01	0.01	0.01
Season	0.10	0.47	0.08	0.29	0.55	0.74	0.89	0.28	0.39	0.68	0.17
Landuse-depth	0.01	0.95	0.34	0.22	0.39	0.20	0.28	0.09	<0.01	0.68	0.01
Landuse-season	0.01	0.77	0.12	0.12	0.98	0.36	0.83	0.38	<0.01	0.17	0.11
Depth-season	0.09	0.23	0.15	0.58	0.67	0.39	0.38	0.09	0.78	0.55	0.33
L*D*S <sup>1</sup>	0.04	0.88	0.14	0.24	0.77	0.20	0.40	0.17	0.01	0.32	0.14

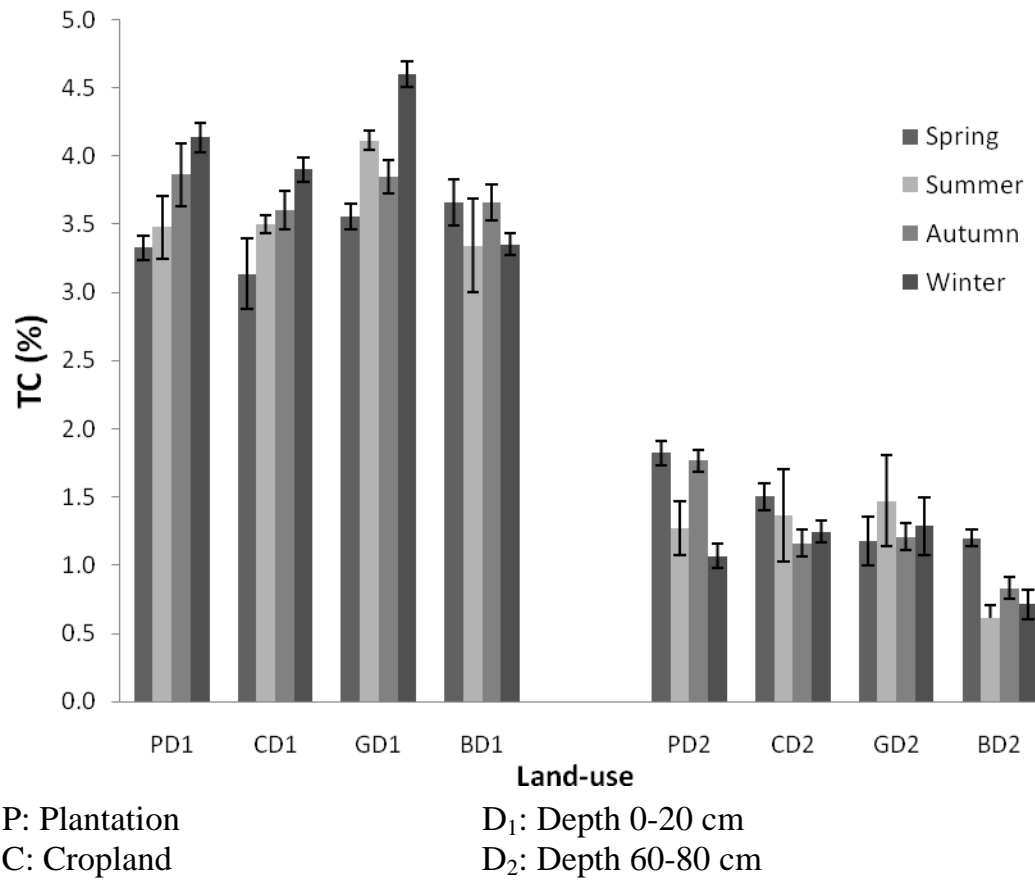
1- Landuse-Depth-Season

**Figure 5.1.** Seasonal fluctuations of the temperature and soil moisture in the Mackenzie Basin



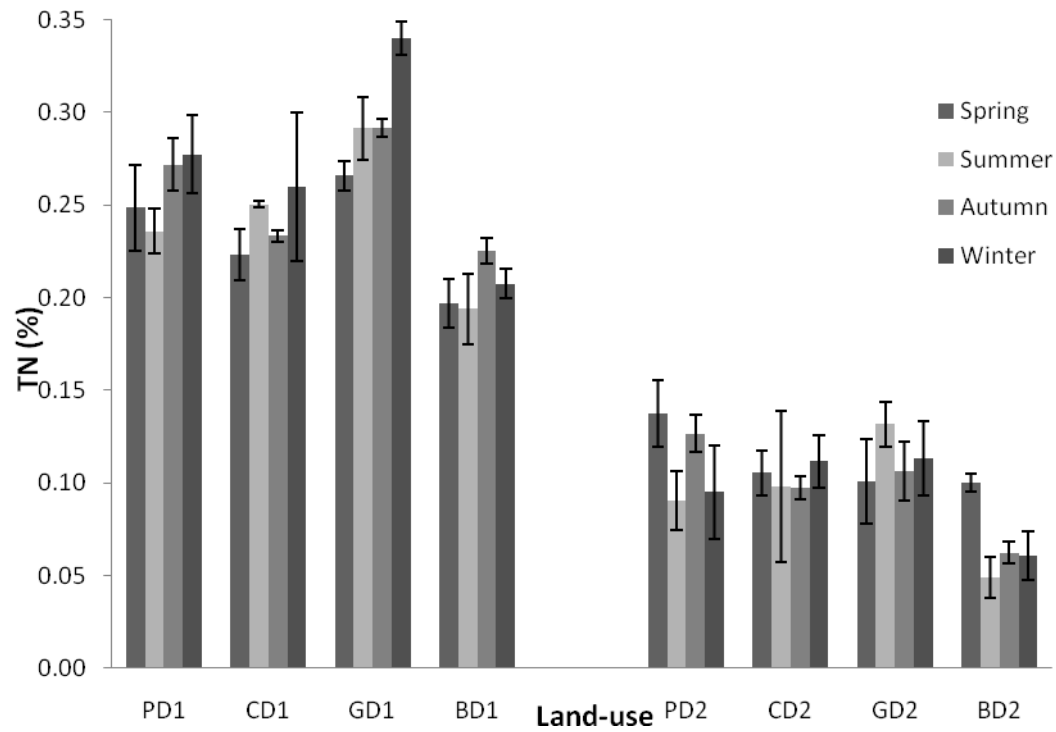
Data of 13-year period; source NIWA ([www.niwa.co.nz](http://www.niwa.co.nz))

**Figure 5.2.** Seasonal variation of the concentration of the total C of the soils under different land uses and at different depths



\* Data are mean $\pm$ SD (n=3)

**Figure 5.3.** Seasonal variation of the concentration of the total N of the soils under different land uses and different depths



P: Plantation

C: Cropland

G: Degraded grassland

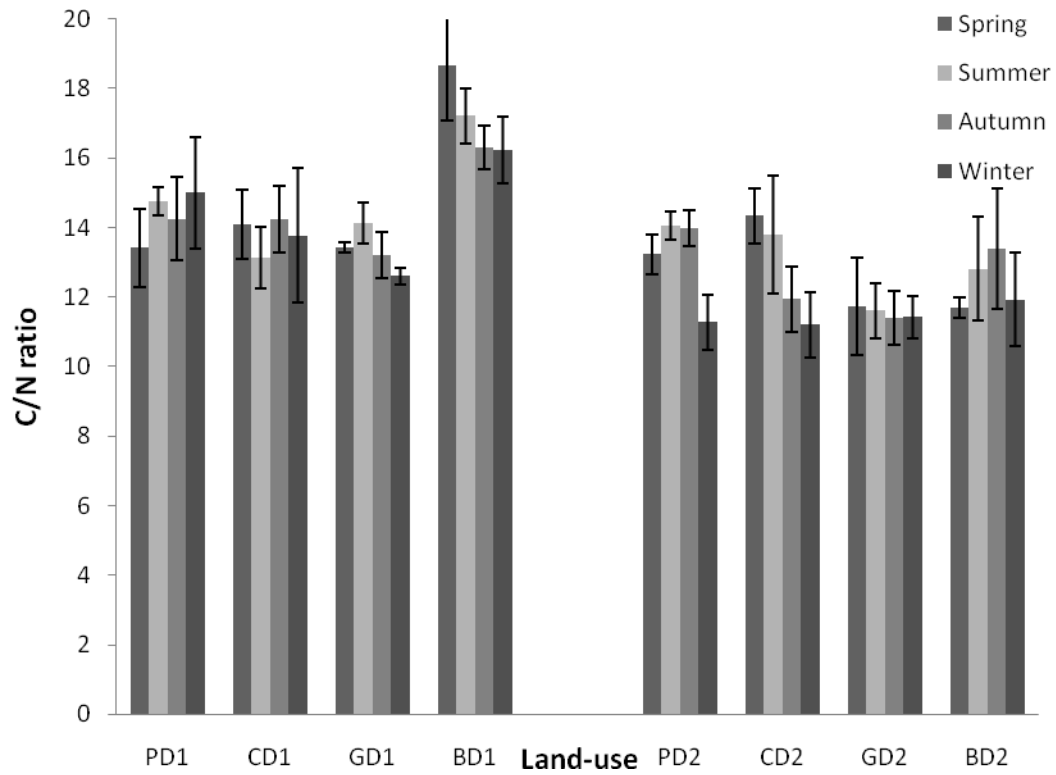
B: Bog pine

\* Data are mean $\pm$ SD (n=3)

D<sub>1</sub>: Depth 0-20 cm

D<sub>2</sub>: Depth 60-80 cm

**Figure 5.4.** Seasonal variation of the C/N ratio of the soils under different land uses and at different depths



P: Plantation

C: Cropland

G: Degraded grassland

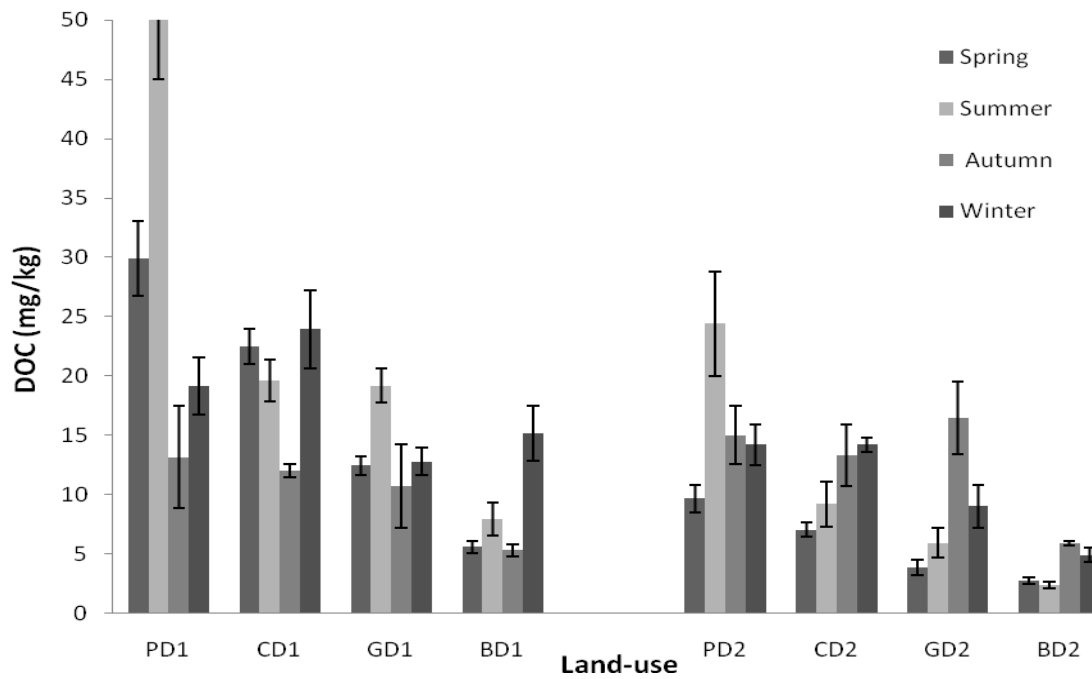
B: Bog pine

\* Data are mean $\pm$ SD (n=3)

D<sub>1</sub>: Depth 0-20 cm

D<sub>2</sub>: Depth 60-80 cm

**Figure 5.5.** Seasonal variation of the concentration of the WEOC of the soils under different land uses and at different depths



P: Plantation

C: Cropland

G: Degraded grassland

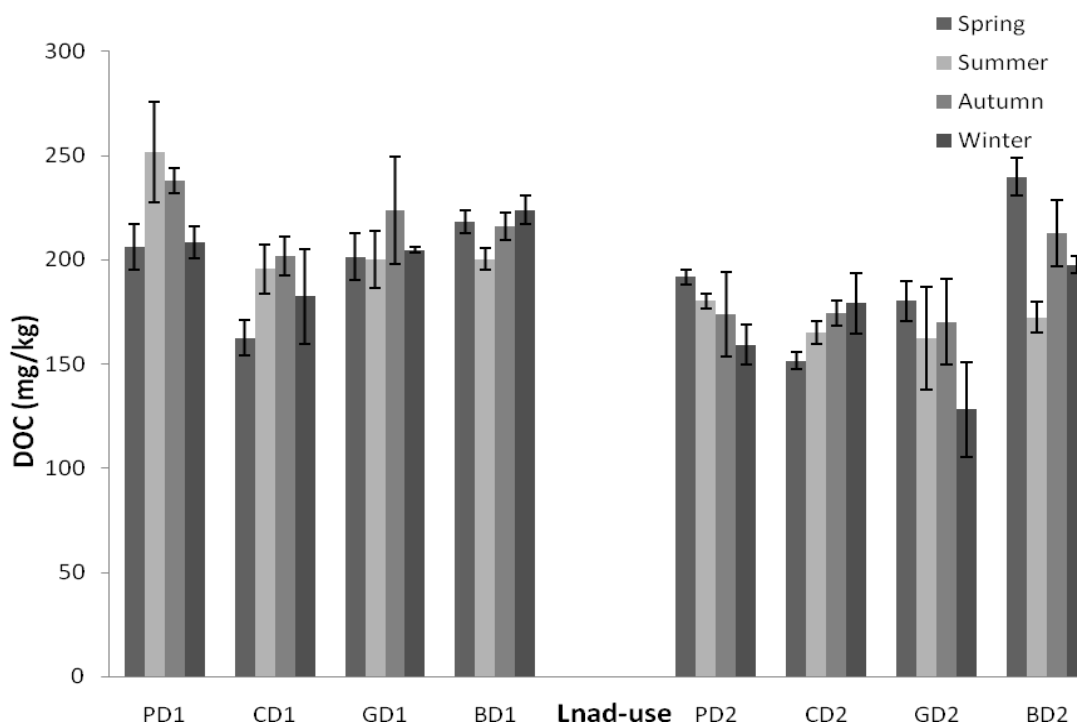
B: Bog pine

\* Data are mean $\pm$ SD (n=3)

D<sub>1</sub>: Depth 0-20 cm

D<sub>2</sub>: Depth 60-80 cm

**Figure 5.6.** Seasonal variation of the concentration of the SEOC of the soils under different land uses and at different depths



P: Plantation

C: Cropland

G: Degraded grassland

B: Bog pine

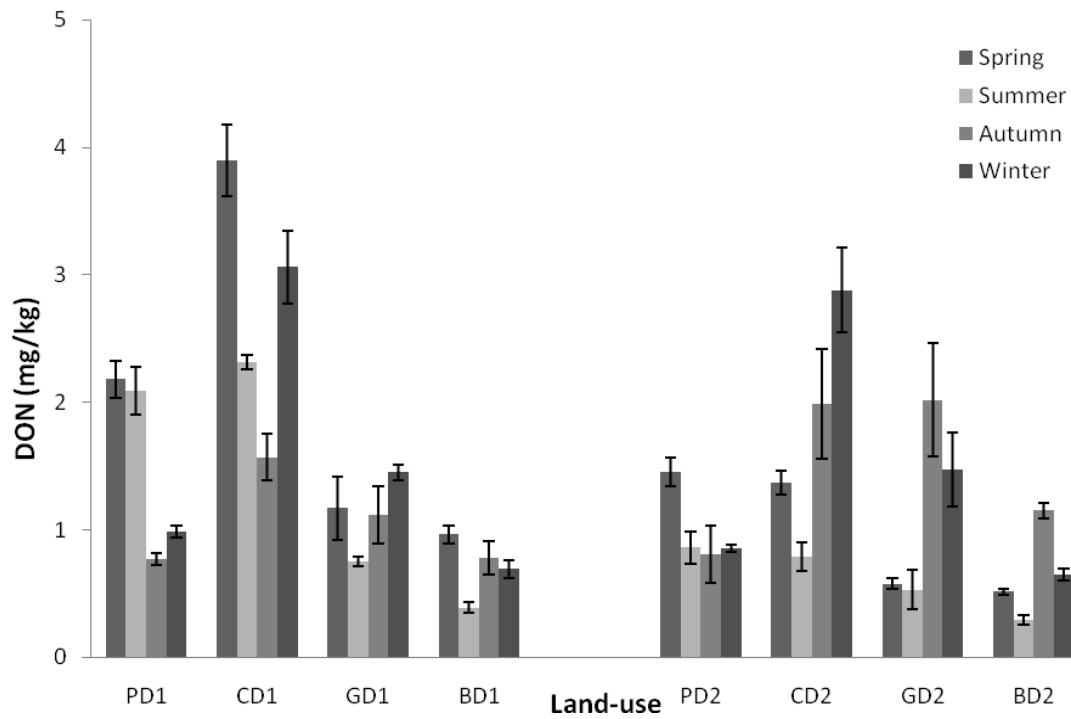
\* Data are mean $\pm$ SD (n=3)

D<sub>1</sub>: Depth 0-20 cm

D<sub>2</sub>: Depth 60-80 cm



**Figure 5.7.** Seasonal variation of the concentration of the WEON of the soils under different land uses and at different depths



P: Plantation

C: Cropland

G: Degraded grassland

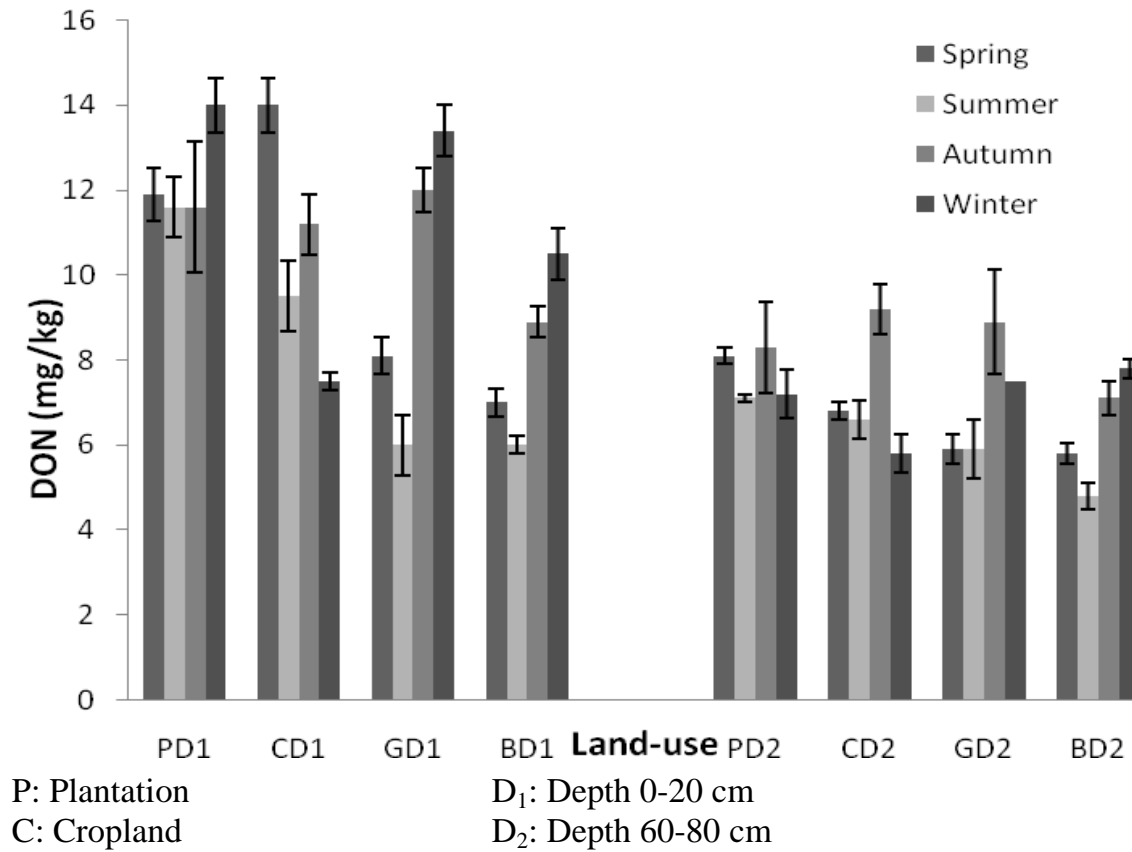
B: Bog pine

\* Data are mean $\pm$ SD (n=3)

D<sub>1</sub>: Depth 0-20 cm

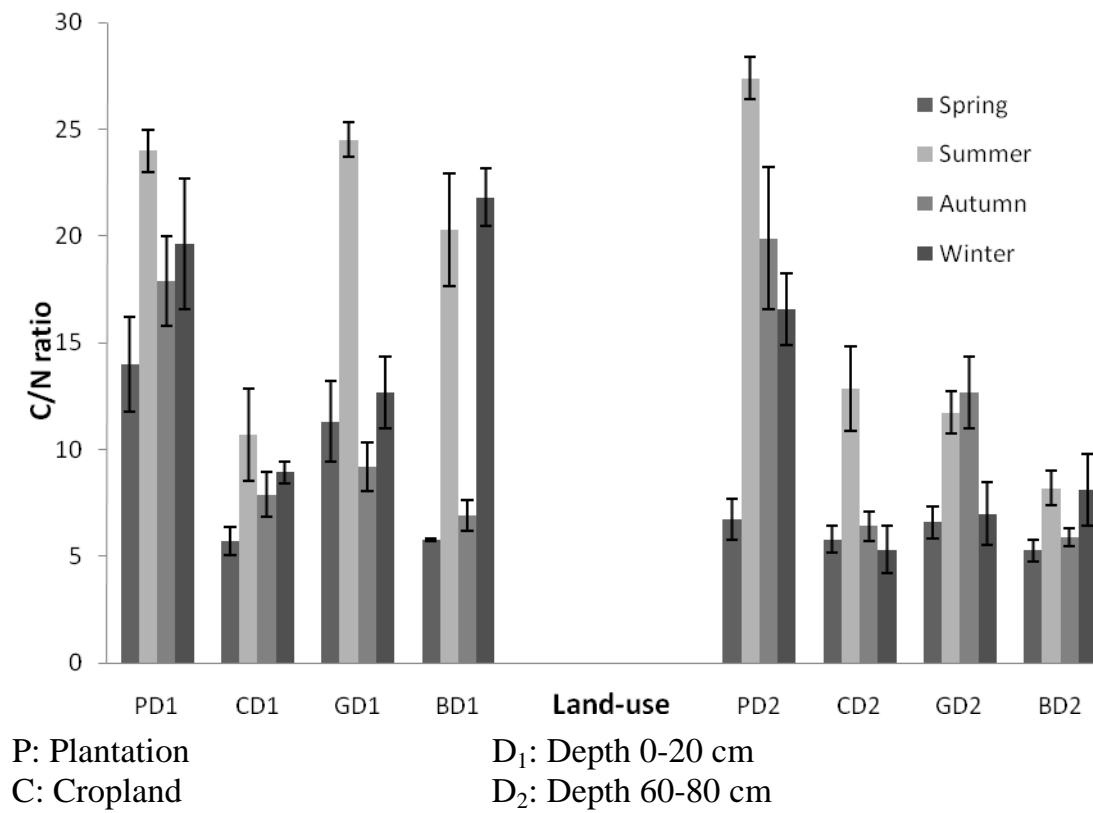
D<sub>2</sub>: Depth 60-80 cm

**Figure 5.8.** Seasonal variation of the concentration of the SEON of the soils under different land uses and at different depths



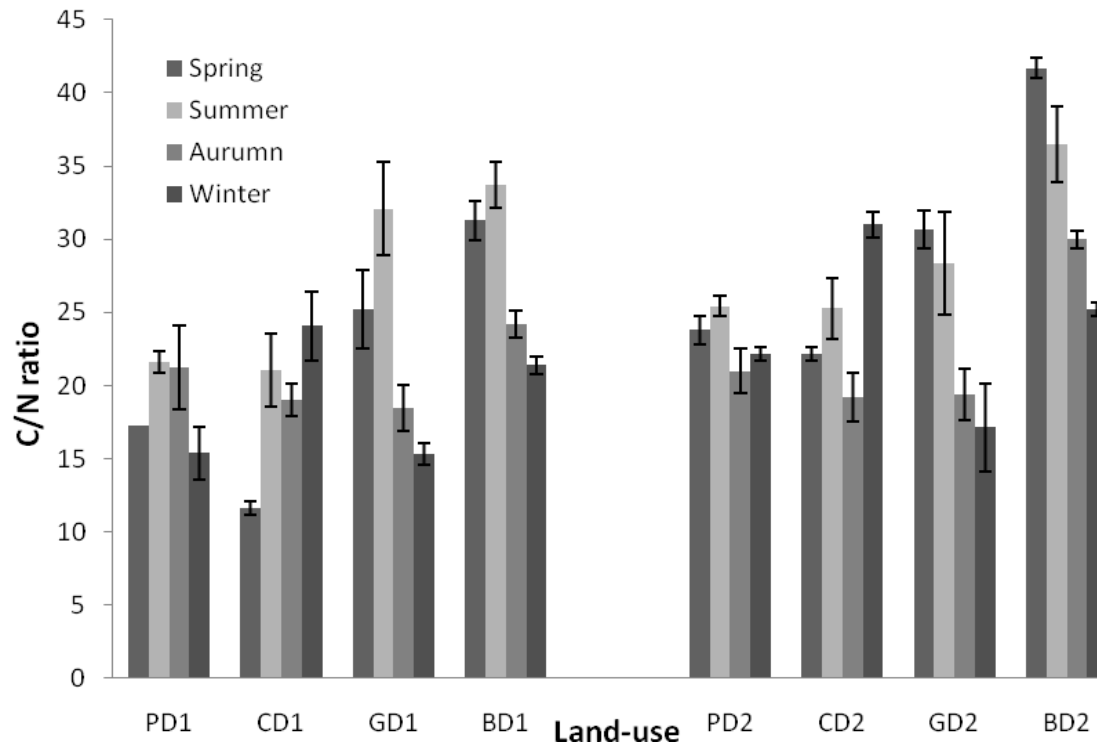
P: Plantation  
C: Cropland  
G: Degraded grassland  
B: Bog pine  
\* Data are mean±SD (n=3)

**Figure 5.9.** Seasonal variation of the C/N ratio of the WEOM of the soils under different land uses and at different depths



\* Data are mean±SD (n=3)

**Figure 5.10.** Seasonal variation of the C/N ratio of the SEOM of the soils under different land uses and at different depths



P: Plantation

C: Cropland

G: Degraded grassland

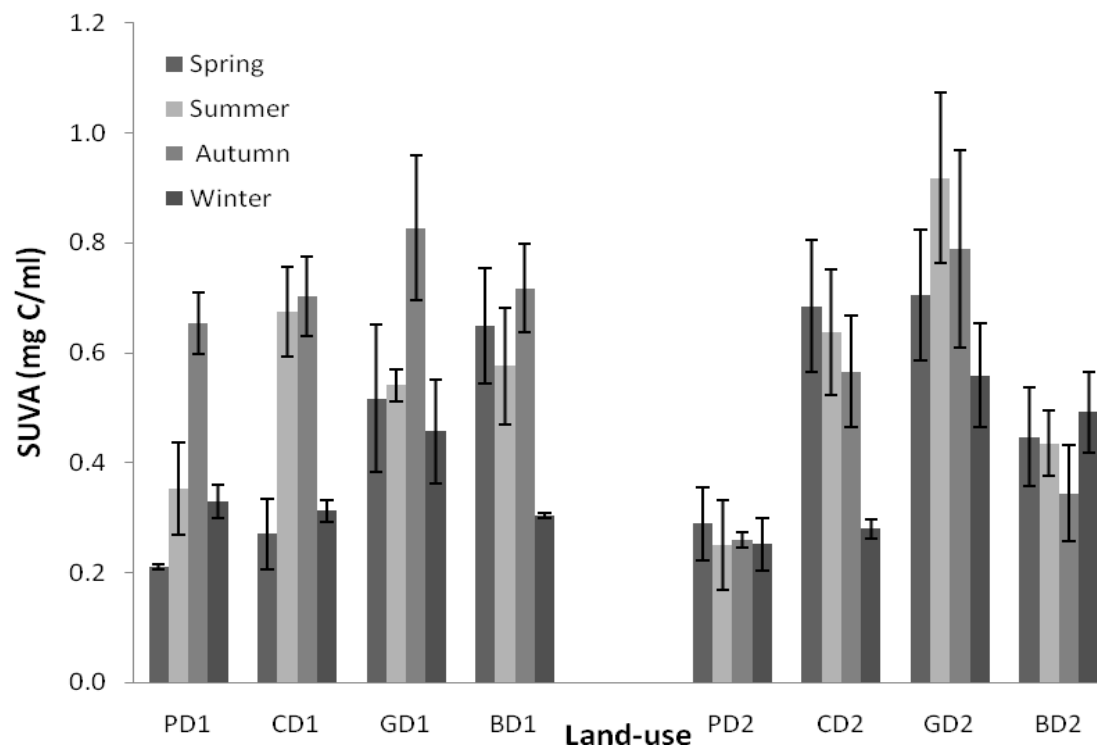
B: Bog pine

\* Data are mean $\pm$ SD (n=3)

D<sub>1</sub>: Depth 0-20 cm

D<sub>2</sub>: Depth 60-80 cm

**Figure 5.11.** Seasonal variation of the SUVA value of the WEOM of the soils under different land uses and at different depths



P: Plantation

C: Cropland

G: Degraded grassland

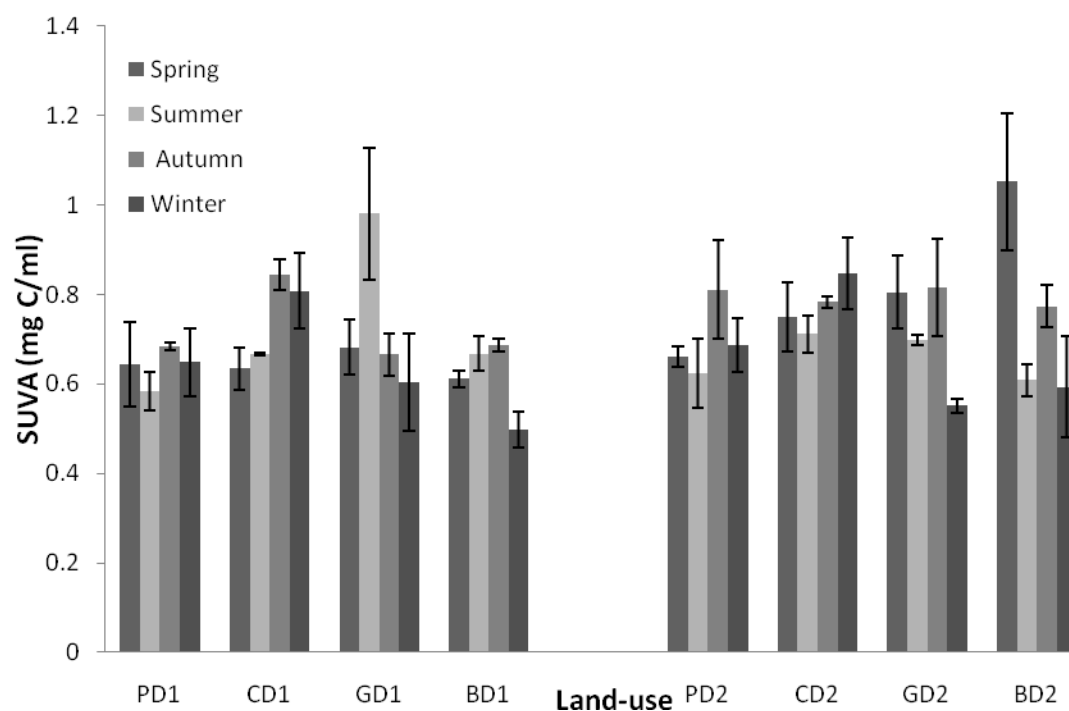
B: Bog pine

\* Data are mean $\pm$ SD (n=3)

D<sub>1</sub>: Depth 0-20 cm

D<sub>2</sub>: Depth 60-80 cm

**Figure 5.12.** Seasonal variation of the SUVA value of the SEOM of the soils under different land uses and at different depths



P: Plantation

C: Cropland

G: Degraded grassland

B: Bog pine

\* Data are mean $\pm$ SD (n=3)

D<sub>1</sub>: Depth 0-20 cm

D<sub>2</sub>: Depth 60-80 cm

## **Chapter Six**

# **Abiotic solubilization of soil organic matter, a less-seen aspect of dissolved organic matter production**

### **6.1. Introduction**

Stabilization and de-stabilization mechanisms of soil organic matter (SOM) are increasingly examined to understand the effects of land use and climate change on soil C. Examining the factors affecting the immense pool of soil C; estimated to be greater than 1500 Gt; is critical to understand the terrestrial C cycle (Jobbagy and Jackson, 2000; von Lützow et al., 2006). One of the most elusive soil C pools -dissolved organic matter (DOM)- is thought to be the central link among the continuum of labile to stable SOM pools that control soil C dynamics. The extent to which biotic and physiochemical factors influence DOM dynamics has been vigorously debated (Kemmitt et al., 2008).

DOM is the most mobile and potentially reactive pool of SOM (Neff and Asner, 2001; von Lützow et al., 2007). Though dwarfed in size by total SOM, it plays an important role as the intermediary between physically stable and labile C pools (Zsolnay, 1996). DOM contributes considerably to many soil processes through its' fast turnover rate, high mobility and broad reactivity in the soil environment (Boddy et al., 2007; Ellert and Gregorich, 1995; Neff and Asner, 2001). Although little is known about actual DOM fluxes, it has been suggested to be an important component of terrestrial C dynamics (Neff and Asner, 2001). The production and flux of DOM in soil is considered to be largely controlled by microbial activity acting on plant litter and SOM as a substrate (Park and Matzner, 2003).

Numerous studies have addressed the dynamics of DOM in terrestrial ecosystems, yet there is still much debate on mechanisms leading to its production. The dearth of information on DOM production is largely attributable to the lack of knowledge on mechanisms controlling OM solubilization and subsequently its release into soil solution

(DOM). There is growing evidence indicating that native SOM is the primary source of DOM within the mineral soil (Froberg et al., 2006; Kalbitz et al., 2000b). Physicochemical factors like temperature (Christ and David, 1996; Moore et al., 2008), oxidation-reduction regime (O'Connell et al., 2000), and water flux (Moore and Dalva, 2001; Park and Matzner, 2003) are important regulators of the production rate of DOM. Currently, the consensus is that the rate of OM solubilization (DOM production) is determined as a result of the interactions among biotic and abiotic processes. However, contrasting views exist concerning the relative significance of biotic and abiotic controls on DOM release (Guggenberger et al., 1994; Kemmitt et al., 2008; Moore et al., 2008; Neff and Asner, 2001).

Winogradsky's (1924) theory on soil substrate utilization proposes that microbial activity can be partitioned into autochthonous and zymogenous habits, which are responsible for the biodegradation and consumption of recalcitrant and labile SOM, respectively. However, abiotic processes act indirectly on microbial activity to control the source and fractions of mineralized SOM. Regardless of the conceptual schemes of SOM turnover proposed to date, the activities of the microbial decomposer community are compartmentalized to account for observed differential turnover of SOM fractions. Apart from some ambiguities and questions concerning this concept (e.g. Carney and Matson, 2005; Langer, 2004; Strickland et al., 2009), Kemmitt et al. (2008) recently challenged this well-established theory, proposing the "regulatory gate" hypothesis. They posited that SOM is mineralized during a two-step process with non-bioavailable compounds initially transformed to bioavailable OM (DOM), solely as a result of abiotic processes. The altered (bioavailable) compounds are metabolized by the decomposer community (the second step). Despite the supporting evidence provided by their experiment, the hypothesis has been questioned mainly because of the extra emphasis on the role of non-biological factors controlling the mineralization of OM (Kuzyakov et al., 2009; Paterson, 2009).

The factors controlling the production of DOM can be divided into microbially driven and abiotic processes. We tested the regulatory gate hypothesis, using a  $^{13}\text{C}$



isotope pool dilution approach to determine the source production and consumption of DOM in soil solution. The objective of this study was to evaluate the impact of the microbial activity on the net production of DOM from the native SOM in the presence of added DOM and plant residue.

## **6.2. Materials and methods**

### **6.2.1. Soil sampling**

Soils were selected to reflect differences in mineralogy with similar total soil C content and chemistry (Table 1). Topsoil (0-15 cm) samples were collected from two soils under i) oak woodland (Sierra Field Station, 39.278 N and 121.289 W) and ii) permanent pasture (Yolo Land, 38.650 N and 122.066 W). The woodland (Sierra) soil is derived from schist (metavolcanic), dominated by 2:1 clay minerals including mica and chlorite, with a high iron oxide content. The pasture (Yolo) soil is derived from mixed alluvium and dominated by montmorillonite. The visible litter of the soils was removed, the soils were mixed and sieved (2 mm). Soils were adjusted to optimum water status (50% of the water holding capacity; WHC) by addition of deionized water and mixing the moistened soil followed by a 10 day pre-incubation period at room temperature (22°C). The pre-incubated soils were stored in the refrigerator (4°C) for about two weeks until the start of the experiment.

### **6.2.2. OM and DOM source**

The above-ground part (shoot and leaves) of ryegrass (*Lolium perenne* L.) grown in  $^{13}\text{CO}_2$  enriched conditions was used as the source of plant residue (Experiment A) and DOM (Experiment B). Plant material was oven dried at 50 °C, ground and sieved (mesh size 40). In Experiment A the prepared plant residue was mixed into the soil at a ratio of 1% (w/w, dry based). In Experiment B, DOM was prepared by extracting plant residue with 0.01 M  $\text{CaCl}_2$  (0.5:100 ratio) at 75°C for 6 hr followed by shaking (15 min). The extract was filtered through a 0.45  $\mu\text{m}$  filter to obtain DOM. DOM was prepared fresh before each leaching event (see Experiment B). As shown in Table 2 the properties of

plant residue changes substantially during extraction (e.g. C/N ratio of 27 vs. 15 in residue and DOM, respectively). This provides two different levels of lability of the added OM for decomposer community to help for further assessment of the impact of the quality of the added OM (residue vs. its extract) on the regulatory gate hypothesis.

### 6.2.3. Sample preparation and incubation experiment

#### 6.2.3.1. Experiment A

To ensure proper infiltration and leaching during the experiment, soils were mixed with sand (0.4:1, sand-to-soil ratio, dry based). The mixed soils were wetted to 50% of WHC and pre-incubated for 7 d. Ryegrass residue was uniformly mixed into the soil-sand mixture and 70 g of the mixture was packed into 100 ml polyethylene leaching columns. A subset of soils containing the plant residue was sterilized by autoclaving at 121°C (1 hr). Soil without added ryegrass served as controls. Each treatment was replicated three times. Each leaching column was placed in a one-quart (946 ml) Mason jar containing about 5 ml water to prevent soil desiccation, sealed and placed in the dark at 22°C. The lids of the jars contained septa for headspace sampling to monitor CO<sub>2</sub> production using an infrared gas analyzer (Qubit CO<sub>2</sub> analyzer, model S-151, Qubit systems, Kingston, ON, Canada). The duration of incubation was 90 d (78 d for control soils) to provide sufficient time for the assessment of DOM production dynamics. The sampling of headspace CO<sub>2</sub> occurred regularly to ensure the CO<sub>2</sub> concentration did not exceed 2-4% by volume in the non-sterile samples. At each CO<sub>2</sub> sampling point, a sample of 12 ml for <sup>13</sup>CO<sub>2</sub> was transferred to a Vacutainer and analyzed on a SerCon Cryoprep TGII trace gas concentration system interfaced on a PDZ Europa 20-20 isotope ratio mass spectrometer (Sercon Ltd., Cheshire, UK). After each CO<sub>2</sub> sampling, the jars were opened and aerated. Headspace samples from blank jars were used to correct the sample CO<sub>2</sub> concentration. Ambient concentration for CO<sub>2</sub> ranged from 0.03 to 0.05%.

The soil columns were leached on 1, 6, 18, 30, 42, 54, 66, 78 and 90 d. The leaching solution contained CaCl<sub>2</sub> (0.01M) at a ratio of 1:1 (solution-to-soil) to maintain the soil structure and consistent leaching characteristics (0.5 ml l<sup>-1</sup>). To maintain the

sterility of the autoclaved soils during the incubation,  $\text{HgCl}_2$  ( $0.7 \text{ mg-Hg g-soil}^{-1}$ ; Wolf and Skipper, 1994) was added to the leaching solution. Following each leaching event, the soil columns were placed under 1 atm of vacuum, weighed and additional water was added as necessary to maintain soil moisture content at 50% of WHC throughout the incubation. The leachate was filtered through a  $0.45 \mu\text{m}$  filter (DOM) and stored at  $4^\circ\text{C}$  (<7d) until analyzed.

#### 6.2.3.2. Experiment B

The same soil preparation, sterilization procedures, leaching columns, and  $\text{CO}_2$  and DOM analysis was carried out except than the plant residue was replaced by DOM obtained from plant residue (part 2.2.). The DOM (Table 2) was applied in the leaching intervals mentioned above.  $\text{HgCl}_2$  ( $0.7 \text{ mg-Hg g-soil}^{-1}$ ) was added to the DOM. Prior to the experiment it was demonstrated that no precipitation or fluctuation occurred between Hg and DOM at the given concentrations (data not shown).

#### 6.2.4. Analytical methods

Total soil C was determined on an elemental analyzer (Costech ECS 4010, Valencia, CA). Dissolved organic C (DOC) was determined by UV-persulfate digestion (Teledyne-Tekmar Phoenix 8000). Total dissolved nitrogen (TDN) was determined by persulfate oxidation (Cabrera and Beare 1993). Mineral N ( $\text{NO}_3^-$  and  $\text{NH}_4^+$ ) was determined colorimetrically (Verdouw et al. 1978; Doane and Horwath, 2003). Organic N was calculated as the difference between the concentrations of total N and total mineral N.

UV absorbance of DOM was determined in 1 cm quartz cuvettes at room temperature, on a Shimadzu UV spectrophotometer (UV-Mini 1240) at 280 nm to estimate the average degree of aromaticity of DOM. Specific UV absorption (SUVA) was calculated after normalizing UV absorption value for the DOC concentration (Weishaar et al., 2003). The samples with a SUVA value of greater than 0.3 were diluted to ensure comparability of results among samples. No interference by Hg on DOM UV

absorption at 280 nm was observed following separate addition of HgCl<sub>2</sub> (data not shown).

Fluorescence measurements were carried out in 1 cm quartz fluorescence cells at room temperature, using a Cary Eclipse spectrofluorometer (Varian Inc, CA, USA). The samples containing more than 10 mg l<sup>-1</sup> DOC were diluted before fluorescence analysis. The excitation wavelength and the emission wavelength range were set at 280 nm and 400-470 nm (5 nm increments), respectively. Humification Index (HI) was calculated as the ratio of fluorescence intensity at 470:400 nm (Kalbitz et al. 2000).

The DOM samples were analyzed for <sup>13</sup>C content using a TOC analyzer (1010 OI Analytical, College Station, TX, USA) coupled to a PDZ Europa 20-20 isotope ratio mass spectrometer (Sercon Ltd., Cheshire, UK). Each run of samples included working DOC standards prepared with glucose of known <sup>13</sup>C enrichment in order to ensure accuracy of the <sup>13</sup>C determination within and between runs. At each CO<sub>2</sub> sampling point, a sample of 12 ml for <sup>13</sup>CO<sub>2</sub> was transferred to a Vacutainer and analyzed on a SerCon Cryoprep TGII trace gas concentration system interfaced on a PDZ Europa 20-20 isotope ratio mass spectrometer (Sercon Ltd., Cheshire, UK).

The fraction of C originating from added ryegrass residue or DOM (F) in leachate or CO<sub>2</sub> was calculated according to the mixing model as:

$$F = (\delta^{13}\text{C}_{\text{sample}} - \delta^{13}\text{C}_{\text{control}}) / (\delta^{13}\text{C}_{\text{substrate}} - \delta^{13}\text{C}_{\text{control}}) \quad (1)$$

Where  $\delta^{13}\text{C}_{\text{sample}}$  is the  $\delta^{13}\text{C}$  value of the leached or respired CO<sub>2</sub> from treated soils,  $\delta^{13}\text{C}_{\text{control}}$  is the  $\delta^{13}\text{C}$  value in the leachate or respired CO<sub>2</sub> from control soils, and  $\delta^{13}\text{C}_{\text{substrate}}$  is the  $\delta^{13}\text{C}$  value of the labelled substrate. All data are reported as mean±standard error of three replicates.

## 6.3. Results

### 6.3.1. CO<sub>2</sub> efflux in all experiments

We observed regular fluctuations in CO<sub>2</sub> efflux in both experiments, corresponding to leaching events (Fig. 1). The ryegrass-amended soils and controls soils showed a declining valley and peak cycle in CO<sub>2</sub> efflux subsequent to leaching events

(Fig. 1a). ). In DOM-amended soils, CO<sub>2</sub> production was characterized by pronounced valley and peak cycles as a result of the added DOM at each leaching event (Fig. 1b). The CO<sub>2</sub> efflux variations between replicates were very small at both experiments (data not presented). Despite the initially larger concentrations of CO<sub>2</sub> produced in ryegrass-amended compared to DOM-amended soils, the respiration rate of ryegrass-amended soil declined considerably (89% and 75% in Sierra and Yolo soil, respectively) by the end of the incubation period (Fig. 1a). The respiration rate decline of control soils was 76% in Sierra and 72% in Yolo soil. The CO<sub>2</sub> efflux was greater in Yolo than Sierra soil in control, and both residue- and DOM-amended soils (Figs. 1a and 1b). While total CO<sub>2</sub>-C losses was 468 and 357  $\mu\text{g-C g-soil}^{-1}$  in control Yolo and Sierra soils respectively; it was 3028 and 2525  $\mu\text{g-C g-soil}^{-1}$  in residue amended, and 2387 and 1714  $\mu\text{g-C g-soil}^{-1}$  in DOM amended soils (Yolo and Sierra soils, respectively). We did not observe any CO<sub>2</sub> production in sterilized soils in either ryegrass- or DOM-amended soils throughout the incubation period (data not shown).

### 6.3.2. Experiment A

The concentration of the DOC leached from sterilized soils was consistently greater compared to non-sterile soils (Fig. 2a). By day 50, DOC release from sterilized soils had stabilized, but remained about 10  $\mu\text{g-C g-soil}^{-1}$  greater than the non-sterile soils. The concentration of DOC leached from non-sterile soils was consistently small and averaged 10  $\mu\text{g-C g-soil}^{-1}$  throughout the experiment. Despite the overall lower concentrations of DOC leached from control than ryegrass-amended soils, the pattern of DOC changes was very similar between the control and ryegrass-amended soils (Fig. 2a). In control soils, DOC decreased substantially in sterilized soils (86 and 90% in Yolo and Sierra, respectively), but it changed considerably less in non-sterile soils (28 and 37% in Yolo and Sierra, respectively). The amounts or pattern of leached DOC between the two soils (Yolo and Sierra) were very comparable (Fig. 2).

The pattern of DON release was similar to DOC (Fig. 3a). We observed a substantial decrease in the concentration of DON leached from both non-sterile (88% and 95% in Yolo and Sierra soil, respectively) and sterilized soils (91% and 92% in Yolo and Sierra soils, respectively) during the incubations.

Apart from a few exceptions, the proportion of the DOC derived from the added ryegrass was less than 25% of the total DOC leached from the soils (Fig. 4). Despite slight fluctuations in the proportion of the DOC derived from the added ryegrass in non-sterile soils, this proportion was largely constant in sterilized soils during the experiment. The results (Fig. 4) clearly revealed that the proportion of the DOC derived from the added ryegrass was very similar among sterilized and non-sterile soils, especially after day 50 of the incubation. Data presented in Table 3 indicate that proportion of  $^{13}\text{C}$  content of leachate originated from the added residue ranged from 8 to 14.5% and was comparable between the sterilized and non-sterilized soils. However, the proportion  $^{13}\text{C}$  originated from residue which remained in soil at the end of the experiment was almost 2 times more in the sterilized than non-sterilized soils. The difference was accounted as respired C (46-52%) from ryegrass residue in non-sterile soils.

The pattern of changes in C/N ratio of the leached DOM between non-sterile soils was different, but by the end of the experiment, C/N ratio was more stable and similar between soils (15.0 and 14.3 in Yolo and Sierra soil, respectively) (Fig. 5a). In sterilized soils, the initially constant C/N ratio (ranged 13.0 - 16.8) increased sharply by the end of the experiment (Fig. 5a).

The SUVA values were constantly greater in DOM leached from non-sterile than sterilized soils, indicating a higher degree of aromaticity (Fig. 6a). The proportion of SUVA values of sterilized soils gradually increased during the incubation period (48% and 83%, in Yolo and Sierra, respectively). In non-sterile soils, SUVA values fluctuated, increasing (115%) in Sierra soil or decreasing (16%) in Yolo soil. By the end of the experiment, SUVA values of both soil reached similar levels (Fig. 6a).

Similar to SUVA, HI of DOM was constantly greater in non-sterile than sterilized soils (Fig. 7a). HI of the DOM leached from non-sterile soils was almost stable. In

contrast, HI gradually increased (14% and 12% in Yolo and Sierra soil, respectively) in sterilized soils (Fig. 7a).

### 6.3.3. Experiment B

The concentration of DOC leached from sterilized soils was consistently greater than non-sterile soils (Fig. 2b). In contrast to sterilized soils, the pattern of the DOC release between non-sterile soils differed, with the Sierra soil releasing greater concentrations of DOC after day 20. By the end of the experiment, the concentration of leached DOC was similar between soils and regardless of sterility conditions. DOC leached from sterilized soils increased slightly (6% and 17% in Yolo and Sierra soil, respectively) during the experiment (Fig. 2b). This increase was, however, considerably greater in non-sterile soils (89% and 93% in Yolo and Sierra soil, respectively).

A similar pattern of DON release to that of DOC (Fig. 3b) occurred with DOM addition. The concentration of the DON leached from non-sterile soils increased substantially (from 2.4 and 5.5 to 18.0 and 17.5  $\mu\text{g-N g-soil}^{-1}$ , in Yolo and Sierra soil, respectively) following continuous application of DOM (Fig. 3b). This led to a similar concentration of the DON obtained from sterilized and non-sterile soils at the end of the experiment. The concentration of DON leached from sterilized soils was consistently less than the concentration of N of the applied DOM ( $25.6 \pm 0.7 \mu\text{g-N g-soil}^{-1}$ , Table 2).

The proportion of the leached DOC derived from the added DOC was fairly constant for both soils and sterility conditions (Fig. 4). We observed that in non-sterile soils at least 92.0% (Yolo) and 88.7% (Sierra) of the DOC derived from the added DOC (Fig. 4). This proportion was less in sterilized soils, but increased slightly (18% in both soils) during the experiment. After successive addition of C as DOM (end of the experiment), there was not a considerable difference between the proportion of DOC that was derived from the applied DOC in sterile and non-sterile soils (Fig. 4). Fig. 4. suggests that under the experimental conditions (high DOM input), soil mineralogy had only a limited impact on the proportion of the DOC derived from the added DOC. Table 3 shows that the majority (53-72%) of the DOM derived  $^{13}\text{C}$  was recovered in leachate of

both sterilized and non-sterile soils, whereas proportion of DOM derived  $^{13}\text{C}$  that was remained in soils at the end of experiment was similar between Yolo and Sierra soils (17%), but substantially smaller in non-sterile than the sterilized soils (28 and 29% in Yolo and Sierra, respectively). The difference was amounted as respired C in non-sterile soils (14-30% of added C).

Despite some differences in the initial C/N ratio of the leached DOM, this was largely constant during the experiment in both soils and sterility status (Fig. 5b). The C/N ratio of the DOM leached was slightly wider than that of the added DOM (mean  $15 \pm 0.6$ , Table 2).

We observed a gradual increase in SUVA values of DOM leached during the incubation period (Fig. 6b). However, the overall increase was greater in DOM leached from non-sterile soils (26% and 23% in Yolo and Sierra soil, respectively) compared to sterilized soils (7% and 21% in Yolo and Sierra soil, respectively). The SUVA values of the leached DOM remained less than that of the added DOM ( $0.86 \pm 0.03 \text{ L mg-C m}^{-1}$ , Table 2) throughout the experiment.

Although HI of DOM did not change in the DOM derived from non-sterile soils, it increased 18% and 19% (Yolo and Sierra soil, respectively) in DOM derived from sterilized soils (Fig. 7b). The HI of DOM derived from non-sterilize soils was larger than sterilized soils throughout the experiment. HI of the applied DOM ( $0.61 \pm 0.02$ , Table 2) increased when passing through either of sterilized or non-sterile soil columns.

## **6.4. Discussion**

### **6.4.1. Soil respiration**

Addition of either of ryegrass residue or DOM to soil caused a significant initial pulse in soil respiration (Fig. 1) which often occurs after addition of labile substrates to soil (e.g. Bingemann et al., 1953). We observed a considerable decrease in the basal respiration of the control and ryegrass-amended soils during the incubation as anticipated. This has been attributed to the gradual consumption of the more labile portion of the native OM and/or added substrate and decrease in the pool size overtime (Chow et al.,



2006; Park et al., 2002). The addition of the labile DOM input in separate leaching events caused spikes in CO<sub>2</sub> production. The change in the basal soil respiration was considerably larger (up to 4 times) in DOM- compared to residue-amended soils, as a result of frequent addition of labile input (DOM). A considerable portion (46-52%) of the added ryegrass residue was metabolized (CO<sub>2</sub> loss), amounted to 67 and 71% of the total respired C in Yolo and Sierra soils, respectively. In comparison, a smaller proportion (14-30%) of the applied DOM respired as CO<sub>2</sub>, equivalent with 42 and 27% of the total C loss (CO<sub>2</sub>) in Yolo and Sierra soils, respectively. Compared to control soils, the amount of respired C from SOM pool (CO<sub>2</sub> primed) was 2.1 times (both soils) larger in residue-amended and 3.0 and 3.5 times larger in DOM-amended soils (Yolo and Sierra soils, respectively). The accelerated decomposition of native SOM in response to added labile OM, termed priming effect, has been long observed (e.g. Bingemann et al., 1953; Löhnis, 1926).

While the majority (~70%) of DOC (see part 4.3) was derived from the humified OM, the majority (67-71%) of mineralized C derived from ryegrass residue. Similarly, Hagedorn et al. (2004) reported that recently added C was preferentially respired as CO<sub>2</sub>, with the majority of DOC originating from the humified SOM. This is line with recent evidence (e.g. Chow et al., 2006) and in contrast with the concept that solubilized OM constantly serves as the primarily source of C for decomposing community (e.g. Kalbitz et al., 2000b).

#### 6.4.2. Dynamics of DOC and DON

The initially great concentrations of the DOC and DON leached from sterilized ryegrass- or DOM-amended soils (Fig. 2 and 3) were likely due to the (i) release of the lysed microbial biomass (Warcup, 1957), ii) release/detachment of the physically bound/trapped OM (Berns et al., 2008), and iii) solubilization of the OM as a result of the autoclaving process (Alef, 1995; Salolius et al., 1967). The initial few leaching events appeared sufficient to remove the DOC and DON produced as a result of the autoclaving. Thus, the subsequent lower concentrations of DOC and DON leached from non-sterile

soils; that have been observed in similar experiments (e.g. Stutter et al., 2007); are attributed to CO<sub>2</sub> mineralization and microbial assimilation in non-sterile soils.

The soils examined had similarities in texture and other characteristics of the soils (Table 1), but differed in mineralogy. We assumed that the different trends of DOC and DON release in DOM-amended Sierra than Yolo soil was mainly due to its more complex mineralogy, particularly its large content of iron oxides (Table 1). However, similar amounts of DOC and DON leached from the soils at the end of the incubation. Other studies have shown the considerable influence of mineralogy on DOM adsorption (e.g. Benke et al., 1999). In addition to mineralogy, the different dynamics of C and N release could have been due to different decomposer community structures of the soils. Decomposition rate of added organic matter (grass vs. forest litter) to a soil has been shown (Cookson, W. R, 1998) that is affected by the structure of the native decomposing community of the soil. Therefore, at the initial stage of the incubation, the microflora and related enzymes of Sierra soil (woodland) may have been less efficient at decomposing ryegrass extract (DOM) compared to that of Yolo soil (grassland).

#### 6.4.3. Abiotic solubilization of OM

We observed that with a few exceptions, the contribution of C derived from the added ryegrass was not more than on the average 25% of leached C (DOC). In other words, in the presence of labile OM (ryegrass residue), 60-80% of the solubilized C originated from native SOM, with no considerable effect of microbial activity (Fig. 4). Paterson et al (2007) quantified the accelerated solubilization of the native SOM by microorganisms in the presence of labile OM input. They reported that after 4 weeks more than 80% of bacterial biomass C within the rhizosphere originated from native C source (SOM). Other studies suggested that compared to native SOM, fresh OM input (throughfall, rhizodeposition, litter layer) may have minor contribution to DOM production in topsoil (e.g. Hagedorn et al., 2002) and specifically subsoil (e.g. Froberg et al., 2006). For example, Froberg et al. (2006) using <sup>14</sup>C and Sanderman et al. (2008) with <sup>14</sup>C and <sup>13</sup>C-NMR techniques showed that the solubilized OM within the mineral soil

layers was derived substantially from the humified SOM, rather than fresh OM sourced from upper horizons. They concluded that DOM originates as a consequence of i) substantial microbial processing of OM and particularly ii) exchange (sorption–desorption) reactions between incoming soluble OM and intact SOM. In our experiment, under sterilized conditions even after successive addition of DOM (380 mg-C l<sup>-1</sup>), 10–20% of DOC consistently derived from native SOM (Fig. 4). In other words, in DOM-amended soils despite a consistently larger DOC release from sterilized than non-sterile soils (Fig. 2a), a larger proportion of C was derived from SOM in sterilized soils (Fig. 4). This suggests that the presence of microorganisms reduced the size of native OM solubilized as a result of exchange reactions. This along with the results observed in the ryegrass-amended soils highlight the contribution of abiotic processes (e.g. exchange and/or solubilization reactions) in DOM-SOM interactions.

In addition, our results did not show a considerable difference over time in the proportion of DOC derived from the applied ryegrass/DOM, regardless of soil microbial activity (Fig. 4). These results show that the presence of the microorganisms initially enhanced the production of the DOM from the added ryegrass and its DOM. However, the initial stronger impact of microbial activity was largely attenuated over time as evidenced by only small difference between the proportions of the DOC derived from ryegrass or DOM in sterilized and non-sterile soils (Fig. 4). Although it has been commonly accepted that the combination of biotic and abiotic factors determine the production of DOM in the soil (Kuzyakov et al., 2009), some researchers have indicated that the process of DOM export within soil is controlled primarily by abiotic factors (Michalzik and Matzner, 1999; Neff et al., 2001). For example, diffusion of OM from immobile to mobile phase (potentially solubilisable pool) has been suggested to act as a constant DOM replenishing process in soil (Tipping, 1998; Stutter et al., 2007). The diffusion process itself is largely regulated by soil water flux (Park and Matzner, 2003). Similarly, through comparison of a large number of soil samples collected across a global latitudinal gradient, Jones et al. (2009) reported that the conversion of high- to low-

molecular weight OM is the rate limiting step in SOM breakdown and is primarily controlled by abiotic factors (moisture, temperature, etc.). Our results do not contradict the close relationship between DOC production and microbial activity in the soil, but they clearly suggest that the primary control over DOM production is physical rather than biological. Despite some ambiguities raised from the experimental conditions in the study undertaken by Kemmitt et al (2008), (Kozyakov et al., 2008), our findings are consistent with the “regulatory gate” hypothesis proposed by Kemmitt et al. (2008). This hypothesis states that in the absence of fresh OM, the solubilization of the Native SOM is regulated primarily by abiotic factors. Our results showed that even in the presence of fresh OM, the solubilization rate of native SOM (DOC production) was not affected by microbial activity (Fig. 4). Therefore, microbial activity appears to be largely regulated by the delivery rate of the abiotically solubilized OM.

In defence of the biological production pathway, the production of DOM in sterilized soils could have resulted from the activity of residual extracellular enzymes. The activity of residual extracellular enzymes, released from i) the lysed microbial biomass during the sterilization processes, and/or ii) disruption of the aggregates after successive leaching experiments (wetting-drying), could have been attributed to abiotic factors. In this case, one could expect a decrease in the trend of the DOC production from either ryegrass- or DOM-amended soils because of the consumption and turnover of the enzymes during the experiment. However, the almost constant trend of the DOC production from DOM- and specifically ryegrass-amended soils suggests that extracellular enzymes played a minor role in the decomposition of OM under our experimental conditions. The extracellular and released enzymes were largely denatured during autoclaving (Carter et al., 2007; Tiwari et al., 1988). Furthermore, given the strong interference of Hg with extracellular enzymes (Baldrian, 2003), the continuous addition of Hg in the sterilized soils should have further reduced any activity of extracellular enzymes remaining in the soils after autoclaving.

#### 6.4.4. DOM properties

The initial lower C/N ratios in the leached DOM from ryegrass-amended Yolo soil (Fig. 5a) are likely due to the enhanced microbial activity. The lack of this C/N ratio trend in DOM leached from Sierra soil (ryegrass-amended) appears to be related to the adsorption of the solubilized DOM (Benke et al., 1999; Kaizer and Zech, 2000). Although the initial C/N ratio of DOM leached from sterilized ryegrass-amended soils (Fig. 5a) was almost stable, it appears that the C/N of the abiotically produced DOM may have decreased due to the turnover of microbial biomass with low C/N ratio (Vance et al., 1987). We assume that the increase in the C/N ratio of both soils at the end of the experiment (Fig. 5a) is due to the greater contribution of the humified OM that has been abiotically solubilized (the major source of DOM).

The C/N ratio of DOM leached from the ryegrass-amended soils was considerably less than the C/N ratio of the applied OM (C/N: 27.6, Table 2). In fact, given that the majority of the DOM leached from the ryegrass-amended soils originated from the bulk SOM, the range of C/N ratio of leached DOM was similar to C/N ratio of the soils (10 and 13.7 in Yolo and Sierra soil, respectively) and not of the applied plant residue. Other researchers have reported a stronger relationship between the C/N ratio of DOM with that of the SOM compared to fresh plant residue input (Michalzik and Matzner, 1999; Park and Matzner 2003; Smolander et al., 2002).

The C/N ratio of DOM leached from the DOM-amended soils was almost stable throughout the experiment (Fig. 5b) and similar to C/N ratio of the applied DOM (C/N:  $15 \pm 0.6$ , Table 2). This is consistent with the large contribution of the added DOM to the leached DOM in both sterilized and non-sterile soils (Fig. 3b). Indeed, it appears that the microbial effect on C/N ratio of the leached DOM was largely attenuated by the large DOM input to the soils, resulting in the lack of difference in C/N ratio of the DOM leached in the presence or absence of the microorganisms.

Specific UV absorption (SUVA) at 280 nm has been widely shown to correlate strongly with the aromatic property of DOM (e.g. Chin et al., 1994). We observed that SUVA values of the DOM leached from sterilized or non-sterile soils (either ryegrass- or

DOM-amended) gradually increased during the experiment (Fig. 6). This trend reflects the constant release of DOM from humified SOM. This is consistent with the results reported by Schaumann et al (2000), suggesting a gradual increase in the aromaticity of DOM released during long-term incubations. In the DOM leached from sterilized soils, SUVA values may also increased partly due to the gradual depletion of the lyzed microbial biomass components (low aromatic content) during subsequent leaching events. This is consistent with the increase of the C/N ratio of the DOM leached from sterilized ryegrass-amended soils. The greater SUVA values of the DOM leached from non-sterile soils compared to sterilized soils appears to be a result of the microbial uptake of available compounds in DOM, leaving more aromatic compounds in the leached DOM (Stutter et al., 2007). In addition, with regard to the increased recalcitrance of the microbially produced DOM (e.g. Ogawa et al., 2001), this may result in larger SUVA values in the DOM leached from non-sterile soils. The greater SUVA values of the DOM leached from the ryegrass-amended soils compared to DOM-amended soils (Fig. 3) can be explained by the source of the released DOM (humified OM *vs.* applied DOM with mean SUVA value of  $0.86 \text{ l mg}^{-1} \text{ C m}^{-1}$ ). The results confirm that microbial activity leads to formation of more recalcitrant DOM.

Humification index (HI) has been used as a sensitive indicator for DOM characterization (Lombardi and Jardim, 1999; Zsolnay et al., 1999). This property is believed to reflect the degree of polycondensation of DOM (Kalbitz et al., 2000a). Similar to SUVA value, HI value of ryegrass-amended soils was greater in the DOM leached from non-sterile than sterilized soils (Fig. 7a). The lower HI of the DOM leached from sterilized ryegrass-amended soils is assumed to be partly associated with the presence of the lyzed microbial biomass due to sterilization processes. The lyzed microbial biomass has been reported to substantially decrease the HI of the extractable OM (Akagi et al., 2007; Burns et al., 2008). While HI values were largely constant in DOM leached from non-sterile ryegrass- or DOM-amended soils, it gradually increased (12-20%) in the DOM leached from sterilized soils (Fig. 7). This is likely due to the gradual removal of the lyzed microbial biomass (low HI) from sterilized ryegrass-

amended soils. Given the large input of DOM in the DOM-amended soils, the gradually increased HI of sterilized soils which was constantly larger than non-sterile soils seems to be less affected by released microbial biomass, but highly related to the larger contribution (10-25%) of native soil organic C (high HI) in DOM leached from sterilized DOM-amended soils (Fig. 4). HI has been shown to be strongly correlated with SUVA values and C/N ratio of DOM (Kalbitz et al., 2000a). Although we observed a similar trend of HI and SUVA values, we did not see a similar pattern of HI or SUVA value changes with that of C/N ratio of the DOM.

### **Conclusion:**

DOM production in soil is believed to be regulated by a range of processes that are affected by biotic-abiotic interactions. The commonly accepted paradigm based on the strong influence of the microbial activity and extracellular enzymes on the solubilization of the native SOM has been recently challenged by the “Regulatory gate” hypothesis (Kemmitt et al., 2008). The hypothesis proposes that microbial activity is primarily controlled by “abiotically solubilized OM”. In our experiment, we observed that biological activity did not have a considerable effect on the quality and especially the proportion of the DOM produced from added plant residue or DOM during a 90-day incubation experiment. Although in our experiment the impact of the microbial activity on the properties of DOM was attenuated by a relatively large flux of DOM, microbial transformations affected the properties of the solubilized OM (DOM).

**Table 6.1.** Properties of the soils used in the experiment

Soil	pH <sup>1</sup>	CEC (Cmol Kg <sup>-1</sup> )	EC <sup>1</sup>	C <sub>(%)</sub>	N <sub>(%)</sub>	Texture <sup>2</sup>			Soil Classification <sup>3</sup>
						Sand	Silt	Clay	
Yolo Land	5.7	15	860	1.6	0.16	26	51	23	mixed, thermic Mollic Haploxeralf
Sierra Field Station	5.5	16	1280	2.4	0.18	36	45	19	mixed, thermic Mollic Haploxeralf

<sup>1</sup> Saturated paste<sup>2</sup> Hydrometer method<sup>3</sup> USDA soil Classification System**Table 6.2.** Properties of the OM and DOM added to the soils

Source	C	N	P	C to N	<sup>13</sup> C <sub>(atom %)</sub>	SUVA (l mg <sup>-1</sup> C m <sup>-1</sup> )	HI
OM	38.9*	1.41*	0.26*	27.6	1.819	—	—
DOM <sup>a</sup>	382±18**	25.6±1.2**	2.3±0.3**	15±1.0	1.681±0.01	0.86±0.05	0.61±0.03

a: Data are mean±SD of 9 extraction sets

\* %

\*\* mg l<sup>-1</sup>

Table 6.3. The contribution (%) of C derived from ryegrass residue or DOM to different pools at the end of experiment.

Soil	residue-amended soil			DOM-amended soil		
	Respired	Retained	Leached	Respired	Retained	Leached
Yolo	52.4±0.7	37.2±0.7	10.4±0.4	30.1±2.1	17.0±0.4	52.9±2.5
Yolo (St)	—	85.5±0.4	14.5±0.4	—	27.7±0.6	72.3±0.6
Sierra	45.9±2.3	46.2±2.1	8.0±0.2	14.2±0.9	16.6±0.5	68.0±1.2
Sierra (St)	—	87.9±0.2	12.1±0.2	—	28.8±0.9	71.2±0.9

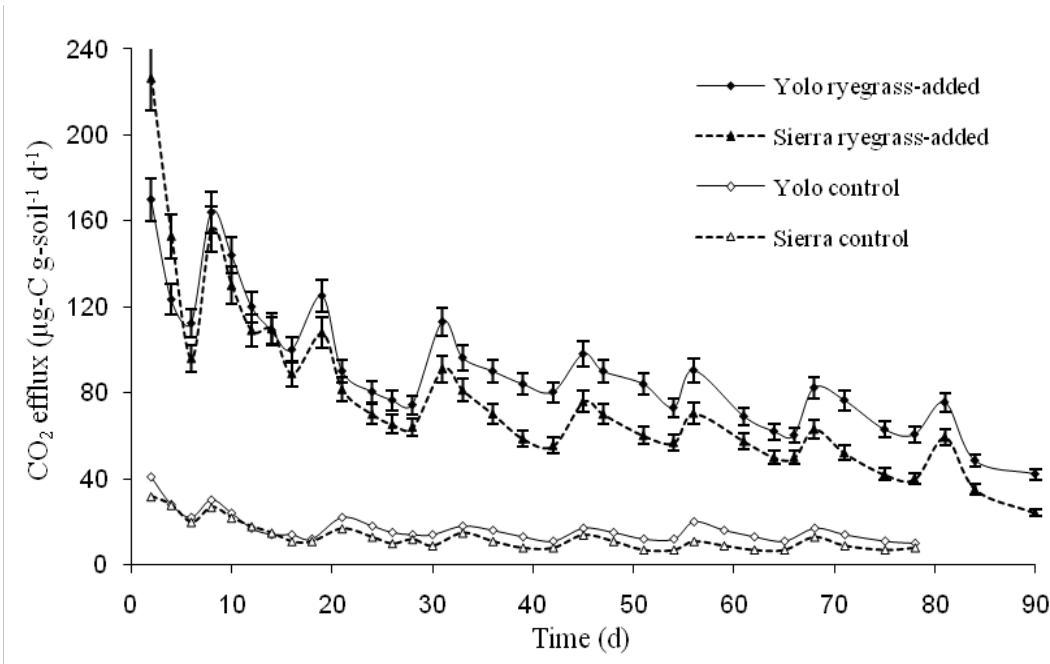
St: sterilized soils

(data are mean±SE, n=3)

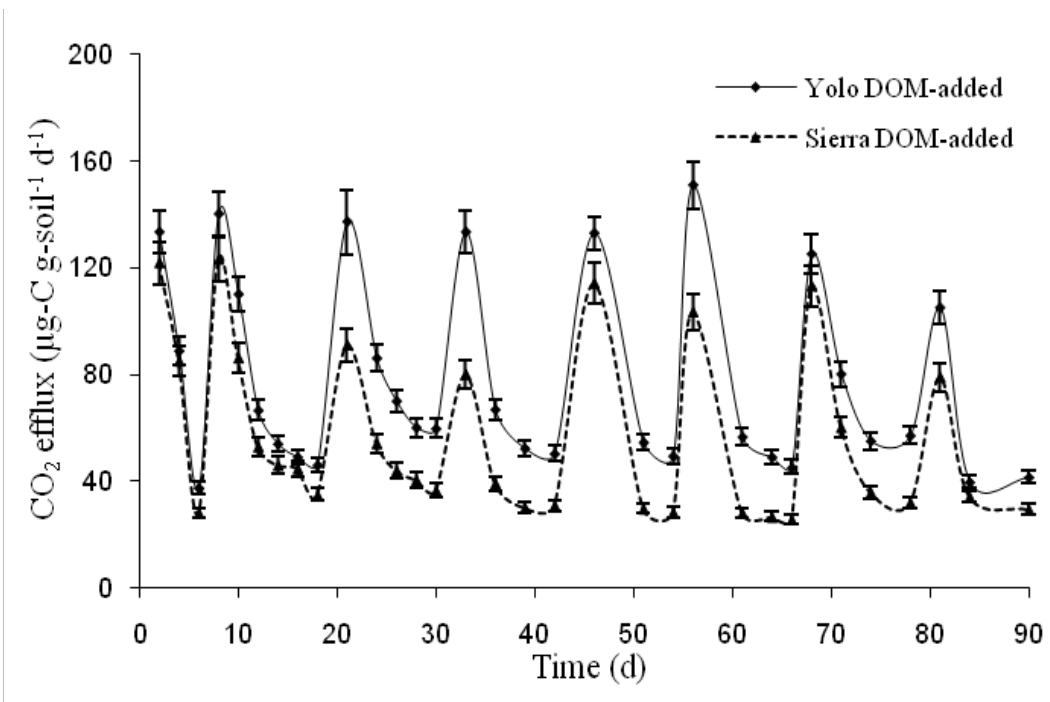


**Figure 6.1.** CO<sub>2</sub> efflux from the OM (Fig. 6.1a) and DOM (Fig. 6.1b) amended soils (data are mean±SE, n=3)

**Fig. 6.1a**

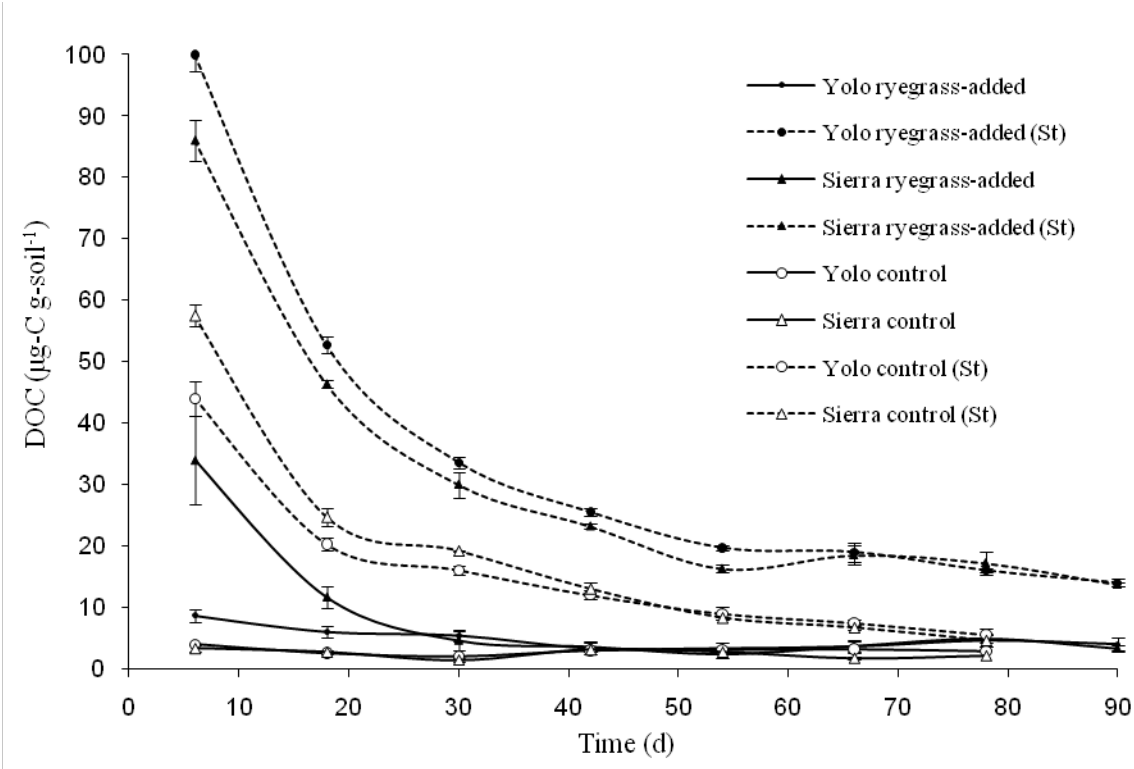


**Fig. 6.1b**

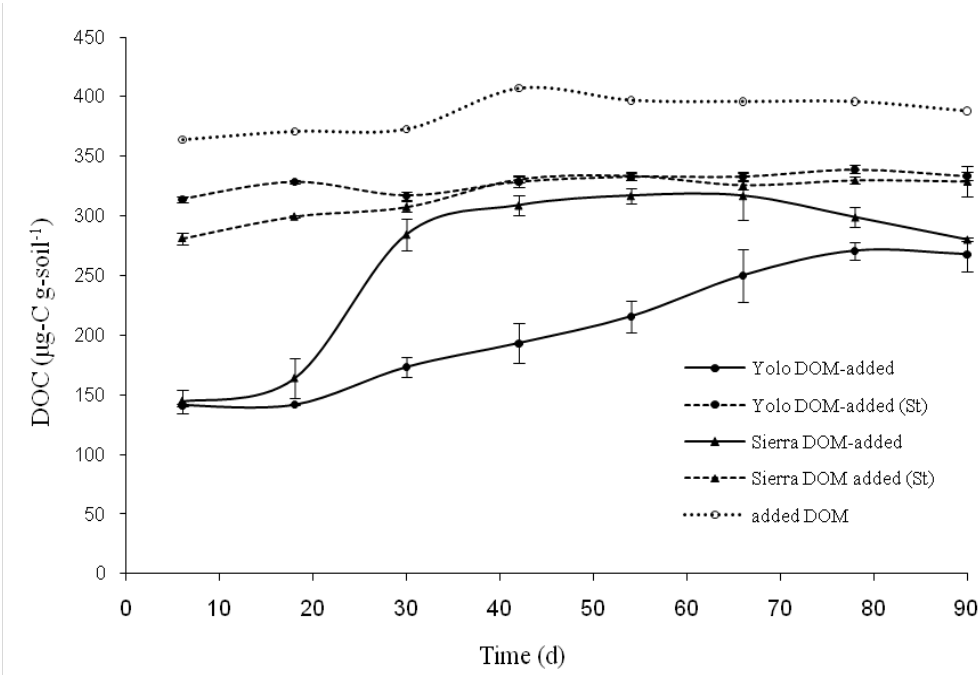


**Figure 6.2.** The concentration of the DOC obtained from the OM (Fig. 6.2a) and DOM (Fig. 6.2b) amended soils (data are mean±SE, n=3)

**Fig. 6.2a**

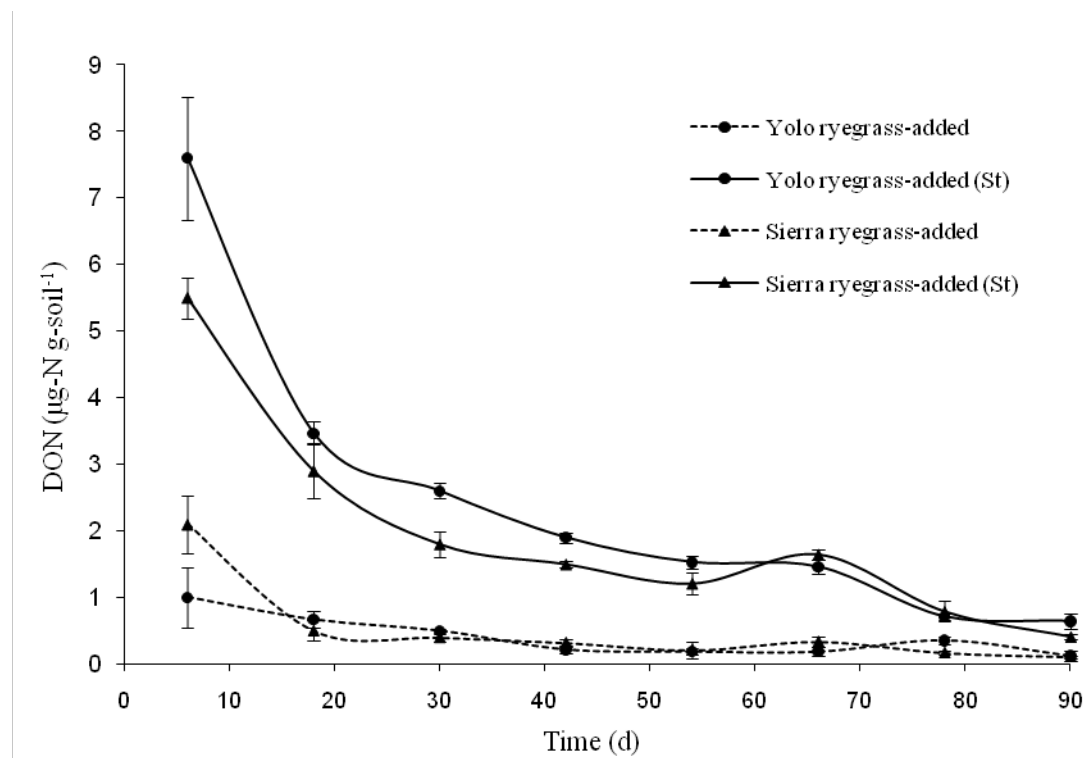


**Fig. 6.2b**

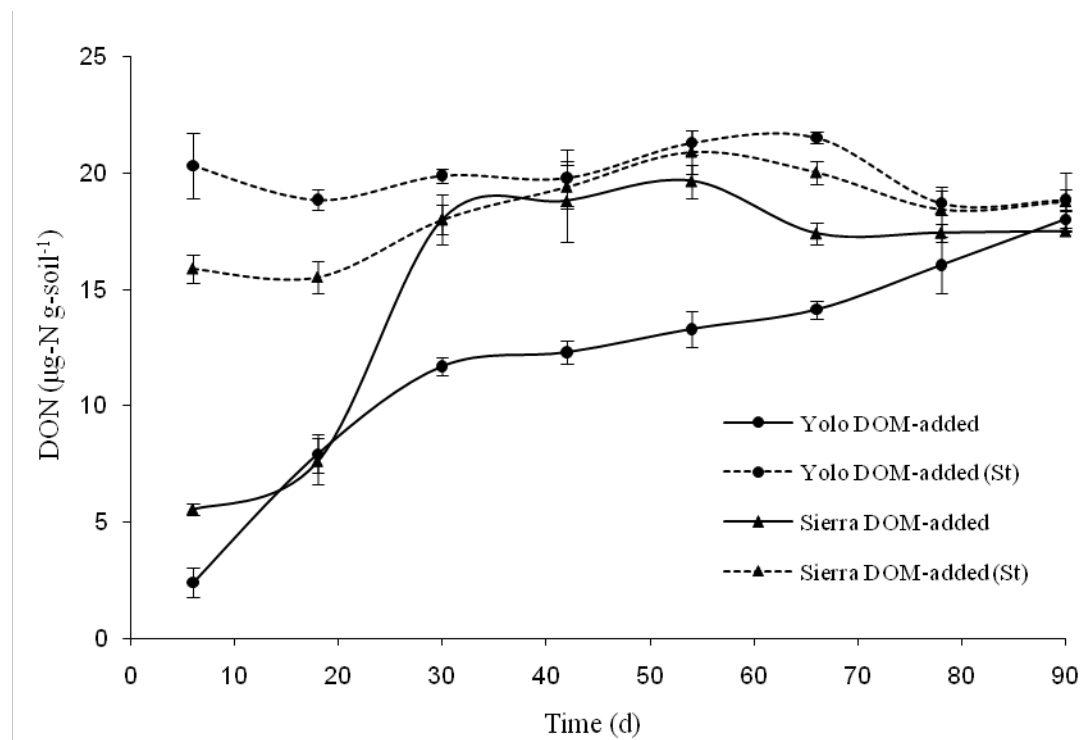


**Figure 6.3.** Concentration of DON leached from ryegrass-amended (a) and DOM-amended (b) soils (St: sterilized soil). (data are mean $\pm$ SE, n=3)

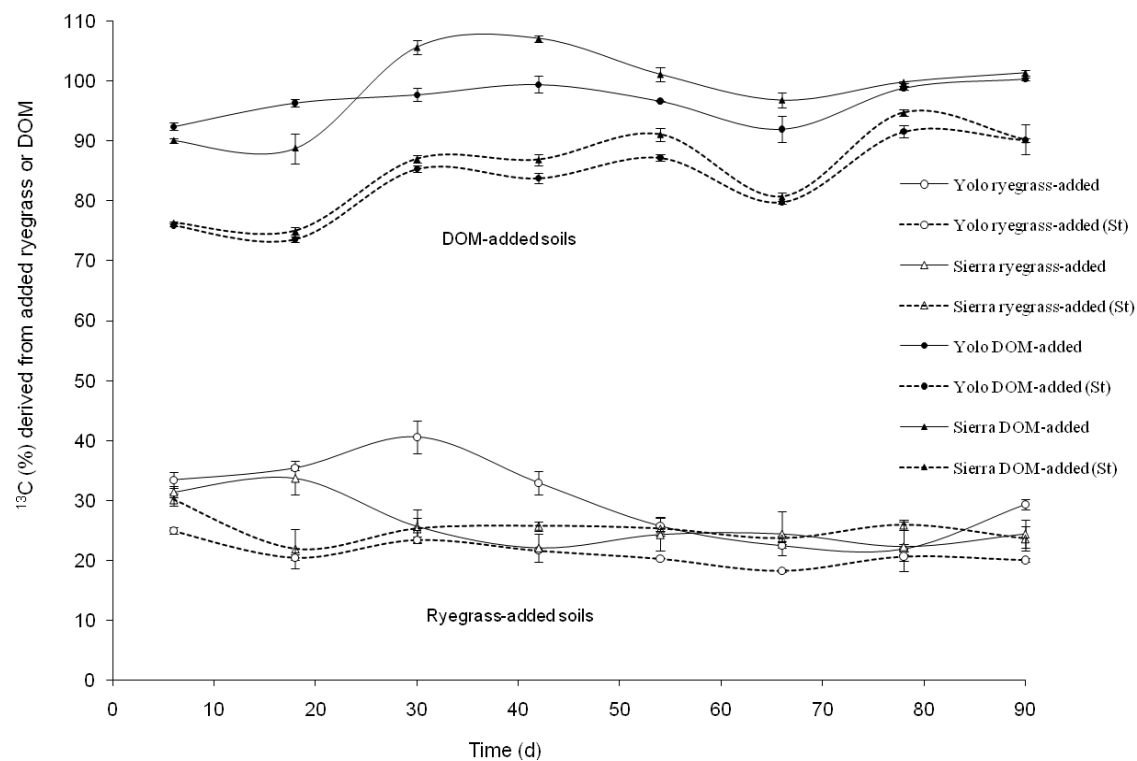
**Fig. 6.3a**



**Fig. 6.3b**

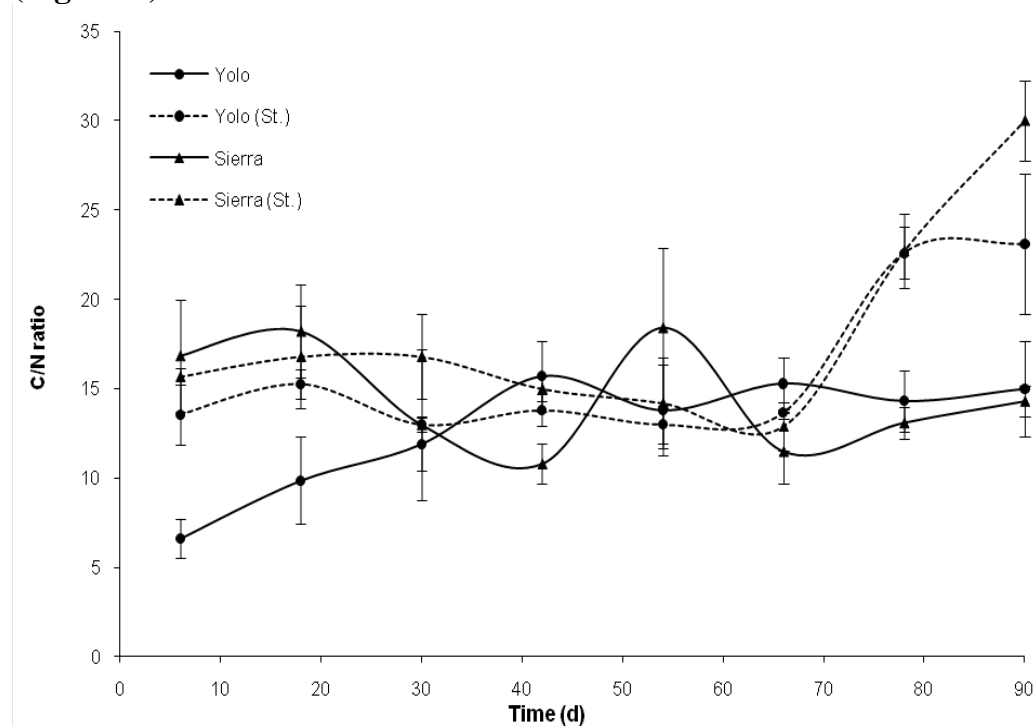


**Figure 6.4.** Proportion of  $^{13}\text{C}$ -DOC derived from added ryegrass or DOM (St: sterilized).  
(data are mean $\pm$ SE, n=3)

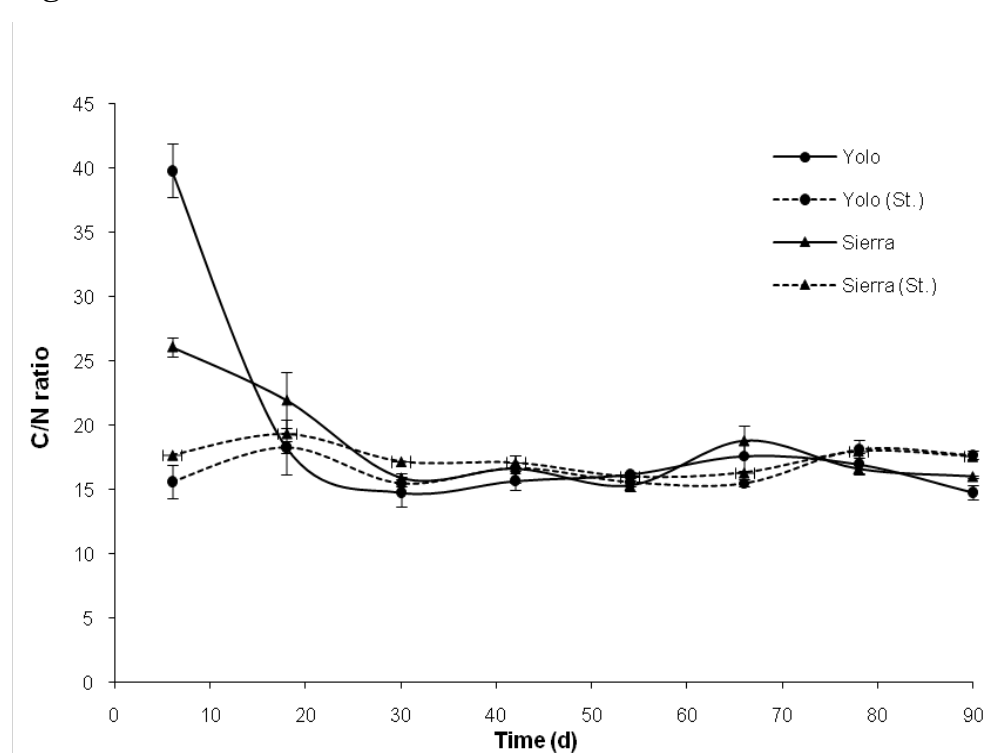


**Figure 6.5.** Changes in the C/N ratio of the DOM obtained from the OM (Fig. 6.5a) and DOM (Fig. 6.5b) amended soils (data are mean $\pm$ SE, n=3).

**(Fig. 6.5a)**

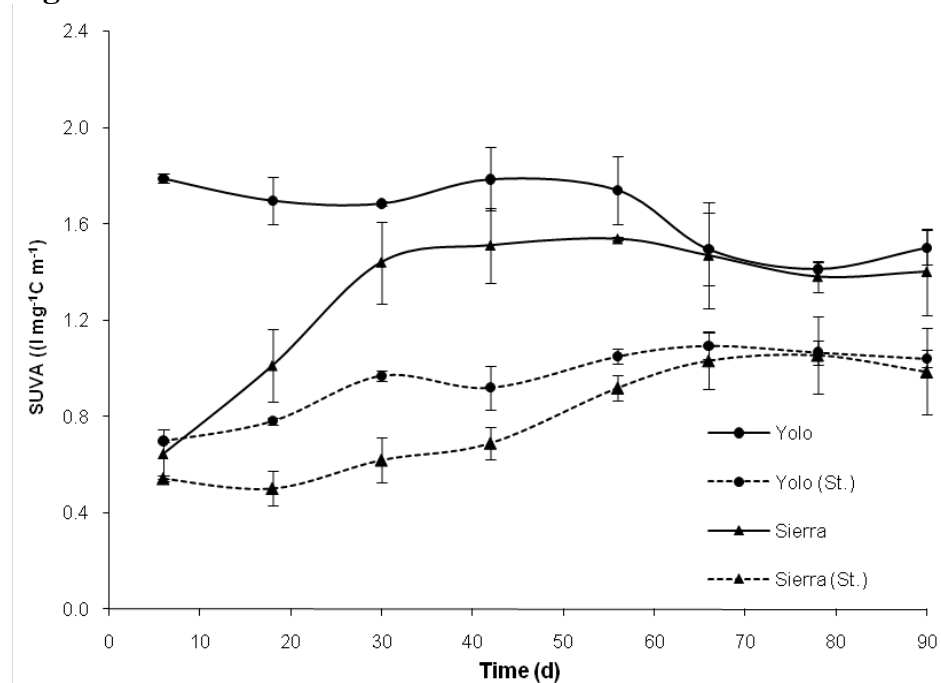


**Fig. 6.5b**

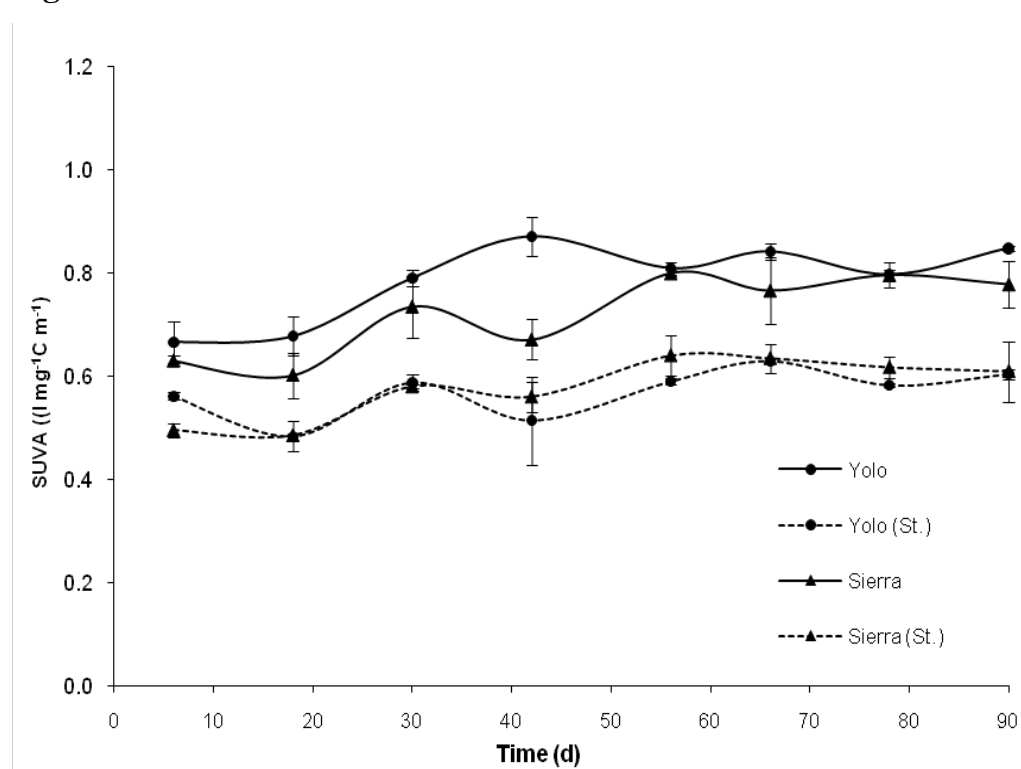


**Figure 6.6.** Specific UV absorption of the DOM obtained from OM (Fig. 6.6a) and DOM (Fig. 6.6b) amended soils (data are mean $\pm$ SE, n=3).

**Fig. 6.6a**

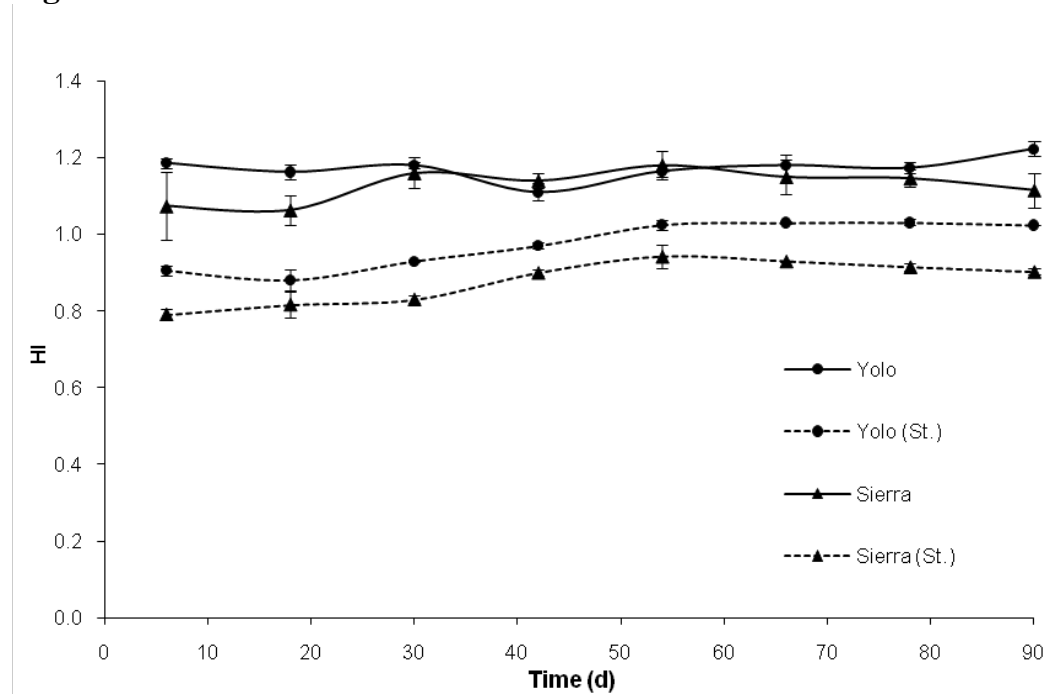


**Fig. 6.6b**

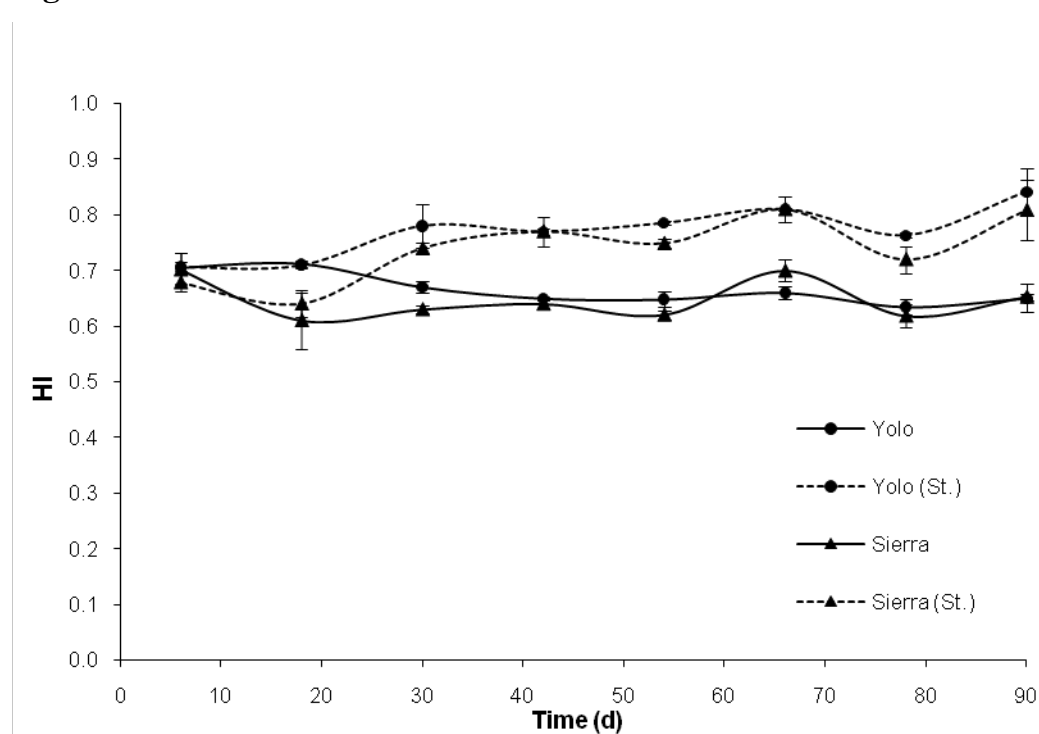


**Figure 6.7.** Humification Index (HI) in the DOM obtained from the OM (Fig. 6.3a) and DOM (Fig. 6.3b) amended soils (data are mean $\pm$ SE, n=3).

**Fig. 6.7a**



**Fig. 6.7b**



## Chapter Seven

### CONCLUDING REMARKS

As discussed in the literature review (Chapter 2), the OM that exists in the soluble form is clearly of significant ecological importance. However, there is some contradictory evidence in the literature that appears to stem from a lack of success in developing and applying a standard method to obtain this OM pool from the soil. In this study, I developed and evaluated a fractionation procedure to reduce a part of the uncertainties observed in the size and components of EOM by others (e.g. Kalbitz et al., 2000b; Rennert et al., 2006). I assessed the biodegradation potential of each of the two suggested fractions -WEOM and SEOM- in a 90-day laboratory experiment to compare the dynamics of each of the fractions with respect to their elemental (C and N), spectroscopic (UV abs), and isotopic ( $\delta^{13}\text{C}$ ) characteristics. In addition, I monitored the seasonal changes in C and N content, the C/N ratio, and aromaticity index (SUVA) of the fractions. In the last part of the study, I tested a hypothesis recently proposed by Kemmitt et al. (2008) termed “regulatory gate” in order to evaluate the role of abiotic factors in solubilization of SOM, a key process in the production of soluble OM in soil system. The abiotically-driven solubilization of SOM is not only of great importance in the dynamics of soluble OM at soil depth (Sanderman et al., 2008), but has great implications in relation to the impact of temperature on SOM dynamics since this has been largely disregarded in the existing SOM models. The key findings are discussed as follows.

#### 7.1. Fractionation of EOM

Although dilute and concentrated aqueous solutions (e.g. 0.01M  $\text{CaCl}_2$ , 0.5M  $\text{K}_2\text{SO}_4$ ) have been widely used to obtain EOM, very few attempts have been made to compare the properties of the solutions obtained by different procedures (e.g. Rennert et al., 2006; Burton et al., 2007). Depending on the concentration of the aqueous extractant being used, the soil extract obtained by dilute or concentrated solutions represents the



OM existing in the soil solution or that bound with mineral particles, respectively (Jones and Willet, 2006). To obtain a fraction of EOM which represents the properties of soil solution *in situ* (WEOM), I extracted the soil samples with 0.01M CaCl<sub>2</sub> during a weak extraction procedure. Such a commonly-used procedure has been proposed to best reflect the *in situ* properties of the soil solution (Zsolnay, 2003). I also extracted the soil samples with 0.5M K<sub>2</sub>SO<sub>4</sub> under relatively harsh conditions to obtain the salt extractable organic matter (SEOM). The SEOM has been recently suggested as a suitable soil quality indicator (Matlou and Haynes, 2006; Llorente et al., 2010). This process used to obtain SEOM, is assumed to maximize the release of the OM from the exchangeable sites to the solution, while reduces the release of the OM trapped in aggregates (Haney et al., 1999). However, due to the extraction conditions, the release of OM from other pools (e.g. biomass, aggregate protected, thermally solubilized) seems unavoidable, as this is an artefact of the fractionation, occurring during many fractionation procedures that are commonly used in OM separations (see Olk and Gregorich, 2006).

The repeated monitoring of the size and properties of the two fractions at different times of the year indicated that regardless of the land use and soil depth, not only the size (C and N), but also the properties (C/N ratio and SUVA value) of the WEOM and SEOM vary considerably in time and spatial scale. Comparison of <sup>13</sup>C natural abundance in the two fractions supports my initial assumption that the WEOM and SEOM fractions originate from different pools of OM. <sup>13</sup>C natural abundance implied that the SEOM originates from and represent an older pool of SOM. Together, I can conclude that the fractionation procedure I used in this thesis separates two almost genuine soluble OM and therefore, it has a potential for further studies. However, the approach can be improved with an easy modification.

Since I did not follow a sequential extraction procedure, a part of the SEOM was also comprised of the WEOM. Although, comparison of the proportion of the two fractions indicated that the WEOM constitutes only a very small portion of the SEOM, carrying out the extraction procedure in a sequential order (soil extraction with a dilute -WEOM-followed by a concentrated solution -SEOM-) can exclude the presence of the

WEOM from the subsequently obtained SEOM fraction and thus, reflect a more genuine, less “contaminated” SEOM fraction. This modified fractionation procedure may better correspond to the two fully different fractions of EOM, when comparing the effects of external factors (e.g. land-use or soil depth etc.) on SOM size and properties.

Since the high salt concentration of the applied aqueous solution (e.g. 0.5M  $K_2SO_4$ , 1:5 soil-to-extractant) has an adverse impact on the activity of the decomposer community and thus, the results the biodegradation experiment, I modified the SEOM obtaining procedure for the biodegradation assay. I increased the ratio of the soil-to-extractant from 1:5 to 1:1 (w:v), along with an increase in the extraction temperature (25 to 75°C). This was achieved by the incubation of the centrifuge tubes -containing suspension of soil and extractant- in a water bath at 75°C. The extraction under the warmer temperature was aimed to compensate a part of the observed lower recovery of OM extracted in 1:1 instead of 1:5 ratio (soil-to-extractant) (see Appendix A). My initial experiments revealed that given the relatively short period of the heat treatment during the extraction, the increase of the temperature (25-75°C) and the change of the soil-to-extractant ratio, increased the recovery of the obtained SEOM (Appendix A). In a separate experiment undertaken to assess the microbial biomass of the soils, I observed that OM released due to the applied temperature accounted for 8.1-13.4% of the pool of microbial biomass (Appendix B). Although previous researchers have shown the influence of such temperatures on the release of OM from microbial biomass (e.g. Sparling et al., 1998), recent evidence suggest that the thermally-induced soluble OM released by even longer heat incubation under high temperature, is unlikely to be largely made up of microbial biomass pool (Chantigny et al., 2010). Instead, it may contain a remaining part of the exchangeable OM now released to the extractant solution and to some extent the thermally solubilized OM.

The quality and size of the obtained SEOM varies as the ratio of soil-to-extractant and extraction temperature changes (Jones and Willet, 2006; Chantigny et al., 2010) and thus, affects the biodegradation dynamics of desired OM pools. Desalination of the SEOM could have been considered as an alternative approach. However, the minimum cutoff

size for most available cellulose membranes that are often used during desaltation is 3000 Da, meaning that during such a procedure a part of the soluble OM will be eliminated. The small OM molecules removed mainly comprise a highly biodegradable pool of OM and thus, lead to misinterpreting the biodegradation dynamics. Another technique is passing the SEOM samples through a size exclusion chromatography (SEC) to remove the salt ions. Similarly, this results in loss of a portion of small size OM compounds absorbed by the resin bed in SEC and consequently affects the properties of the “refined” OM, as mentioned. Both techniques are widely applied for a small size samples, often in biochemical studies, and a large volume of SEOM needed for a biodegradation experiment may limit applicability of either desaltation or SEC techniques.

The final aim of a successful fractionation protocol for obtaining extractable OM is separating a genuine and representative soluble pool of SOM in order to advance our understanding of the dynamics of soluble OM (often reported as DOM) in terrestrial ecosystems. This will minimize the controversial results reported in the literature addressing soluble OM and its dynamics.

## **7.2. Biodegradation of EOM**

The results of the bioassay experiment indicated a similar biodegradation pattern for both C and N of the SEOM as for the previously shown pattern for DOM and WEOM (e.g. McDowell et al., 2006). I included the data of the loss of C and N during the biodegradation incubation in a double-exponential model to assess the biodegradation dynamics of the WEOM and SEOM. I observed that based on the biodegradation model the differences in the chemical properties of the water and salt extractable OM was because of different half-lives (HL) of the slowly decomposable pool ( $K_2$ ) of the WEOM and SEOM. As mentioned, the lack of sequential extraction may have resulted in leakage of some WEOM into SEOM fraction. However, given the largely smaller proportion of the WEOM to that of SEOM, this doesn't seem to affect particularly the calculated HL of the two pools. The different HL of slowly decomposable pool of WEOM and SEOM suggests that regardless of the landuse and soil depth, the EOM is comprised of the

fractions relatively similar in the “fast” but different in the “slowly” biodegradable pool. Given the lack of sequential extraction procedure used for obtaining the WEOM and SEOM, a small part of the SEOM was comprised of the WEOM. This may have been caused a larger biodegradation of both C and N of the SEOM as an artefact of presence of labile OM compounds existing in the WEOM. However, this does not affect the observed pattern of the biodegradation to a large extent. Not to be mentioned that in reality, the soluble pool of OM is a continuum comprised of existing to potentially soluble forms of OM with decreasing bioavailability on timescales varying as short as seconds to the order of years (Hopkinson and Vallino, 2006).

In agreement with the literature, I observed that the biodegradation of the fractions of EOM occurred along with a considerable increase in  $SUVA_{254}$  value. This occurred in a similar pattern as seen for C and N biodegradation (a fast followed by a slow trend). The change in SUVA value of the WEOM was far more than that of the SEOM, supporting the greater biodegradability of the WEOM. The increase in SUVA reflects the proportional increase of aromatic property of both fractions of EOM during the biodegradation, largely as a result of transformation (assimilation and C loss) of labile simple compounds. The relatively strong correlation ( $R^2=0.66$  and  $0.74$  for WEOM and SEOM, respectively) between the amount of biodegraded C and SUVA value increase supported the results reported in the literature that  $SUVA_{254}$  can be used as a simple, low-cost but reliable approach, reflecting the intensity of biodegradation of EOM.

Although I observed a greater biodegradability of the EON than EOC; mainly due to a longer HL of the slowly biodegradable pool of C than N pool; the C/N ratio of the samples did not change very much during the biodegradation. This is in agreement with pervious findings (e.g. Qualls and Haynes, 1992), suggesting that the biodegradation of EOM may occur as function of N availability. Although the OM dynamics in our laboratory study are likely to be very different to OM dynamics in natural systems, it is becoming increasingly apparent that “N dynamics drive C transformations, rather than the other way round” (Sollins et al., 2007).

$^{13}\text{C}$  natural abundance of DOM has been shown as a sensitive (Kabitz et al., 2003b) or non-sensitive (Cleveland et al., 2004) indicator that reflects the intensity of decomposition rate of OM in laboratory studies. I observed that a significant depletion of  $^{13}\text{C}$ , mainly in the WEOM samples occurred during the biodegradation assay, and in accordance with the amounts of the biodegraded C. This confirms the preferential biodegradation of simple organic constituents e.g. monosaccharides, amino acids and amino sugars ( $^{13}\text{C}$  enriched) that leads to the accumulation of more depleted  $^{13}\text{C}$  compounds of a recalcitrance nature (Kalbitz et al., 2003b). Moreover, the results of the  $^{13}\text{C}$  isotope technique revealed that the relatively greater enrichment of  $^{13}\text{C}$  in the WEOM obtained from subsoil (vs. topsoil), seems to be due to the presence of root exudates (often highly enriched from carbohydrates and amino acids; Bowling et al., 2008), while the proportionally greater depletion of  $^{13}\text{C}$  of the SEOM particularly subsoil samples, suggests that there is a close relationship between the SEOM and the typically depleted native SOM.

The OM obtained from soil depth has been commonly referred to as being less biodegradable than that obtained from the topsoil. However, the results of the biodegradation model (HL of C and N) in addition to the characteristics (SUVA<sub>254</sub> value and  $\delta^{13}\text{C}$ ) of the EOM did not show any significant difference in the biodegradability of the EOM obtained from top or subsoil. This suggests that the potential biodegradability of soluble OM under laboratory conditions does not necessary reflect its *in situ* lower biodegradability at soil depth. This is in accordance with the recently suggested hypothesis (Fontaine et al., 2007) that the reported longer HL of the OM at soil depth could be largely due to the lack of optimum conditions (oxygen, nutrients, and moisture) for decomposer community instead of its intrinsic recalcitrance.

It has been commonly suggested that land use considerably affects the size of the EOM (Zsolnay, 1996; Chantigny, 2003). Very little information, however, is available on the impact of land-use on the composition of soluble OM and specifically the interaction with soil. Although I observed a generally larger biodegradability of the samples from the soils under the crop land (WEOC and SEOC) along with a relatively greater increase in

SUVA, I did not observe a consistent trend of the impact of land use on the biodegradation of the fractions of EOM. The lower C/N ratio of the soils under the crop land to some extent explains the proportionally greater biodegradability of these soils that I observed. Given the large differences (e.g. vegetation, microclimate, management) among the selected land-uses, the *in situ* biodegradability of EOM and therefore, the CO<sub>2</sub> efflux from the sampled soils under field conditions are expected to be different than the potential biodegradability of the EOM determined under lab conditions. Previous studies have shown that the microbial activity of these soils and thus, a part of OM dynamics are strongly limited by the lack of available water and low temperature in a significant period of time at the Mackenzie basin (Hunt et al., 2002 and 2004). Thus, the biodegradability of the EOM of the soils is expected to vary considerably during the year and among the land uses.

From the methodological perspective, soluble OM has been widely characterized by the elemental (e.g. C and N), spectroscopic (e.g. UV, fluorescence, <sup>13</sup>C-NMR), and isotopic (e.g.  $\delta^{13}\text{C}$ ) approaches. In contrast, there are few studies that have focused on the characterization of the chemical constituents of soluble OM (e.g. Fischer et al., 2007; Meyer et al., 2008; Jandle et al., 2002). In fact, apart from indirectly obtained data (spectroscopic/isotopic), very little is known about the dynamics of individual non-humified compounds (e.g. proteins, lipids, carbohydrates, etc.) during biodegradation of soluble OM. The transformation of such compounds has recently been suggested as being more complicated than previously thought (Bowen et al., 2009). Based upon my literature review, I believe that better understanding the constituent components of the soluble OM and their alterations during the dynamics of this substance (e.g. decomposition, absorption) has a great potential for future studies. Undertaking such researches has been hampered due to the lack of standardized and efficient instrumental methods (Jones et al., 2005a), namely chromatographic approaches.

Including S and P measurements in routine soluble OM analyses has been suggested by McDowell (2003). Given the importance of C/N/P and C/N/S ratios in biological systems, I assume that such measurements can address biological dynamics of

soluble OM using stoichiometric principles to further reveal biogeochemical cycles of C, N etc. Such indices (e.g. Redfield ratio) have been widely used in marine ecology studies but are lacking in terrestrial studies. It appears that the high variability of S and P in the dissolved phase in soil in addition to their low concentration in the soluble OM could be of the major constraints for routine analyses of these elements.

### **7.3. Dynamics of EOM in relation to land use, soil depth and season**

The large temporal and spatial variability of the soluble OM has been well documented in the literature (see chapter 5). Given this, the selection of the soils from different land uses, soil depths, and also sampling at different times of the year that represent seasonal contrast helped me to i) better assess the proposed fractionation procedure developed in chapter 3 and ii) address the impact of human activity (land use), soil depth and season on the size and selected properties of the EOM.

In agreement with the literature (e.g. Burton et al., 2007), I observed that generally the OM obtained by dilute solution (WEOM) is very variable among the land uses, seasons and between the topsoil and subsoil but there is far less alteration in the size and properties of the OM obtained by concentrated salt solution (SEOM). This is somewhat in contrary with the commonly belief that the soluble OM (size  $<0.45\mu$ ) is the most dynamic pool of the SOM. In turn, my study suggests that the large variability in the size and properties of the extractable OM is affected by the extraction procedure.

I observed similar results to Davis et al., (2007) who showed after 14 years of vegetation alteration from degraded grassland to pine plantation and to a relatively productive cropland (alfalfa), the C content of the topsoil did not change relative to that of degraded grassland. This is more likely related to the limited moisture supply (MAP:  $646\text{ mm yr}^{-1}$ ) and low temperature (154 days of the year ground frost) of the area for the optimum plant growth. Such finding supports the suggested hypothesis by Gue and Gifford (2002) that under low-rainfall conditions, afforestation may not necessarily lead to an accumulation of C in soil for a period of time.

Despite variability in the fractions of EOM due to soil depth and land use, the size of the C and N and also the C/N ratio of the SOM were significantly affected by these factors. Our results indicated that both landuse and soil depth influence the amount of the C of the WEOM. In turn, only soil depth has affected the properties of the SEOM. The lack of sufficient time since the establishment of the plantation and cropland could be an explanation for the lack of significant impact of the land use on the size or properties of the SEOM. However, the lack of enough replicates in the factor of landuse in our study has restricted the certainty level of the interpretation of the impact of the land use.

Our results indicated that the size of the WEOM varied according to landuse in the order plantation>cropland>degraded grassland>bog pine for both topsoil and subsoil. This is in agreement with the literature (e.g. Zsolany, 1996), suggesting that the size of the soluble C decreases from forest to degraded lands or soils under unimproved vegetation. Litter layer and the abundance of the root exudates in soils under plantation are two important sources of soluble OM that contribute to the greater amount of WEOM observed in plantation site (Kalbitz et al., 2000b). The greater amount of the WEOM obtained from soils under the plantation probably reflects differences in the properties of this material such as a lower biodegradability of the leachate sourced from the coniferous litter (Kuiters, 1993, Ganjegunte et al., 2006). The proportionally larger amount of the N in the WEOM obtained from the cropland (alfalfa) soil was related to the fast turnover of its roots (Gou et al., 2006) and thus, the considerable production of the soluble organic N (Yano et al., 2005). In addition, the greater N input in these soils under legumes (Macdonald et al., 2007), and the presence of the N rich root exudates (van Hees et al., 2005) may have contributed to the larger size of the WEON pool in the soils under crop land. In contrast, the low-productivity (Webb, 1992) and therefore, the low OM input (Hunt et al., 2004) and its fast decomposition in the soils under degraded grassland (Hunt et al., 2002; Hunt et al., 2004) and bog pine appear to result in the observed low WEOM content of these soils.

Although SEOM has been shown to reflect the impact of management (Matlou and Hynes, 2005) and thus, it may serve as an indicator of soil quality (Llorent and



Turrion, 2010), some researchers have shown that it has large variability compared to that of WEOM/DOM (Haynes, 2005) and therefore may not be easy to interpret. In accordance with Matlou and Hynes (2005) and Burton et al. (2007), I observed that the components (C and N) of the SEOM were far less affected by land use than those of the WEOM. Therefore, I suggested that regarding large similarities among the soils, the lack of a considerable difference in the amount of the SEOM in the soils under different land uses indicates that the SEOM the potentially soluble OM (Jones and Willet, 2006) is less affected by vegetation than the edaphic (CEC, pH, etc.) properties. Similar conclusion had been suggested for the impact of physicochemical properties of soil compared to vegetation for dynamics of DOM (Don and Schulze, 2008).

In agreement with the literature, I observed that the concentration of the C and N and the C/N ratio of the SOM in the topsoil were substantially larger than in the subsoil. Similarly, the amount of either of C and N content of the topsoil was at least two times more than the subsoil. Furthermore, the results indicated that the alteration of vegetation from Bog pine to other land uses led to a significant increase of C and N content of the both topsoil and subsoil and this was related to the low growth rate of Bog pine and thus, its proportionally low above- and below-ground biomass production. The results of the study also suggested that not only in the topsoil but also the C and N stock of the subsoil have not increased after 14 years of alteration of vegetation from low productive tussock grass land to pine plantation.

The observed decrease in the size of the soluble C (WEOM) from the topsoil down to the subsoil has been related to the less input of the soluble OM at soil depth and its sorption during passage through the soil profile (e.g. Guggenberger and Kaiser, 2003). However, this was not observed for the C pool of the SEOM which has been suggested as “potentially soluble” (Jones and Willet, 2006), probably due to its higher stability and different mechanisms controlling dynamics of this OM compared to those of the WEOM. Despite the lack of the effect of the soil depth on the properties of the WEOM, the C/N ratio and index of aromaticity of the SEOM obtained from the subsoil were significantly larger than those of the topsoil. This may imply the more humified nature of the SEOM

(or perhaps a reflection of OM sourced from early vegetation of the site) at soil depth and is in line with the observed greater depletion of the SEOM at subsoil (Chapter 4). Such data suggest that the properties and transformation of the SEOM are different with those of the WEOM and clearly affected by soil depth.

There is a growing focus in the literature on the limited impact of land use on the soluble OM (WEOM/DOM) dynamics in the subsoil. Some recent evidence suggest that the WEOM/DOM in the subsoil originate not only simply from the topsoil or litter layer, but also *in situ* and from the turnover of the biomass and transformation of the native SOM and thus, is little affected by land use (e.g. Chen et al., 2004; Sanderman et al., 2008; Hassouna et al., 2010). However, our results indicated a significant interaction of land use-soil depth for the size of the C of the WEOM, in addition to its effect on the size and properties of the total SOM. Given the pronounced role of the fresh OM input in the biotic solubilization of the intact SOM at soil depth (Fontaine et al., 2007), and the impact of the size and properties of the OM on the solubilized WEOM (Sanderman et al., 2008), land use appears to partly control the rate limiting step in the production of the soluble OM at soil.

A consistent effect of season on the size of the C and N pools and properties (SUVA and HI) of the WEOM/DOM, has been of debate in the literature (e.g. Embacher et al., 2007; Fellman et al., 2008). In my study, I did not observe any significant impact of the season on either of the components or properties of the SOM and fractions of the EOM. This is along with observations reported by Boyer and Groffman (1996) and Clarke et al. (2007). This appears to be related to the low productivity of the site and thus, lack of a significant seasonal change in the SOM content and properties. In addition, the large temporal variability in the temperature and specially soil moisture (Appendix 3) and the impact of these fluctuations on the size and properties of the WEOM may result in the observed lack of consistent seasonal trend.

It appears to me that regarding the large spatial-temporal variability of the soluble OM, a more accurate seasonal assessment of this pool of the SOM requires a multi-year (instead of one year) monitoring of each of the fractions of the EOM. Instead the

observed results in this study should be considered with. The pseudo-replicates in land-use factor certainly reduced the confidence of the interpretation of the data related to the impact of land use. This was however, difficult to overcome, given the lack of sufficient “true” replicate of land-use at the study site. This appears to be a common shortage in many OM dynamic studies when assessing the impact of land-use and has commonly restricted the interpretation of similar studies (e.g. Sanderman et al., 2008; Kalbitz, 2000).

I observed a large variability in the size of the C and specifically N content of SEOM and in particular WEOM, among replicates. Such a large variability further restrains application of statistical methods and their interpretations. This is of more importance when addressing organic N since it is calculated based on the difference of the total and mineral N (nitrate and ammonium). This is further propagated when calculating the C/N ratio of soluble OM by including errors associated with determining organic C plus mineral and organic N and thus, largely affects the results of organic N and C/N ratio parameters and their interpretations (Kaiser et al., 2004). Therefore, along with Rees and Parker (2005), I suggest considering at least 4 true independent replicates when designing a study related to soluble OM. This reduces the errors arising from lack of sufficient replicates and results in more consistent and reliable data.

In the recent literature, there has been further focus on SOM dynamics through the profile than only in litter or topsoil layer. Thus, sampling from- and including different soil depths; instead of only topsoil and subsoil; certainly helps to reveal how dynamics of the soluble OM is affected through the soil profile. This could expand our understanding of how biotic and abiotic interactions, land-use and management, etc. may affect the size and properties of the soluble OM down the soil profile and particularly at soil depth. Understanding the dynamics of SOM deeper in soils has been proposed as a priority and a challenge to soil and environmental scientists (Rumpel and Kogel-Knabner, 2011).

I see a potential for future studies to focus more on the chemical characteristics of the soluble OM (see chapter 2) when the impact of land use or soil depth is considered. Such studies may address how the individual compounds and thus, chemical properties of this substance switch in response to the vegetation and soil depth. Such data may be of

great value regarding the increasingly focused importance of the SOM in soil related studies.

#### **7.4. The production of the soluble OM from the native and added OM**

The various mechanisms involved in the solubilization of native SOM or freshly added OM are poorly understood. The common belief is that the SOM is solubilized as a result of biotic (mainly enzymatic) and abiotic interactions (e.g. temperature, moisture and mineralogy). It has been noted that diffusion may play a substantial role in the solubilization rate, particularly of recalcitrant fraction OM (Moore, 2001). The properties of the SOM are also known to largely determine its biodegradability and thus, the rate of DOM production (Cleveland et al., 2004; Moore et al., 2008). Biotic solubilization is commonly explained based on Winogradsky's theory (Winogradsky, 1924). The theory states that the decomposer community is comprised of autochthonous and zymogenous microorganisms, mainly responsible for decomposition of the recalcitrant and labile pools of OM, respectively. However, this largely-established perspective has been recently challenged by evidence observed by Kemmitt et al. (2008). Their results suggested that solubilization of the humified SOM is regulated by a two-step process, the first abiotic step followed by biotic, processes. The paper has been criticised (Kozyakov, 2009; Paterson, 2009) due to the lack sufficient evidence and explanations for the suggested "regulatory gate" hypothesis. The regulatory gate hypothesis, however, has not been yet critically assessed by other researchers. While in their experiment Kemmitt et al (2008) had focused on the solubilization of the intact SOM, the results of the isotope dilution technique in my experiment suggested that solubilization of the freshly added OM is also largely unaffected by the presence of microorganisms. I observed that even following addition of large amounts of fresh OM, the soil microbial community acts rather passively during the production of DOM. In addition, my results indicated that even in the presence of the fresh OM, a quantitatively important proportion of the solubilized OM (averaged 70% of the produced DOM) originated from the humified SOM, solely as a result of abiotic interactions. The great contribution of the humified SOM in the presence

of the fresh OM (e.g. rhizodeposits) has recently been reported (Paterson et al., 2007) in normal soil conditions (non-sterile soils). Furthermore, my findings indicated that in contrast to the size of the solubilized OM, microbial activity affects the quality of the abiotically solubilized OM (produced DOM). In fact, the larger proportion of aromatic and humified (assessed by SUVA and HI) compounds of solubilized DOM in non-sterile soils implied the influence of microorganisms on the quality of the produced DOM during its assimilation.

The study undertaken by Kemmitt et al. failed to consider the function of extracellular enzymes (Kozyakov et al., 2009). I assumed that regarding the length of the incubation experiment (90 days) in sterile conditions, the high concentration of Hg (as  $\text{HgCl}_2$ ) in the soil solution, the adverse impact of autoclaving on the extracellular enzymes function, and the relatively stable trend of OM solubilization during the experiment, the extracellular enzymes had a small, if any, impact on DOM production in the sterilized subsamples. Thus, my results confirm that in normal soil conditions (non-sterile), the primary step of the solubilization process could be abiotically driven. This is somewhat in contrast with our widely accepted understanding of DOM production through SOM solubilization. Figure 7.1 demonstrates how the “conventional”, “modified” and “regulatory gate” hypotheses address the OM mineralization.

Despite the very contrasting mineralogy of the two soils (1:1 vs. 2:1 clay) used in this study, there was no evidence, indicating the influence of soil mineralogy on abiotic solubilization. Soil mineralogy affects the stabilization-destabilization of OM, and as a result controls DOM production (Sollins et al., 1995; Russmusen et al., 2007). More likely the large concentration of the applied OM (both residue and its extract) masked the effect of clay mineralogy in this experiment. Soil sterilization, may affect the physical and chemical properties of soil, depending on the applied method. Addition of  $\text{HgCl}_2$  at the applied level, has been suggested as a successful sterilization method (as observed), with minimum consequence on soil properties. Nonetheless, this could have had some artefacts on soil conditions, resulting in altered solubilization rate or properties of DOM as measured by UV and fluorescence. The results of the preliminary experiment showed

that at the applied level,  $\text{HgCl}_2$  did not affect either SUVA or HI properties of the obtained DOM. Most soil incubation studies under laboratory conditions have been carried out over a period of a few months, including this study. However, setting up the experiment for a longer period of time, could have further support the results of the experiment, by providing more consistent data (specifically C/N ratio). Application of two different forms and levels of lability of OM (residue vs. its extract) as shown by the C/N ratio (Table 6-2), was aimed to further elucidate whether the regulatory gate hypothesis occurs dependent of source of OM as suggested by Paterson (2009). Although this could not be compared quantitatively but, under the experimental conditions, solubilization of both plant residue and its extract appeared to be primarily controlled by abiotic factor. It would be well if future experiment address the impact of different levels and qualities (inherent composition) of OM on the SOM solubilization. Despite the observed results of the experiment, I agree with Paterson (2009) that solubilization of SOM is driven by both biologic and non-biologic factors, depending on the size and availability of OM to microorganisms. This merged model further builds on the “energy demand-supply” model suggested by Ekschmitt et al. (2005). The model suggests that given that process of enzyme synthesis by microorganisms demands large levels of energy, it only takes place in conditions where the outcome of enzymatic reaction (broken down/solubilized OM available for metabolism) overrides the energy consumed during metabolic cycles. Based on the model, enzyme synthesis should be minimal, if any; either in the presence of readily available source of energy/carbon or rigorous conditions (very limited sources of energy/carbon). Together, this suggests that solubilization of SOM occurs as a function of biotic, abiotic or their interaction, depending upon environmental conditions, including substrate availability/quality.

Focus on solubilization mechanisms remains a priority in SOM stabilization-destabilization studies. Further determination and quantifying how of biotic and abiotic factors interact under different soil conditions may change our present understanding and thus, update the SOM models that are currently in use.

As mentioned, the current information (including this study) supports the regulatory gate hypothesis, indicating a strong contribution of the abiotic factors to the DOM production. However, how abiotic factors (temperature, moisture, mineralogy, SOM pool size and properties etc.) regulate the solubilization process of either of the humified or fresh OM in soil remains unknown. Kemmitt et al. (2008) have suggested a few possible abiotic mechanisms (abiotic condensation, hydrolysis, etc.) that need to be proven. They do not, however, address some important factors including water flow rate and diffusion, and solubility constant of OM in soil solution as key factors influencing the non-biological release of the OM from solid to the liquid phase.

I used very common chemical, physico-chemical based methods (C/N ratio, SUVA and HI) to assess the quality of the abiotically solubilized OM (DOM). Alternative tools (e.g. PLFA, biomarkers, etc.) can develop our understanding of the contribution of specific microbial communities to the transformation/metabolism of the produced DOM. The value of the isotopic and sterilization techniques and particularly in combination to PLFA technique is unquestionable.

Given the observed large contribution of the humified SOM to the solubilized OM from one side and the function of the SEOM as a pool of OM which appears to be at least partly responsible for replenishing the readily soluble OM pool from the other side, I am convinced that there could be a relationship between these two pools (SEOM and solubilized OM). Future studies are needed to clarify the strength of relationship between these two pools, using isotope and chemical characterization of OM. This perhaps would be of great help in further explaining how OM is solubilized under natural soil conditions and its main controlling mechanisms.

I have represented a mechanistic synthesis of the different processes and pools related to the dynamics of soluble OM in the soil system in Figure 7.2. The diagram illustrates how the biogeochemical processes (arrows) interact with the pools (ovals) that are related to the dynamics of the soluble OM in terrestrial ecosystems. This pool of OM is the product but also the driving substance for many processes in soil. The amounts and properties of soluble OM are controlled by simultaneous interactions of environmental

(moisture and temperature), edaphic (e.g. texture, mineralogy, pH,  $\text{CaCO}_3$ ), and biological (vegetation, microorganisms and soil fauna) processes. Soluble OM is responsible for a part of C and N losses from soil to the atmosphere (biodegradation) and water bodies (surface runoff/subsurface leaching) because of its very labile and mobile nature. Thus, it has been suggested that the soluble OM acts as bottleneck of organic matter and its constituents (e.g. C and N) between soil and water ecosystems (Zsolnay, 1996). However, soluble OM may stay in soil for extended periods of time (Kaiser and Guggenberger, 2000), undergoing a number of biotic and abiotic processes to varying degrees which depending on the conditions, result in its further decomposition/recalcitrance or its mobilization and release from solid to liquid form.

The role of soluble OM in meso-scale (soil profile) is well appreciated. Good examples are its functions in soil genesis, pollutant dynamics and currently, as an active SOM pool being included in SOM models. I suggest a few aspects of soluble OM related studies that especially soil scientists in the future should focus on and address.

#### 1- Global scale

- Role of soluble OM, as a pool containing both C and N, in soil C sequestration under different land-uses with focus on quantification of soluble OM contribution to the C storage.
- Impact of soluble OM on the reduction of N compound to nitrous oxides and conversion of OM to  $\text{CO}_2$  and  $\text{CH}_4$  (greenhouse gases), in particular in arable lands.

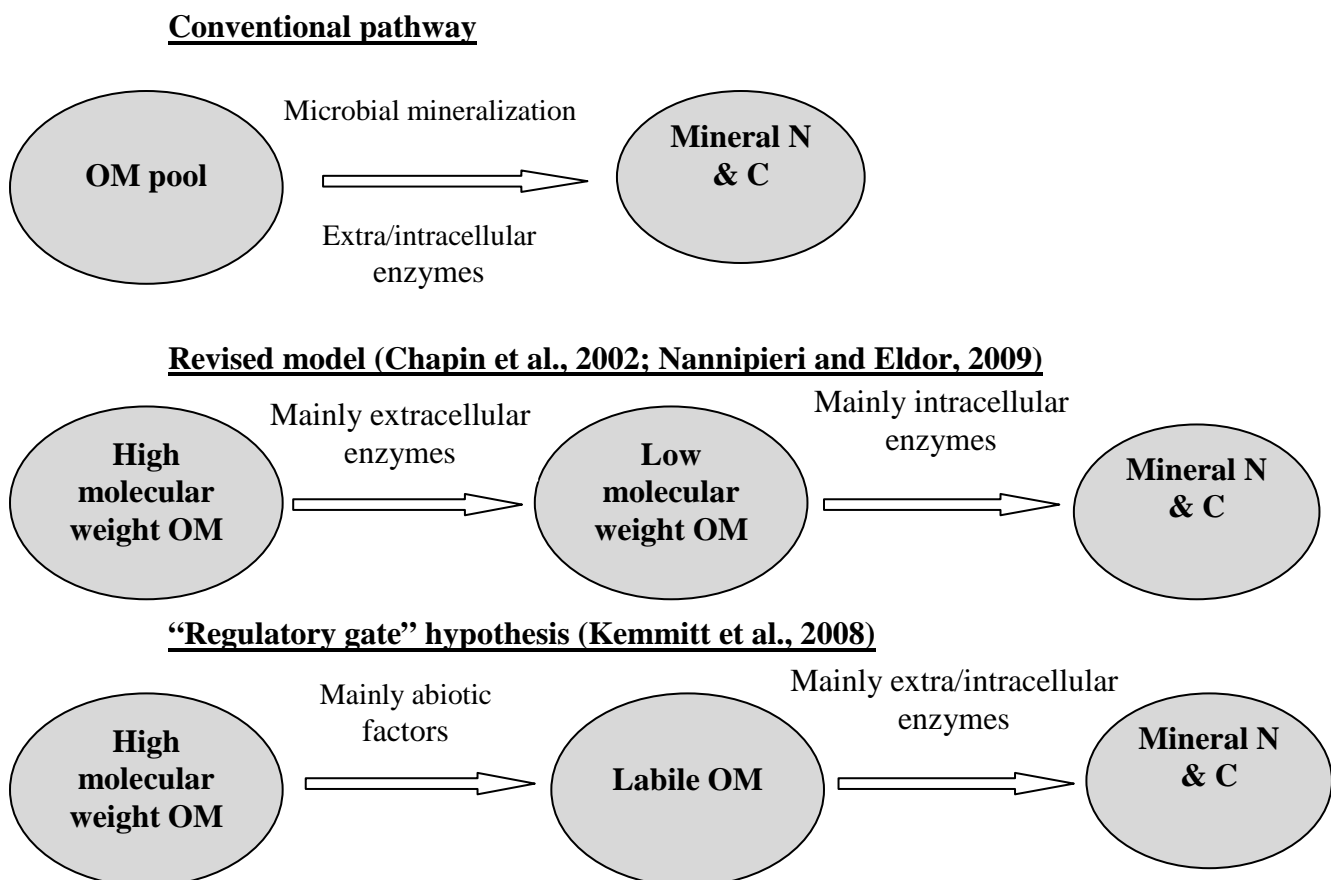
#### 2- Molecular scale

- Nature of soil minerals–soluble OM–microorganisms/enzymes interactions and its further assessment under different land-uses and soil depths.
- Interactions of soluble OM with increasingly focused mineral/organic nanoparticles (colloids), and its implications in plant nutrition and soil-pesticide interactions.

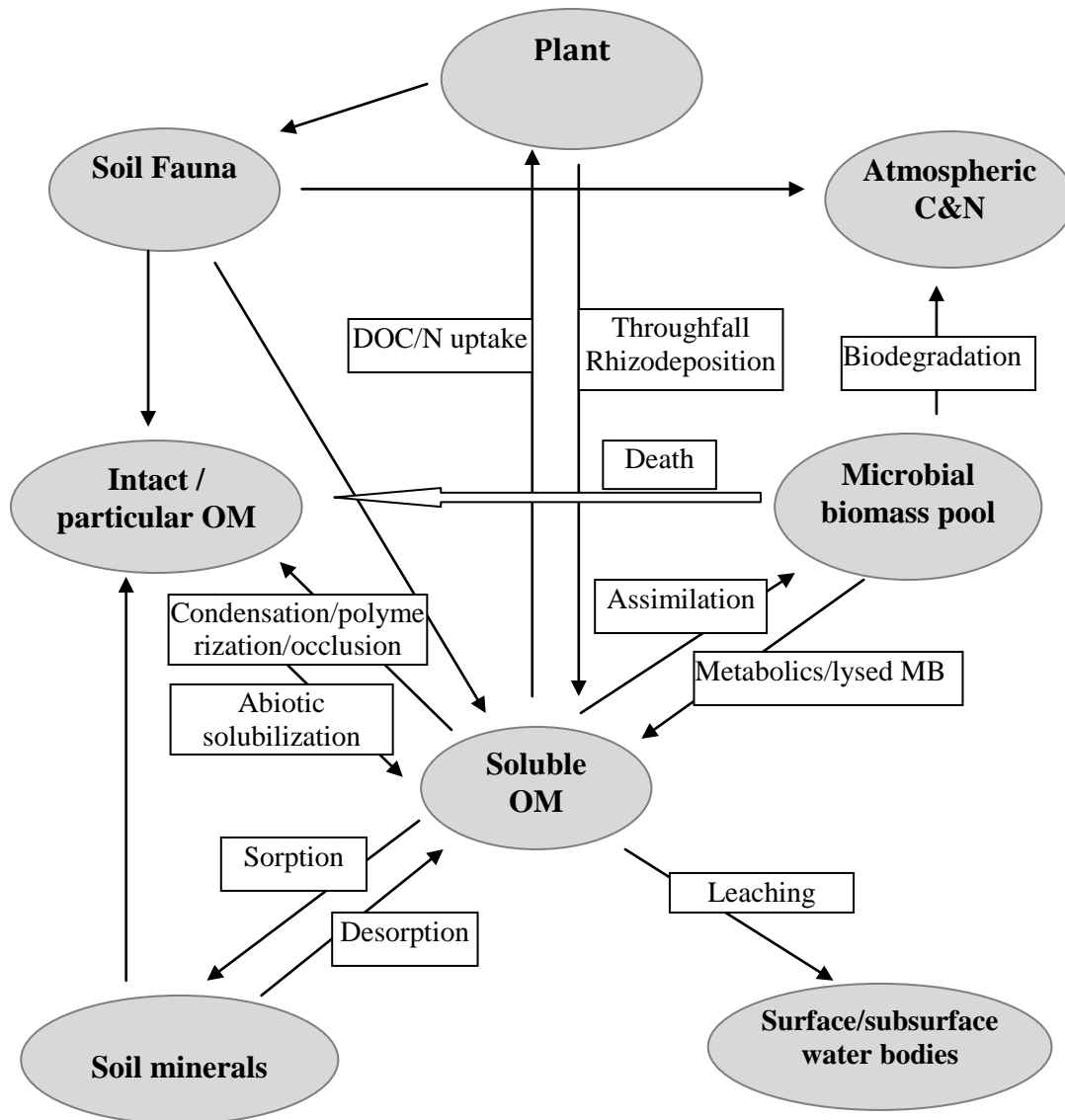


- Improving and developing microscopic, spectroscopic and particularly chromatographic methods needed in soil science studies in order to characterize specific compounds or functional groups of soluble OM and their interactions with minerals.

**Figure 7.1.** Suggested pathways by which DOM is produced during solubilization of soil OM. While the conventional pathway (top) does not discriminate between the functions of extra- and intra-cellular enzymes, in the “more realistic” model (middle) extra- and intra-cellular enzymes contribute to different stages of solubilization (Chapin et al., 2002; Nannipieri and Eldor, 2009). From both perspectives, abiotic factors have an indirect impact on the solubilization through microbial activities. Based on the “regulatory gate” hypothesis confirmed by my experiment (bottom), solubilization process can be primarily driven by abiotic factors. The abiotically solubilized OM (labile) then becomes biologically metabolizable/decomposable.



**Figure 7.2.** A synthetic representation of the major processes and pools that control the flux and size of the soluble OM in terrestrial ecosystems.



## **Appendix A**

### **Sampling and sample preparation procedure**

The selection of the studied sites was based on similar rainfall (MAP: 646 mm yr<sup>-1</sup>), temperature (MAT: 9.1°C), elevation (600-700 m), topography, and soil type. All the sites have been under the same vegetation for at least the past ten years. Due to lack of replication for most of the sites, the soil samples were collected from the same paddocks at each site (pseudo-replication). In contrast to the grass land, bog pine and arable land sites, the plantation site had a limited size (~30\*30 m). The same point within the same area (Cropland: S 44° 01.867, E 170° 24.679; Plantation: S 44° 02.004, E 170° 25.030; Grassland: S 44° 01.728, E 170 23.904; Bog pine: 44°24.405, E 169° 52.766) was used for the samples collected at different seasons, within distance smaller than three meters as signed by a GPS.

Soil samples were collected using a 10 cm diameter stainless steel auger from 0-20 cm and 60-80 cm at three separate locations at each site. Three subsamples were collected from topsoil at each location and pooled together, but only one sample was collected from subsoil at each location. Any litter layer present in the plantation site was removed before the sampling. To assess seasonal variation of soluble OM, soil sampling was carried out at the end of each season (November, February, May and August). Soil samples were transferred in an icebox to the laboratory less than 12 hr from collection. The soils were mixed well after sieving (2 mm) and visible impurities (litter, organisms, etc) were removed before storage at 4°C until the extraction procedure. To avoid differences in the quality and quantity of the extractable OM obtained from field-moist samples collected during different seasons and from different depths, all soil samples were adjusted to the same water status (60% of water holding capacity) by addition of the required deionized water to the soil following by mixing well and overnight incubation prior to extraction (see below). Extractable OM was obtained in less than 5 d after sample collection.

In all soil studies the moisture content of soil needs to be carefully adjusted to ensure comparability of results reported for soils collected from different sites, depths, etc. Depending upon the purpose of the experiment, soil analyses are carried out under different initial moisture content of soil including: i) field-moist conditions, ii) air-dried soils and iii) certain moisture content (commonly practiced 40-60% of water holding capacity; WHC). In soil OM fractionation/characterization studies its well-know that different levels of soil moisture content affect the size and properties of soluble OM mainly due to i) changing in the solubility of SOM (Haynes and swift, 19997), and ii) desiccation of microbial biomass under air-drying conditions, resulting in the release of microbial cell to soil solution (Zsolnay, 2003). As a result, the moisture level of soil being analyzed may affect the size and composition of soluble organic matter. For most soil chemical analyses (elemental/nutrients), air-drying is the preferred method to bring soil moisture and its chemical properties in almost same conditions. In contrast, adjustment of soil moisture to 40-60% of WHC is the most appropriate preparation method for biological/microbiological soil assessments (Wilke, B. M., 2005). This avoids changes in the size and activity of microorganisms as an artifact of air-drying. Despite being commonly practiced, “adjustment of soil moisture to 40-60% of WHC” is of little applicability when soil biological/microbiological properties (including soluble OM) are monitored on a regular basis (e.g. seasonality studies) at field conditions. “Adjustment of soil moisture to 40-60% of WHC” usually takes 7-14 d (Wilke, B. M., 2005) and more likely reduces the differences among the samples collected at different times. This is due to a substantial change in soil microbial growth, community composition, and activity in laboratory conditions during the incubation period. This results in consumption of residual soluble OM simultaneous with a release of “recently produced soluble OM” during the incubation period.

In my experiment where the “obtained” soluble OM was intended to best reflect the size and properties of soluble OM under “field” conditions at different seasons, I incubated different soils (collected from land uses and depths) under the same (50% of WHC) moisture content for a short period (overnight) to prevent the changes in the size

and properties of soluble OM as a result of long-term incubation (7-14 d). The WHC of soils and their initial moisture content has been reported below. Table C.2 indicates that the soils collected at different seasons, were sampled when minimal moisture manipulation was required from obtaining soluble OM. For the part of the thesis where the influence of field conditions were not of interest (Chapter 6), the laboratory protocol of “soil incubation at 50% of WHC for 10 d” was carried out for both soils (Sierra and Yolo) before start of main incubation period (90d).

## **Appendix B**

### **Water holding capacity of the soils and soil's moisture content at the sampling time**

There is a very close relationship between the microbial biomass (MB) of the soil and soil moisture content. At low soil moisture contents, a part of the MB is lysed due to the difference between the water potential of the soil and vial cells. Depends to the initial moisture content of the soil, this may cause a large flush of the soluble OM to the soil after re-moistening of the soil (Zsolnay, 2003). From the other hand, WEOM obtaining procedure is carried out under specific ratio of soil-to-extractant (e.g. 1:2). Thus, the initial moisture content of the sampled soils should be considered for comparability of the results of WEOM obtained from different soils (e.g. locations, depths, and times). Adjusting the water content of the sampled soils based on their water holding capacity (WHC) is one of the most common preparation methods in many microbiological and biochemical experiments (e.g. obtaining WEOM) (Wilke, 2005).

Because of the differences in the moisture content of the soils sampled from different depths, vegetations and seasons in our experiment, the moisture content of the sampled soils was adjusted based on 60% of the WHC. This was performed by addition of water to the samples before obtaining WEOM. In a few cases when the water content of the field-moist soils was larger than the standard (60% WHC), the additional water was removed by gentle aeration of soils along with continuous mixing of the soil to avoid the release of MB. WHC was determined using protocol proposed by Wilke (2005). Briefly, sieved fresh soil samples were filled homogenously into perforated metal cylinders (10\*7.5cm). The cylinders were submerged in the water bath with the soil surface lower than water level (room temperature) over night. The cylinders were removed from the water bath and placed on a pre-moistened sand surface. The samples were weighed regularly and after reaching constant weight, were placed into a beaker and put in the oven (105°C) for 24 h. WHC was calculated as follows:

$$WHC_{max}(\% \text{ dry mass}) = 100 * (m_s - m_t) / (m_t - m_b)$$

*Ms*: mass of beaker containing water saturated soil (g)

*Mt*: mass of beaker containing oven-dried soil (g)

*Mb*: mass of beaker (g)

**Table B. 1.** Water holding capacity of the soils

Soil	Core (g)	Saturated core (g)	Drained core (g)	Dried core <sup>1</sup> (g)	WHC (%)	60% WHC
PD <sub>1</sub>	79.4	143.1	133.4	112.3	64.1	38.5
PD <sub>2</sub>	79.8	143.4	137.1	121.7	36.8	22.0
CD <sub>1</sub>	80.0	166.1	153.1	127.2	54.9	32.9
CD <sub>2</sub>	79.6	166.7	159.9	136.8	40.4	24.2
GD <sub>1</sub>	80.0	154.6	142.8	119.0	61.0	36.6
GD <sub>2</sub>	81.8	170.1	164.6	144.8	31.4	18.8
BD <sub>1</sub>	80.2	155.0	139.1	119.9	48.4	29.0
BD <sub>2</sub>	83.1	163.6	151.8	134.0	35.0	21.0

1- Oven dried (105°C)

2- Each soil is comprised of three replicates

**Table B. 2.** Moisture content of the soils (%) at the sampling time based on the WHC of the soils

Soil	Spring	Summer	Autumn	Winter
PD <sub>1</sub>	8.3	5.6	37.1	27.2
PD <sub>2</sub>	11.1	8.3	34.3	24.9
CD <sub>1</sub>	8.1	6.0	31.3	21.3
CD <sub>2</sub>	11.2	6.3	29.9	22.1
GD <sub>1</sub>	7.6	6.9	29.8	21.3
GD <sub>2</sub>	10.6	11.5	24.3	22.9
BD <sub>1</sub>	7.8	9.0	27.6	19.8
BD <sub>2</sub>	12.9	12.6	22.4	22.4

P: Plantation

C: Cropland

G: Degraded grassland

B: Boggpine

D<sub>1</sub>: Depth 0-20cm

D<sub>2</sub>: Depth 60-80 cm



## Appendix C

### **The impact of temperature and soil-to-extractant ratio on the concentration of organic C and N extracted by 0.5M K<sub>2</sub>SO<sub>4</sub>**

Given the nature of biodegradation assay, there was a tendency to minimize the concentration of salt in the SEOM fraction to avoid biological stress due to high osmotic pressure. Thus, the commonly used proportion of soil-to-extractant 1:4 (w:v) was increased to 1:1 (w:v). However, to compensate the reduction in the amount of OM obtained by this new soil-to extractant ratio, the extraction temperature was increased from room temperature to 75°C (by putting the tubes containing soil-extractant suspension in a water bath and increasing the temperature to 75°C for total length of 90 min) , in order to maximize the amount of obtained OM. The maximum OM along with a minimum concentration of extractant (0.5M K<sub>2</sub>SO<sub>4</sub>) was desired for the bioassay experiment, since the concentration of both organic C and N decreases (due to microbial respiration and assimilation), while the concentration of salt remains stable during the experiment. The pros and cons of this procedure have been explained in the last Chapter (Concluding remarks). Following shows the impact of soil-to-extractant ratio and extraction temperature on the recovery of organic C and N of SEOM.

**Table C.1.** The impact of temperature on the release of organic C and N extracted by 0.5 M K<sub>2</sub>SO<sub>4</sub> (1:1 soil to extractant ratio)

Land use	Depth (cm)	Carbon (mg kg <sup>-1</sup> )		Nitrogen (mg kg <sup>-1</sup> )	
		25°C	75°C	25°C	75°C
Plantation	0-20	151.6±6.4	176.0±15.4	8.7±1.2	9.8±0.9
	60-80	121.7±12.0	154.7±5.0*	4.8±0.3	6.4±0.4**
Cropland	0-20	107.3±12.9	129.0±11.5	8.3±1.0	10.6±0.8*
	20-80	96.7±14.4	126.3±6.1*	4.3±0.6	5.8±0.3*

\*: P<0.05

\*\* P<0.01

**Table C.2.** The impact of different soil:extractant ratios on the recovery of organic C and N extracted by 0.5 M K<sub>2</sub>SO<sub>4</sub>

Soil	Soil:extractant ratio				F	P>F
	1:1	1:2	1:3	1:5		
	Carbon (mg kg <sup>-1</sup> )					
PD1	151.6±6.4 <sup>B</sup>	195±14.2 <sup>A</sup>	204±14.2 <sup>A</sup>	206±18.4 <sup>A</sup>	9.9	<0.01
PD2	121.7±12.0 <sup>B</sup>	173±6.8 <sup>A</sup>	183±6.7 <sup>A</sup>	191±6.2 <sup>A</sup>	45.1	<0.01
CD1	107.3±12.9 <sup>B</sup>	143±13.0 <sup>A</sup>	154±14.5 <sup>A</sup>	162±14.5 <sup>A</sup>	9.3	<0.01
CD2	96.7±14.4 <sup>B</sup>	138±5.1 <sup>A</sup>	145±5.6 <sup>A</sup>	151±7.3 <sup>A</sup>	23.6	<0.01
	Nitrogen (mg kg <sup>-1</sup> )					
PD1	8.7±1.2 <sup>B</sup>	10.9±0.9 <sup>AB</sup>	11.4±1.0 <sup>A</sup>	11.9±1.1 <sup>A</sup>	5.2	0.03
PD2	4.8±0.3 <sup>B</sup>	7.5±0.5 <sup>A</sup>	7.9±0.4 <sup>A</sup>	8.1±0.3 <sup>A</sup>	55.1	<0.01
CD1	8.3±1.0 <sup>B</sup>	12.4±1.3 <sup>A</sup>	13.4±1.1 <sup>A</sup>	14.0±1.1 <sup>A</sup>	15.3	<0.01
CD2	4.3±0.6 <sup>B</sup>	6.4±0.5 <sup>A</sup>	6.8±0.6 <sup>A</sup>	6.8±0.4 <sup>A</sup>	16.7	<0.01

## Appendix D

### Soil Microbial biomass

Microbial biomass was determined by fumigation-extraction method (Vance et al., 1987) modified by Solaiman (2007). Briefly, sieved soil samples were adjusted with water at 60% of WHC and incubated at room temperature for 12 days. 20 gr incubated soil samples were transferred to a beaker and placed in a dessicator. A beaker containing 50 ml ethanol-free chloroform was also placed in the dessicator along with a moist wet paper (avoid soil drying). The dessicator was sealed and a vacuum was applied to let the chloroform boil from about 2 min. The dessicator was left for 10 min, unsealed and the vacuum was re-applied, and the system was left under vacuum for 24 hr. The fumigated and non-fumigated soil samples were extracted by 0.5 M K<sub>2</sub>SO<sub>4</sub> (1:4 soil:extractant) at room temperature followed by filtration (Whatman No.42). The C in the extracts was analysed using TOC analyser. The biomass C was calculated as

$$BC = EC : K_{EC} w$$

Where EC = [extractable C in the fumigated – non-fumigated soil extracts]  
k<sub>EC</sub> = 0.45 (extractable part of microbial C after fumigation).

**Table D.1.** Microbial biomass content of the selected soils

Land use	Depth (cm)	Extractable C (mg kg <sup>-1</sup> )		Microbial biomass C (mg kg <sup>-1</sup> )
		NF	F	
Plantation	0-20	157.4±14.9	306.1±39.1	330.4± 54
	60-80	129.1±9.2	252.1±33.7	273.5±52.8
Cropland	0-20	114.7±12.0	247.0±41.5	294.0±67.7
	20-80	109.5±3.6	215.8±19.9	236.2±38.4

Data are mean± SE (n=3)

## Appendix E

### Comparison of one-pool vs. two-pool models for assessing mineralization rate constants of C and N

The pool size and mineralization rate constant/s of biodegradable organic matter is assessed using decomposition models. Two-pool model has been suggested to adequately represent the size and mineralization rate constants of biodegradable organic matter, specifically in long-term incubation experiments (Robertson et al., 1999). A number of studies have used two-pool decomposition model in order to assess decomposition dynamics of soluble organic matter (e.g. Gregorich et al., 2003; Kalbitz et al., 2003b; Kiikkilä et al., 2006; McDowell et al., 2006). However, to my knowledge, only one study (Wickland et al., 2007) has used one-pool model to evaluate the size and rate constant of biodegradable C of soluble OM. Given the frequently observed non-linear change in C losses during decomposition experiments, the two-pool model appears to better assess the size and mineralization rate constants of biodegradable OM.

#### Models and model components

##### 1-Two-pool

$$\text{Mineralized C or N} = (100-a) (1-e^{-k_1 t}) + a (1-e^{-k_2 t})$$

“100-a” (%): Fast decomposable pool

“a” (%): Slow decomposable pool

$k_1$  : mineralization rate constants ( $d^{-1}$ ) of the fast decomposable pool

$k_2$  : Mineralization rate constant ( $d^{-1}$ ) of the slow decomposable pool

“t” : Incubation period (d)

##### 2- One-pool

$$\text{Mineralized C or N} = (a) (1-e^{-k t})$$

“a” (%): Decomposable pool

$k$  : Mineralization rate constant ( $d^{-1}$ ) of the decomposable pool

“ $t$ ” : Incubation period (d)

Based on the literature review, I determined the size and rate constants of mineralizable pools of C and N of WEOM and SEOM by the two-pool model (Chapter 3). However, the following Tables represent the mineralization rate constants determined by both (one- vs. two-pool) models. The total amount of mineralized C or N is the same both models. The Sum of Squared Errors (SSE) of each model -as a measure of model fitness- suggests that the applied two-pool model fits much better with the loss of C or N during the incubation experiment.

Table E-1. Mean of biodegradation rate constants of the mineralized C during 90d incubation experiment as assessed by one-pool and two-pool decomposition models

Land-use	Two-pool model						One-pool model			
	SSE		K <sub>1</sub>		K <sub>2</sub>		SSE		K	
	Mean	S.E.	Mean	S.E.	Mean	S.E.	Mean	S.E.	Mean	S.E.
<b>Water extractable organic carbon</b>										
0-20 cm										
Plantation	57.5	22.6	0.154	0.025	0.0042	0.000489	573.7	31.4	0.0132	0.00043
Cropland	19.1	7.8	0.119	0.006	0.0049	0.000070	344.2	64.7	0.0138	0.00185
Grassland	27.0	11.4	0.283	0.048	0.0042	0.000582	522.2	35.7	0.0097	0.00066
Bogpine	56.8	22.3	0.232	0.039	0.0044	0.000312	428.9	137.5	0.0088	0.00137
60-80 cm										
Plantation	15.7	5.2	0.128	0.013	0.0062	0.000299	336.0	65.5	0.0262	0.00325
Cropland	77.2	31.1	0.202	0.019	0.0063	0.001418	1738.6	750.1	0.2557	0.07465
Grassland	27.4	9.9	0.142	0.003	0.0055	0.000967	419.5	172.7	0.0287	0.00998
Bogpine	62.3	15.6	0.112	0.004	0.0058	0.000631	320.6	155.7	0.0775	0.02444
<b>Salt extractable organic carbon</b>										
0-20 cm										
Plantation	9.8	3.9	0.290	0.008	0.0017	0.000284	465.0	21.8	0.0050	0.00044
Cropland	12.3	3.8	0.067	0.014	0.0022	0.000374	70.4	26.4	0.0039	0.00022
Grassland	5.3	3.0	0.132	0.019	0.0016	0.000107	136.2	63.7	0.0034	0.00029
Bogpine	10.7	3.8	0.095	0.006	0.0019	0.000389	173.5	62.0	0.0046	0.00004
60-80 cm										
Plantation	19.6	4.1	0.116	0.022	0.0022	0.000238	232.9	13.6	0.0054	0.00023
Cropland	17.2	5.2	0.056	0.001	0.0029	0.000405	71.4	8.6	0.0054	0.00047
Grassland	5.9	4.0	0.157	0.014	0.0012	0.000349	118.3	34.5	0.0028	0.00069
Bogpine	3.1	0.8	0.103	0.011	0.0010	0.000166	88.1	24.4	0.0025	0.00006

SSE: Sum of Squared Errors

SE: Standard Error (n= 3 replicates)

Table E-2. Mean of biodegradation rate constants of the mineralized N during 90d incubation experiment as assessed by one-pool and two-pool decomposition models

Land-use	Two-pool model						One-pool model			
	SSE		K <sub>1</sub>		K <sub>2</sub>		SSE		K	
	Mean	S.E.	Mean	S.E.	Mean	S.E.	Mean	S.E.	Mean	S.E.
<b>Water extractable organic nitrogen</b>										
0-20 cm										
Plantation	29.7	4.4	0.042	0.004	0.0060	0.000454	34.0	7.7	0.0098	0.00054
Cropland	42.3	16.7	0.073	0.009	0.0067	0.000655	130.1	46.5	0.0292	0.00681
Grassland	41.7	10.0	0.122	0.019	0.0086	0.000699	581.2	293.0	0.0759	0.02051
Bogpine	35.8	23.8	0.123	0.015	0.0053	0.000892	337.4	47.8	0.0142	0.00207
60-80 cm										
Plantation	35.8	11.3	0.120	0.010	0.0073	0.000434	221.0	19.8	0.0306	0.00965
Cropland	35.2	6.8	0.128	0.016	0.0073	0.000290	252.9	22.8	0.0357	0.00613
Grassland	55.9	13.5	0.131	0.023	0.0081	0.000135	179.2	29.0	0.0378	0.00610
Bogpine	68.5	25.9	0.086	0.009	0.0114	0.001172	904.7	598.4	0.0721	0.00847
<b>Salt extractable organic nitrogen</b>										
0-20 cm										
Plantation	18.8	7.9	0.151	0.020	0.0017	0.000086	742.7	238.4	0.0413	0.02269
Cropland	18.3	11.5	0.224	0.007	0.0030	0.000256	277.8	60.9	0.0060	0.00069
Grassland	31.8	13.4	0.159	0.010	0.0039	0.000205	288.2	178.2	0.0081	0.00251
Bogpine	6.5	0.9	0.092	0.012	0.0021	0.000268	179.7	73.5	0.0052	0.00073
60-80 cm										
Plantation	12.3	5.2	0.190	0.005	0.0025	0.000253	800.2	200.3	0.0162	0.00667
Cropland	30.0	15.2	0.176	0.027	0.0025	0.000342	671.5	190.0	0.0088	0.00132
Grassland	4.6	0.8	0.104	0.013	0.0016	0.000546	233.7	79.9	0.0050	0.00093
Bogpine	7.5	0.2	0.114	0.009	0.0013	0.000311	216.5	38.6	0.0042	0.00063

SSE: Sum of Squared Errors

SE: Standard Error (n= 3 replicates)

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