AN ABSTRACT OF THE THESIS OF

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Abstract approved: _____

Phillip Sollins

Most soil organic C is in a stable form, associated with clay minerals in the upper soil profile. Upon environmental and land-use changes, stable soil C is subject to losses, which influence the global C cycle and the productivity of terrestrial ecosystems. However, mechanisms controlling soil C destabilization are not well understood. Microbial respiration and leaching are the two major fates of destabilized C. I hypothesized that water-soluble C plays an important role in soil C turnover and studied the susceptibility of water-soluble C both to leaching and to microbial degradation. Furthermore, I studied factors influencing the amount of water-soluble C and its biodegradability using five soils from a wide fertility gradient.

First, soil columns (20 cm of top soil) with and without Douglas-fir seedlings were leached every 2 weeks for 20 weeks and the soils were extracted with water before and after the 20-week study period. Extracts contained 8 to 17 times more dissolved organic C (DOC) than did leachates. Similarly, biodegradable DOC (BDOC) in extracts was 4 to 96 times that in leachates. The leachable C pool was constant over the 20 weeks regardless of the presence of tree seedlings in soil leaching columns, while the extractable C pool declined by 31 to 40% over time. A soil C destabilization model was proposed based on the changes in measured C pools (particulate, water-extractable, and leachable C) and the estimates of soil respiration and microbial biomass from a previous work. Microbial respiration accounted for 86 to 92% of the C destabilized. Leached C accounted for the remaining 8 to 14%, indicating its minor role in C mobilization in the

system studied. However, the model didn't rule out the possibility that extractable C accounts for a significant portion of microbial respiration.

Second, the hypothesis that water-extractable C is a major energy source for microbes was tested using Douglas-fir forest soils from two sites (Cascade Head and Wind River) and with and without red-alder influence. Despite a large range of soil fertility (C:N ratio of 13 to 31), C extracted before and after the 2-month soil incubation showed a strong correlation with the C respired during the incubation period (r^2 = 0.71 and 0.92), suggesting the efficacy of extractable C pools to predict the availability of C to soil microbes. Furthermore, DOC and BDOC pool were relatively constant during the 2-month incubation, indicating the possibility that a rapid regeneration and degradation of extractable C explain the measured microbial respiration during the study period. No clear correlation was found between DOC and dissolved organic N. Across all stands, soil C:N ratio showed a strong correlation with the relative amount of BDOC in the DOC incubation (r^2 = 0.72) and with the relative amount of C respired from bulk soil (per g C basis) at the end of incubation period (r^2 = 0.72), suggesting a strong influence of soil C:N on the overall C availability to soil microbes both in solution and solid phase.

Water-extractable C deserves continued attention as a strong predictor of available C for soil microbes and as a potentially significant energy source for microbial respiration. More information is needed to estimate the regeneration rate of labile extractable C and to identify factors controlling soil C degradation.

Dynamics of Water-soluble Carbon in Forest Soils of Contrasting Fertility

by

Rota Wagai

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I understand that my thesis will become part of the permanent collection of Oregon State University libraries. My signature below authorizes release of my thesis to any reader upon request.

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Rota Wagai, Author

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DEDICATION

This thesis is dedicated to the memory of my grandfather, Taiji Tomita, and my parents, Fusayo and Michiumi Wagai.

Dynamics of Water-soluble Carbon in Forest Soils of Contrasting Fertility

CHAPTER 1
INTRODUCTION

Overview

Loss of soil organic C greatly influences the global C cycle and the productivity of terrestrial ecosystems. On a global scale, soil contains 1500 Pg C, which is roughly three times the terrestrial plant C pool and twice the atmospheric CO_2 pool (Schlesinger, 1991). After the atmospheric greenhouse effect was recognized, an increasing effort has been made to quantify pools and fluxes of C and to assess the potential influence of climate change on terrestrial element cycles (e.g., Perry *et al.*, 1991). Soil organic C is in a stable form, associated with clay minerals in the upper soil profile to form soil organic matter (SOM). Microbial degradation of SOM results in a continuous production of nutrients for plant growth and thus strongly influences the productivity of a terrestrial ecosystem. The importance of SOM to forest productivity is well established (e.g., Binkley 1986). Land-use changes such as conversion of forest to agriculture, for example, causes up to a 50% loss of SOM in the first year (Coleman *et al.* 1984). However, the mechanisms as well as the factors controlling SOM destabilization are poorly understood (Sollins *et al.* 1996).

Enormous heterogeneity in composition of SOM limits our methods to study SOM. In general, SOM is broken down into various pools (e.g., biologically active, labile pool and passive, recalcitrant pool). Some operationally define such

pools by experimentation, for example, mineralizable, KCl-extractable, and biomass pool (e.g., Hart *et al.*, 1994). Others mathematically derive the pools to explain the empirical data on SOM loss: active, slow, and passive pool (e.g., Parton *et al.,* 1988). Here, I hypothesized that water-soluble C plays an important role in soil C turnover. As soil heterotrophic microbes utilize a portion of soil organic C as their energy source, they respire CO_2, build biomass, and release metabolites including organic acids, polysaccharides, polyaromatics, and extracellular enzymes. Some of these metabolites contribute to stabilize SOM (e.g., binding mineral particles). Others, particularly extracellular enzymes, depolymerize portions of the SOM. The SOM then becomes lower in molecular weight and more water-soluble. These soluble compounds are then susceptible to microbial degradation and leaching loss. When soils are disturbed by agricultural or forestry practices, SOM is likely to be more available to microbes, creating a positive feedback toward increased losses of water-soluble C. Thus, water-soluble C is viewed as a key pool resulting from soil C destabilization. Consequently, studying the factors that influence the quantity and quality of water-soluble C may help us to understand the SOM destabilization mechanisms.

Water-soluble C as a Microbial Energy Source

A mechanistic understanding of heterotrophic microbial respiration, which accounts for roughly half of the CO_2 evolution from vegetated soil surfaces and all the CO_2 evolution from bare soils, is critical to predict the response of the soil C pool to anthropogenic and natural disturbances including any potential climate change. Furthermore, microbial respiration associated with soil enzymatic activity is a major driver of soil C destabilization. Despite the vast amount of organic C present in soil, microbial activity is often energy limited, presumably because most of the soil C is chemically recalcitrant and/or physically inaccessible to microbes. Then, how do heterotrophic microbes gain energy in soil? I hypothesized that these

microbes gain a significant portion of energy from water-soluble C pool because of its low molecular weight and easy accessibility to microbes. A strong correlation between water-extractable soil C and microbial respiration has been reported from a wide range of soils in temperate regions (Burford and Bremner, 1975; Powlson and Jenkinson, 1976; Seto and Yanagiya, 1983; Davidson *et al.*, 1987; Zak *et al.*, 1990; Cook and Allan, 1992 a). For water-soluble C to be the main energy source for soil microbes, one has to compare the pool size and turnover rate of water-soluble C pool with the amount of C required to support soil microbial biomass. Although previous workers proposed the same hypothesis (Seto and Yanagiya, 1983; McGill *et al.*, 1986; Cook and Allan, 1992 a; Jandl and Sollins, 1998), no study has investigated a soil's capacity to produce water-soluble C sufficient for microbial substrate needs. Thus there is no consensus whether soil microbes gain their energy from particulate C or water-soluble C. We have very little understanding of the mechanism resulting in half of the CO_2 flux from land surfaces to atmosphere.

Biodegradability of Water-soluble

For water-soluble C to be the main energy source for heterotrophic microbes, one also needs to study its degradability (i.e., availability to microbes). Biodegradability of dissolved organic C (DOC) can be assessed either by measuring the degradation loss of DOC during the incubation of soil solution or by chemically characterizing DOC. I chose the former because it was technically simpler and a more direct estimate of the biodegradability than the latter technique. The amount and biodegradability of DOC (including both extracts and leachates) in soil are known to be affected by the depth in soil profile (Zsolnay and Steindl, 1991; Qualls and Haines, 1992; Nelson *et al.*, 1994), season (Qualls and Haines, 1992; Nelson *et al.*, 1994; Yano, 1997), soil type (Boissier and Fontville, 1993), land-use (Boyer and Groffman, 1996), N fertilization level (Yano, 1997), and

nature of extractant (Nelson *et al.*, 1994). The degradation rate of DOC is not constant over time and the labile portion of DOC (BDOC) can be consumed very rapidly. Roughly 22% (deciduous forest) and 29% (corn field) of the C extracted from top soil can be degraded within two weeks (Boyer and Groffman, 1996). In an Appalachian forest, 5 to 10% of C leached at different depths was degraded within 48 hours (Qualls and Haines, 1992). For C leached from the A-horizon of a mixed forest, up to 28% was lost in 48 hours and 50% after 4 days (Boissier and Fontville, 1993). Dahm (1981) reported that roughly 85% of the freshly leached C from alder foliage was degraded in 48 hours. Yano (1997) reported 10 to 45% degradation of leached C collected beneath an O-horizon in 5 hours using a flow-through bioreactor with a column colonized by native microbes. The readily available portion of DOC is likely to be lost rapidly unless the solution is filtered, or DOC is analyzed immediately after production. Thus, underestimation of biodegradable DOC (BDOC) is a possibility in many of the above studies. Such loss was minimized in this study by centrifugation at 5 ^{o}C (30 min.) immediately after leaching and extraction of soil followed by 0.2 μm filter-sterilization (taking approx. 3 to 4 hrs).

A Technical Concern in DOC Analysis

Another concern in DOC studies is inconsistency in defining DOC. Dissolved organics span a continuum from small molecules to colloids and investigators need to choose an approximate pore size and type of filter. Polycarbonate membranes (0.2 μm) were used in this study, following Jandl and Sollins (1997), to assure removal of microbes as well as sub-micron organo-mineral particles (although viruses cannot be excluded). Because filtered samples might have lost some DOC upon sitting for a few hours, all samples were frozen after filtration. More commonly, 0.45-μm pore-size membranes or 0.7-μm glass fiber filters are used. Compared with glass fiber or paper filter, pores in

polycarbonate membrane are made by gamma rays, creating a much more accurate and homogeneous size distribution of pores. Effects of pore-size and filter type on DOC value can be quite significant (e.g., Dahlin, 1994). Difference in filtration methods should be considered for detailed comparison of DOC values in literature.

Forest Floor VS. Mineral Soil C

Here, I studied C dynamics of the mineral soil because (1) much more C is present in mineral soil than forest floor, thus more CO_2 is produced by the mineral soil, and (2) C mobilization and destabilization mechanisms are more complex and less understood in mineral soil than in the litter layer. Binkley and Sollins (1990), working in Douglas-fir forests of contrasting soil fertility with and without red alder (the same sites as my study, Chapter 3), reported that the top 15 cm of mineral soil contained 5 to 11 times more C than the litter layer. Jandl and Sollins (1997) found that the mineral soil and litter layer contributed roughly 60% and 40% of annual soil respiration in a secondary Douglas-fir forest where I sampled the soil for a repeated leaching study.

Carbon in the litter layer is relatively unstable and susceptible to both microbial degradation and leaching (Cronan, 1985; Vance and David, 1991; Guggenberger, 1992; Gödde *et al.*, 1996). Most of the C that leaches from forest floor is stabilized in mineral soil (Cronan and Aiken, 1985; McDowell and Liken, 1988; Qualls and Haines, 1992), resulting in an accumulation of SOM in the upper mineral horizons. While plant-derived C continues to be destabilized in litter layer, organic C of both plant- and microbial-origin interacts with organo-mineral complexes and is thus subject to both stabilization and destabilization in mineral soils. These concurrent processes and their mechanisms in mineral soil are largely unstudied. By looking at the biodegradability and dynamics of water-soluble C in the top 8 to 10 cm mineral soil, I attempted to find some patterns in the complex stabilization/destabilization processes.

Nitrogen Connection

Douglas-fir forest ecosystems in the Pacific Northwest are known to be N-limited (e.g., Sollins *et al.*, 1980). Hydrologic N losses from undisturbed temperate forested watersheds are dominated by organic forms rather than ammonium and nitrate (Sollins and McCorison, 1981; Hedin *et al.*, 1995). Although a close link between soil C and N during SOM decomposition is well established (e.g., McGill and Cole, 1981), the relationship between DOC and dissolved organic N (DON) is largely unstudied. Both DOC and Kjeldahl N (ammonium + DON) concentration in leachates decreased with soil depth in a clear-cut and old-growth Douglas-fir forest soils (Sollins and McCorison, 1981). During an incubation of soil water-extracts, DOC:DON ratio of the whole extracts didn't change but the proportion of the hydrophobic acid fraction decreased over the incubation time (Allan and Cook, 1992 b). The biological reason for the observed change is unclear. I hypothesized that a close link between C and N exists in water-soluble pools and expected that more DON is utilized by soil microbes in the soils of low fertility (e.g., high C:N ratio).

Scope

I attempted to approach soil C dynamics with a mechanistic conceptual model (Fig. 1.1) that I developed based on previous conceptual models (McGill *et al.*, 1981; Zsolnay and Steindl, 1991; Boyer and Groffman, 1996; Sollins *et al.*, 1996) instead of empirically-based mathematical models (e.g., Parton *et al.*, 1988).

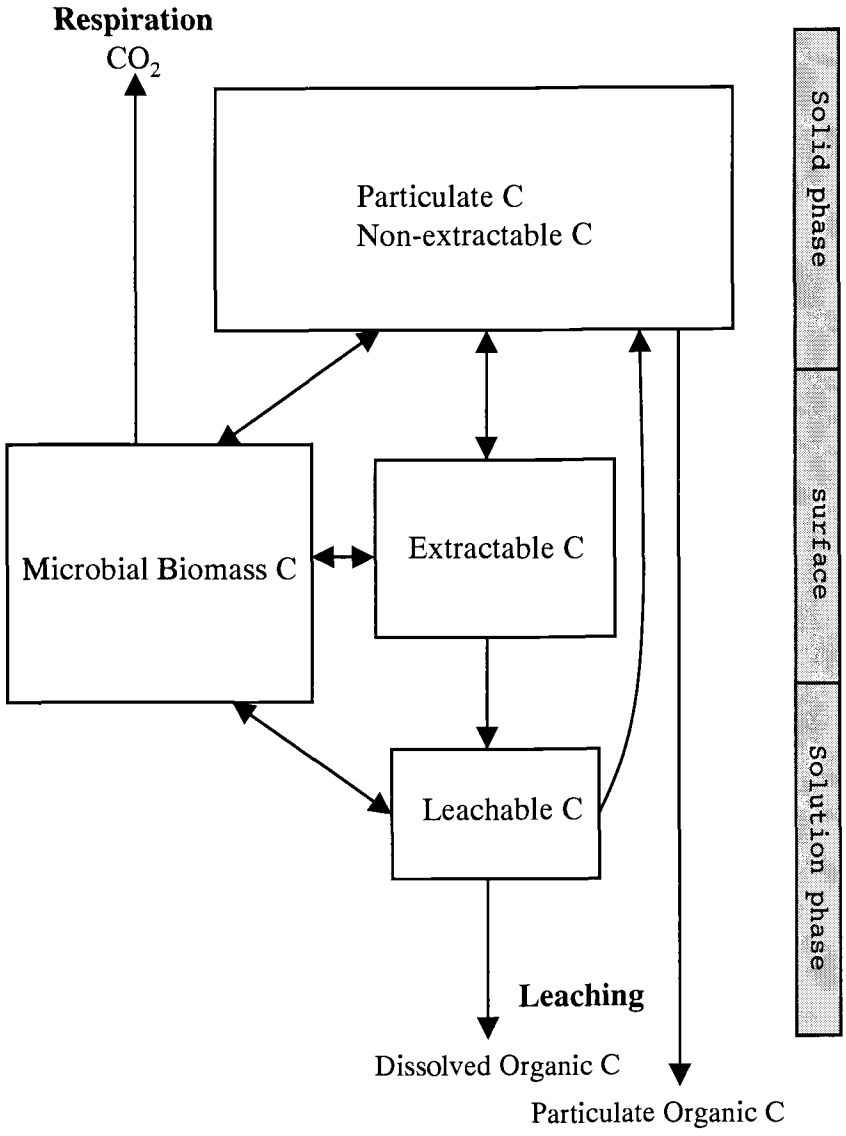

Fig. 1.1. A conceptual model of soil C mobilization following destabilization of particulate C. No C input from roots or litter layer was considered. The shaded bar on the right side represents the conditions of soil C.

In the first experiment (Chapter 2), I defined pools by using two contrasting extraction methods (leaching and shaking). I studied the total amount, biodegradability, regeneration rate, and contribution of the leachable and extractable C pool to the total C loss by leaching soil columns every 2 weeks for 20 weeks and by extraction of the soil before and after the repeated-leaching experiment. In the second experiment (Chapter 3), the importance of extractable C as a microbial energy source was studied using soils from two locations (Cascade Head coastal forest and Wind River upland forest) and total of five forest stands. I conducted a laboratory soil incubation experiment and extracted the soils before and after the incubation. Correlation analysis was used to find patterns between water-soluble C and soil C degradation measures (e.g., BDOC and microbial respiration) and to find important factors controlling DOC dynamics.

CHAPTER 2

DYNAMICS OF WATER-SOLUBLE CARBON IN A FOREST SOIL: SOIL LEACHING COLUMN STUDY

Rota Wagai

To be submitted to Biology and Fertility of Soil

Introduction

General

As water percolates through a soil profile, organic C dissolves and is translocated downward. It is well documented that dissolved organic C (DOC) concentrations are the highest in the litter layer and that more than 80 to 95% of litter-layer leachate is adsorbed or immobilized in the mineral soil horizons (Cronan and Aiken, 1985; McDowell and Liken, 1988; Qualls and Haines, 1991). Leaching of DOC from litter layer has been examined with respect to microbial availability (Qualls and Haines, 1992; Yano *et al.*, 1999), process controls and mechanisms (Christ & David, 1996 a, 1996 b), its role in C mobilization (Gödde *et al.*, 1996), and mobilization of other dissolved organic nutrients (Qualls and Haines, 1991; Qualls *et al*, 1991). The dynamics of DOC in mineral soil, where a much larger C pool is present, has been less studied, especially with respect to soil C destabilization.

Here, I viewed water-soluble C as a key pool resulting from soil C destabilization, with microbial respiration and leaching as the two major fates of destabilized C in mineral soil. A conceptual model was developed as a tool to study the movements of destabilized C and the factors influencing those processes by separating water-soluble C into two operationally definable pools (Fig. 1.1).

Leachable vs. Extractable Pool

Among a vast literature on DOC from soils collected either from leachates or by extraction, two conflicting views can be seen on the biological nature of DOC in mineral soil. Some regard DOC as a labile substrate for soil microbes (Chapter 1, Water-soluble C as Microbial Energy Source) and others consider DOC as recalcitrant compounds, by-products of microbial degradation of soil particulate C. Studies of soil DOC degradability have shown that DOC contains compounds of

different recalcitrance and that the amount and degradability are influenced by various factors (Chapter 1, Biodegradability of Water-soluble C). Thus, DOC in soil is likely to be susceptible to both microbial respiration and leaching loss.

Here, water-soluble C was separated into two operationally defined pools by using two contrasting extraction methods (i.e., leaching and shaking). A leachate contains the readily soluble C from soil aggregate surfaces along preferential flow pathways, whereas an extract (obtained after shaking) also includes soluble C from within aggregates because shaking disrupts at least the less stable aggregate structures. The activity of microbes and extracellular enzymes is expected to be high on soil aggregate surfaces because of higher probability of interaction between soil C and the enzymes on the surface than within aggregates. Depending on their size, soil aggregates can either protect microbes from predators or prevent microbes' access to labile C within the aggregates (Young and Spycher, 1979; Elliott, *et al.*, 1991, Ladd *et al.*, 1993). Thus, there is a potentially large difference between leachable and extractable C pools, with the extractable pool having larger and more labile C than leachable pool.

I hoped to gain some insights on the mechanism of soil C mobilization by assessing the dynamics of these two contrasting water-soluble C pools in relation to the total C loss from a soil system.

Soil Columns with and without Tree Seedlings

Sieved soil was used to make leaching columns to avoid spatial heterogeneity of soil C. Laboratory leaching experiment was designed to look at the potential contribution of water-soluble C to the soil C losses (i.e., respiration and leaching) instead of measuring the actual C mobilization fluxes in the field. Laboratory experiment was more practical to conduct frequent leaching treatments and to minimize the loss of labile DOC during the handling time before the filter-sterilization of soil solution. In addition, replicated columns were planted with Douglas-fir seedlings and compared with soil-alone columns under a controlled

environment to study the influence of plants on the amount and degradability of leachable and extractable C pools. Biological, chemical, and physical effects of the plant root system (i.e., rhizosphere effects) on soil and soil microbes are well known (e.g., Killham, 1994). For example, microbes are more abundant around root systems compared to bulk soil because roots release some labile C (e.g., root exudates). It is, however, unclear how roots affect the leachable and extractable C pools. I hypothesized that both soil C pools would increase due to the labile C input from roots and that these pools account for a significant portion of microbial respiration.

Objectives

The objectives of this study were;

(1) to test the hypothesis that water-soluble C is a significant portion of available substrates for microbial respiration in soil,

(2) to compare the amount and biodegradability of DOC between leachable and water-extractable pool.

(3) to assess the significance of leachable C and extractable C with respect to the soil C losses.

Materials and Methods

Pilot Experiments: Soil Leaching and Extraction

Soil Sample Source

The study site, at the Andrews Experimental Forest in the central Cascade Mountains of Oregon, lies on a gentle north-facing slope at approximately 630 m elevation and 44°13' N latitude. Annual temperature averaged 9.0 °C and 9.5 °C at

the two nearest reference stands. Precipitation, mostly falling in winter, has averaged 2200 mm $year^{-1}$ over the past 41 years at the main meteorological station about 6 km SW of the study site. The soil is a clay loam Inceptisol developed in alluvial and colluvially deposited andesitic parent material. The soil profile was described by Jandl and Sollins (1997). The stand is dense second-growth Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) averaging 25-30 cm diameter. After old-growth was clear cut and slash-burned in 1952, Douglas-fir seedlings were planted and some established naturally. Douglas-fir was replanted in fall 1956 and in 1957.

Soil Sampling

Surface mineral soil (0 to 8 cm in depth, October 1996) was collected after careful removal of the litter layer. The soil was brought back to the laboratory and stored at 5 °C in a refrigerator for several days. Identifiable litter, woody debris, and roots were removed by hand and the soil sieved (5 mm) and homogenized. A 5-mm sieve was used (instead of 2-mm sieve) to minimize soil handling effects on aggregates, to hasten the sieving process and avoid soil drying (Hart *et al*, 1994). The sieved soil was stored at 5 °C until it was used for the soil water-extraction experiment.

Soil Water-extraction

Soil was extracted with deionized water (soil:water = 1:3 by weight) on an orbital shaker (approx. 40 rpm) for 30 minutes. Immediately after shaking, the extracts were centrifuged at 5 °C for 30 minutes (maximum g = 9,715, Sorvall). The centrifuged samples were filter-sterilized through a 0.2 μm polycarbonate membrane (Poretics). The membrane was acid-soaked and rinsed, and the filtration unit was rinsed with deionized water prior to sample filtration. A vacuum suction of less than 0.68 (atm) was applied for the filtration to avoid lysing microbial cells.

The membrane size of 0.2 μm was used instead of more commonly used 0.45 μm to exclude microbes and submicron organomineral particles (Jandl and Sollins, 1997).

Incubation of Extracts

The filtered solutions were incubated for 3 months at room temperature (24 to 27 °C) to determine the biodegradability of DOC. Filtrate was separated into three 200-ml aliquots and NH_4Cl was added to achieve a final N concentration of 0, 1, and 5 mg L^{-1} to examine any effect of N limitation on DOC degradation. Flasks were covered with aluminum foil. Flasks were placed on an orbital shaker (roughly 40 rpm) and kept in the dark to prevent CO_2 fixation by algae and to allow oxygenation. Dissolved oxygen content was measured after 3-month incubation by a dissolved oxygen meter with a two-electrode probe (model 840, Orion). The values ranged between 83 to 86% saturation (6.6 to 7.0 mg L^{-1}) suggesting that enough oxygen was available to the heterotrophic microbes for DOC degradation.

Inoculum was prepared by mixing 10g of the sieved moist soil with 50 ml of deionized water. The mixture was shaken for 30 minutes and left at room temperature overnight in a gentle shaker. Soil solution was filtered through a 5-μm membrane that keeps bacteria and fungal spores/fragments in the solution but removes the major grazers (Frey *et al.* 1985). Filtrate was added to each incubation flask (inoculum:sample = 1:100, volume basis). One 12-ml aliquot for DOC analysis and two, 15-ml aliquots for dissolved N analysis were removed before inoculation. Another 12 ml was removed immediately after inoculation, then 6, 12 hours and 2, 4, 7, 21, 42, and 90 days later for DOC analysis. For dissolved N analysis, another two, 15-ml aliquots were collected immediately after inoculation, then 7 and 90 days later. All samples were stored frozen in glass scintillation vials until analysis for DOC and dissolved N.

Pilot Leaching Experiment

A single soil leaching column was repeatedly leached every two weeks for ten times and the biodegradability of leachates was examined. The soil column was constructed with a tissue culture filter unit (12 cm tall, 1 L, Nalgene). Surface mineral soil (0 to 8 cm) was collected from the same location in Andrews Forest (January, 1997) and sieved (5-mm mesh) as described above. After acid-washing the column, a 1-mm mesh nylon sheet was placed at the bottom and acid-washed silica sand (200 ± 5 g, 16 mesh) was placed on the sheet to minimize filter clogging and particle loss during the repeated leachings. Then the field-moist soil (670 g) was gently packed into the column above the sand and nylon mesh layer.

A synthetic throughfall solution contained 0.24 mg Na L^{-1}, 0.040 mg K L^{-1}, 0.050 mg Mg L^{-1}, 0.15 mg Ca L^{-1}, 0.16 mg SO_4^{2-}-S L^{-1}, and 1.55 mg Cl^- L^{-1}, similar to the field throughfall solution measured at a nearby site (Sollins *et al.* 1980). The solution pH was adjusted to 5.287 (5.28 to 5.31) by adding HCl, that is the same pH as the field throughfall. Organic nitrogen, and total P concentration in the field throughfall were very low (0.047 and 0.006 mg L^{-1}) and thus those weren't added to the synthetic throughfall solution. Similarly, no inorganic N was added to avoid the effect of N addition on dissolved N species in leachates and extracts. The column was leached with the throughfall solution (470 ml) every two weeks for 20 weeks. The column was kept at room temperature in the laboratory with a lid and the moisture loss between leaching was roughly 2.5% of the total soil weight. Leachates were centrifuged, filtered through 0.2-μm membrane filters, and incubated up to 2 to 3 months. Aliquots were taken for DOC and dissolved N analysis during the incubation as described in the incubation of extracts above.

Replicated Leaching Experiment

Soil Sampling

Surface mineral soil (0-8 cm in depth) was collected in June 1997 from the same stand in Andrews Experimental Forest. The soil was brought back to the laboratory, stored at 5 °C for four days, sieved (5 mm), and then stored at 5 °C in the refrigerator for 30 days until the preparation of soil leaching columns.

Soil Column Preparation

Soil columns, 33.6-cm long with a bottom chamber for leachate collection, were constructed by attaching 21.6-cm long PVC pipe (10.1 cm inner diameter) to the upper chamber of a tissue culture filter unit (12 cm tall, 1 L, Nalgene) with a PVC bushing. Silicone caulk was applied outside to seal the connected parts of bushing. An acid-washed nylon sheet and silica sand (500 ± 1 g, 16 mesh) were placed at the bottom of each column as described for the pilot leaching experiment. After mixing the soil thoroughly, 2.1 to 2.3 kg of field-moist soil was gently transferred to each soil column. Soil depth in the columns was 25 to 28 cm. Six columns were planted with tree seedlings. Three 1-0 seedlings of Douglas-fir (seeds from southwest Oregon) were planted in each of the six columns after gently washing off the peat moss medium by quickly soaking roots in water. Three soil columns contained soil but no seedlings as controls. Two columns contained only nylon mesh and the sand layer to control for background contamination.

All eleven columns were kept in a greenhouse from July to October, allowing time to recover from the soil disturbance, stabilize soil structure, and establish a root system. Air temperature in the greenhouse fluctuated between 18 and 34 °C. Soil moisture was brought back to 80% of field capacity every 3 to 14 days depending on season with the synthetic throughfall solution. Leaching losses from the columns were minimum during this period (total of 2.2 to 15.2 mL per

column). All columns were covered with a shade cloth between August 1 and October 15 to reduce solar radiation to seedlings.

Leaching Procedure

Roughly 470 ml was applied to each column every two weeks from November 10, 1997 to March 14, 1998. The total volume of throughfall solution applied during the repeated leaching study was approximately 20% of the annual precipitation for the Andrew study area. The throughfall solution was applied gently to minimize soil compaction. Leachate was collected from the bottom chamber of each column by applying a gentle vacuum (less than 0.14 atm). Soils were in contact with the throughfall solution for 6 to 25 minutes (15 minutes on average). The leachates were immediately centrifuged at 5 °C for 30 minutes (maximum g = 9,715, Sorvall). The centrifuged samples were filter-sterilized through a 0.2-μm membrane (Poretics) as described for the incubation of extracts above. Soil particles were collected after the centrifugation, air-dried, and the weight of leached particles was measured. Between leaching events, the soil columns were kept in the green house where temperature fluctuated between 11 and 18 °C.

Incubation of Leachates

Biodegradability of leachates was measured after the first through sixth leachings. The incubation experiment was not conducted for the 7, 8, and 9th leachings. Filtrate from each soil column was transferred to an incubation jar and incubated for 3 months as described above for the incubation of extracts. Due to the limited space, Mason jars (476 mL) with aluminum foil cover were used for the solution incubation instead of flasks (1000 mL) with parafilm. Because of the wider mouth and the loose aluminum cover, unexpected evaporation of water occurred during the leachate incubation. Thus I was unable to measure the

biodegradability of leachates except for four jars from the sixth leachings for which I could account for the evaporation loss by adding water back before removing aliquots for DOC and dissolved N analysis.

Inoculum was prepared by mixing 10g of surface soil from one extra soil+tree column (prepared for this purpose) with 50 ml of deionized water after each leaching. The mixture was shaken for 30 minutes, left at room temperature overnight in a gentle shaker, and filtered through a 5-μm membrane as described for the pilot study above. Samples for DOC and dissolved N were taken at the same intervals used in the pilot-study incubation. Unlike the pilot study, all samples were filtered (0.2 μm) each time to remove particulate organic C from the incubated solution. All the filtered samples were stored frozen in glass scintillation vials until analysis for DOC and dissolved N.

Soil Water-extraction

The soil remaining after column preparation was stored at 5 °C until March 1998, homogenized, and extracted with deionized water (soil:water = 1:5, weight basis) on a shaker (roughly 350 rpm) for 30 minutes (initial extraction). The extract was centrifuged and filtered as described above for the leachate samples.

At the end of the repeated-leaching experiment, soils were extracted with deionized water (final extraction). After the ninth leaching, the soil moisture of the columns was maintained at roughly 80% of field capacity until the columns were dismantled. Soils from all columns were sieved (2 mm) to remove any fine roots. An extensive root system was present and the tap root from each seedling was 14-28 cm long, often coiling at the bottom of the column. A 2-mm sieve was used instead of 5-mm to minimize addition of fine roots into soil. The field-moist soil from each column (100 g) was extracted, centrifuged, and filter-sterilized as in the initial extraction.

Subset of the sieved soil was oven-dried (60 °C) to estimate soil moisture content. The dried soil was mixed with deionized water (1:10) to measure soil pH.

Incubation of Extracts

Immediately after the extraction of refrigerated pre-leaching soil (initial extraction), the extract was separated into three, 370-ml aliquots. The first and second aliquots received the inoculum extracted from the refrigerated soil in the same way as for leachate inoculation. Before inoculation, K_2HPO_4 was added to the second aliquot (final phosphate concentration of 0.25 μmol L^{-1}) to avoid phosphorus limitation of DOC degradation. Inoculum for the third aliquot was prepared by rinsing the 0.2-μm membrane with the filtrate to collect the material caught by the membranes. This was done to look at the effect of the two inoculum types on DOC degradation.

The soil from each leaching column was used as inoculum for the DOC incubation of extracts at the end of the repeated-leaching experiment (final extraction). The inoculum was prepared in the same way as for the leachate inoculation. During the incubation of both initial and final extracts, samples for DOC and dissolved N analysis were collected as described above for incubation of leachates.

DOC Analysis and Biodegradable DOC Estimate

DOC was calculated as the difference between total C and inorganic C in solution based on platinum-catalyzed combustion/non-dispersive infrared gas analysis (TOC-5000A, Shimadzu Co.). Samples in scintillation vials were thawed and the biodegradable DOC (BDOC) was determined as the decrease in DOC between the pre-inoculation sample and the sample after a 3-month incubation. Percent BDOC (%BDOC) was calculated as BDOC / $DOC_{pre\text{-}inoculation}$ x 100.

A few flasks from the incubation showed small increases in DOC over time: theoretically DOC should not increase over the incubation time due to no C input. The higher values may have been caused by contamination during the 2-month

incubation or by heterogeneity of the incubated solution: the solution was mixed only gently to reduce damage to microbial population. Analytical errors are also possible for extracts incubated longer, which had DOC concentrations close to the detection limit of the Shimadzu TOC analyzer.

Dissolved Nitrogen Analysis

Ammonium and nitrate samples were thawed and analyzed with a Lachat autoanalyzer (QuikChem 4200, Lachat Instruments). Nitrate and ammonium standards were prepared using both deionized water and the artificial throughfall solution. The solution matrix had no effect on Lachat results, thus deionized water was used to make standards.

A persulfate digestion method (Koroleff, 1983; Qualls, 1989) was used to convert organic N and ammonium to nitrate, which was measured with a Lachat analyzer (see above). The digestion reagent was made of 10 g low-N potassium peroxydisulfate ($K_2S_2O_8$) and 6 g boric acid, and then was brought to 1000 mL in 0.075 M NaOH. Sample (10mL) and the digestion reagent (10mL) were pipetted into the test tube and weighed. The tubes were placed in a water bath (10cm deep) and autoclaved on liquid cycle (30 minutes, 120 °C). When cool and dry, the tubes were weighed to determine the evaporative moisture loss, which was accounted for after Lachat analysis. Although recovery of organic N in extracts might have been incomplete, the observed relative pattern in DON should be valid because all 25 samples were treated identically, thus the recovery rate should have been similar for all the samples.

Other Soil Analysis

Total C and N in each soil column and seedlings were determined with a Carlo-Erba CNS analyzer after dismantling of the leaching columns. Soil was air-

dried, manually ground, and sieved (60 mesh) before analysis. All three seedlings from each column were air-dried and ground. The soil used to make the columns in the beginning of study was also analyzed for total C and N. Soil pH from each column was determined with a pH/ISE meter (Orion) after mixing air-dried sieved soil with deionized water (soil:water = 1:10, weight basis) for 60 minutes.

Statistical Analysis

Each column was leached repeatedly and leachates were separately analyzed for DOC and dissolved N. Because the same columns were measured over time, a repeated measures design (univariate and multivariate, SAS Institute, 1992) was used to assess the pattern over the nine leachings. Due to the limited replication of soil columns, only three leachings (i.e. 1st, 5th, and 7th leachings) were selected for the repeated measures analysis. The results didn't change when other sets (e.g., 2nd, 6th, and 9th leachings) were used. The treatment effect (i.e. addition of tree seedlings to soil column) was examined by t-test assuming an unequal variance. The degradation of C during DOC incubation was regressed against incubation time (least-square linear regression, SAS institute) after log transformation of both DOC and time. There is no biological basis for such a transformation; it simply allowed a comparison of the slope of degradation curves among columns and between initial and final extraction.

Unless noted, soil C and N variables were all expressed on a per gram oven-dried soil basis (e.g., $\mu g\ g^{-1}$ soil).

Results

Pilot Experiments

The amount of leached DOC was roughly constant over the 10 successive leachings, while BDOC as well as %BDOC were higher for the first two leachings than for the remaining eight leachings in the pilot study (Table 2.1). Water extracts (shaken) contained approximately 12 times more DOC and 20 times more BDOC than did leachates (Table 2.2).

Table 2.1. DOC, BDOC, and %BDOC over the pilot repeated-leaching experiment.

leaching number	leaching interval[a]	volume	incubation period	DOC leached	BDOC[b]	%BDOC
	Day	mL	day	------- μg g^{-1} soil ------		%
1[c]	0	443	95	2.36	1.49	63.2
		443	95	-----	1.17	49.6
2	14	462	80	6.92	4.84	70.0
3	15	454	66	3.49	0.34	9.7
4	16	450	90	4.57	0.21	4.7
5	16	446	90	5.62	0.44	7.9
6	15	442	92	5.55	0.60	10.8
7	14	465	70	5.12	0.34	6.7
8	14	456	66	4.19	0.27	6.5
9	14	454	42	4.22	0.41	9.7
10	22	454	21	4.22	0.48	11.4

[a] Number of days between leachings.

[b] After the DOC incubation, leachates were analyzed for DOC without filtration.

[c] The first row had regular inoculum and the second row had inoculum made from a fresh litter layer.

Table 2.2. Summary of DOC incubation experiments (both leachates and water-extracts) from the pilot experiments and replicated leaching study. Mean and (standard error).

Type of sample	Incub. period	n [a]	DOC	BDOC	%BDOC	Remarks
	days		--- μg g^{-1} soil ---		%	
pilot extracts-N effect [b]	29.2	3	51.84	20.05	38.67	soil:water=1:3 by wt., n=1 for extraction.
				(1.05)	(2.03)	incubated samples unfiltered before DOC analysis
pilot repeated leachings [c]	21-90	10	4.42	0.96	22.74	incubated samples unfiltered before DOC analysis
			(0.42)	(0.41)	(7.55)	
6th leachates-soil only	73.8	2	1.65	0.26	15.93	
			(0.12)	(0.03)	(0.95)	
6th leachates-soil+trees	73.8	2	1.47	0.19	12.96	
			(0.03)	(0.01)	(0.84)	
initial extract	45.4	3	24.79	6.81	27.36	solution contained different inoculum and
			(0.58)	(0.80)	(2.62)	phosphorus levels.
final extracts- soil only	89.5	3	17.01	4.02	23.13	
			(1.24)	(0.88)	(3.40)	
final extracts-soil+trees	89.5	5	14.97	2.66	17.69	
			(0.54)	(0.23)	(0.83)	

[a] Sample number for each solution incubation experiment.

[b] Single soil water-extraction. The extract was separated into three aliquots to see N effect on DOC degradation.

[c] Average value over 10 leachings. (see Table 2.1 for detail)

[d] Only 6th leachate was used for DOC incubation.

Leached C and Water-extractable C

In the replicated study, the soil continued to lose a relatively constant level of C over the nine repeated leachings, and water extracts (shaken) contained 10-20 times more C than did leachates (Fig. 2.1). Similarly the initial extracts had roughly 30 times more labile C than the leachate (Table 2.2). The sum of leached C in all nine leachings was still less than the amount of extractable C at both extraction times (Fig. 2.2). The initial extracts contained 48 to 56% more DOC and 81 to 174% more BDOC than the final extracts. The relative proportion of DOC degraded (%BDOC) was 5 to 16% higher in extracts than in leachates (Table 2.1).

The soil-alone columns released more C than soil+tree columns consistently for all leachings (Fig. 2.1). However, significant difference between the two column types was detected only for the 1st, 3rd, 5th, and 6th leaching ($p < 0.1$). Soil water extracts at the end of the leaching experiment also showed a significantly less DOC in the soil+tree columns than in soil-alone columns ($p = 0.005$). Biodegradable DOC in the sixth leachates and final extracts was less in the soil+tree columns than in soil-alone columns (Fig. 2.2) but the difference was not significant. Although biodegradability was successfully measured only at the sixth leaching, earlier leachates were expected to contain an equal or greater amount of BDOC than sixth leachates considering the pattern found in the pilot study (Table 2.2).

Dissolved organic C at both initial and final extraction time showed a rapid degradation in the earlier stage followed by a slow degradation (Fig. 2.3). The percent of DOC biodegradable (%BDOC) appeared to be higher and the slope of the DOC degradation curve appeared more negative (i.e., faster degradation) in the soil-alone columns than in the soil+tree columns (Table 2.3), however no significant difference was detected. Perhaps with higher replication, these differences would be significant. Production of BDOC from the roots of seedlings was expected but not detected in this study. Soil pH didn't differ between the two column types at the final extraction time.

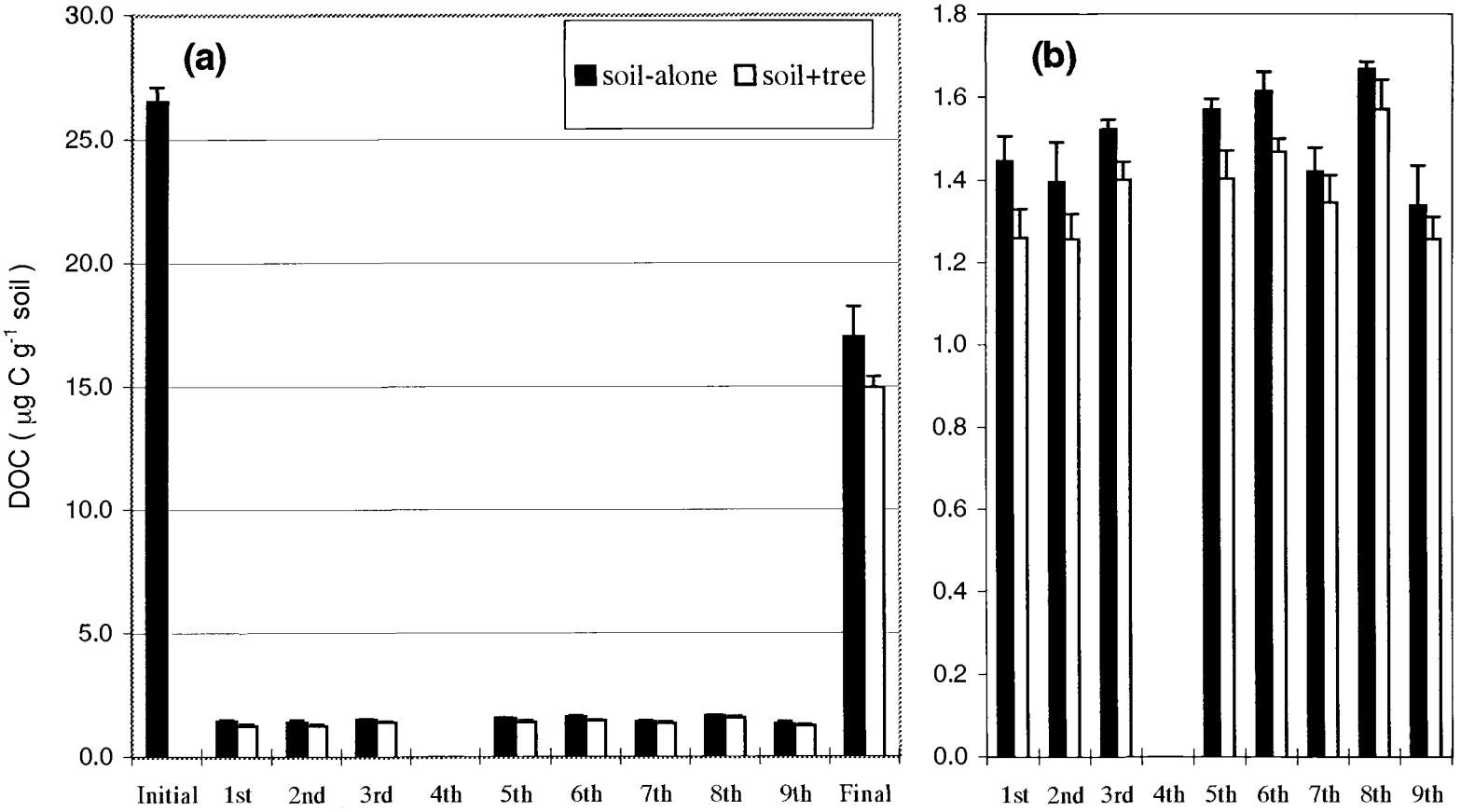

Fig. 2.1 Changes in DOC among (a) the extractions (initial and final extraction times) and repeated leachings, and (b) among the nine repeated leachings. Error bar shows standard error (n = 3 for soil-alone column, n = 5 for soil+tree column). All the soil columns were leached at the fourth leaching but leachates were not analyzed.

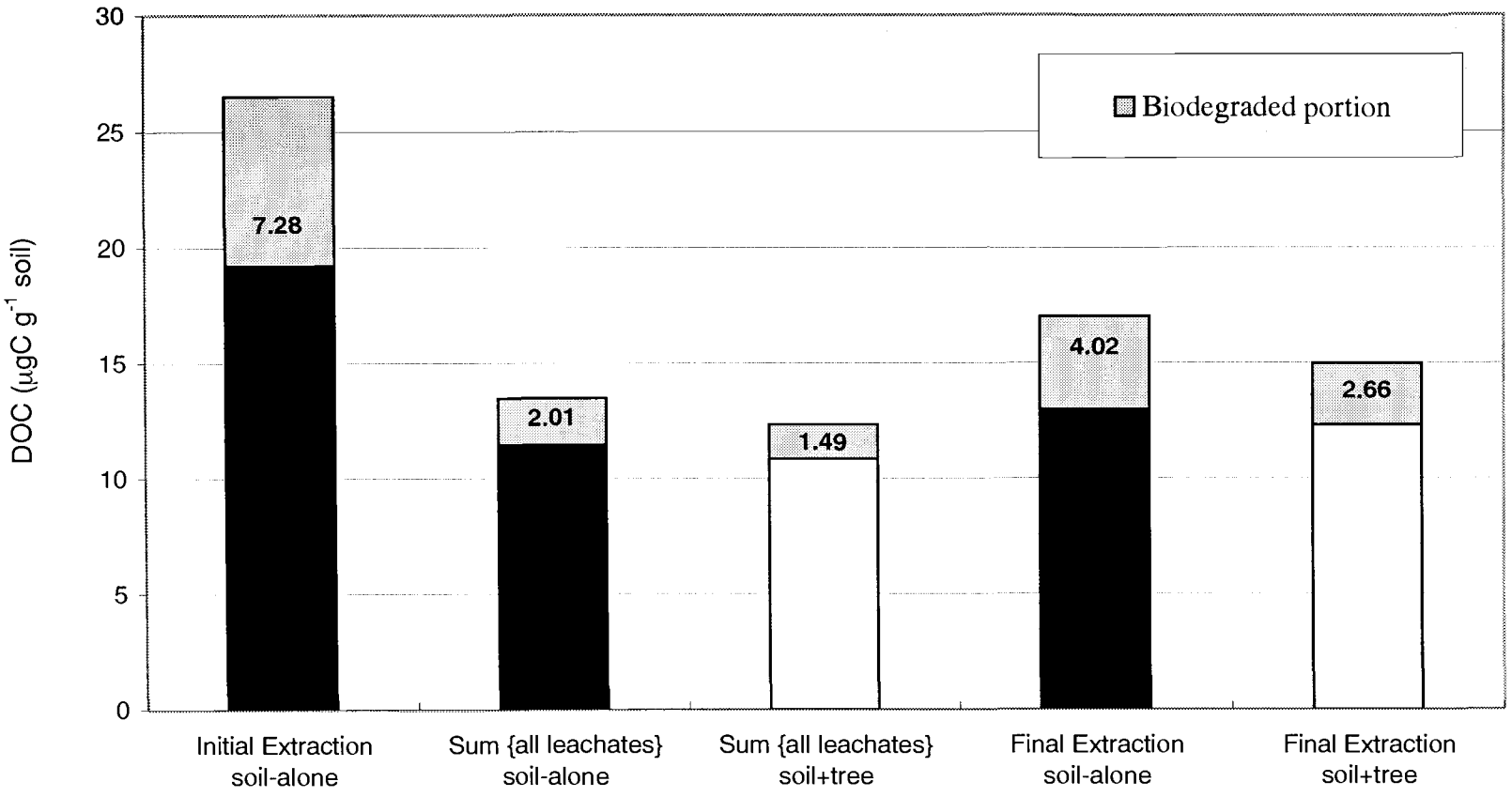

Fig. 2.2. Comparison of DOC level among the initial extraction time, the sum of all nine leachings, and the final extraction time. Dark bar is the soil-alone columns and open bar is the soil+tree columns. Shaded part represents the biodegradable portion of DOC (value is biodegradable portion in $\mu gC\ g^{-1}$ soil)

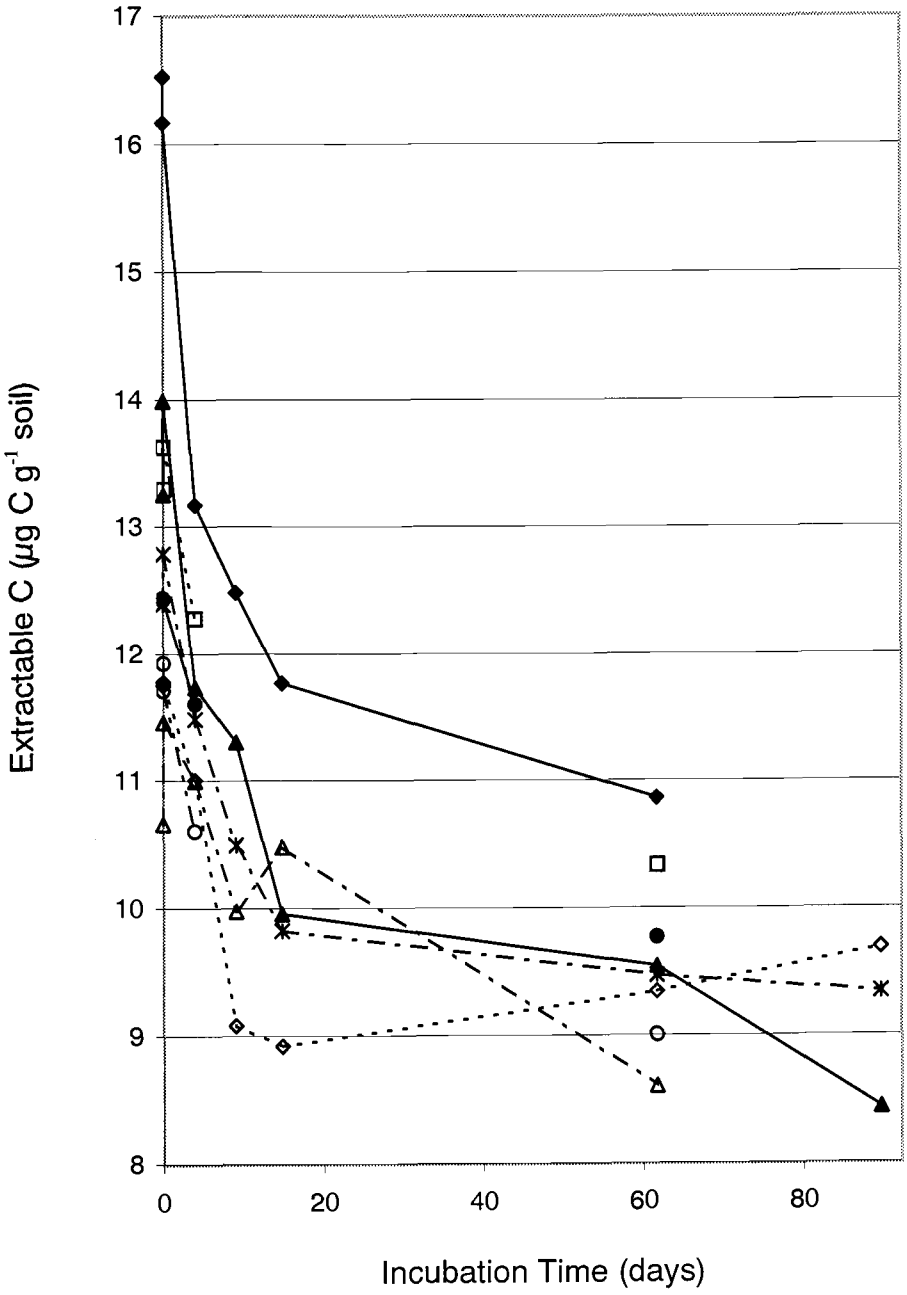

Fig. 2.3. Degradation of water-extractable C from each soil column after the final extraction. Solid lines with filled symbol are extracts from the soil-alone columns and broken lines with open symbols are from the soil+tree columns. Each symbol represents indivisual flasks.

Table 2.3. Degradation of water-extractable C at the final extraction and the regression analysis of individual degradation curves after log transformation of both DOC and incubation time.

	column number							
	1	2	3	4	5	6	7	8
	soil-alone columns			soil + tree columns				
total DOC*	16.53	13.25	12.44	12.39	13.30	11.92	11.78	10.66
avg	14.07			12.01				
std error	1.25			0.43				
BDOC**	5.67	4.81	2.67	3.05	2.96	2.92	2.09	2.05
avg	4.38			2.62				
std error	0.89			0.22				
%BDOC***	34.28	36.33	21.46	24.62	22.25	24.50	17.78	19.26
avg	30.69			21.68				
std error	4.65			1.38				
Regression Model Fit †								
r^2	0.951	0.799	0.798	0.850	0.8223	0.8911	0.726	0.405
avg	0.85			0.74				
std error	0.05			0.09				
Intercept	2.573	2.413	2.410	2.385	2.475	2.336	2.327	2.330
avg	2.47			2.37				
std error	0.05			0.03				
Slope	-0.037	-0.035	-0.019	-0.026	-0.021	-0.023	-0.023	-0.014
avg	-0.030			-0.021				
std error	0.006			0.002				

* DOC concentration in the extracts prior to inoculation for DOC degradation (μg C g^{-1} soil).

** The loss of C during the DOC incubation experiments (μg C g^{-1} soil).

*** BDOC relative to DOC (%).

† Both DOC concentration and incubation time were log-transformed.

Ammonium and Nitrate

Ammonium in leachates significantly increased in the soil-alone columns and decreased in the soil+tree columns over the nine successive leachings ($p = 0.040$ and 0.013, respectively, Fig. 2.4). The treatment effect (of seedlings in soil columns) was significant for all leachings except the first. The final extraction also showed significantly more extractable NH_4^+ in the soil-alone columns than in the soil+tree columns.

Leached NO_3^- decreased significantly across the nine leachings for the soil-alone columns ($p = 0.009$), while leached NO_3^- in the soil+tree columns was relatively constant (Fig. 2.4). The treatment effect was present only at 1st and 2nd leachings ($p = 0.05$ and 0.08). In contrast to ammonium, the final extraction of nitrate didn't show any difference between the soil-alone and the soil+tree columns.

Mass Balance of Soil Column

There was no statistically significant change in total C and N from the start to end of the repeated leaching study (Table 2.4). The study period was a total of 242 days including a 4-month incubation of columns in the greenhouse prior to the 20-week repeated leaching. Total leaching of DOC during the nine leachings was 14.1 and 14.6 mg C column^{-1} for the soil-alone and soil+tree column (Table 2.4). Soil-alone column lost more N than the soil+tree column did primary in the form of ammonium. Leaching loss of soil particles (i.e. particulate organic C and N) was estimated from the measurement at the fifth leaching. During the repeated leaching, more C was lost as particulates but more N was lost as dissolved species in both types of column (Table 2.4).

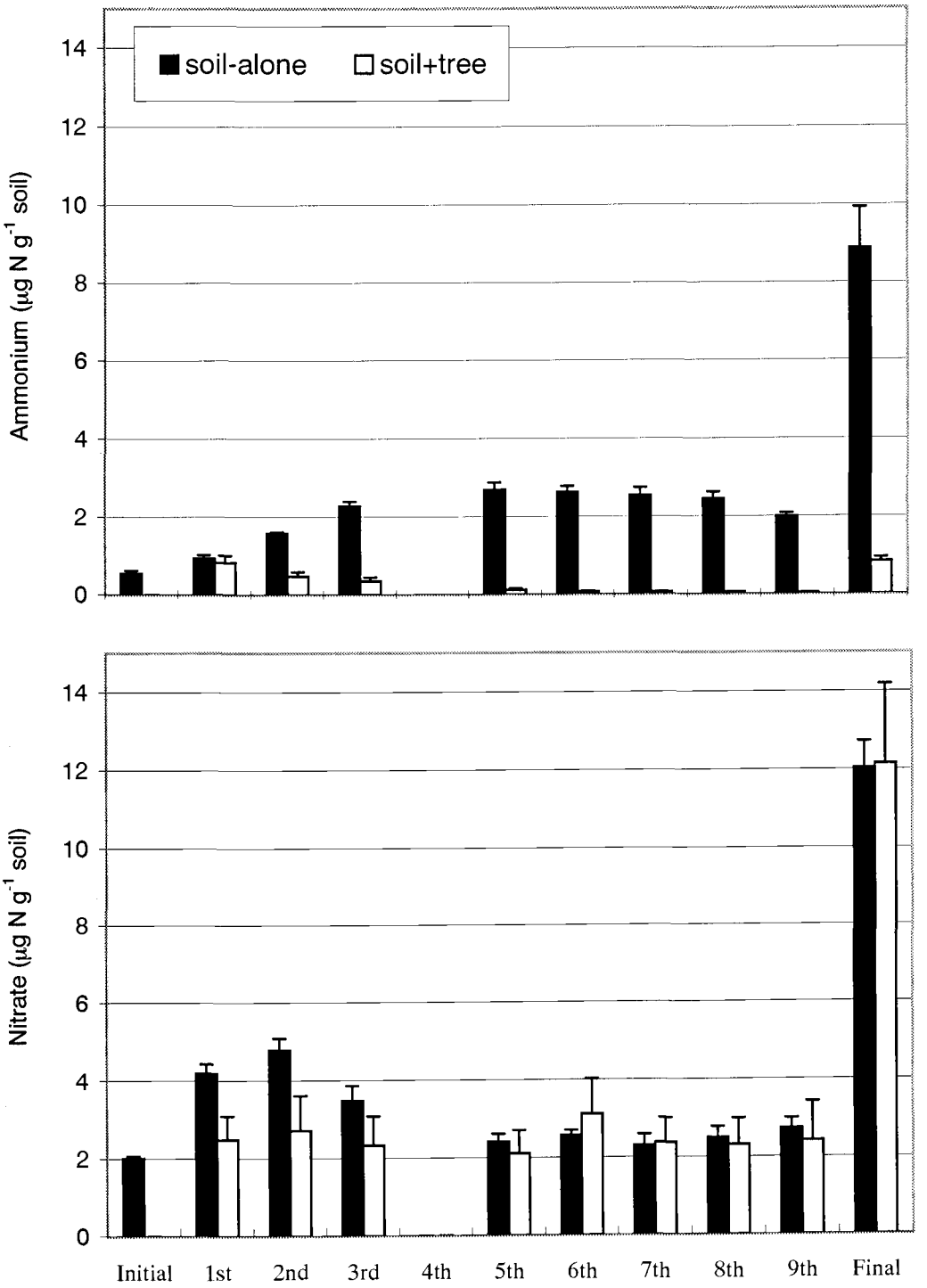

Fig. 2.4. Changes in ammonium (a) and nitrate (b) between the initial and final extraction time as well as the nine repeated leachings. Error bar shows standard error (n = 3 for soil-alone column, n = 5 for soil+tree column).

Table 2.4. C and N budget of soil leaching column during the 242-day incubation period. Mean and (standard error).

	Carbon				Nitrogen				
	soil-alone column		soil+tree column			soil-alone column		soil+tree column	
	mg C per column					mg N per column			
Bulk soil at day 0	72901	(2688)	75128	(1690)		2712	(100)	2795	(63)
Bulk soil at day 242	69191	(4876)	71730	(4764)		2661	(131)	2671	(163)
The difference	3709	(2319)	3398	(3681)		52	(44)	124	(120)
Loss of dissolved organic matter over the nine leachings	14.2	(1.9)	14.8	(1.8)	NH_4^+	20.8	(0.4)	2.6	(0.5)
					NO_3^-	30.7	(0.3)	26.8	(8.0)
					DON*	1.2	(0.3)	1.9	(0.6)
Leaching loss of particulate**	21.6		21.6			0.8		0.8	
Total C and N loss by leaching	35.8		36.4			53.5		32.0	

* Dissolved organic N may be underestimated due to a possible imcomplete recovery in the persulfate digestion method.

** The leaching loss of soil particles was measured only at the fifth leaching. The amount of leached particles was higher in the earlier leachings and, thus the weight of leached particles at the 4th, 3rd, 2nd, and 1st leaching was assumed to be 2, 4, 8, and 16 times that of the fifth leaching. The amount of leached particles at the 6th and later leachings was assumed to be the same as in the fifth leaching. The concentration of C and N in the particles was assumed to be the same as that of the bulk soil at day 0.

Discussion

DOC Production over Repeated Leachings

The soil studied here produced a constant level of DOC (avg. 4.2 mg C l^{-1} leaching^{-1}) for 18 weeks without inputs of C from plants and O-horizon (Fig. 2.1). Similarly, Vance and David (1991) reported that 3.6 mg C L^{-1} leaching^{-1} of DOC were continuously leached from spodosol soil columns (O + B horizon) during weekly repeated leachings over a year.

There are at least two explanations for the constant production of leachable DOC. The first assumes that soil holds a large leachable C pool and a small amount of DOC is leached every time, thus the pool doesn't diminish during the successive leachings. This view implies a hydrological control on DOC leaching (i.e., the amount of water or the length of leaching time, when water is in contact with soil, determines the amount of C leached). A second possible explanation is that a constant amount of leachable C is regenerated between leachings, even though each leaching removes all or at least a major portion of the leachable C from the soil. A more realistic view may be a combination of the above two explanations: the production of leachable C exceeds the leaching loss, resulting in a constant release of C.

At the ninth leaching, a 470-mL throughfall solution was separated into three portions, leached sequentially, and three leachate samples were collected from one soil-alone column and two soil+tree columns. No consistent pattern of C concentration was found among the three successive leachates and, so the first explanation was not supported. Among the nine repeated leachings, the first leaching released the same amount of DOC as the second one despite the fact the soils had been incubated for four months before the first leaching and thus had more time to generate leachable C. This may support the first explanation:

hydrological control of leachable C production. However, this doesn't rule out the second explanation because it is possible that microbial consumption of regenerated leachable C had kept the pool at a low level.

Christ and David (1996 a) studied the mechanisms of leaching from Spodosol forest-floor materials by using different concentrations of litter-derived DOC extractant and a wide range of leaching intervals. Hourly successive leachings of their red spruce litter showed a continuous decline in leached C followed by a steady level, supporting the second explanation: a small but regenerating C pool. When the litter samples were incubated at 10 °C for different period up to 14 days after initial intensive leaching, more C was leached from the samples incubated longer but the increase in leached C was not proportional to the incubation time. Thus the calculated rate of leachable C production decreased from 220 to 40 μgC g^{-1} soil day^{-1} for 1-day to 14-day incubated samples. Although not mentioned by the authors, this decline in DOC production rate may be explained by microbial consumption of leachable C in excess of production during the incubation period (i.e. the longer the leaching interval, the more leachable C consumed, and thus the less C leached). In a companion study, respiration rate of the litter materials from the same site was 70 μgC g^{-1} soil day^{-1} at 10 °C (Gödde *et al.*, 1996). Assuming the initial fast rate of leachable C production continued for 14 days (i.e., 3220 μgC g^{-1} soil), the microbial population depended solely on leachable C for their substrate, and the respiration rate was constant, then the microbial degradation of leachable C accounted for only 30% of the potentially produced leachable C. Thus, the microbial consumption of leachable C would explain only up to 30% of the decline in DOC production rate. Then other mechanisms such as lower accessibility of microbes and extracellular enzymes to substrates due to accumulation of biomass and metabolites on litter surfaces over time have to be considered. Christ and David (1996 a) also chemically fractionated the DOC and found that the relative ratio of hydrophilic to hydrophobic acids increased over the 8-week incubation period. An increase in this ratio suggests an

increase in microbial metabolites relative to plant-derived degraded compounds in the leachable C pool during that period (Guggenberger *et al.,* 1994).

Biodegradability of DOC

Only 13 to 23% of the leachable C was biodegraded in my study, suggesting that the leachable C pool is not highly labile (Table 2.1). In contrast, 18 to 39 % of the extractable C was degradable. Jandl and Sollins (1997) found on average 15% BDOC in water-extracts of a soil from the same location (near the same soil pit). Their method was identical to mine except that soil was shaken overnight and the filtration took more than 4 hours (C. Glassman, pers. comm.). Moreover, their DOC incubation was done after extracts were separated into hydrophobic acid (HoA), hydrophilic acid (HiA), and hydrophilic neutral (HiN) fractions. Loss of labile DOC during the extraction and fractionation processes may account for the lower %BDOC. Temporal change (e.g., seasonal change) in soil C dynamics may also affect the level of BDOC. Biodegradable DOC was concentrated in the HiN fraction, in which most degradation occurred within 3 days.

The DOC solution incubation technique provided an artificial aquatic environment for microbes. Thus it is possible that some labile DOC wasn't utilized as efficiently as in a field condition. This and similar techniques used by other workers, however, have shown degradability values ranging up to 85% (see Chapter 1, Biodegradability of Water-soluble C), demonstrating the usefulness of the technique for measurement of labile DOC. Incubation of extracts in my study showed 4.7 to 70 %BDOC (Table 2.1 and 2.2).

Leached C vs. Water-extractable C

Amounts of water-extractable C and BDOC were 10 to 30 times that of leached C. In addition, %BDOC was consistently higher in extracts than in

leachates for both pilot and replicated experiments (Table 2.2. and Fig. 2.1), suggesting that a significant amount of water-soluble C and BDOC was present within aggregate rather than on the surface of aggregates. Similar pattern in DOC has been seen previously. The top 25-cm of Mollsols from Iowa corn and soybean fields produced roughly 25 times more C by water-extraction than by leaching on a dry soil basis (McCarty and Bremner, 1992), assuming the field moist soil had 30% moisture. Greater release of DOC by extraction can be explained not only by disruption of aggregate structure but also longer dissolution time. The soils in my study were shaken for 30 minutes during the extraction before centrifugation at 5 °C. The leaching time among the eight columns varied between 6 to 25 minutes but no clear relation was found between leaching time and DOC production. Thus disruption of aggregates is more likely than leaching to be the dominant factor causing greater release of DOC and BDOC during extraction. Soil aggregates can physically prevent microbial access to labile C (Amato and Ladd, 1992). A soil incubation study of various aggregate size class showed that more C was respired from the crushed macroaggregates (>250 μm) than intact macroaggregates, suggesting the aggregate physical protection of labile C from microbial degradation (Beare *et al.*, 1994). Microbial cell lysis and consequent flush of labile C by the crushing treatment, however, might confound their result. Presence of labile C within aggregates could explain the higher %BDOC in extracts than leachates observed in my study. Chotte *et al.* (1998) demonstrated that added labile DOC was rapidly consumed by the microbes associated with soil microaggregates (i.e. heavy clay fraction, 2 to 50 μm in size) of Vertisols. A labile portion of leachable C in my study may have been consumed before the water percolated through the columns, resulting in a lower amount of BDOC in the leachate than in the extracts.

Qualls and Haines (1992) found that %BDOC in leachate decreased with soil depth and suggested that O-horizon leachate is adsorbed rapidly at first, after which microbes degrade the adsorbed compounds slowly. If that is true, then leached C beneath A-horizon is likely to be dominated by recalcitrant compounds

left over after microbial degradation, agreeing with lower %BDOC of leachates than extracts observed in my study. Degradation of adsorbed DOC can be large in the long term but its significance in soil C dynamics in unclear.

Patterns of Dissolved N Species over Successive Leaching

The columns with and without seedlings showed a contrasting pattern of leached NH_4^+ (Fig. 2.4). The fact NH_4^+ was detectable in leachates implies that the gross mineralization rate exceeded the sum of gross nitrification, immobilization, plant uptake (in case of the soil+tree column), and abiotic fixation and volatilization. The soil+tree columns released significantly less NH_4^+ than did the soil-alone columns but not NO_3^-, suggesting preferential uptake of ammonium by plant roots. The increase in leached ammonium over time in the soil-alone columns could be due to an increase in mineralization and/or decrease in the loss processes over the nine leachings. Nitrate in the soil-alone columns increased, suggesting an increase in gross nitrification and/or decrease in immobilization and denitrificaiton. These N transformation processes weren't, however, examined in this study.

Treatment Effect (soil-alone vs. soil+tree columns) on DOC

Extracted and leached C were lower in the soil+tree columns than in the soil-alone columns (Fig. 2.1). In addition, final extracts from the soil+tree columns had a lower %BDOC than initial extracts or final extracts from the soil-alone columns (Table 2.3). These results contradict the hypothesis that the soil+tree columns should have more leachable and extractable C than the soil-alone columns because of C inputs from roots of the seedlings. Live plant root systems release low molecular weight organic C, provide habitats for microbes, and break some of the soil aggregates during growth. Two hypotheses can be proposed to explain my results. First one would be that the microbial population, primed by the root C

inputs, consumed both the labile C from roots and less labile native soil C, resulting in the lower leachable and extractable C from the soil+tree columns than the soil-alone columns. Alternatively, added C from roots might have been stabilized in non-extractable forms. The latter hypothesis is very unlikely. For the lack of any other explanations, I use the former one as a working hypothesis. The first hypothesis implies that the microbes in soil+tree columns actively utilized leachable and extractable C on aggregate surfaces in addition to the labile C from roots. Such additional microbial consumption of soil C may be possible because of the fact that roots grow between aggregates and even penetrate aggregates, providing microbial habitats for microbes, where they couldn't have colonized otherwise. Extensive root systems were observed for all the soil+tree columns in my study. If the first hypothesis is true, my results imply that the root-derived C was not stabilized in soil but rapidly utilized by soil microbes and rather enhanced soil C destabilization. Using a ^{14}C pulse labeling technique, Norton *et al.* (1990) found that 31% of photosynthesized ^{14}C in a Ponderosa pine seedling was found in a bulk soil after a 5-day labeling period and over a half of the labeled C in the soil was respired during a 7-day chase period. Although plant litter contributes to C accumulation in mineral soil (Cronan and Aiken, 1985; McDowell and Liken, 1988; Qualls and Haines, 1991), root-derived C such as root exudates may enhance the microbial destabilization of soil C.

Similarly, ammonium was lower in the soil+tree columns than in soil-alone columns in all leachates except for the first leaching as well as at the final extraction time. Both microbial immobilization and plant uptake of ammonium in the soil+tree columns could have caused the lower ammonium in both leachates and extracts.

Integrated View: Significance of Water-soluble C with respect to Soil C Destabilization

A conceptual model of soil C mobilization was proposed and the pool sizes and fluxes were estimated based on the measurements of particulate, water-extractable, and leachable C pools from the soil-alone columns except respired CO_2 and microbial biomass C (Fig. 2.5). No inputs from litter layer or plant roots were considered. Major factors influencing the destabilization of each phase (solid, surface, and solution phase) were hypothesized (Fig. 2.5). All the values were extrapolated to an annual basis to compare the size of different pools and fluxes. I make no claim that these values are predictions of field conditions. The model is rather an attempt to integrate measurable pools and fluxes with mechanisms of soil C destabilization and to identify important pools and fluxes for further study. Because I wasn't able to detect the decrease in total C in the columns over a 242-day study period (Table 2.4), the exact amount of CO_2 respired during this period is unknown. Thus I used a field measurement of soil respiration from a litter-removed plot (226 gC m^{-2} $year^{-1}$) in the same stand as my soil sample source to estimated the respired CO_2 from the columns (Jandl and Sollins, 1998). The respiration rate may be overestimated by roughly 50% due to root respiration in the field. Microbial biomass C was also estimated from Jandl and Sollins (1998) at the same soil pit 3 years prior to my study using a microscopic method (Frey *et al*, 1985; Ingham *et al*, 1991).

Microbial respiratory loss of C was far more than leaching loss (Fig. 2.5). This is true even after accounting for a possible overestimation of respiration rate (up to 50%) due to root respiration in addition to microbial respiration. Leaching loss of C (including both DOC and POC) accounts for 8 to 14% of total C loss (i.e., leached C plus respired C) in my study depending on the estimated respiration rate. Leaching loss of N was greater than that of C mostly due to high leaching of NH_4^+ and NO_3^-. Gaseous loss of N wasn't measured and thus the significance of N leaching loss relative to gaseous loss is unknown.

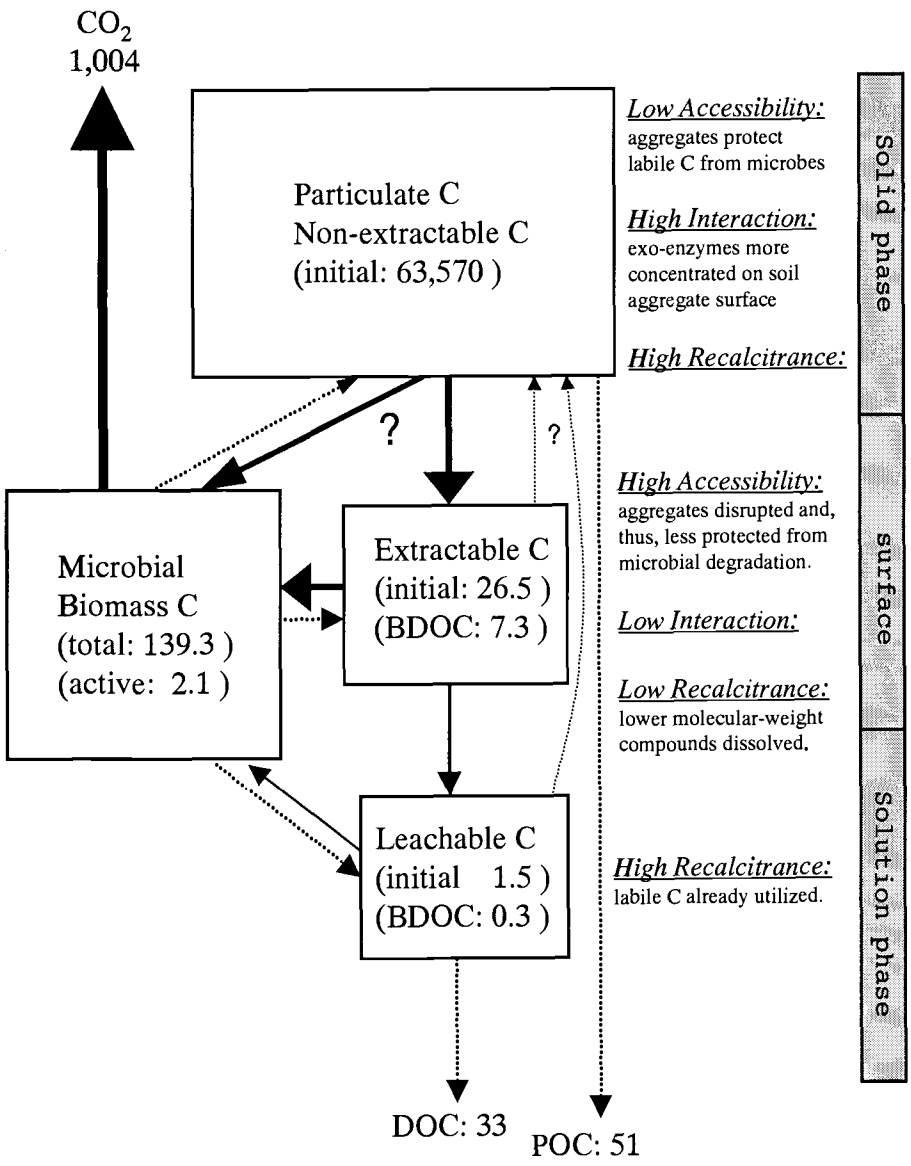

Fig. 2.5. Soil organic C destabilization model. No C input from roots or litter layer was considered. Values are means from the soil-alone columns (n = 3). All the fluxes were expressed in mg C kg^{-1} soil $year^{-1}$ and all the pool sizes were in mg C kg^{-1} soil. The width of fluxes shows relative importance in C mobilization. Microbial biomass and respiration data were from the same study location (Jandl and Sollins, 1997).

The contribution of DOC leaching to soil C loss was also relatively low in other forest systems. Cronan (1985) conducted a weekly leaching of intact soil columns from three forest types in New England for one year in greenhouse. The contribution of DOC leaching to total C loss was 30 to 44, 16 to 28, and 6 to 14% for the columns made of O-horizon, O + A, and O + A + B-horizon, respectively. Leached C varied by a factor of two among the forest types, even between two sites with the same soil type (Haplorthod). Vance and David (1991) conducted a one-year weekly leaching study using reconstructed Haplorthod soil columns under northern hardwood. DOC leaching accounted for roughly 6, 9, and 30% of total C loss for the O-horizon, O + B, and O + doubled B-horizon columns, respectively. It should be noted that neither study measured particulate C loss and both used less conservative definitions of DOC, 0.45 μm (Cronan, 1985) and 0.7 μm (Vance and David, 1991), than I did. Such coarse filtration should have increased the measured amount of leached C. Also, Vance and David (1991) spent 2 to 3 days for leaching, during which leached C was not sterilized and a large portion of BDOC might have been lost.

I concluded that leachable C pool in my study was constant during the study period because of the constant level of leached C over the successive leaching (Fig. 2.1). Because of low amount and biodegradability of leachable C, it is very unlikely that microbial degradation of leachable C explains a significant portion of the CO_2 respired from the soil I studied.

In contrast, the extractable C and its biodegradable portion were much greater than leachable C. The size of labile extractable C was comparable to that of active portion of microbial biomass C. Depending on the turnover rate of extractable C pool, a significant portion of the respiration may be explained by microbial consumption of extractable C. Other workers have speculated that extractable C is an immediate energy source for soil microbes. A laboratory incubation of wheat straw materials showed 2 to 4 times faster respiration rate in the non-leached straw than in the straw that has been intensively leached prior to

the straw incubation (Reinertsen *et al.*, 1984). This difference in respiration rate was greatest in the first three days, supporting the idea that extractable C is a readily available substrate for soil microbes (Seto and Yanagiya, 1983; McGill *et al.*, 1986; Hart *et al.*, 1997; Jandl and Sollins, 1998). In a 456-day laboratory incubation of old-growth Douglas-fir soil from Andrews experimental forest, Hart *et al.* (1997) observed that the decline in respiration generally corresponded with the decline in K_2SO_4-extractable pool, suggesting that K_2SO_4-extractable pool as a main C and N source for microbes. Furthermore, a strong correlation between extractable C and microbial respiration has been reported from a wide range of mineral soils in temperate regions (Burford and Bremner, 1975; Powlson and Jenkinson, 1976; Seto and Yanagiya, 1983; Davidson et al., 1987; Zak *et al.*, 1990; Cook and Allan, 1992a).

Then, to what extent could extractable C account for the microbial respiration? From the amount of extractable C degraded during the DOC incubation and its degradation rate, one can assess the potential contribution of labile extractable C to total soil respiration. Because natural DOC tends to contain compounds of different recalcitrance, DOC degradation is better explained by assuming more than one DOC pool with different decay rates (Qualls and Haines, 1992). Based on the fitted decay curves for the initial extracts (least-square linear regression), roughly 80 and 10% of BDOC were degraded within 6 and 12 days, which can be translated to 3.4 and 0.2 mg CO_2-C kg^{-1} soil day^{-1}. Assuming that BDOC is kept replenished over time, these respiration rates extrapolate to 98.1 gC m^{-2} $year^{-1}$ from the top 10 cm mineral soil. This estimate of extractable C degradation is reasonably close to the field respiration rate (226 gC m^{-2} $year^{-1}$, Jandl and Sollins, 1998) given the fact that root respiration constitutes up to 50% the field respiration. Using the same approach, Jandl and Sollins (1998) estimated 120 gC
m^{-2} $year^{-1}$ for the annual degradation rate of extractable C based on DOC degradation curve.

Validity of the annual degradation estimate above depends on the assumption of constant BDOC over time. There was a net decrease in extractable C from the start of the leaching study to the end (Fig. 2.1 and Fig. 2.2), suggesting that the loss of extractable C via leaching plus microbial consumption exceeded the production of extractable C. During the 242-day study period, BDOC in extracts declined 45% and 63% for the soil-alone and soil+tree columns, respectively. Because leaching loss was small, microbial consumption of extractable C may account for most of the observed decrease. The assumption of constant BDOC may still be reasonable in the field condition where top mineral soils receive a large amount of DOC from litter layer (e.g., Qualls and Haines, 1992; Yano, 1997).

The storage of soil might have affected the initial level of extractable C. My initial extraction was conducted after 7-month storage of moist soil at 5°C. Degradation of extractable C during this period is possible, which may explain the lower value of initially extracted C compared to the pilot extraction experiment (Table 2.2). Alternatively, the difference in extractable C between the pilot and initial extract (33 μgC g^{-1} soil) could be due to the seasonal change in extractable C, which ranged from 17 to 37 μgC g^{-1} soil across the season at the same site (Jandl and Sollins, 1998).

The extent to which extractable C becomes a major energy source for microbes can also be considered from the microbes' need for labile substrate. Assuming water-extractable C as a sole substrate for heterotrophic microbes, McGill *et al.* (1986) estimated that the extractable C needed to be replenished 26-39 times annually to support the measured size of microbial biomass in Alberta agricultural soils. Assuming that the active portion of microbial biomass C (2.1 mgC kg^{-1} soil, Fig. 1.6) is in steady-state and the microbial assimilation efficiency, the amount of C assimilated into new microbial biomass divided by the amount of total C utilized, is 0.4, microbes require 5.3 mgC kg^{-1} soil of labile C to maintain the active microbial biomass. Such substrate need is quite similar to the

biodegradable portion of extractable C (7.3 mgC kg^{-1} soil, Fig. 1.6), supporting the possibility that extractable C is a major energy source for soil microbes.

Roughly 1.1% of particulate C was destabilized and lost from the studied system on an annual basis (Fig. 2.5). Microbial respiration accounted for approximately 86 to 92% of the destabilized C and leaching loss constituted the remaining 8 to 14%. Due to its small size and low biodegradability, I concluded that leachable C in the studied soil wasn't a major energy source for soil microbes. Although it is unclear whether microbes gain energy directly from particulate C or from extractable C, my result and its comparison with respiration and biomass data didn't rule out the possibility that extractable C constitutes a major energy source for microbes. If microbial respiration is driven by microbes' need of labile extractable C, then extractable C pool must turn over very rapidly since the extractable C pool is relatively small at any point in time (Seto and Yanagiya, 1983; Cook and Allan, 1992 a; Hart *et al.*, 1997; my study).

Further study on the turnover rate of extractable C pool is necessary to determine whether extractable C can support soil microbial biomass and thus explains a significant portion of soil microbial respiration.

CHAPTER 3

PRODUCTION AND BIODEGRADABILITY OF DISSOLVED ORGANIC CARBON (DOC) IN FOREST SOILS OF CONTRASTING FERTILITY: CAN DOC SUPPORT MICROBES?

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To be submitted to Soil Biology and Biochemistry

Introduction

The amount of soil C available to heterotrophic microbes may influence the decomposition of stable SOM and, consequently, nutrient availability for plants and microbes. Thus, identification and operational separation of labile C from bulk SOM is of great interest. Labile C is only a fraction of total soil C because most soil C is recalcitrant and/or physically inaccessible to microbes. Density fractionation studies reveal that the heavy fraction associated with clay minerals holds the majority of soil C compared to the light fraction which is primary more recent plant-derived compounds (Strickland and Sollins, 1987). I hypothesized that the water-soluble portion of C in mineral soil accounts for a significant portion of available C to microbes. Two lines of findings in literature are consistent with this hypothesis: (1) solution incubation studies showed a high biodegradability of water-soluble C in soil (see the citations in Chapter 1), and (2) laboratory soil incubation studies showed a strong correlation between respired C and the amount of C extractable among a wide range of soils (see the citations in Chapter 1). The missing information between the two is whether soil is capable of regenerating labile DOC to support the microbial needs for energy over the long term.

The repeated leaching study in Chapter 2 ruled out the possibility that leachable C was the main energy source for microbes. Water-extractable C was, however, contained a relatively large amount of labile C and might be replenished quickly, suggesting its potential as a main energy source for heterotrophic microbes. The studied soil in Chapter 2 was, however, from one location and a larger generalization cannot be made.

Main objective of this study was to test the hypothesis that extractable C is the main energy source for microbes using five Douglas-fir forest soils from two sites of contrasting fertility. In addition, I attempted to study the relationship between dissolved N species and the degradation of DOC as well as to find important factors controlling DOC dynamics.

Materials and Methods

Soil Sample Source

Soil was sampled from a total of five stands from two sites. A contrast in soil fertility and forest productivity between the two sites, Wind River and Cascade Head, was well documented (Binkley *et al.* 1992 a, b; Hart *et al.*, 1994). Each site has a conifer stand dominated by Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) and a mixed stand, Douglas-fir and red alder (*Alnus rubra* Bong.). Cascade Head site also has a pure alder stand. Thus, there is also a large difference in soil fertility between the stands within each site due to N-fixation by red alder (e.g., Bormann and DeBell, 1981). The two sites, U.S. Forest Service Experimental Forests, are described in detail in Binkley *et al.* (1992 a).

The Wind River site is located in southwestern Washington (45°49'N latitude) on rolling terrain with slopes up to 60% at about 625-m elevation. Mean annual temperature is about 9 °C; precipitation averages 2500 mm yr^{-1}, about three-quarters falling as snow between November and March. The soil is an unnamed series of silty clay loam Andic Haplumbrepts, developed in Tertiary andesitic or rhyolitic parent materials with some pumice and basaltic gravel of Pleistocene origin (Miller and Tarrant, 1983). After successive wildfires (120,000-ha Yacolt burn) in 1902, 1922, and 1927, Douglas-fir seedlings were planted at a density of 1700
trees ha^{-1} in 1929. Two-yr.-old red alder seedlings were planted in 22-m wide strips at a density of 3000 trees ha^{-1} to provide a firebreak across the plantation in 1933. The site index (expected height of dominant tree species) for Douglas-fir without red alder is 25 m at 50 yr. The understory was notably denser at the Douglas-fir stand than the alder stand.

The Cascade Head site is near the coast of Oregon at approximately 180-m elevation and 45°03' N. The mean annual air temperature is 15 °C and freezing temperatures are uncommon. Precipitation, mostly rain, averages 2400 mm yr^{-1}. The soil is a well drained silty clay loam Typic Dystrandept of the Astoria series, developed on tuffaceous siltstone overlying basalt. The land was cleared for agriculture then abandoned in 1925. By 1935, an 8-yr-old mixed stand had naturally established with roughly 4500 conifer/ha, a mixture of Douglas-fir, western hemlock (*Tsuga heterophylla* (Raf.) Sarg.), and Sitka spruce *(Picea sitchensis* (Bong.) Carr.), and 3000 red alder ha^{-1}. Between 1935 and 1936, three plots were established. A 0.2-ha Douglas-fir stand was established by removing all alders and smaller conifers to achieve a density of about 2800 trees ha^{-1}. A 0.2-ha pure alder stand with a density of about 1800 trees ha^{-1} (2.4 x 2.4 m spacing) was created by removing all conifers and some alders. A 0.4-ha alder-conifer mixed stand was left unthinned as control. The three stands are aligned on a south west facing slope. The mixed stand lies on a relatively flat ridge top, the Douglas-fir stand is on shoulder (roughly 15% slope), and the alder stand on the toe slope. Notable differences in understory were found. False lily-of-the-valley (*Maianthemum dilitaum*) was abundant in the mixed stand. The conifer stand had less ground cover but dominated by Sword fern (*Polystichum munitum*). At the alder stand, death of matured alders caused partial canopy opening and a dense understory of Salmon berry established. Some bryophytes were present on the ground of all stands. The site index for Douglas-fir in the absence of alder is 40 m at 50 yr.

Soil Sampling

Soil was sampled on June 8, 1998 (Wind River) and July 8, 1998 (Cascade Head). Five mineral soil samples (0-10 cm) were taken from each stand (i.e. conifer, alder, mixed stand, at Cascade Head; conifer, alder stand, at Wind River) at random distances from points along a transect through the middle of each stand.

Soil samples were kept in polyethylene zip lock bags in a cooler, returned to the laboratory within 12 hours, and stored in a refrigerator (5 to 7 °C). Soils were sieved (5 mm) on June 14, 1998 (Wind River) and July 9, 1998 (Cascade Head) as described above for leaching column preparation, and refrigerated.

Initial Soil Water-Extraction and DOC Incubation

Soil water-extraction and DOC incubation were carried out as described above for the leaching study (Chapter 2). The water-extraction was conducted on June 18-19, 1998 (Wind River) and July 9-10, 1998 (Cascade Head). Filtrates were stored at 5 to 7 °C for a maximum 15 hours before inoculation. A common inoculum was used for the five extracts in each stand, prepared from a composite of the five soil samples. Inoculum was prepared by mixing 10 g of the composite soil with 50 ml of deionized water, then the mixture was shaken for 30 minutes, left at room temperature for several hours, and filtered through a 5-μm membrane as described in Chapter 2. Extracts were incubated for up to 95 days for Wind River extracts and 111 days for Cascade Head extracts. Samples for DOC and dissolved N were taken in the similar interval to the repeated leaching study (Chapter 2). All samples were filtered (0.2 μm) each time to remove particulate organic C (e.g., biomass C) from the incubating solution.

Soil Incubation

Soil water content was determined for all five soil samples from each stand as the weight difference between the field-moist soil and oven-dried soil (24 hours at 105 °C) after sieving. Another 25 to 37 g aliquot of moist soil was transferred from each Wind River sample to an incubation jar (473 mL) on July 1, 1998. Soil water content was adjusted to 0.46% (wt/wt) soil by air drying or addition of deionized water. High-vacuum silicone grease (Dow Corning) was applied on the top edge of each jar to ensure sealing. Immediately after the soil transfer and

moisture adjustment, the incubation container was sealed and placed in an incubator (25 °C). CO_2 concentration in the headspace of each container was measured every 4-6 days for two months. The headspace gas was mixed 8 to 10 times with a 20-ml syringe, then the headspace gas was sampled with a 0.5-mL syringe, and injected into a gas chromatograph equipped with a thermal conductivity detector (Model 5700A, Hewlett-Packard). After the CO_2 measurement, all jars were opened and flushed with ambient air. Water loss when the jars were open was accounted for by bringing the weight back to the weight before opening. During the two-month monitoring, the headspace CO_2 concentration never exceeded 4% by volume except five cases (Wind River, alder stand samples -- maximum 5.2%. The rate of CO_2 evolution (microbial respiration) was calculated from the increase in headspace CO_2 concentration during the two months.

Cascade Head soils were prepared for soil incubation on July 13, 1998 as described above except that the soil water content was not adjusted. Instead the field moisture level was used for the incubation because the significant difference in soil water content between stands would have created water logging for the WR soils which would have resulted in irrelevant comparison between the stands. Such difference in the soil water content is partly due to the abundance of aluminum-rich amorphous clay (organo-aluminum complex) at the Cascade Head site (Strickland *et al.*, 1988).

Final Soil Water-extraction and DOC Incubation

After the 2-month soil incubation, the soils in all incubation jars from Wind River and Cascade Head were extracted with deionized water as described above. Due to the small soil volume, volume of extracts was limited. Thus dissolved N samples were taken only at pre-inoculation stage. Dissolved organic C samples were taken immediately after inoculation and 0.3, 5.5, 16.7 and 36.5 days later for Wind River soils, and 5 and 19.5 days later for Cascade Head soils. Due to the

limited volume of extract, DOC incubation for the mixed stand, Cascade Head wasn't conducted successfully.

DOC Analysis and Biodegradable DOC Estimate

Dissolved organic C samples were kept frozen in scintillation vials until DOC analysis. The concentration of DOC was calculated as the difference between total C and inorganic C in extracts, as measured by platinum-catalyzed combustion/non-dispersive infrared gas analysis (TOC-5000A, Shimadzu Co.). The biodegradable portion of DOC (BDOC) was determined as the decrease in DOC between the pre-inoculation sample and the sample from the end of DOC incubation. Percent BDOC (%BDOC) was calculated as

$$BDOC / DOC_{\text{pre-inoculation}} \times 100$$

A few data from the DOC incubation showed an increase over time: theoretically DOC should not increase over the incubation time due to no C input. The higher values may have been caused by contamination during the 2-month incubation or by heterogeneity of the incubated solution: the solution was mixed only gently to reduce damage to microbial population. Analytical errors are also possible for low-concentration extracts incubated longer, which had DOC concentrations close to the detection limit of the Shimadzu TOC analyzer.

Dissolved Nitrogen Analysis

Ammonium and nitrate samples were thawed and analyzed with a Lachat autoanalyzer (QuikChem 4200, Lachat Instruments). Nitrate and ammonium standards were prepared using both deionized water and the artificial throughfall solution. The solution matrix had no effect on Lachat results, thus deionized water was used to make standards.

Persulfate digestion method (Koroleff, 1983; Qualls, 1989) was used to convert organic N and ammonium to nitrate, which was measured with a Lachat

analyzer (see above). The digestion reagent was made of 10 g low-N potassium peroxydisulfate ($K_2S_2O_8$) and 6 g boric acid, then was brought to 1000 ml in 0.075 M NaOH. Sample (10mL) and the digestion reagent (10mL) were pipetted into the test tube and weighed. The tubes were placed in a water bath (10cm deep) and autoclaved on liquid cycle (30 minutes, 120 °C). When cool and dry, the tubes were weighed to determine the evaporative moisture loss, which was accounted for after Lachat analysis. Although recovery of organic N in extracts might have been incomplete, the observed relative pattern in DON should be valid because all 25 samples were treated identically, thus the recovery rate should have been similar for all the samples.

Other Soil Analysis

Total soil C and N were determined with a Carlo-Erba CNS from each stand (n=5) after soils were air-dried, sieved (60-mesh), and ground. Soil pH was determined with a pH/ISE meter (Orion) from each stand (n=5) after mixing air-dried sieved soil with deionized water (soil:water = 1:10 in weight basis) for 60 minutes.

Statistical Analysis

A least-squares linear regression was used to compare the relationship between extractable C and the incubation time among stands and extraction dates after log transformation of both dependent variable (i.e., DOC) and independent variable (i.e., time). Although there is no biological basis for log transformation of the incubation time, it allowed a comparison of the slope of degradation curves among stands.

A protected Fisher's least square difference tests (SAS Institute, 1992) were used to compare the means among the five stands (alpha = 0.05 and critical value

of T = 2.09). For the variables that had high variability (i.e., more than one order of magnitude), data were log transformed for Fisher's test. Correlation analysis was used to examine relationships among the variables across all stands and within each site. I used the significant level of 0.0001 for the correlation analysis unless stated specifically.

The stands of alder, Douglas-fir, and the mixture of the two species at Cascade Head are abbreviated here as CH-alder, CH-df, and CH-mix. Likewise, the Douglas-fir stand and mixed stands at Wind River are called WR-df and WR-mix. The three stands in Cascade Head or the two in Wind River studied here may not be a good representation of entire Cascade Head and Wind River area, as no reconnaissance survey of stands in the region was one prior to stand selection. It is possible that the observed higher value for CH-mix, for example, is due to environmental factors specific to that particular location and that another mixed Douglas-fir/alder stand at Cascade Head would not have yielded a similar value. Thus I make no claim that any differences among the five stands studied here are caused by the differences in site characteristics between entire Cascade Head and Wind River area. It is possible to examine the effect of stand type by treating the location as block and by excluding the CH-alder (no replication of pure alder stand type). This study rather focuses on ranges of and correlations among the measured variables.

Unless noted, soil C and N variables are all expressed per gram oven-dried soil (e.g., μg C g^{-1} soil).

Results

Respiration

Microbial respiration rate was measured every 5 to 8 days for each soil sample. Respiration rate for WR soils slowed toward the end of the 2-month soil incubation more than CH soils did (Fig. 3.1). Daily respiration rate was calculated between each measurement, was expressed relative to the total soil C, and was defined as %respired C (Fig. 3.2). The soils at the WR stands consistently had higher %respired C than the CH soils during the incubation period. At both sites, the soils at Douglas-fir stand showed faster decline over the incubation period than did the mixed stand.

Extractable C and its degradation

Water-extractable C at the initial extraction time (initial DOC) and final extraction time (final DOC) was significantly higher in the mixed stand than in the Douglas-fir stand at both sites (Table 3.1). The mixed stand had a higher DOC and biodegradable DOC (BDOC) than did the pure alder stand at Cascade Head at both extraction times (Table 3.2). In contrast, the %BDOC was higher without alder for the final extraction. During the soil incubation period, DOC increased for the WR-mix soils and decreased for the CH-df and CH-alder soils, while the rest of the stands showed inconsistent trends (Table 3.1). To compare the changes in BDOC pool size, BDOC for the initial extraction time was calculated using the incubation period similar to that of the final extraction (Table 3.1). After the 2-month incubation, BDOC pool stayed relatively constant for all the stands except an increase for the WR-mix. The relative amount of BDOC (%BDOC) increased consistently for all the samples except the two samples from the CH-mix (Fig. 3.1). Although difficult to see from the DOC degradation curves (Fig. 3.3-3.6), the initial extracts had faster biodegradation (i.e. more negative slope) than did the final

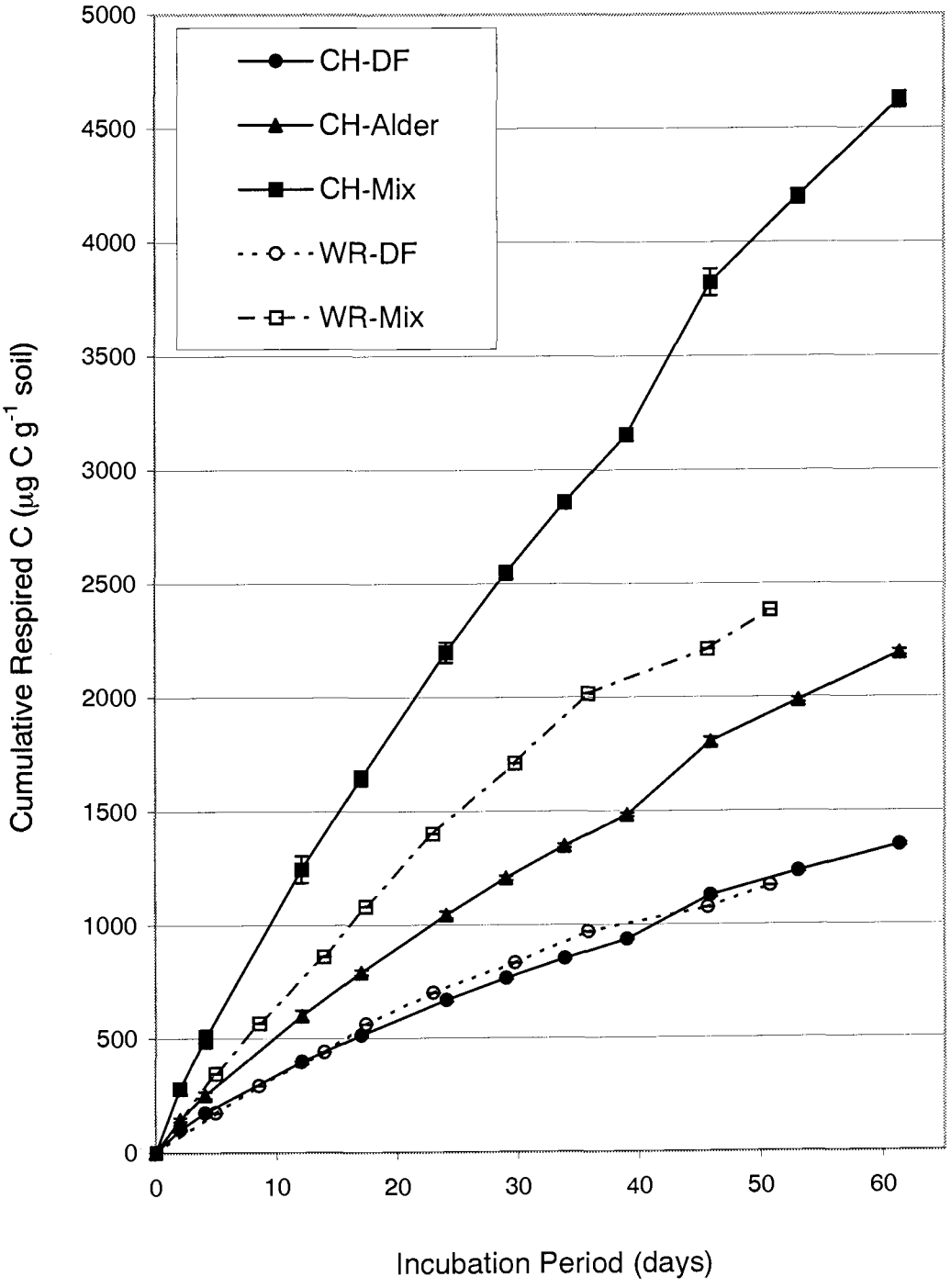

Fig. 3.1. Cumulative Carbon respired during the 2-month soil incubation (with standard error bars, n = 5).

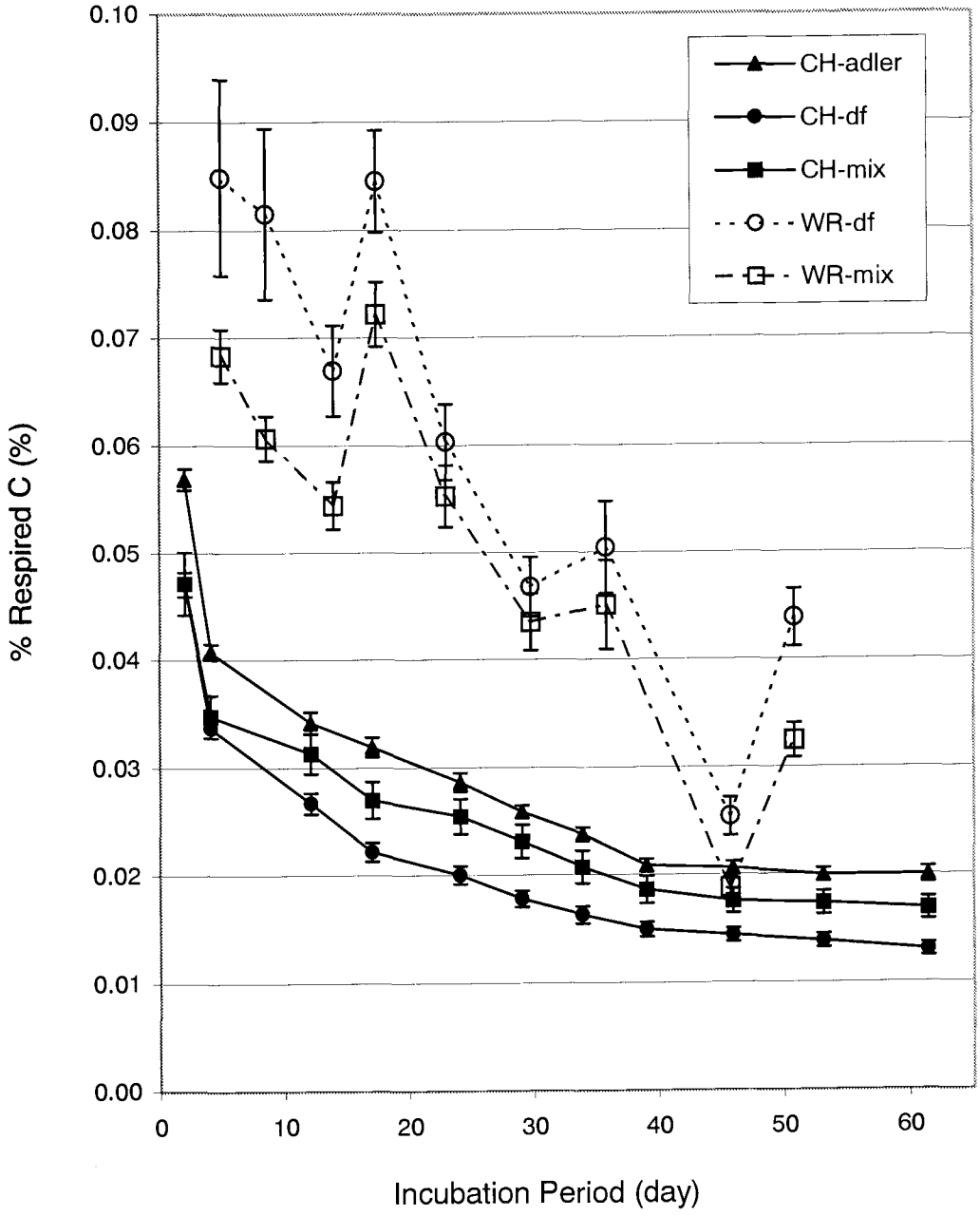

Fig. 3.2. Relative amount of respired C during the 2-month soil incubation. %Respired C = Daily respired C (mgC g^{-1} soil day^{-1}) / Total soil C (mgC g^{-1} soil) *100. The daily respired C was calculated from the accumulation of CO_2 in each incubation jar between the measurements (5-14 days interval). Error bar represents standard error (n = 5).

Table 3.1. Descriptive statistics of the results from extraction and degradation of DOC before and after the 2-month soil incubation. Significant difference statistics from Fisher's protected least significant difference test was indicated by letters (a, ab, b, etc.)

	stand									
soil source	WR-df		WR-mix		CH-df		CH-mix		CH-alder	
				Initial Extraction[a]						
*DOC ($\mu g\ C\ g^{-1}\ C$)	21.0 c	(4.7)	40.9 b	(10.4)	43.5 b	(2.0)	185.5 a	(27.5)	63.5 b	(11.7)
*BDOC ($\mu g\ C\ g^{-1}\ C$)	12.9 bc	(3.0)	13.3 bc	(3.5)	8.0 c	(0.8)	53.4 a	(8.9)	15.4 b	(4.2)
%BDOC	60.7 a	(1.6)	32.7 b	(3.7)	18.6 d	(2.0)	28.5 bc	(1.4)	24.9 c	(4.4)
r^2 [d]	0.81	(0.03)	0.63	(0.10)	0.68	(0.08)	0.90	(0.02)	0.86	(0.08)
Intercept [d]	2.8	(0.2)	3.4	(0.3)	3.7	(0.1)	5.1	(0.2)	4.0	(0.2)
k (10^{-3}) [d]	-8.6 c	(0.4)	-3.1 b	(0.5)	-1.8 a	(0.3)	-2.7 ab	(0.1)	-2.7 ab	(0.3)
				Final Extraction[b]						
*DOC ($\mu g\ C\ g^{-1}\ C$)	18.7 c	(2.5)	71.9 b	(27.7)	20.1 c	(2.5)	180.6 a	(42.5)	52.7 b	(9.3)
*BDOC ($\mu g\ C\ g^{-1}\ C$)	11.7 ab	(2.1)	24.2 a	(8.3)	5.3 c	(1.0)	19.2 a	(4.7)	8.7 bc	(1.8)
%BDOC	61.4 a	(4.4)	37.5 b	(3.2)	26.0 c	(1.6)	11.2 e	(1.3)	17.2 d	(2.7)
r^2 [d]	0.71	(0.04)	0.89	(0.05)	0.81	(0.08)	0.43	(0.08)	0.83	(0.04)
Intercept [d]	11.7	(2.1)	3.9	(0.4)	2.9	(0.1)	5.0	(0.3)	3.9	(0.2)
k (10^{-3}) [d]	-28.5 d	(2.4)	-21.6 c	(2.8)	-13.8 b	(0.8)	-6.5 a	(0.7)	-9.4 ab	(1.1)
				Changes between the two extraction[c]						
DOC ($\mu g\ C\ g^{-1}\ C$)	-2.3 ab	(6.0)	31.0 a	(17.8)	-23.4 b	(1.2)	-4.8 ab	(23.3)	-10.8 b	(4.0)
BDOC[e] ($\mu g\ C\ g^{-1}\ C$)	4.0 b	(4.0)	21.6 a	(7.7)	1.3 b	(1.3)	4.3 b	(4.1)	4.0 b	(1.7)
%BDOC[e]	27.1 ab	(8.0)	30.8 a	(4.2)	16.4 bc	(2.6)	4.1 c	(2.7)	8.8 b	(2.0)

[a] Initial extracts were incubated for 111.2 days.

[b] Final extracts were incubated for 16.7 days for Wind River soils and 19.5 days for Cascade Head soils.

[c] The changes in DOC, BDOC, and %BDOC were calculated as "Final amount - Initial amount".

[d] Biodegradation of DOC was regressed against the incubation time (day) after log-transformation of both DOC and Time (e.g., log DOC = k*log(Time) + Intercept)

[e] BDOC and %BDOC at the initial extraction was calculated using the DOC value incubated for 21.8 days for Wind River soils and 17.9 days for Cascade Head soils to mutch with the final extraction.

* DOC and BDOC values were log-transformed before Fisher's LSD test to meet the assumption of normality and homogeneity of variance.

Table 3.2. Descriptive statistics of selected soil characteristics and dissolved N. Significance statistics based on Fisher's protected least square difference test was indicated by letters. Standard errors are in prentices.

	stand				
	WR-df	WR-mix	CH-df	CH-mix	CH-alder
[α] total soil C (mg C g^{-1} soil)	40.7 (2.0) c	97.9 (21.5) b	105.6 (5.0) b	313.9 (57.6) a	127.0 (15.6) b
[α] total soil N (mg N g^{-1} soil)	1.34 (0.05) e	4.08 (0.80) d	6.26 (0.09) c	18.19 (2.95) a	9.42 (0.95) b
total soil C:N	30.5 (1.2) a	23.7 (1.9) b	16.9 (0.8) c	17.1 (0.5) c	13.4 (0.5) d
soil pH	5.13 (0.06) a	4.88 (0.07) b	4.73 (0.06) b	3.64 (0.08) c	3.75 (0.11) c
respired C[β] (μg C g^{-1} soil)	1299 (240) bc	2718 (759) b	1241 (126) c	4285 (714) a	2051 (246) bc
early respiration[χ] (μg C g^{-1} soil day^{-1})	35.2 (9.0) b	70.1 (18.6) b	49.7 (3.6) b	139.3 (20.4) a	72.0 (8.3) b
late respiration[δ] (μg C g^{-1} soil day^{-1})	18.1 (3.0) bc	33.5 (8.6) b	14.6 (1.7) d	52.4 (8.9) a	25.1 (3.0) bc
[α] initial NH_4^+ (μg N g^{-1} soil)	0.06 (0.04)	1.23 (0.68)	0.35 (0.11)	4.26 (2.38)	1.13 (0.19)
[α] initial NO_3^- (μg N g^{-1} soil)	0.11 (0.03)	1.69 (0.97)	1.08 (0.47)	24.46 (8.21)	26.08 (6.46)
[α] initial DON (μg N g^{-1} soil)	5.43 (0.09)	7.14 (0.70)	7.84 (0.09)	7.29 (1.21)	2.64 (1.09)
[α] DOC:DON	3.8 (0.8)	6.1 (1.24)	5.6 (0.3)	33.1 (12.8)	35.7 (21.2)

[α] The variables that were log-transformed for the Fisher's LSD test.

[β] Respired C is the total amount of CO_2-C respired during the 61.4-day soil incubation.

[χ] Early respiration is the daily respiration rate calculated from the first 2.0 days of the 2-month laboratory incubation of each soil sample (5 replicates per stand).

[δ] Late respiration is the daily respiration rate calculated from the last 7.2 days of the incubation.

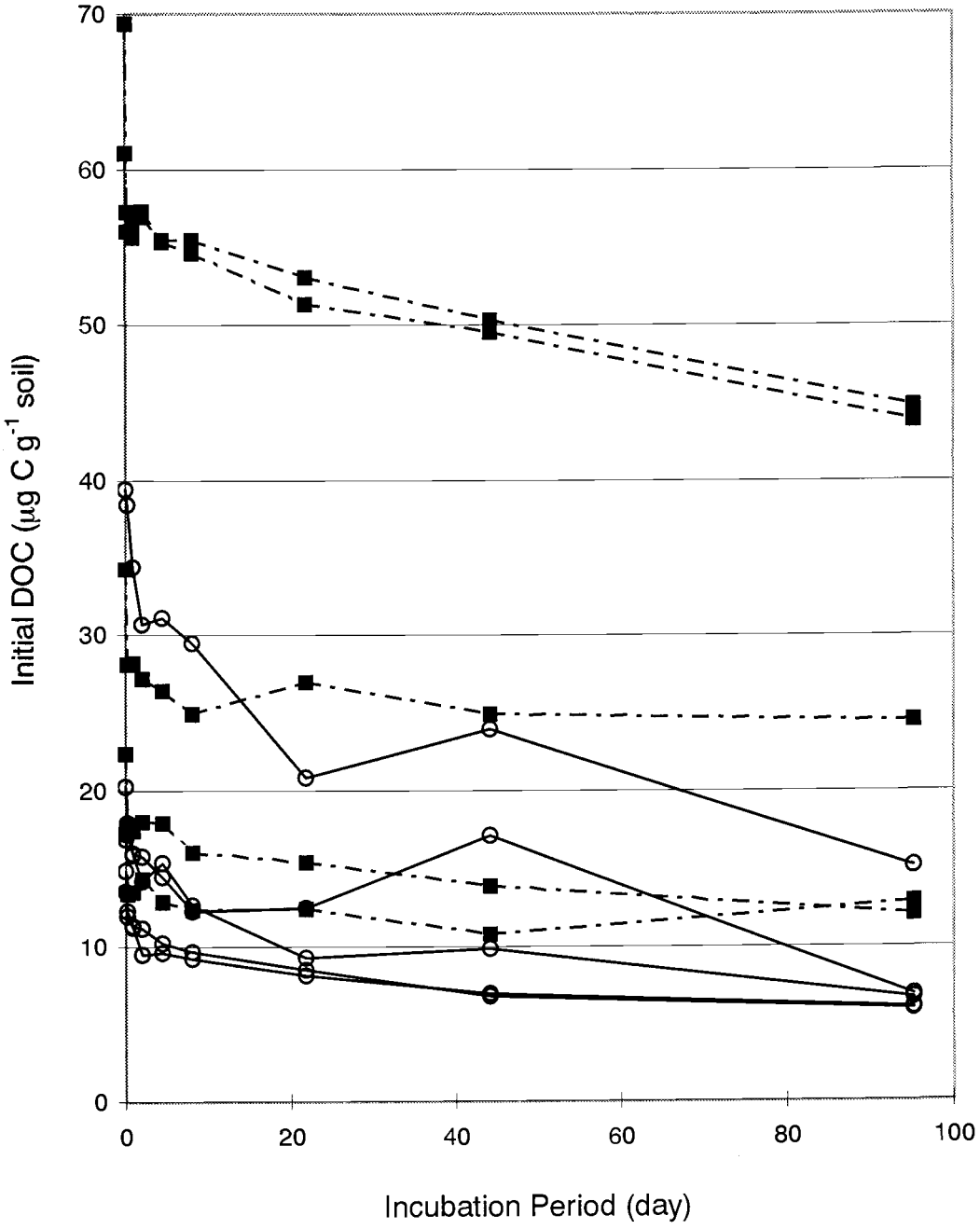

Fig. 3.3. DOC degradation curves for the initial extraction from Wind River soils. Five replications per stand. Square symbols are the mixed and circles are the Douglas-fir stands.

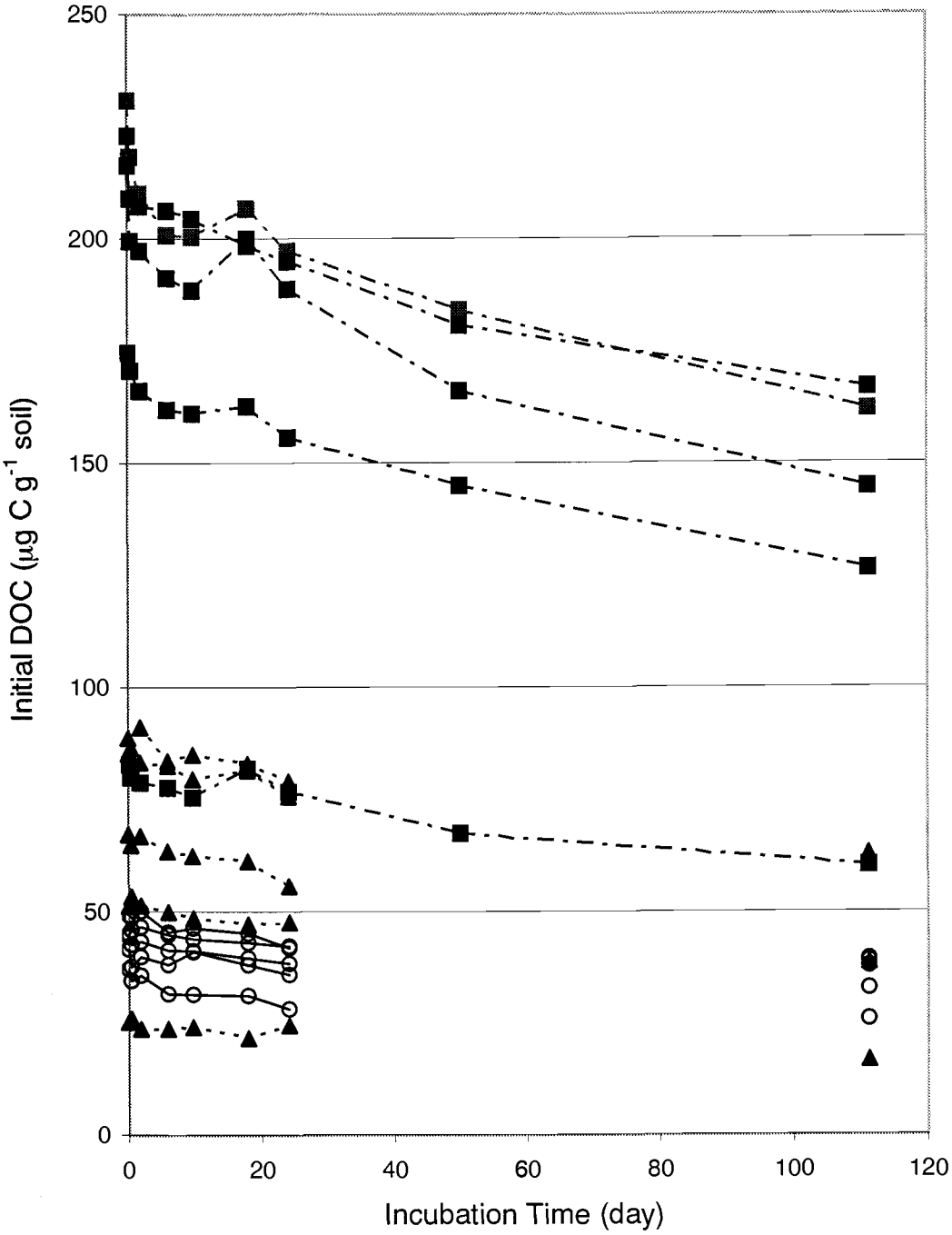

Fig. 3.4. DOC degradation curves for the initial extraction from Cascade Head soils. Five replications per stand. Square symbols are the mixed, circles are the Douglas-fir stand, and rectangles are the alder stand. Values at day 50 are missing for all the alder and Douglas-fir samples.

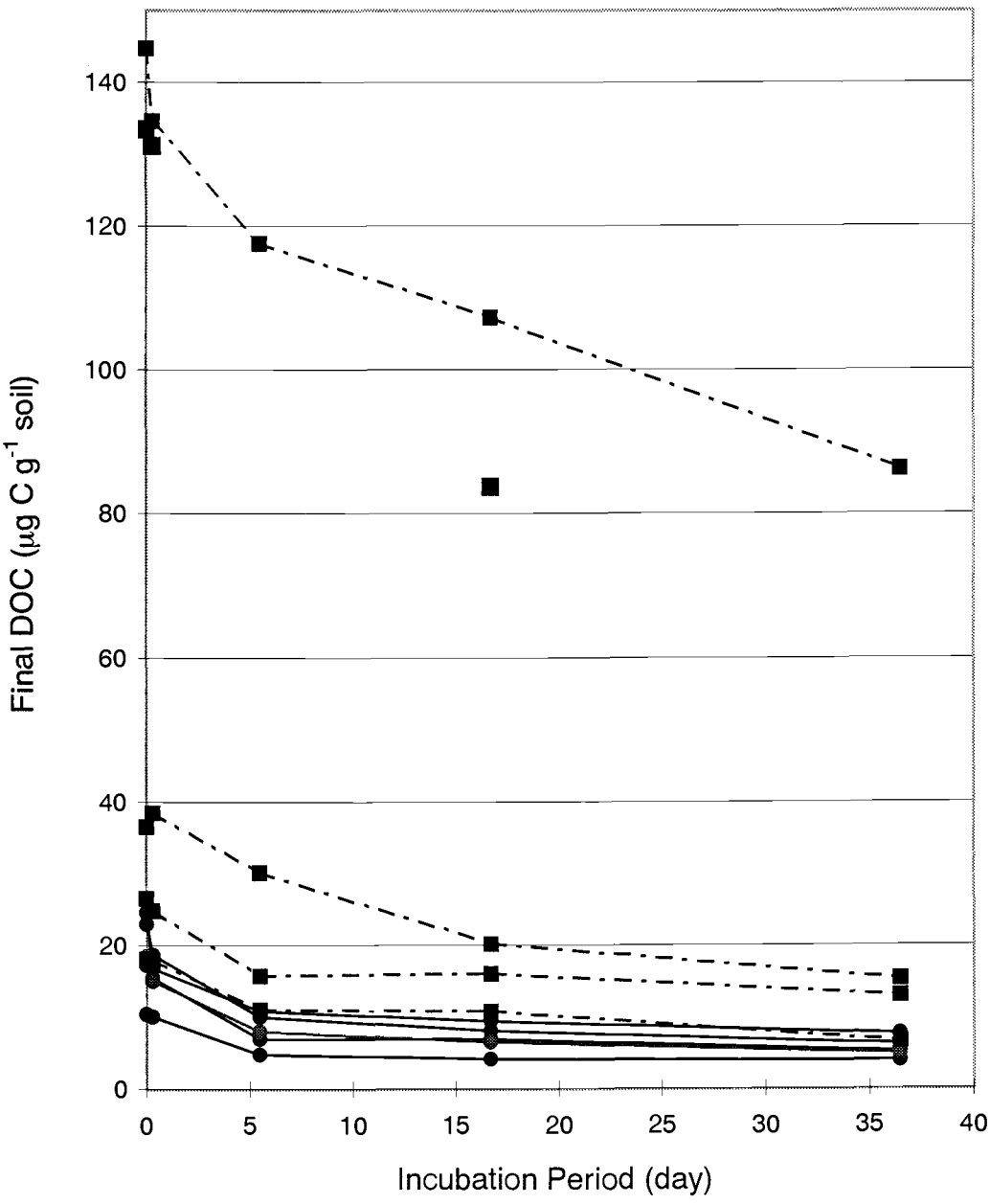

Fig. 3.5. DOC degradation curves for the final extraction from Wind River soils. Five replications per each stand. Square symbols are the mixed and circles are the Douglas-fir stand. The values at day 5.5 and 36.5 are missing for one sample from the mixed stand. DOC value at day 16.5 was used to estimate biodegradable DOC to make close comparison with the final extracts from Cascade Head (Fig. 3.6).

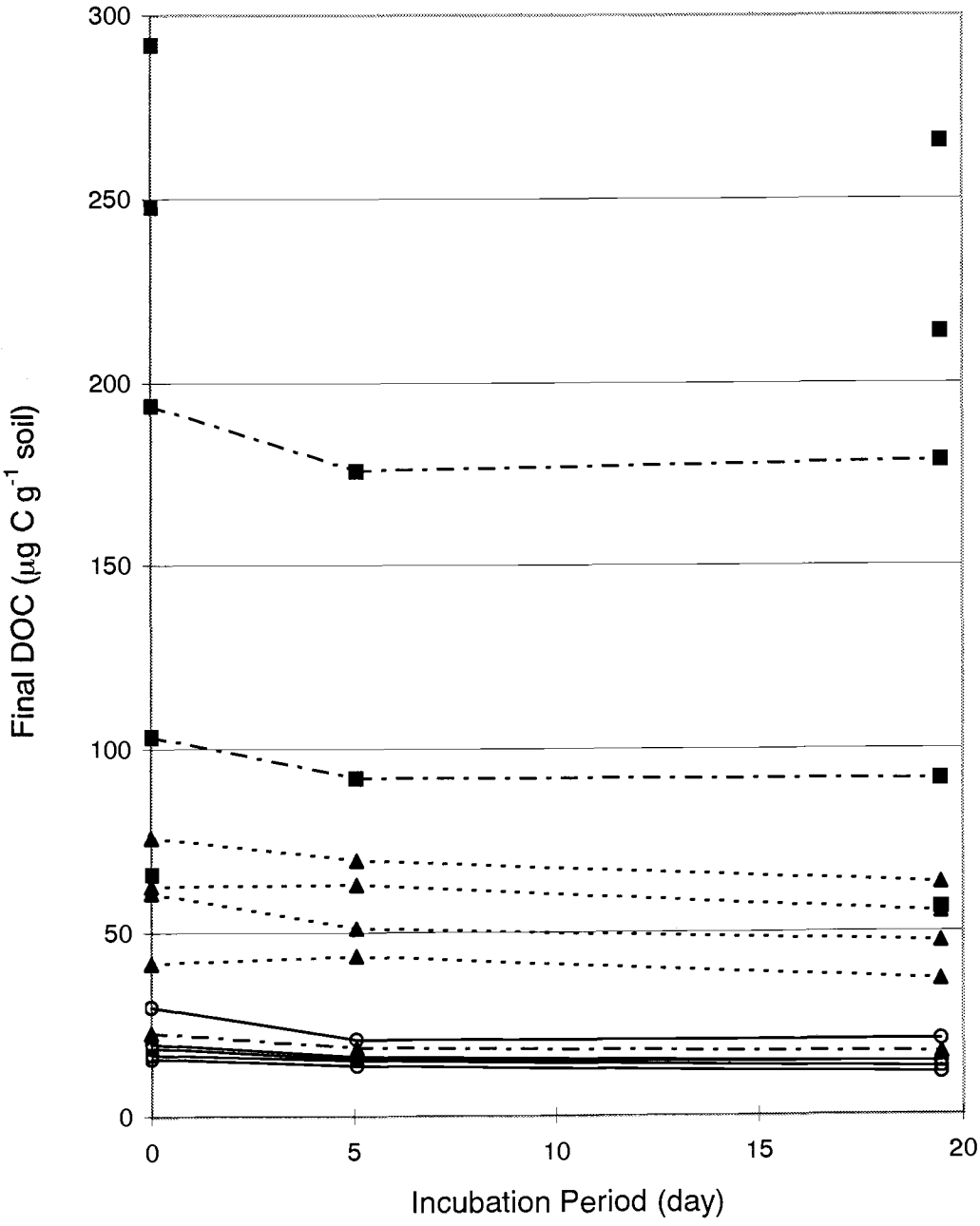

Fig. 3.6. DOC degradation curves for the final extraction from Cascade Head soils. Five replications of each stand. Square symbols are the mixed, circles are the Douglas-fir stand, and rectangles are the alder stand. Values at day 5 are missing for three out of five curves for the mixed stand.

extracts across all stands when a regression model was fit to each DOC degradation curve (Table 3.1). The degradation was faster (i.e., more negative slope) for the Douglas-fir stands than for the alder stands both sites at the final extraction time (Table 3.1).

Comparisons among the Five Stands

Soil characteristics were compared among the five stands and statistically significant differences were found for most variables (Table 3.2). Total soil N decreased significantly among all the stands in the following order: highest at CH-mix, then CH-alder, then CH-df, then WR-mix, and lowest at WR-df. A similarly clear pattern was found for total soil C except that WR-mix was as high as CH-alder and CH-df. A nearly opposite pattern was seen for soil C:N ratio and soil pH. Extractable ammonium at the initial extraction time (initial NH_4^+) also showed a trend similar to total C. At the initial extraction time, DOC:DON ratio was significantly higher for CH-alder and CH-mix than for the other stands.

When the same variables were expressed relative to the total amount of each element (e.g., μg C g^{-1} C and μg g^{-1} N), the patterns among the five stands changed for most variables (Table 3.3). Extractable C was the highest for CH-mix and lowest for WR-mix at the first extraction time. There was, however, no statistical difference in extractable C among stands at the final extraction time. Biodegradable DOC and %BDOC were higher for WR stands than CH stands especially at the final extraction time. For the same two variables, WR-df was higher than WR-mix. Such pattern was seen in initial DON as well as the total amount of CO_2 evolved during the 2-month incubation.

Table 3.3. Descriptive statistics of selected soil characteristics and dissolved N expressed relative to the total amount of each element. Significance statistics based on Fisher's protected least square difference test was indicated by letters. Standard errors are in prentices.

	stand				
	WR-df	WR-mix	CH-df	CH-mix	CH-alder
respired C ($\mu g\ C\ g^{-1}\ C$)	31.39 (4.47) a	26.16 (2.70) a	11.74 (0.92) b	14.21 (1.79) b	16.22 (0.84) b
[α] initial NH_4^+ ($\mu g\ N\ g^{-1}\ N$)	0.04 (0.03)	0.24 (0.12)	0.06 (0.02)	0.20 (0.10)	0.12 (0.02)
[α] initial NO_3^- ($\mu g\ N\ g^{-1}\ N$)	0.09 (0.02)	0.30 (0.14)	0.18 (0.08)	1.25 (0.29)	2.68 (0.57)
[α] initial DON ($\mu g\ N\ g^{-1}\ N$)	4.08 (0.11)	1.82 (0.27)	1.25 (0.02)	0.46 (0.12)	0.41 (0.16)
initial DOC ($\mu g\ C\ g^{-1}\ C$)	0.50 (0.09) ab	0.40 (0.03) b	0.41 (0.01) b	0.61 (0.06) a	0.49 (0.06) ab
[α] initial DOC:DON	0.12 (0.02)	0.25 (0.06)	0.33 (0.01)	1.99 (0.83)	2.10 (1.28)
initial BDOC ($\mu g\ C\ g^{-1}\ C$)	0.31 (0.06) a	0.14 (0.01) bc	0.08 (0.01) c	0.18 (0.02) b	0.13 (0.02) bc
initial %BDOC (%)	60.71 (1.63) a	35.13 (3.25) b	18.55 (1.98) d	28.53 (1.37) bc	26.71 (2.79) c
final DOC ($\mu g\ C\ g^{-1}\ C$)	0.47 (0.08) a	0.63 (0.14) a	0.19 (0.02) a	0.57 (0.10) a	0.41 (0.05) a
final BDOC ($\mu g\ C\ g^{-1}\ C$)	0.30 (0.06) a	0.22 (0.03) a	0.05 (0.01) b	0.07 (0.01) b	0.07 (0.01) b
final %BDOC (%)	61.44 (4.39) a	37.50 (3.15) b	17.22 (2.72) c	11.45 (1.08) c	17.22 (2.72) c

[α] The variables that were log-transformed for the Fisher's LSD test.

Correlation Analysis

Overall relationships

Considerable variation was present within each stand for most of the measured variables. Thus, correlation analyses were conducted using all the individual soil samples (n = 25) to assess the overall relationships among the measured variables across all the stands. Correlation was examined in two ways, per gram soil and per gram C and N (Table 3.4). As expected, total soil C and N had a strong linear relationship (r^2 = 0.94). Total C and N correlated better with initial DOC (r^2 = 0.89 for both) than with final DOC (r^2 = 0.73 and 0.68). The variation in the amount of C respired during the 2-month incubation (respired C) was explained more by final DOC and initial DOC (r^2 = 0.92 and 0.71) than by soil C and N (r^2 = 0.60 and 0.53), suggesting that water-extractable C pools are useful measures of C availability to soil microbes. The CO_2 evolution rates during the early and late part of the 2-month soil incubation were calculated from the first 2 days and last 7.2 days of the respiration measurements, respectively. Initial BDOC correlated better with the early respiration rate (r^2 = 0.83) than with the total C respired (r^2 = 0.76). Similarly, final BDOC correlated slightly better with the late respiration rate (r^2 = 0.60) than with the total C respired (r^2 = 0.59).

Initial NH_4^+ was moderately correlated with the recalcitrant portion of DOC (RDOC ≡ DOC - BDOC) at the final extraction time and with respired C (r^2 = 0.67 and 0.52). Initial NO_3^- showed a strong correlation with DOC:DON ratio at the initial extraction time (r^2 = 0.78) and a moderate correlation with soil pH and soil N (r^2 = 0.67 and 0.58).

When the above variables were expressed relative to the total amount of each element, correlation patterns changed dramatically (Table 3.4). No variables were significantly correlated with initial or final DOC. The variation in respired C was explained most by initial BDOC, C:N ratio, and initial %BDOC (r^2 = 0.72, 0.66, and 0.66). Carbon to nitrogen ratio also showed a significant correlation with

Table 3.4. Selected significant correlations among soil variables across all five stands. Best five predictors of each dependent variable, with r^2 of more than 0.5, are shown. Obvious high correlations (e.g., DOC and BDOC from the same extraction) were excluded from the list. Initial and final DOC represent DOC at the initial and final extraction time.

Dependent Variable	explanatory variable / r^2 [a]				
respired C (mg C g^{-1} soil)	final DOC 0.92	final RDOC 0.88	initial BDOC 0.76	initial DOC 0.71	initial RDOC 0.67
initial DOC (mg C g^{-1} soil)	total C 0.88	total N 0.88	final RDOC 0.87	final DOC 0.81	respired C 0.71
final DOC (mg C g^{-1} soil)	respired C 0.92	initial BDOC 0.83	initial DOC 0.81	initial RDOC 0.77	total C 0.71
initial BDOC (mg C g^{-1} soil)	final RDOC 0.87	early resp. [b] 0.83	initial RDOC 0.83	final resp.[c] 0.78	respired C 0.76
final BDOC (mg C g^{-1} soil)	delta-BDOC [d] 0.7	delta-DOC [e] 0.64	final resp.[c] 0.60	respired C 0.59	
initial %BDOC [f] (%)	initial k [g] 0.94	total C:N 0.66	final %BDOC [f] 0.63		
final %BDOC [f] (%)	initial k [g] 0.92	total C:N 0.72	soil pH 0.65	initial %BDOC 0.63	initial slope 0.61
initial ammonium (mg N g^{-1} soil)	final RDOC 0.67	final DOC 0.64	early resp. 0.55	late resp. 0.55	respired C 0.52
initial nitrate (mg N g^{-1} soil)	initial DOC:DON 0.78	soil pH 0.67	total N 0.58		
respired C (mg C g^{-1} C)	initial BDOC 0.61	total C:N 0.53	initial %BDOC [f] 0.52		
initial %BDOC (%)	initial DON 0.75	late resp. 0.67	total C:N 0.66	final %BDOC [f] 0.63	respired C 0.52
final %BDOC (%)	initial DON 0.92	total C:N 0.72	soil pH 0.65	initial RDOC 0.65	initial %BDOC [f] 0.63
initial nitrate (mg C g^{-1} N)	initial DOC:DON 0.70	soil pH 0.68			
initial DON (mg C g^{-1} N)	final %BDOC 0.92	total C:N 0.78	initial %BDOC 0.75	soil pH 0.68	late resp. 0.58

[a] Variables with obvious high correlations (e.g., DOC, BDOC, and RDOC at the same extraction) were excluded.

[b] Early resp. is the daily respiration rate calculated from the first 2.0-day of the soil incubation of each soil samples.

[c] Late resp. is the daily respiration rate calculated from the last 7.2 days of the soil incubation period.

[d] delta-BDOC = final BDOC - initial BDOC

[e] delta-DOC = final DOC - initial DOC

[f] Initial %BDOC = (initial BDOC / initial DOC)* 100, and the same calculation for the final %BDOC.

[g] initial k is the slope of regression line fitted to each DOC degradation curves after log- transformation of both DOC and incubation time (see Table 3.1).

final %BDOC, initial %BDOC, and final BDOC (r^2 = 0.61, 0.53, and 0.52). Initial extractable NH_4^+ didn't correlate with any of these variables. Initial NO_3^- pool was strongly correlated with initial DOC:DON and soil pH (r^2 = 0.70 and 0.68, respectively). In contrast to the lack of significant correlation with any variables in per weight soil basis, initial DON was strongly correlated with several variables including final %BDOC, total C:N, initial %BDOC, and soil pH (r^2 = 0.92, 0.78, 0.75, and 0.68).

Site specific relationships

A stronger correlation was found between respired C and extractable C pools within each site than across sites (Fig. 3.7). For CH soils, the variation in respired C was most explained by final DOC, final RDOC, initial BDOC, initial DOC, and initial RDOC (r^2 = 0.98, 0.97, 0.95, 0.92, and 0.89), the same variables that correlated most with respired C in the cross-site analysis (Table 3.4). In contrast, for WR soils, the respiration was best explained by initial DOC, initial RDOC, final DOC, final RDOC, and total soil C (r^2 = 0.97, 0.96, 0.90, 0.90, and 0.90).

Initial DOC:DON ratio was strongly correlated with initial DOC, respired C, and initial RDOC at WR (r^2 = 0.92, 0.89, and 0.81), while only initial NO_3^- had a significant correlation for CH site. Initial DON had a high correlation with total C, initial NH_4^+, and initial DOC (r^2 = 0.92, 0.90, and 0.85) only for WR.

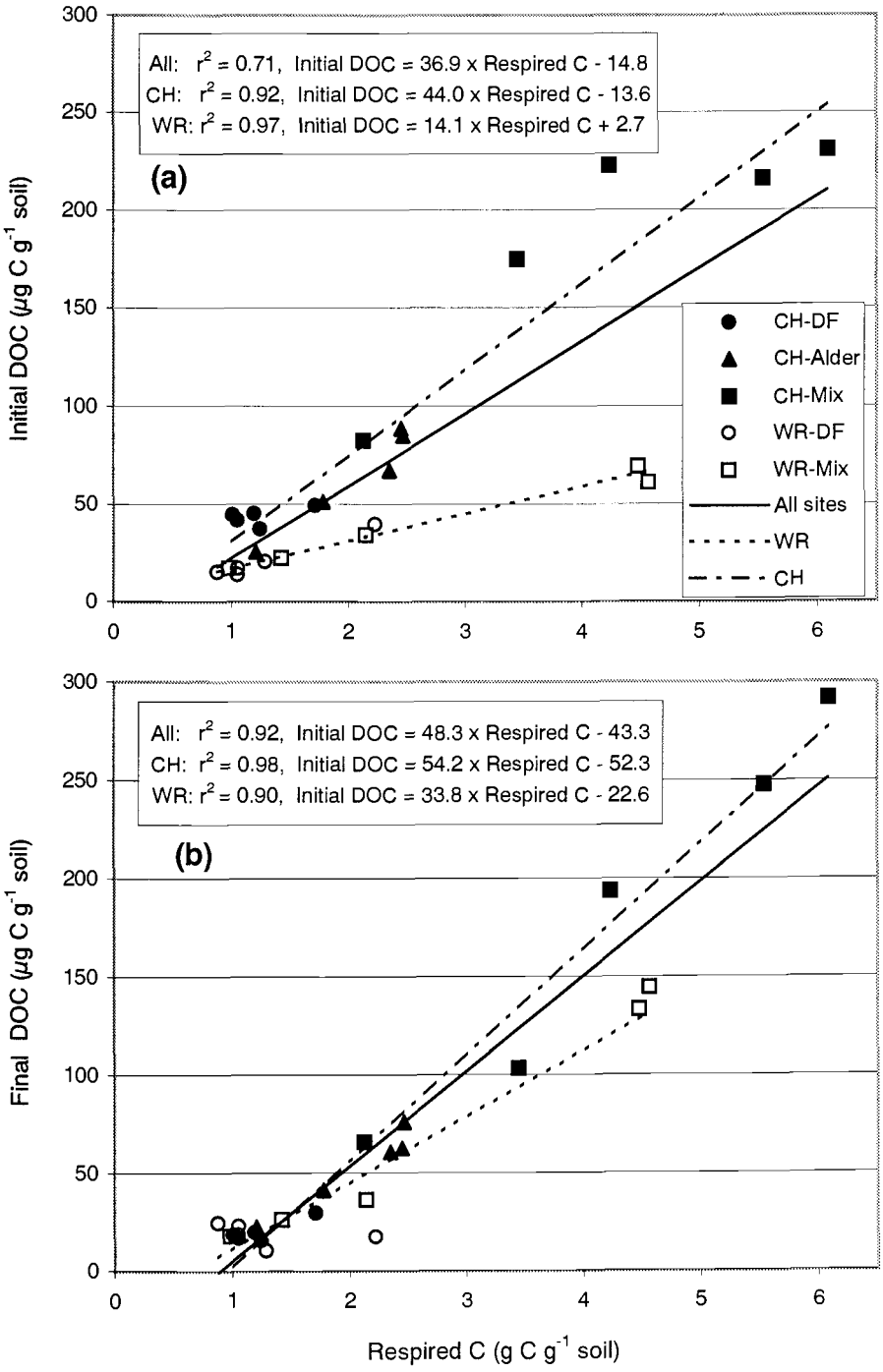

Fig. 3.7. Regression of DOC for the initial extracts against respired C (a) and for the final extracts against respired C (b). "All" represents all five stands including both sites. "CH" represents all three stands at Cascade Head site. "WR" represents all two stands at Wind River site.

Discussion

Relationship between Extractable C and Respired C

Water-extractable C pools were stronger predictors of the respired C (r^2 = 0.71-0.92) than total soil C or N (r^2 = 0.60 and 0.53; Table 3.4), suggesting that the extractable C pool is more closely linked with microbial metabolic activity and a better predictor of the mineralizable C than either total soil C or N. Soil C extracted soon after the field sampling (initial DOC) should best reflects the field condition. In contrast, DOC extracted after the 2-month incubation (final DOC) should reflect the environmental conditions during the laboratory incubation. Thus it is reasonable that the mineralizable C pool was predicted better by final DOC (r^2 = 0.92) than by initial DOC (r^2 = 0.71; Table 3.4). For WR, however, the available C correlated slightly better with initial DOC (r^2 = 0.97) than final DOC (r^2 = 0.90). CH soils showed the same relationship as seen across all sites.

When extractable C pools were regressed against respired C within each site, the correlation improved (Fig. 3.7). The slope of the regression line (i.e., ratio of extractable C to respired C) generally increased from initial to final extraction and the increase was more than 2-fold for WR soils. This was mainly due to the net increase in extractable C pool during the 2-month soil incubation. A consistent net increase was, however, found only for WR-mix (Table 3.1). The difference in slope between the two sites became smaller after the incubation, resulting in a stronger overall correlation of the respired C with final DOC than with initial DOC (Fig. 3.7). These results suggest that environmental differences between the two sites caused a unique slope for each site at the initial extraction time(Fig. 3.7.a) and such difference became smaller after the 2-month incubation under the same controlled environment (Fig. 3.7.b) and, thus final DOC predicted the respired C better than did initial DOC across all stands. It is unclear what factors caused the increase in extractable C for WR-mix and the decrease for CH-df and CH-alder during the 2-month lab incubation (Table 3.1). The changes in DOC between the

two extraction times had a significant positive correlation with final DOC and initial NO_3^- for WR soils (r^2 = 0.93 and 0.86) but not for CH soils.

Factors Controlling Biodegradability of DOC

At both initial and final extraction times, the correlation between DOC and BDOC of the extracts was quite strong for each stand despite the larger range in values (up to 6-fold in DOC for WR-mix; Fig. 3.8), resulting in low variation in %BDOC (Table 2.1). Compared to the initial extraction time, the slope (i.e., %BDOC) significantly decreased for the two most fertile stands (CH-mix and CH-alder) at the final extraction time (Fig. 3.8). For example, the % BDOC was significantly correlated with soil C:N ratio at the initial (r^2 = 0.66; Table 3.4) and final extraction time (r^2 = 0.72; Fig. 3.9) across all stands. These significant correlations across stands, however, require careful interpretation because there was little overlap in both soil C:N and Final %BDOC values among the five stands (Fig. 3.9). The correlation pattern within stands and sites didn't follow the one found across sites. Thus it is possible that other factors controlling both final %BDOC and soil C:N ratio, resulting in a clustering of both variables among the five stands. The results still imply that soil C:N ratio can be a major factor influencing the overall availability of DOC for microbes across stands, though it was not able to explain the variation in biodegradability within each site or stand.

Final %BDOC correlated significantly with the initial DON (expressed in μgN g^{-1} N) across all stands (r^2 = 0.92; Fig. 3.10). This same DON (μgN g^{-1} N) was again highly correlated with soil C:N ratio (r^2 = 0.78). Thus, as soil total N decreased relative to soil C in a stand, more of the soil N was extractable as DON, and more of the DOC in the extracts was biodegradable. Although a causal relationship cannot be tested by a correlation analysis, it is reasonable to speculate that soil microbes had to degrade more dissolved organic compounds (i.e., DOC) in

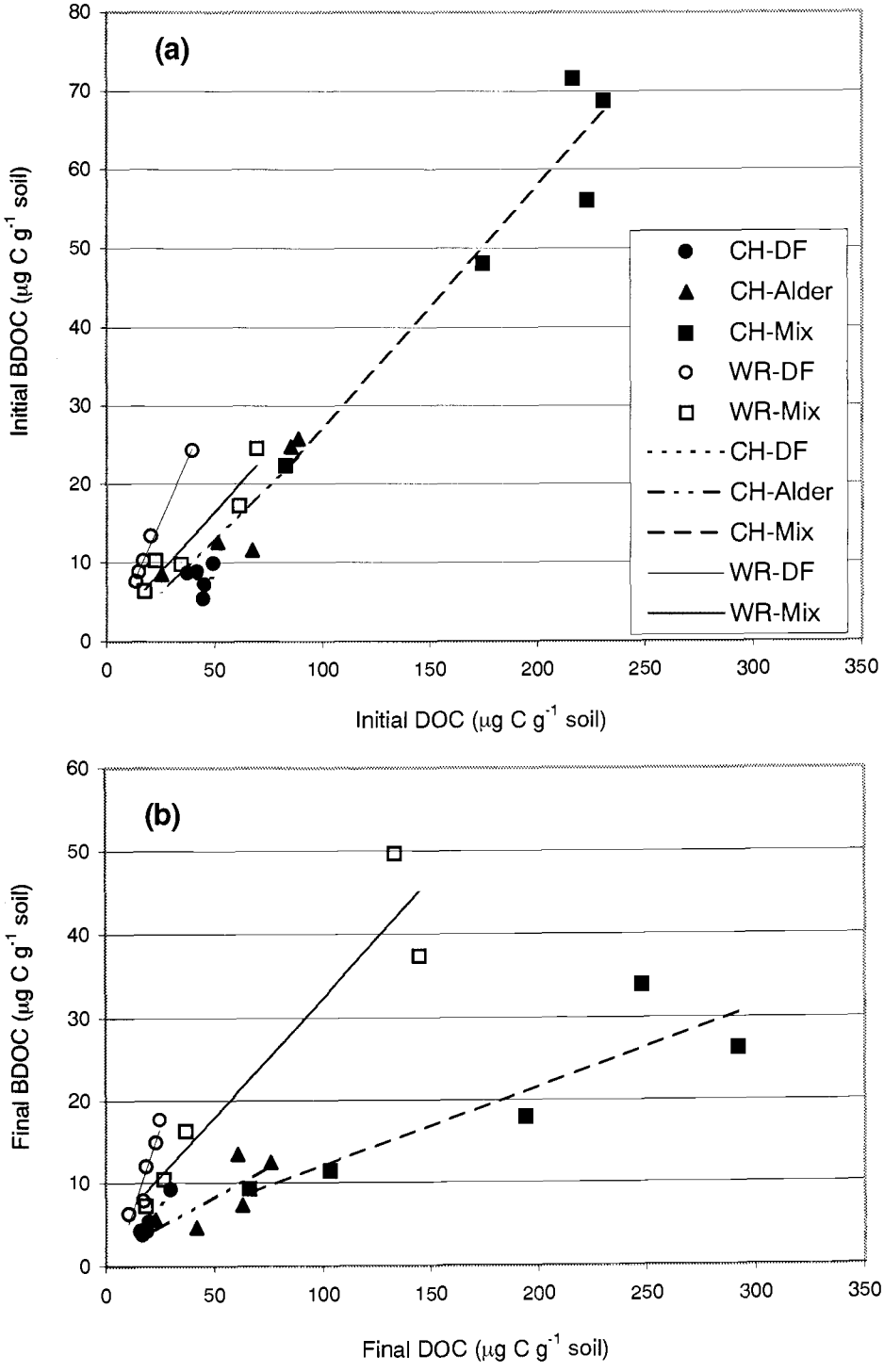

Fig. 3.8. Correlation between DOC and BDOC at the initial extraction time (a) and final extraction time (b). The regression lines were fitted to the five samples from each stand.

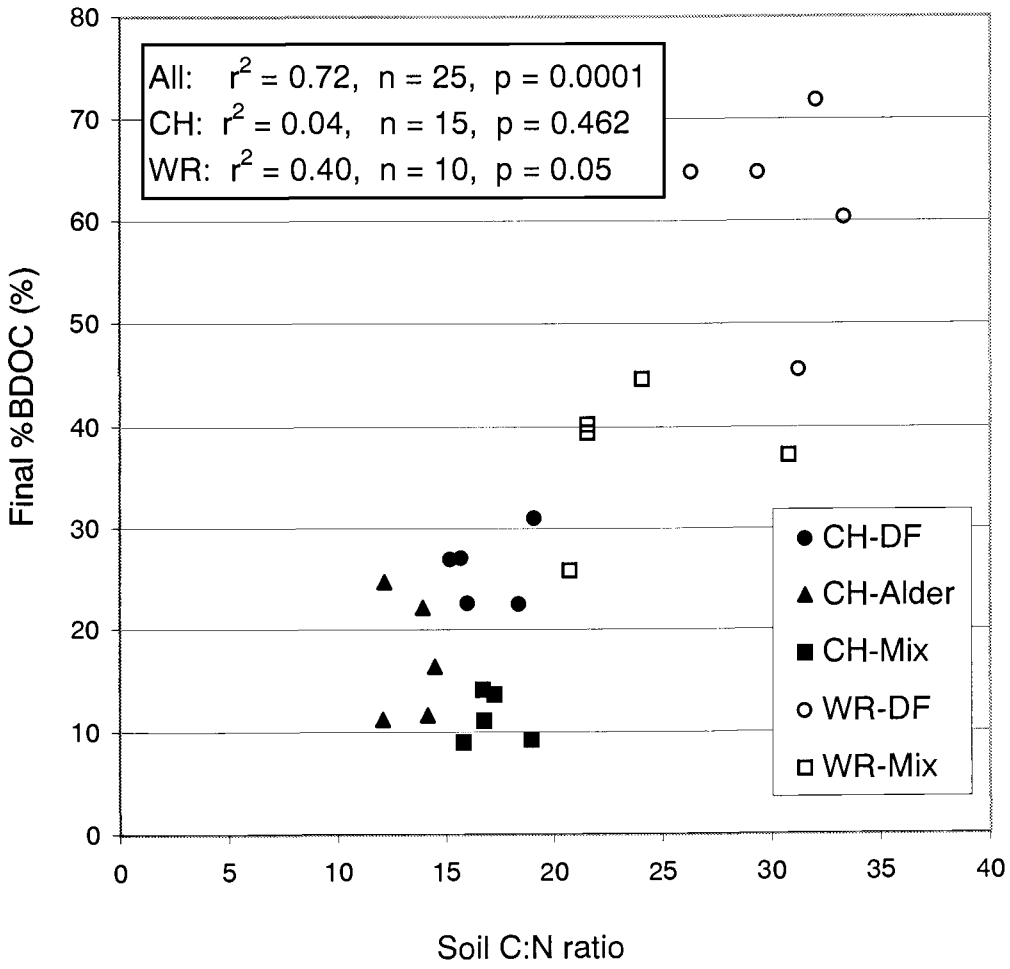

Fig. 3.9. Correlation between %BDOC at the final extraction time and C:N ratio of the bulk soil.

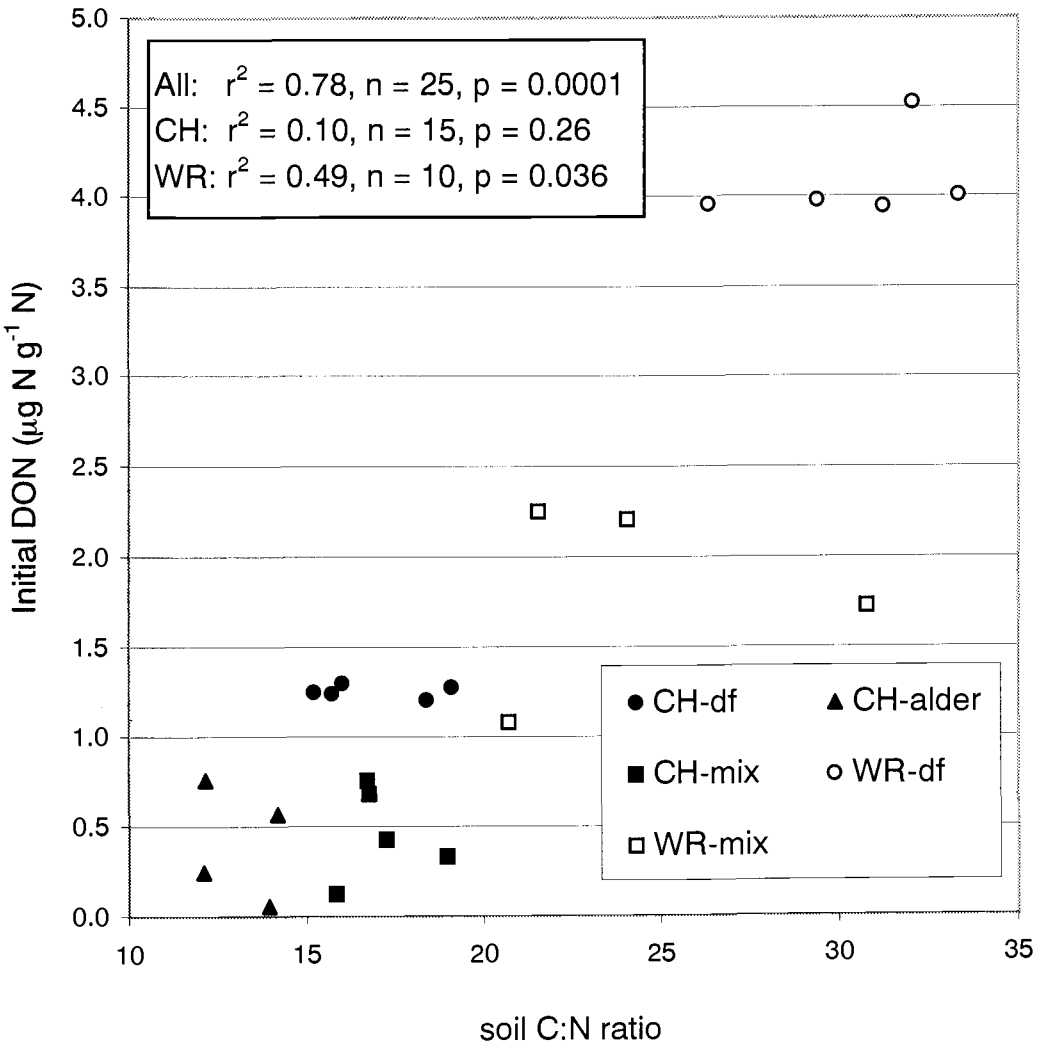

Fig. 3.10. Correlation between dissolved organic N at the initial extraction time and C:N ratio of bulk soil. The concentration of DON was expressed as a percent of the total N in soil.

order to gain enough N from the soil poor in N relative to C. Such a hypothesis could be tested in future research by an incubation experiment using extracts from the five stands with addition of inorganic N or labile organic N. Zsolnay and Steindl (1991) found a relatively constant %BDOC (85%) from the top mineral soil to 1 m depth in an agricultural loess soil where a large fertility gradient is presumably present across the depth.

It is difficult to determine whether the higher %BDOC in low fertility soils was due to dissolution of more labile C from soil or by a better ability of the inoculated microbial communities to degrade the DOC. In other words, the higher %BDOC could result from microbial activity either before the extraction or after the extraction (i.e., during the DOC incubation). Because the extracts were inoculated with native soils from each stand, these two possible causes could not be separated.

A high correlation between initial DON and respired C was expected because dissolved organic compounds in extracts contain both C and N and DOC had a strong correlation with the available C. There was, however, no correlation between initial DON and respired C across stands ($r^2 = 0.01$). Significant positive correlation was found for WR soils ($r^2 = 0.81$) and among the five samples for CH-df ($r^2 = 0.53$). The most fertile soils, the alder and mixed stand for CH, showed a weak negative correlation between initial DON and respired C. These results are consistent with the view that microbes gain more N by degrading DOC in extracts from an N poor site than from an N rich site. Thus, soil microbial activity in CH-mix and CH-alder soils are not likely to be N limited. The soil C:N ratio was lower for CH-alder than CH-df and CH-mix, while DOC:DON ratio at the initial extraction time was lower for CH-df than CH-alder and CH-mix (Table 3.2). It is unclear why the extracts enriched with organic N (i.e., all stands except CH-mix and CH-alder) had a strong positive correlation with the respired C. The extracts from the CH-mix and CH-alder soils may contain large amounts of recalcitrant DON.

N Transformation with respect to C Dynamics

Hart *et al.* (1997) measured respired C (over 10-day incubation) in relation to various N transformation rates and microbial biomass by a laboratory incubation of soils from the same five stands as the ones in my study. In their study, the best predictors of respired C were microbial biomass C, biomass N, gross N immobilization, and gross N mineralization (r^2 = 0.37, 0.28, 0.22, and 0.16, all at 0.01 significance level). Thus, the water-extractable C pools measured in my study were much better predictors of available C than the variables measured by Hart *et al.* (1997), suggesting that extractable C pool was more closely associated with microbial respiration than any N transformation processes. In my study, soil C was slightly more correlated with the available C than soil N (r^2 = 0.60 and 0.53). Furthermore, the variation in the available C was explained more by extractable C pools than extractable N pools (Table 3.4). These results suggest that microbial activity is controlled more by the demand for C than for N, at least in the soil systems I studied.

Reinertsen *et al.* (1984) conducted a month-long laboratory incubation of wheat straw, with and without removing extractable C beforehand, and monitored respired C and total extractable N. They suggested that net N immobilization was dependent on the available C that consists of both the initially extractable C and the non-extractable C. Chantigny *et al.* (1999) suggested that N availability for microbes may influence the amount of extractable C. They found a unique logarithmic relationship between DOC and dissolved inorganic N for the two soils with different level of inorganic N fertilization. In contrary, Zsolnay and Gorlitz (1994) reported that the amount of water-extractable C was not affected by inorganic N fertilizer but had a strong positive correlation with manure N application level among three agricultural fields.

DOC as a Labile Substrate or Left-over Recalcitrant Compounds?

Extractable C contains organic compounds of different origins and availability to soil microbes. The DOC incubation experiments showed a wide range of degradability (19 to 61 %BDOC) among the five stands (Table 3.2). Extractable C can be separated into two biologically distinct pools, BDOC and recalcitrant DOC (RDOC). At final extraction time, the variation in respired C was better explained by RDOC (r^2 = 0.88) than by BDOC (r^2 = 0.59) across all stands. A similar pattern was seen for each site separately. This implies that extractable C at the final extraction time was not a major labile substrate for microbial respiration but was rather by-products (or end-products) of microbial activities.

The microbial respiration rate (i.e., the slope in Fig. 3.1) and the amount of respired C relative to the total C present in soil declined over the 2-month incubation period (Fig. 3.2). Such decline may be due to the increasing dominance of RDOC in extractable pool. Regardless of lability, I expected to see a decrease in extractable C pool corresponding the decline in the microbial respiration. Microbial respiration rate indicates the magnitude of heterotrophic microbial activity. Thus, as the microbial activity decreases, smaller amount of soil C is expected to be destabilized and become extractable. On contrary to above view, the decrease in DOC was not seen for most of the soils except for CH-df and CH-alder (Table 3.1). A clear increase in DOC was seen for WR-mix soil. Furthermore, the BDOC pool increased over the 2 months for all five stands. Possibly, persistent extracellular enzyme activity continued to depolymerize the particulate C while the declining microbial population had less need for substrate, resulting in more BDOC extractable.

Similar decline in the respiration rate during a soil incubation is typical and is often attributed to the decrease in labile C in soil over the incubation period (Reinertsen *et al.*, 1984; Davidson *et al.*, 1987; Allan and Cook, 1992 a; Hart *et al.*, 1994). Such decline could have been due to either lower production rate of the available C or faster consumption of the available C under the laboratory

incubation environment compared to a steady-state field system. In a 456-day laboratory incubation of old-growth Douglas-fir soil, Hart *et al.* (1994) suggested that the decline in microbial respiration rate was due to a decrease in the quality and size of available C and N pools. They considered K_2SO_4-extractable organic pools (DOC and DON) as the major source of C and N for microbes based on the observation that the changes in biomass C and N (measured as $CHCl_3$-labile C and N) had a inverse relationship with that of the extractable pool. Carbon-to-nitrogen ratio of the K_2SO_4-extractable pool increased from roughly 14 to 21, implying that the substrate quality decreased over time. During the early part of their incubation (e.g., first 80 days), however, neither the size nor the C:N ratio of K_2SO_4-extractable pool nor the biomass pool changed while the respiration rate declined.

Lack of a clear decline in the total amount of extractable C in my study and that of Hart *et al.* (1994) seems to contradict the strong correlation between DOC and respired C across all stands. Further study is necessary to understand the relation between extractable C and microbial respiration.

DOC as a Major Substrate for Microbial Respiration

The role of water-soluble C as a major energy source for microbial respiration has been the subject of several previous studies. A laboratory incubation of wheat straw materials showed 2 to 4 times faster respiration rate in the non-leached straw than in the straw that was intensively leached prior to incubation (Reinertsen *et al.*, 1984). Such difference in respiration rate was greatest during the first three days, supporting the speculation that extractable C pool is an immediate energy source for soil microbes (Seto and Yanagiya, 1983; McGill *et al.*, 1986; Cook and Allan, 1992 a; Jandl and Sollins, 1998). As in my study, a strong correlation between extractable C and microbial respiration in laboratory soil incubations was reported from a wide range of mineral soils in temperate regions (Burford and Bremner, 1975; Powlson and Jenkinson, 1976; Seto and Yanagiya, 1983; Davidson *et al.*, 1987; Zak *et al.*, 1990; Cook and Allan,

1992). However, the high correlations alone don't confirm that extractable C is the main energy source for the heterotrophic microbes in soil. The actual magnitudes are critical: does extractable C provide enough labile substrate to sustain microbial biomass and, more specifically, are the soils studied here capable of regenerating labile extractable C fast enough to meet the microbes' demand for substrate?

To answer this, I compared my estimate of total amount BDOC degraded with the microbial substrate demand over the soil incubation period (Table 3.5). To make this comparison, I assumed that the biodegradable portion of extractable C is always replenished (i.e., constant pool of BDOC). This assumption can be justified because the BDOC pool was roughly constant or slightly increased from the initial to the final extraction time (Table 3.1). Similarly, Cook and Allan (1992 a) found that the extractable C pool remained roughly constant or increased over the course of five extractions during a 210-day laboratory incubation of Entisols that contained 10 to 30% of total C found in Wind River soils. From the same study, Cook and Allan (1992 b) found a constant ratio of various chemical fractions including hydrophilic neutral fraction, which is considered to be highly degradable (e.g., Jandl and Sollins, 1998), in the extractable C pool. Other studies have also found no change or a small decline in the extractable C pool over a soil incubation period but the lability of extracts was not studied (Seto and Yanagiya, 1983; Hart *et al.,* 1994).

First, the total amount of CO_2 respired by the degradation of BDOC during the soil incubation period (61.4 days) was estimated. The BDOC measured at the final extraction was separated into two pools based on the DOC degradation curve from each stand because the slope of curve was significantly different among the stands (Table 3.1). Early BDOC was defined as the amount of DOC degraded in the first several 5.1-5.5 days of the incubation (Table 3.5). The early BDOC was 40 to 80% of total amount of BDOC depending on the stand. Late BDOC was defined as the rest of BDOC degraded in the first 17 to 20 days of the DOC incubation. The late BDOC accounted for another 10 to 35% of total BDOC.

Table 3.5. Estimates of total amount of extractable C degraded and microbial substrates during the 2-month soil incubation. (Standard errors in prentices for respired C.)

	stand				
	WR-df	WR-mix	CH-df	CH-mix	CH-alder
Early BDOC production [a] ($\mu g\ C\ g^{-1}$ soil day^{-1})	2.7	7.1	2.6	14.2 [b]	6.6
Late BDOC production [c] ($\mu g\ C\ g^{-1}$ soil day^{-1})	0.1	0.9	0.4	5.6 [b]	1.0
Estimated total BDOC [d] ($\mu g\ C\ g^{-1}$ soil 61.4 day^{-1})	726	2029	724	4684	1875
Respired C [e] ($\mu g\ C\ g^{-1}$ soil 61.4 day^{-1})	1299 (240)	2718 (759)	1241 (126)	4285 (714)	2051 (246)
Estimated substrate [f] ($\mu g\ C\ g^{-1}$ soil 61.4 day^{-1})	2165	4530	2068	7142	3418
Est. BDOC/Est. substrate [g]	0.34	0.45	0.35	0.66	0.55

[a] Early BDOC: the amount of DOC degraded in the first 5.1 days (CH soils) and 5.5 days (WR soils) divided by the incubation time (day) based on the DOC incubation at the final extraction time.

[b] No degradation rate of DOC was obtained due to a lack of DOC solution and, thus 40 % and 60 % of the total BDOC were assumed to be in primary and secondary BDOC pools, respectively.

[c] Late BDOC: early BDOC of each stand was subtracted from the amount of DOC degraded in the first 19.5 days (CH soils) and 16.7 days (WR soils), and then averaged over the incubation time.

[d] Total BDOC = (Primary BDOC + Secondary BDOC) x 61.4 days.

[e] The total amount of C respired during the 61.4-day soil incubation period.

[f] Estimated amount of substrate microbes would need to respire the observed amount of CO_2 in 61.4 days. thus, Estimated substrate = (Respired C) / (1 - k); where k = microbial assimilation efficiency = 0.4

[g] Estimated total BDOC / Estimated substrate.

Daily production rates of the two pools were calculated for each stand and, then those were multiplied by 61.4 days to estimate how much BDOC was potentially consumed during the 2-month soil incubation (Estimated total BDOC in Table 3.5).

Second, the amount of microbial substrate need during the incubation period was calculated from the CO_2 respired during the soil incubation period assuming that the microbial assimilation efficiency, the amount of C assimilated into new microbial biomass divided by the amount of total C utilized, is 0.4 (Estimated substrate, Table 3.5).

The estimated total BDOC accounted for 34 to 66% of the estimated microbial substrate needs (Table 3.5). More fertile stands appeared to have higher BDOC:substrate ratio (estimated total BDOC / estimated substrate) than poor stands did. However, the correlation analysis of stand means showed weak correlation of BDOC:substrate with soil C:N ratio. Instead, the BDOC:substrate ratio was strongly correlated with the respiration rate at the start of soil incubation, total soil N, soil pH, and NH_4^+, NO_3^-, and DOC:DON at the initial extraction time (r^2 = 0.87 to 0.81). Interpretation of these strong correlations may require further information on soil microbial characteristics. Considering there was no C inputs from litter layer and roots in the soil incubation, the possibility that extractable BDOC pool provides a significant portion of substrate for microbial respiration deserves continued attention.

Possible Differences between the Two Sites

Despite within-site variability of up to a factor of ten, a strong but different relationship was present between DOC and respired C for each site, suggesting a site difference in soil characteristics influencing available C at CH and WR (Fig. 3.7.a). Most of organic C in mineral soil is stabilized in recalcitrant and/or protected forms (i.e., particulate C in Fig. 1.1 and 2.5). Both extractable and respired C can be viewed as results of biological destabilization of soil C. Then, the unique slope found for CH soils and for WR soils (Fig. 3.7.a) implies

differences between the sites with respect to biological destabilization of soil C. Respired C also had a better regression fit against other variables including BDOC, total C and N within each site than across sites. These distinct relationships among the measured variables between the two sites further support the possibility of site specific controls on soil destabilization processes.

Such controls can be inherent differences in microbes' habitat (e.g., aggregate size and structure), microbial metabolism (e.g., assimilation efficiency), or environmental factors besides temperature (e.g., moisture, Al toxicity). Despite the similar geological substrate between the two sites, clay mineralogy for CH soils is quite different from that for WR soils, possibly creating a significant difference in microbes' habitat. Cascade Head soils contain large amounts of amorphous organo-aluminum complexes, creating stable aggregate structure (Strickland *et al.*, 1998). Characteristics of such clay (e.g., high surface area, strong aggregation, and metal-ligand complexation) may result in the higher total soil C and N in CH than in the WR soils. The amount of extractable C on a per gram C basis was similar at the two sites at the both extraction times (Table 3.3). In contrast, the amount of BDOC (per gram C basis) was significantly higher for the WR soils than for the CH soils (Table 3.3). Similarly, the relative amount of respired C (%respired C) was higher for the WR soils than for the CH soils during the incubation period (Fig. 3.2). These results suggest that a larger portion of soil C in both the extractable pool and particulate pool was susceptible to microbial degradation in WR soils than in CH soils, resulting in greater soil C destabilization at WR site. Characteristics of the amorphous clay such as stable microaggregate formation in the CH soils are likely to contribute to the lower destabilization and consequently the greater accumulation of SOM in CH soils than in the WR soils.

The higher %respired C and the relative amount of BDOC mentioned above may also be attributed to a difference in soil microbial community with respect to their C and N utilization. A strong correlation specific to each stand was present between DOC and BDOC (Fig. 3.8) and %BDOC was highest for the WR-df soils

(high C:N ratio) and lowest for the CH-mix soil (low C:N ratio; Fig. 3.9). The %respired C at the end of incubation had a strong positive correlation with soil C:N ratio ($r^2 = 0.72$). Similarly, Gödde *et al.* (1996) reported that both leachable C and respired C from red spruce litter had a strong positive correlation with C:N ratio of the litter across eight sites. These results suggest that the microbial community adapted to low fertility soils (e.g., high C:N soil) would need to degrade more C, either in bulk soil or extracts, to gain enough N. From my study, it is difficult to separate the influence of physical aggregate characteristics from that of microbial metabolism.

CHAPTER 4
SUMMARY

The repeated leaching study in Chapter two illustrated the different roles of leachable and extractable C, the two operationally defined water-soluble C pools (Fig. 1.1), with respect to soil C loss. In a static view, the extractable C pool was 7.5 to 16.9 times larger than the leachable C pool based on both the pilot and replicated experiments (Table 2.1 and 2.2). Similarly, the biodegradable portion of DOC was 4.1 to 95.5 times more in extracts than in leachates. In a dynamic view, the size of leachable C pool was constant over the 20-week study period regardless of the presence of tree seedlings in soil leaching columns (Fig. 2.1). Extractable C declined by 31.4% (soil-alone columns) and 39.6% (soil+tree columns) but didn't diminish during the 242-day incubation period (Fig. 2.1). The inclusion of tree seedlings in the soil column also resulted in the reduction of both DOC and ammonium over the nine leachings as well as at the final extraction time (Fig. 2.1 and 2.2), perhaps due to an enhanced microbial activity associated with rhizosphere in the soil+tree columns.

A soil C destabilization model (Fig. 2.5) was proposed based on the measured changes in particulate, water-extractable, and leachable C pools over the study period (Table 2.4). Microbial respiration accounted for 95% of the C destabilized on annual basis. Leaching of DOC and POC accounted for the remaining 5 %. Similarly, leaching of dissolved and particulate N explained only 11 % and 6 % of total N loss from the soil-alone and soil+tree column, respectively. Together with the fact that the leachable C pool is small and constant over time, I concluded that the leaching process and leachable C pool had a negligible role in C mobilization in the studied soils. Extractable C is highly

accessible by microbes, was relatively labile, and its pool size didn't significantly decline over the 242-day study period, even without C inputs from the litter layer. Based on the rate of DOC degradation and the assumption of a constant pool size, the total amount of extractable C degraded over the study period was extrapolated as 98.1 g C m^{-2}
$year^{-1}$ from the top 10 cm mineral soil, quite close to the estimated C loss from the soil column by respiration (i.e., 118 gC m^{-2} $year^{-1}$). Thus, it was speculated that the destabilized particulate C flows into the extractable C pool and then is consumed rapidly by microbes. This supports the view that the extractable C is a significant energy source for heterotrophic microbial activity.

No statistical inferences could be made from the repeated leaching study because there was no replication of soil type. Thus the importance of extractable C as a microbial energy source suggested in Chapter 2 was tested in the cross-site study by comparing the top 10-cm mineral soils from two locations (Cascade Head coastal forest and Wind River upland forest) and a total of five forest stands. Because of the differences in climate, geology, forest type, and other environmental factors, the studied soils had C contents ranging from 4 to 18 % and N contents from 0.13 to 1.8%. Despite these large differences in fertility among the five soils, C extracted before and after the 2-month soil incubation showed a strong correlation with the C respired during the incubation period (r^2 = 0.71 and 0.92; Table 3.4), suggesting the efficacy of extractable C pools to predict the availability of C to soil microbes.

Dissolved organic C and its biodegradable portion (BDOC) were higher for the soil under Douglas-fir/alder mixed stand than pure Douglas-fir forest soils at both locations at both extraction times. Under the same forest type, Cascade Head soils had more DOC and BDOC than did Wind River soils. Thus, both location and forest type had significant effects on the level of DOC and BDOC in water-extractable pool. The change in extractable C pool during the soil incubation was variable ranging from 54% decline (CH-df) to 76% increase (WR-mix). The

biodegradable portion of DOC, however, remained same or increased slightly for all the stands (Table 3.1), demonstrating that BDOC pool is relatively constant over the 2-month incubation period despite the absence of C inputs from O-horizon and plant roots. Assuming that the constant BDOC pool size, the extent of BDOC contribution to microbial respiration was examined by comparing the estimate of total amount BDOC degraded with the microbial substrate need during the soil incubation period (Table 3.5). The estimated total BDOC accounted for 76, 83, 95% of the estimated substrate need for the CH-df, CH-mix, and CH-alder soils, respectively. For the Wind River soils, only 44 and 57% of the substrate demand was met by the estimated BDOC at Douglas-fir and the mixed stand, respectively. I assumed the same microbial assimilation efficiency (i.e., 0.4) for all stands to estimate the microbial substrate demands (Table 3.5).

Several lines of evidence, however, suggested that microbes in less-fertile soils (i.e., Wind River soils) utilized C more efficiently than the microbes did in the fertile soils (i.e. Cascade Head soils). The amount of respired C relative to the total soil C (%respired C) was higher for the WR soils than in the CH soils over the soil incubation period (Fig. 3.2). The %respired C at the end of incubation had a strong positive correlation with soil C:N ratio ($r^2 = 0.72$), suggesting that microbes' need for N may be affecting the observed difference in % available C between CH and WR soils. Similarly, %BDOC at the final extraction time correlated positively with C:N ratio ($r^2 = 0.72$; Fig. 3.9). The relative amount of degraded C in both bulk soil and extract showed a similar response to the soil fertility gradient (i.e., soil C:N ratio). Similarly, Gödde *et al.* (1996) reported that both leachable C and respired C from red spruce litter had a strong positive correlation with C:N ratio of the litter across eight sites. These results are consistent with the view that extractable BDOC is a major substrate for microbial respiration and implies that the microbial communities adapted to low fertility soils (e.g., high C:N ratio) are more efficient in assimilating soil C and N. In other words, these microbes need to degrade a

larger portion of soil organic matter present in soil to gain enough N from low N soils.

There was no C input to the soil system during the incubation. In field condition, labile C will be added to mineral soil from litter layer and rhizosphere. Thus it is still possible that extractable C pool provided a major portion of substrate for the microbial respiration. Then, the flow of destabilized particulate C to microbial biomass pool through extractable C pool (Fig. 2.5) proposed in Chapter 2, is likely to occur for soils from the five stands in the cross-site study. If microbial respiration is driven by microbes' need of labile extractable C, then extractable C pool must turn over very rapidly since the extractable C pool is relatively small at any point in time (Seto and Yanagiya, 1983; Cook and Allan, 1992 a; Hart *et al.*, 1997; my study).

My study illustrated two aspects of the role of extractable C in soil C dynamics. First aspect is its lability. The biodegradable portion of extractable C (BDOC) may be a major energy source for heterotrophic microbes. The BDOC was roughly 10 to 60% of the total extractable C. Degradation of BDOC is likely to account for 34 to 66% of the observed microbial respiration assuming BDOC is replenished rapidly. No decline in BDOC pool during the 2-month soil incubation period supported the assumption. Second aspect is its recalcitrance. The recalcitrant portion of extractable C (RDOC) constituted 40 to 90% of extractable C. Recalcitrant DOC can be seen as a left-over from microbial utilization of labile C and is likely to have a slow turnover rate.

I speculate that different factors control the dynamics of BDOC and RDOC. My study showed that the dynamics of BDOC was influenced by soil fertility, organic C and N concentrations in soil solution, and microbial assimilation efficiency. The dynamics of RDOC may not be related to microbial activity such as respiration but rather affected by extracellular enzyme activity and clay/aggregate surface characteristics. The role of RDOC was not studied here and would be more important in soil C stabilization processes. In fact, both

stabilization and destabilization of water-soluble C is likely to occur concurrently in soil (e.g., Sollins *et al.*, 1998). Studies of both labile and recalcitrant pools of extractable C will be important to understand the role of water-soluble C in SOM dynamics.

Water-extractable organic C and N accounted for only 0.03 to 0.09% of total soil C and 0.01 to 0.45% of total N among the six soils (including Chapter 2) studied here. The size of these extractable pools was quite comparable to that of microbial biomass. Furthermore, Chapter 3 showed that water-extractable C and N were strongly correlated with soil C degradation measures (e.g., respired C, BDOC, and %BDOC) among the five soils of a wide fertility gradient. Thus, despite those small quantities, water-extractable C and N deserve an attention to understand the mechanism of soil C destabilization. In particular, interactions between water-extractable pool and particulate pool with respect to microbial activity should be studied further. My soil samples covered both low and high end of fertility in forest soils of the Pacific Northwest. It is not clear, however, if these relationship hold true for soils under highly disturbed systems and on different parent materials. One of the important future questions is if these relationships are sensitive to small but critical changes in soil quality such as various intensity of agricultural and forestry management.

Water-extractable C deserves continued attention as a strong predictor of available C for soil microbes and as a potentially significant energy source for microbial respiration. Clearly more information is needed to test the hypothesis that heterotrophic microbes gain a significant portion of energy from water-extractable C and to find out the critical factors controlling soil C degradation in both solution and particulate phase. Future research should include

1) biological and biochemical characterization of dissolved organic matter and bulk soil organic matter together with DOC measurement (e.g., enzyme activities, microbial biomass and respiration),
2) estimation of the regeneration rate of labile extractable C,

3) interaction of litter- and root-derived C with DOC, and

4) comparison of production rate and biodegradability of DOC under laboratory condition with those under field condition.

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