AN ABSTRACT OF THE DISSERTATION OF

Christopher W. Swanston for the degree of Doctor of Philosophy in Forest Science presented on August 14, 2000. Title: Influences of Nitrogen on Carbon Dynamics in Forest Soil and Density Fractions.

Abstract approved: _____

Phillip Sollins

Abstract approved: _____

Peter S. Homann

Application of N fertilizer is a common forest management practice in the Pacific Northwest, yet the long-term influence of fertilization on forest soil properties is not well known. Although elevated N often increases mineralization of C and N from labile organic matter, negative effects have been documented in recalcitrant organic matter and whole soil. Using a series of paired plots in which one of each pair had undergone long-term N fertilization, I investigated the effects of elevated N on C and N mineralization in forest soils and organic fractions. The O2 horizons (O2), whole soils (WS), light fractions (LF), heavy fractions (HF), and physically recombined fractions (RF), from the paired plots were incubated in the laboratory for 300 d. For control soils, an additional "summed" fraction (SF), was computed from LF and HF results. Prior to analysis of the effects of elevated N, a general test of the density fractionation technique was conducted in the control soils. The LF and HF were hypothesized to represent labile and recalcitrant fractions in soil, but C and N were not substantially more stable in the HF

during the incubation. Total cumulative respiration and N mineralization were similar for both the SF and the WS, but C and N mineralization in both fractions were higher than in the RF. The depressed respiration in the RF might be explained by an antagonistic interaction between the varied microbial communities that degrade LF and HF; in the heterogeneous WS, these communities may be spatially separated. The density separation technique appears to be a viable method for isolating and studying different soil fractions, but these fractions should be considered more carefully in the context of microbial interaction and soil spatial heterogeneity. Elevated N depressed cumulative respiration to a similar extent in all substrates. The mechanisms most involved in degrading these substrates are negatively affected by elevated N, but may not be the same in each substrate. While laboratory results may not withstand the variability of the natural environment, the potential for elevated N to stabilize C in soil suggests the need for more detailed field measurements.

Influences of Nitrogen on Carbon Dynamics in Forest Soil and Density Fractions

by

Christopher W. Swanston

A DISSERTATION

submitted to

Oregon State University

in partial fulfillment of

the requirements for the

degree of

Doctor of Philosophy

Presented August 14, 2000

Commencement June 2001

Doctor of Philosophy dissertation of Christopher W. Swanston presented on August 14, 2000

APPROVED:

Co-Major Professor, representing Forest Science

Co-Major Professor, representing Forest Science

Head of Department of Forest Science

Dean of Graduate School

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

Christopher W. Swanston, Author

Acknowledgments

This is my favorite part of the dissertation, my chance to thank the people who guided, cajoled, consoled, inspired, helped, and pushed me through the whole process. The first thanks go to my committee: Phil Sollins and Peter Homann, my co-major professors who didn't really know what they were getting into until it was too late to back out; Dave Myrold, who knew what he was getting into, but apparently considers me a mission of mercy; and Mark Johnson, who stuck with me because I bribed him with a bunch of NaPT. They are all outstanding scientists with big hearts, and I am honored to be associated with them.

How can I adequately thank Bernard Bormann, who gave me dominion over nearly an entire lab for almost four years, and was kind enough not to remind me that he'd really only agreed that I could use a countertop or two? Thanks, Bernard. Kim Mattson generously lent me his gas chromatograph for three years and more than 20,000 samples; his kindness helped make this project possible. Rick Kelsey lent me several large incubators for a couple of years (I only broke one!). Lisa Ganio and Manuela Huso are not only my friends, but also invaluable sources of statistical advice and admonishment. Sandy Lewis and Glenda Serpa kept the bureaucratic monsters at bay, and generally just took good care of me. Elaine Ingham, Becky Norton (slacker), John Cliff, Gody Spycher, Steve Blum, and Nan Ritchie (you can't get rid of me, Nan!) all helped with data or lab analyses. Special thanks go to Will Austin and the folks at CAL (Carol, Ellen, and Sandy). Nick Chappell, Bob Gonyea, and Bert Hasselberg provided copious and valued logistical and field assistance.

Bruce Caldwell is a friend and mentor; nothing like a bit of disrespect and irreverence to put things in perspective. Thanks to Chris K., whose attempts to kill me in rafts or on bicycles helped me appreciate my nice safe office. Reduced to its very essence (ahem...), my friendship with the gang (Rob (you bug me), Rachel, Squeek, Scott (nice tie, buddy), and Liz) has ironically been a source of sanity and rejuvenation. And finally, The Folks: you guys make everything worthwhile, and not just because you fed me for the first two thirds of my life.

I was generously supported by the USDA Cooperative State Research Service National Research Initiative Competitive Grants Program, and the Long-Term Ecosystem Productivity research network at the USFS PNW Research Station in Corvallis, OR.

Contribution of Authors

Dr. Phillip Sollins and Dr. Peter S. Homann, as co-major advisors, were active in all aspects of design, analysis, and manuscript preparation related to this dissertation. Bruce Caldwell was involved in the conception, design, and establishment of the experiments, in addition to lending his expertise on data interpretation. Dr. David D. Myrold was generous with both his time and laboratory facilities, and also provided helpful comments on the manuscripts. Dr. Mark G. Johnson provided scientific guidance and support throughout the course of the experiments, and supplied thoughtful edits of the manuscripts.

Table of Contents

List of Figures

List of Tables

Dedicated to my wife, Elizabeth.

I never knew how good life could be until I met you.

Influences of Nitrogen on Carbon Dynamics in Forest Soil and Density Fractions

1. Introduction

1.1 Topics of Study

I will address two general topics in this dissertation. The first topic gives consideration to a relationship between the conceptual model of soil organic matter dynamics and the methodology used in the density fractionation procedure (Sollins et al. 1999). This method has been used for decades to separate labile and recalcitrant organic matter from soil, and appears to have recently gained in popularity. Unfortunately, relatively few researchers have actually attempted to test whether the separated fractions are truly 'labile' and 'recalcitrant.' A clarification of this point provides a sharper focus to research using this methodology, as well as clarifying inconsistencies in past research.

The second topic examines the influence of long-term fertilization with N on the recalcitrance of C in soil, and in density fractions of soil. Most consideration to forest N fertilization has been given to volume response in the primary tree species, or to increases in N availability. We sought to determine the effects that long-term elevated N might have on the stability of C in soils. That is, is C less 'available' in soils with elevated soil N? Do soil fractions that are chemically different, and have different concentrations of C and N, exhibit different patterns of C mineralization with elevated N?

Ultimately, I believe that it is vital to remain cognizant of the fact that C does not simply cycle. Carbon *is cycled.* The 'lability' or 'stability' of a C compound needs to be judged at least partly on the basis of the ability of the soil biota to degrade it, not simply on its weight or chemical structure. This concept served as the foundation of most of my decisions regarding methodology.

1.2 Soil Organic Matter Fractionation

1.2.1 Choice of method

Numerous methods exist to fractionate soil organic matter (SOM). The common goal of all such methods is to consistently isolate different soil components. The components might be different forms of organic C or N, or even types of microflora. The operative concept, though, is that the procedure used *consistently* isolate the component. If an attempt to isolate a component from an experimentally-treated soil results in a low yield, it is important to know that the method was functional and thereby be able to conclude that the low yield was a result of the treatment. At the very least, a method should be 'consistently inconsistent,' so that the researcher knows when, and to what extent, to expect deviations from common separation efficiency. Ideally, though, once faced with deviations in separation efficiency, a researcher will be able to adjust the method so that it once again isolates the correct soil component. I chose to use density fraction techniques to separate SOM in these forest soils because I believe that, of the physical separation methods, density fractionation has the greatest potential for consistency. I chose physical separation methods over chemical separation methods because I believe that physical separation methods were more appropriate for separating biologically-relevant fractions.

1.2.2 Chemical fractionation

Chemical fractionation separates SOM fractions based on their solubility characteristics in alkali and acid solutions. Stevenson (1994) reviewed the characteristics and separation procedures for the fractions. I will not attempt to duplicate that review here, but only to summarize the major concepts. Humin, operationally defined as being insoluble in alkali (NaOH), is the first fraction separated. It contains any number of organo-mineral complexes, waxes, and highly condensed humic material. The alkali supernatant is then treated with acid to separate humic acid (precipitate) and fulvic acid (soluble). There is a continuum between fulvic and humic acids of increasing C concentration, molecular weight, and polymerization; oxygen concentration decreases

from fulvic to humic acids. A further battery of solvents and resins can be used to separate more fractions (Stevenson 1994). These fractions may be useful in providing 'signatures' for soils, perhaps varying with factors such as vegetation and management (Miglierina and Rosell 1995). But the question is, how relevant are these fractions to the decomposition environment? For example, an organism may inhabit a discrete piece of organic matter, degrading carbohydrates and polyphenolic lignin structures simultaneously in a cometabolic process (Rayner and Boddy 1988, Chotte et al. 1998, Guggenburger et al. 1999). Extraction of the piece of SOM with NaOH may separate some lignin-derived aromatic C, depositing it into the humic acid while the remainder is left in the humin (Kogel-Knabner et al. 1991). Most polysaccharides would presumably reside in the fulvic fraction. Thus, although these C structures are related spatially and biologically in the soil, chemical fractionation procedures may separate them into several fractions, potentially obfuscating their connected roles in soil C cycling. Indeed, a recent, comprehensive review of ^{13}C CPMAS NMR spectra of humic and fulvic fractions led the authors to conclude that these chemical fractions showed few consistent trends with management and were of questionable biological significance (Mahieu et al. 1999). Since determining the effect of elevated N on C stability was a primary goal of this study, and C stability is a function of both soil biology and chemical composition, chemical fractionation alone did not appear to be an appropriate methodology for this study.

1.2.3 Physical SOM fractionation

The most common methods of physical fractionation are of two varieties, particle size fractionation and density fractionation. Particle size fractionation is founded on the concept that different mineral particle sizes are associated with C pools of different stability (Tiessen and Stewart 1983). Operationally, this allows for separation of this C by using sieves of various sizes. The conceptual model for density fractionation is that with increasing molecular condensation and mineral-association, the density of organic matter will increase (Young and Spycher 1979, Spycher and Young 1979). Operationally, density fractions are separated using solutions of different densities. Both

types of methods rely on the degree to which the soil is dispersed prior to fractionation, but for different reasons.

1.2.4 Size-density fractionation

Numerous variations in methodology exist in particle size separation, many of which are described in a comprehensive review by Christensen (1992). Methods typically involve dispersion of soils by sonic dispersion or prolonged shaking in sodium metahexaphosphate. Dispersion is necessary to disrupt aggregates of small particles that would otherwise be retained on larger sieves. The dispersed soil slurry is passed through a series of decreasing sieve sizes. Common fractions are 2000-250 μm (coarse sand) and 250-53 μm (fine sand). Organic matter in these fractions is also commonly referred to as particulate organic matter (POM) (Cambardella and Elliot 1992). Silt and clay fractions are separated by sedimentation of the slurry that passed through the 53 μm sieve. Using non-dispersed soil, Cambardella and Elliot (1993, 1994) isolated different aggregate sizes before separately dispersing the aggregates and sieving the resulting slurries. They further fractionated the size separates in sodium polytungstate (NaPT), a dense liquid medium (i.e.: density fractionation, described below). Meijboom et al. (1995) separated 'macroorganic' matter on a 150 μm sieve. Sand was removed from the macroorganic matter with a panning technique. The resulting organic fraction was further separated in Ludox, a dense liquid medium similar to NaPT, but more viscous.

Typically, C concentration increases with decreasing particle size. Variations in this pattern are often attributed to inadequate dispersion of the soil (Christensen 1992). Alternatively, Cambardella and Elliot (1994) contend that high initial dispersion may redistribute C and N among the particle size classes, resulting in inaccurate estimation of C and N distributions in soil. Their method attempts to compensate for the redistribution problem by separating the soil into various aggregate sizes before dispersion. In doing so, they obtain fine-silt-sized-heavy fractions that are especially enriched in C and N.

Size fractionation certainly has utility. Mahieu et al. (1999) examined over ^{13}C 100 NMR spectra from size separates, and concluded that they were typically different from each other and from the whole soil. However, the main reservation I have with size

fractionation procedures is that although they may identify some useful patterns, the physically separated fractions are essentially arbitrary. Meijboom (1995) used 150 μm as the limit for macroorganic matter because smaller sieve sizes clogged. Other recoveries of 'sand,' 'silt,' and 'clay' will vary based upon whether the sieves used follow USDA or ISSS classification schemes, which define the size of sand, silt, and clay differently. Even within a classification scheme, the researcher is limited by the available sizes of sieves, which may in turn have no relevance to biologically meaningful size ranges of aggregates or particles. In order to compensate for this lack of flexibility in the primary means of separation, researchers are compelled to create increasingly complicated and laborious variations of the basic size separation method (Cambardella and Elliot 1994). Separating a dozen or more fractions with size-density techniques is certain to find one or two notable fractions. However, I believe that such complicated methodology, and the labarynthal conceptual model required to support it, is inherently more brittle when exposed to the great variation in natural systems. More parsimonious and flexible models and methods may be able to adapt much more readily to the variability of the natural environment (Holland 1998). For these reasons, I distrust that size-density fractionation would be certain to separate biologically meaningful fractions. Using the same reasoning, I find density fractionation especially appealing.

1.2.5 Density fractionation

Density fractionation is a method in which the soil is physically split into low- and high-density fractions, referred to as light- and heavy-fractions (Greenland and Ford 1964, Sollins et al.1999). The light fraction (LF) is plant-like material altered slightly by microbial degradation (Spycher et al. 1983, Golchin et al.1994a, Gregorich et al. 1996). Heavy fraction (HF) is typically mineral-associated and more humified (Golchin et al. 1994b, Golchin et al. 1995). Conceptually, the LF is thought of as the more labile component that drives soil heterotrophic respiration and N mineralization (Spycher et al. 1983, Hassink 1995a). The HF serves as a large reservoir of stable C and N; physically protected and chemically recalcitrant, it is thought to provide little to soil heterotrophic respiration and N mineralization (McGill et al. 1986, Alvarez and Alvarez 2000). Some

have proposed and identified an intermediate fraction, similar in density to the LF but occluded, somewhat more degraded, and possibly still active (Strickland et al. 1992, Golchin et al. 1994a, Golchin et al. 1995).

The identification of these 'active-protected' (Strickland et al. 1992) and 'occluded' (Golchin et al. 1994a) LF's illustrates a crucial concept of density fractionation: by varying the level of soil disruption and densimetric solution density, functionally different fractions can be separated. Therein lies the simplicity and enormous flexibility of the method. Simplicity: what floats is structurally and chemically different than what sinks; applying increasing energy to the soil slurry increasingly disperses the aggregates, while applying little or no energy to the soil maintains aggregation. Flexibility: the density of the densimetric solution can be adjusted to avoid mineral contamination while maximizing recovery of the 'plant-like' LF; the degree of disruption can be sequentially adjusted to release SOM from within increasingly strong (small) aggregates.

Ideally, the researcher would separate the soils of interest at several densities. The resulting LF and HF would be analyzed for C, N, and ash content. The density which separates the LF that best matches the conceptual LF would be used for that soil. The conceptual LF would have the highest C concentration and C:N ratio, with the lowest ash content, possible. Visual inspection of the LF and LF-ash is also important, since ash may not always indicate soil minerals. Greenland and Ford (1964) determined that much of the ash remaining after combustion of the LF was actually composed of phytoliths.

A study using several soils with different minerologies may achieve the best recovery of LF at different densities in each soil. This poses a dilemma: can LF's separated at different densities be compared to each other? Most scientists would feel some discomfort comparing fractions obtained with 'different methods.' However, although the LF is operationally defined according to the method of separation, it is also conceptually defined. It is the conceptual model, supposedly, that drives the methodology. It would seem that methodological consistency may produce conceptual inconsistencies. Picking one density and sticking with it may result in LF's that, in some cases, do not meet the conceptual criteria of LF. I think that consistency in densimetric

methods should not be judged on the density of the separation medium, but rather on the qualities of the products of the separation.

Richter et al. (1975) measured the change in C concentration in the LF when the density of the densimetric solution, bromoform-ethanol, was changed. They observed an exponential increase in LF-C between the densities of 1.6 g mL^{-1} and 2.0 g mL^{-1}. Sollins and Spycher (1983), working with andesitic soils, identified pumice fragments in a LF separated at 1.65 g mL^{-1} with NaI. They noted that R. Boone later separated the same soils densimetrically using NaCl at 1.2 g mL^{-1}; the LF contained nearly the same amount of organic matter, but far less pumice. Spycher et al. (1983) chose a density of 1.65 g mL^{-1} based partly upon earlier studies of mineral densities in their region (Young and Spycher 1979, Spycher and Young 1979), and partly upon observations of mineral contamination in LF from previous fractionations of the soils.

The arguments in favor of flexibility in the choice of solution density also apply to the choice of how much energy to dedicate to disaggregation. The goal should be to separate conceptually-defined LF, HF, and intermediate fractions. The energy required to do that will change from soil to soil, and even within a soil type. Boone (1994), working with a sandy soil, only needed to stir with a glass rod for 30 s to achieve adequate disaggregation. Strickland et al. (1992) needed to sonicate to achieve greater than 70 percent dispersion. Greenland and Ford (1964) determined that sonication, in place of shaking by hand, increased the ash-free C concentration in several LF's separated on bromoform-ethanol. Strickland et al. (1992) applied ^{15}N tracer to whole soils, then separated and incubated the HF material in two treatments, sonicated and unsonicated. Dilution of the tracer in the sonicated incubation slurry indicated an 'active protected' fraction. Golchin et al. (1994a) gently shook soils in NaPT, then aspirated the LF. Since microaggregates were still intact, this LF was termed the 'free LF,' and was considered to exist in interstitial spaces without strong mineral association. Subsequent sonication of the soil remaining in NaPT released more LF, which was visually and chemically different from the free LF. This LF was called 'occluded LF.' The concept of occluded LF later formed the core of their conceptual model of aggregation and C cycling (Golchin 1994b)

Although density fractionation of soil has been routinely conducted for nearly forty years (Greenland and Ford 1964), no studies have reported a direct measurement (i.e.: incubation) of the C recalcitrance of both LF and HF (denismetrically-separated). Sollins et al. (1983) incubated LF and HF and reported rates of anaerobic net N mineralization. Greenland and Ford (1964) and Alvarez and Alvarez (2000) incubated the LF and measured O_2 consumption and CO_2 evolution, respectively. Boone (1994) incubated LF and reported net N mineralization potential.

Thus, a fundamental tenet of the density fractionation conceptual model, that the fractions actually differ in recalcitrance, has seldom been directly confirmed or refuted. Without direct confirmation of the basic conceptual model, studies that attempt to use the methodology to determine the effects of some experimental treatment must rely on an uncomfortable degree of circular reasoning. Even as researchers test treatment effects using the fractionation methodology, and explain them using the conceptual model, they attempt to confirm the conceptual model based upon the treatment effects. In general, this kind of reasoning is important to both the continued refinement of a conceptual model and the calibration of the associated methodology ('spiral reasoning'), but it is predicated on the knowledge that the basic tenets of the conceptual model match the products of the methodology.

1.2.6 Goals for Chapter 2

I sought to directly test the relationship of the conceptual model of density fractionation to the fractions actually separated by the methodology. Five substrates were treated to a 300 d aerobic incubation: whole soil, LF, HF, a physically recombined fraction, and a mathematically recombined fraction. Using CO_2 respiration as an index of substrate recalcitrance (Stotzky 1965, Hart et al.1994), two basic questions were addressed: 1) Do LF and HF differ in recalcitrance? 2) Is LF actually the driving force behind soil respiration and N mineralization and what part does HF play?

1.3 Effects of Elevated N on C Stability

1.3.1 Overview

Fertilization with N is an increasingly important and widespread practice in forests of the Pacific Northwest (Allen 1987). The effects of N fertilization on tree growth are generally positive in the first years following amendment (Hopmans and Chappell 1994, Weetman et al. 1997), but the effects on soil nutrient cycling are less well understood (Smolander et al. 1994). Berg (1986) hypothesized that fertilization with N would ultimately depress the rate of nutrient cycling, thereby reducing the relative growth rate of fertilized conifers in the long-run. Fog (1988) addressed the first part of this hypothesis with an exhaustive review covering the effects of increased N on organic matter (OM) degradation in litter and soil. Citing dozens of seemingly contradictory papers, Fog (1988) noted that numerous studies had found a negative N effect, while others reported a neutral or positive effect of N on OM degradation. Through careful consideration of the type of substrate being degraded, the C and N concentrations in the substrate, and the degradation-strategies of organisms thought to be the primary degraders of the substrate, Fog (1988) was able to merge the disparate studies into an integrated empirical model. According to the model, addition of N will increase the rate of degradation of labile OM with low C:N, but will decrease the rate of degradation of recalcitrant OM with high C:N ratios. A broader conceptual model was adapted from Fog's empirical model and is summarized in Figure 1.1. Organic matter destabilization is defined as the potential for OM loss through respiration, leaching, or conversion into another OM component (Sollins et al. 1996). Although OM is often referred to as 'labile' or 'recalcitrant,' a discrete sample of OM may include both labile and recalcitrant components (McClaugherty and Berg 1986, Paul and Clark 1989, Zech and Guggenberger 1996). Measures of mass loss and respiration of a whole sample may be dominated by the activity of one component of the OM at a specific point in time, but both components are probably degraded simultaneously (McGill and Cole 1981, Rayner and Boddy 1988, Harmon et al. 1990, Van Veen and Kuikman 1990, Killham 1994, Entry and Blackman 1995, Andrén and Kätterer 1997). Thus, measures of OM

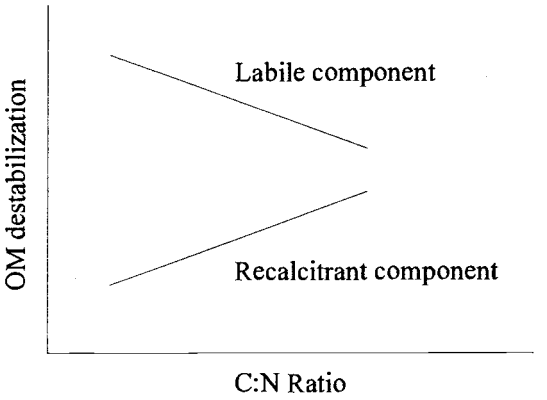

Figure 1.1. Conceptual model describing the destabilization of OM. Increasing C:N ratio affects the destabilization of labile and recalcitrant components in an opposite manner.

destabilization of whole soil are influenced by degradation of both labile and recalcitrant components. Each component, in turn, is probably also composed of labile and recalcitrant components, but in different proportions. Ultimately, the dominant component defines the OM as labile or recalcitrant.

Densimetric methods are commonly used to physically separate two fractions of the soil organic matter considered to represent the labile and recalcitrant components (Christensen 1992, Hassink 1995a). Density fractionation involves disrupting soil aggregation within a dense liquid medium, allowing a 'light fraction' to float to the surface where it is collected, followed by collection of the sedimentary 'heavy fraction' (Sollins et al. 1999). The light fraction (LF) is chemically and visually similar to litter (Spycher et al. 1983, Skjemstad et al. 1986, Golchin 1994a). The heavy fraction (HF) is composed of organo-mineral complexes and humified C (Young and Spycher 1983, Golchin 1994b, Golchin 1995). The LF is typically considered the "active" component and the primary source of heterotrophic respiration and N mineralization in soil (Spycher et al. 1983, Theng et a. 1989, Hassink 1995b). Conversely, the HF is considered to be the more recalcitrant component of soil organic matter. Although the

HF may contain the bulk of the soil C and N, mineralization of the HF is thought to be severely curtailed by physical protection and chemical recalcitrance (McGill et al. 1986, Alvarez and Alvarez 2000).

1.3.2 Goals for Chapter 3

I sought to test whether elevated N resulting from long-term N fertilizatoin increased C stability in litter, soil, and densimetric fractions of soil. Respiration was used as a measure of C stability, under the conceptual model that less CO_2 would evolve from soils with less labile C. Litter and soils were collected from a series of paired N-fertilized and unfertilized plots in second growth Douglas-fir forests. The plots were amended with N periodically between 1969 and 1986, and the soil and litter collected in 1995. The soils were separated into light- and heavy-fractions. The litter, whole soil, and density fractions were incubated for 300 d to determine the effect of elevated N on C lability in the fractions and the whole soil. It was hypothesized that the O2 horizon and LF would exhibit increased degradation with added N, while C in the HF would become more stable. Whole soil, composed primarily of HF, was expected to manifest reduced C destabilization.

CHAPTER 2

2. Carbon and Nitrogen Dynamics in Separate and Recombined Density Fractions from Forest Soils

Christopher W. Swanston, Peter S. Homann, Phillip Sollins
Bruce Caldwell, David D. Myrold, Mark G. Johnson

2.1 Abstract

Although density fractionation of soil has been routinely conducted for nearly forty years, relatively few studies have reported a direct measurement of the lability of the individual density fractions. In a series of long-term laboratory incubations, light fraction (LF), heavy fraction (HF), whole soil (WS), and physically recombined light and heavy fractions (RF), were measured for respiration, net N mineralization, and shifts in microbial biomass. A combined 'fraction' was calculated from the incubation results of the light and heavy fractions, and called the 'summed fraction' (SF). When cumulative respiration was considered per gram of substrate, the physical fractions exhibited a predictable response: LF > RF > HF. However, per gram of initial C, the respiration of the LF was only marginally higher than that of the HF, and the HF not different from the RF. Whether considered per gram of substrate or initial C, the respiration of the SF was not different from that of the WS, and both showed increased respiration over the RF. Net N mineralization showed even less statistical separation of the fractions; LF was greater than RF and HF on a substrate basis, but no differences were evident when net mineralization was normalized by initial N. The WS exhibited twice the net mineralization of the SF and HF, which were not different. Our findings indicate that the difference in intrinsic lability between the LF and HF is not as wide as previously thought. The depressed respiration in the RF might be explained by an antagonistic interaction between the varied microbial communities that degrade LF and HF; in the heterogeneous WS, these communities may be spatially separated to a large degree. The density separation technique appears to be a viable method for isolating and studying different soil organic matter fractions, but these fractions should be considered more carefully in the context of microbial interaction and physical protection.

2.2 Introduction

Numerous conceptual models have been developed to describe the dynamics and interrelationships among different components of soil organic matter. Many modern conceptual models include both a labile and a recalcitrant component, and possibly one or more intermediate components (Christensen 1992, Cambardella and Elliot 1993,

Golchin et al. 1994b, Killham 1994, Stevenson 1994). These models are often methodologically driven. That is, the conceptual model is partly defined by the methodology by which soil components can be separated and analyzed. The nature of the components that are separated mostly depend on the method of separation.

Density fractionation is a methodologically driven model in which the soil can be physically split into low- and high-density fractions, referred to as light- and heavy-fractions (Greenland and Ford 1964, Sollins et al.1999). The light fraction (LF) is plant-like material altered slightly by microbial degradation (Spycher et al. 1983, Golchin et al. 1994a, Gregorich et al. 1996). Heavy fraction (HF) is typically mineral-associated and more humified (Golchin et al. 1994b, Golchin et al. 1995). Conceptually, the LF is thought of as the more labile component that drives soil heterotrophic respiration and N mineralization (Spycher et al. 1983, Hassink 1995a), although others have proposed that HF may play a more prominent role (Sollins et al. 1984, Boone 1994). The HF serves as a large reservoir of stable C and N; physically protected and chemically recalcitrant, it is commonly thought to provide little to soil heterotrophic respiration and N mineralization (McGill et al. 1986, Alvarez and Alvarez 2000). Some have proposed and identified an intermediate fraction, similar in density to the LF but occluded, somewhat more degraded, and possibly still active (Strickland et al. 1992, Golchin et al. 1994a, Golchin et al. 1995).

Although density fractionation of soil has been routinely conducted for nearly forty years (Greenland and Ford 1964), very few studies have reported a direct measurement (i.e.: incubation) of the C recalcitrance of both LF and HF. Sollins et al. (1984) incubated LF and HF, but only measured anaerobic net N mineralization. One of their main findings, that HF had a higher rate of anaerobic net N mineralization than LF, runs contrary to common views of the roles LF and HF. Unfortunately, these findings have not been directly confirmed through replicated experiments, and are seldom discussed. Others have incubated only LF (Barrios et al. 1996, Magid et al. 1996), perhaps calculating values for the HF from the difference between the whole soil and LF (Boone 1994). Thus, a fundamental tenet of the conceptual model, that the fractions actually differ in recalcitrance, has seldom been directly confirmed or refuted. Without direct

confirmation of the basic conceptual model, studies that attempt to use the methodology to determine the effects of some experimental treatment must rely on an uncomfortable degree of circular reasoning.

This study sought to directly test the relationship of the conceptual model of density fractionation to the fractions actually separated by the methodology. Four substrates were incubated aerobically for 300 d: whole soil (WS), LF, HF, a physically recombined LF and HF fraction (RF); additionally, the mathematical sum of LF and HF was calculated (SF). We distinguished between recalcitrance, the inherent chemical resistance of a molecule to microbial breakdown, and physical protection, the degree to which a molecule is physically accessible to microbial attack (Sollins et al. 1996). Using CO_2 respiration as an index of substrate recalcitrance (Stotzky 1965, Hart et al. 1994), two basic questions were addressed: 1) Do LF and HF differ in recalcitrance? 2) Is LF actually the driving force behind soil respiration and N mineralization; what part does HF play?

2.3 Materials and Methods

2.3.1 Site Characteristics

Soils were collected from seven installations of the Stand Management Cooperative Nutrition Project in western Oregon and Washington, USA (Table 2.1, Hazard and Peterson 1984). Although each of the installations included fertilized plots, the soils used in the present study were only collected from the single non-fertilized plot at each installation. The overstory was primarily composed of 46 to 72-year old second-growth Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) at the time of sampling.

2.3.2 Soil Collection and Storage

Soils were collected in August and September, 1995. Plots were sampled using a 5 x 5 grid with a 4.5 m spacing, beginning 1.4 m from the corner of each 20 x 20 m plot. At each sampling point, the O layer was removed, and a 10 x 10 cm section of the 0-5 cm mineral soil was sampled from the side of a mini soil pit. Samples from alternating grid

Table 2.1. Characteristics of seven Stand Management Cooperative installations from which soils were collected.

Installation	Location	Great group	Parent Material	Soil (0-5 cm)			
				Textural class	% C	% N	C:N
Cedar Falls (1)	47.410 N, 121.815 W	Durochrept	Glacial till	gravelly loam	10.60	0.72	15
Deep Creek (20)	45.962 N, 123.307 W	Haplumbrept	Glacial sediments	loam	5.10	0.45	11
Skykomish (43)	47.735 N, 121.249 W	Haplorthod	Granite	fine sand	3.91	0.24	16
Headquarter Camp (57)	46.219 N, 122.732 W	Haplohumult	Igneous	loam	10.30	0.72	14
Fourth Creek (65)	43.804 N, 122.381 W	---[†]	Pumice	loam	8.13	0.47	17
Elk Creek (89)	43.365 N, 123.868 W	Haplumbrept	Sandstone	loam	10.59	0.65	16
Cristy Falls 103)	43.904 N, 122.301 W	---[†]	Pumice and ash	loam	10.81	0.60	18

[†]Great groups have not been classified at these installations.

points were immediately composited and sieved (< 2mm) to yield a homogeneous fine fraction. Samples from remaining grid points were similarly composited and sieved, resulting in two composited samples per plot. Moist subsamples of this fine fraction were removed and transported to the Forestry Sciences Laboratory in Corvallis, OR, where they were tightly sealed in plastic bags and stored frozen at -20°C. Meijboom et al. (1995) and Magid et al. (1996) have determined that freezing does not significantly affect density fractions.

2.3.3 Density Fractionation

The density fractionation methodology was based on techniques described by Strickland and Sollins (1987) and Sollins et al. (1999), but with necessary modifications. All soils were fractionated while 'field moist,' although weights provided are dry weight equivalents unless otherwise specified. The procedure involves mixing soil into a dense liquid medium (sodium polytungstate (NaPT), 1.65 g L^{-1}, Sometu, Van Nuys, CA), destroying macroaggregates with a benchtop mixer, allowing the fractions to separate, aspirating the LF from the surface of the NaPT supernatant, collecting the sedimentary HF, and finally rinsing the separated fractions of NaPT. Most soils were fractionated at a ratio of 1:4 in 400 mL of NaPT, but sandy soils were fractionated at a soil weight to NaPT volume ratio of as high as 1:2.5. The basic requirement of the chosen ratio was that it allow for ample supernatant between the sedimentary HF and the floating LF. Typically, at least 50 mL of NaPT would be drawn off during the aspiration of the LF.

Frozen soils were thawed in August, 1998, the two composites from each installation mixed, and analyzed for gravimetric water content. Between 400 and 700 g of soil from each installation were fractionated in order to obtain the amount of LF required for the subsequent analysis and incubation (see below) (Table 2.2). To fractionate the soil, approximately 100 g (dry weight equivalent) of moist soil of known mass was placed in a 600 mL tall-form beaker, followed by the addition of 250 mL of NaPT at a density of 1.80 g mL^{-1}. Distilled water was added, which in combination with the measured soil moisture, diluted the NaPT to 1.65 mL^{-1}. The slurry was then mixed for 30 s with a Hamilton benchtop mixer on a setting of 2.5 (1800 rpm). The blades of the mixer were

Table 2.2. Percent recovery, C, and N of light fractions, heavy fractions, and recombined fractions.

Installation	Soil g (oven dry)	Recovery %	Light Fraction				Heavy Fraction				Recombined		
			% of recovered	%C	% N	C:N	% of recovered	% C	% N	C:N	% C	% N	C:N
1	417.27	100.74	18.28	28.62	1.30	22	81.72	5.63	0.41	14	10.80	0.63	17
20	691.57	100.13	8.68	25.83	1.11	23	91.32	3.28	0.38	9	4.70	0.40	12
43	704.60	101.65	16.90	16.20	0.68	24	83.10	1.09	0.11	10	3.70	0.20	19
57	423.09	98.69	17.99	28.00	1.50	19	82.01	5.23	0.51	10	9.72	0.70	14
65	423.45	98.98	20.98	23.49	1.05	22	79.02	3.10	0.29	11	9.32	0.50	19
89	500.11	99.03	17.12	26.41	1.24	21	82.88	5.29	0.46	12	10.36	0.61	17
103	366.71	105.06	32.63	24.47	1.00	24	67.37	4.10	0.41	10	11.31	0.58	19

rinsed over the beaker using NaPT (1.65 g mL^{-1}). The beakers were covered with plastic wrap and set aside. After 48 hr, the LF floating on the supernatant was aspirated into a separate container and the remaining sediment was remixed and again allowed to settle for 48 hr. Any floating LF was removed at that time and added to the previously removed LF. The separated LF was rinsed with distilled water at least five times on a Whatman no. 52 filter. Rinsed LF was dried in a forced-air oven at 50°C for 48 hr. Subsamples of the oven-dried (50°C) LF were dried at 105°C to obtain an 'oven dry' weight.

Following the second removal of the LF, the HF was flushed from the beaker into several 250 mL centrifuge bottles, mixed thoroughly with dH_2O and centrifuged. The non-turbid supernatant was aspirated and discarded. This process was repeated at least five times. Rinsed HF was flushed into tins and dried in a forced-air oven at 50°C for 72 hr. Subsamples of the oven-dried (50°C) HF were dried at 105°C to obtain an 'oven dry' weight.

Prior to fractionating the bulk soil samples, small subsamples were collected and fractionated as above, but with the additional step of sonic disruption (156 J mL^{-1} for 2 minutes) following mixing. This step was added to estimate the additional amount of 'occluded LF' (Golchin et al. 1994a) that would be recovered if sonication were included in the fractionation procedure. The sonication added from 0 to 0.2 percent to the recovery of the LF, indicating that the series of mixes adequately disrupted aggregation.

2.3.4 Substrates

Four physical substrates were analyzed: the whole soil (WS), the light fraction (LF), the heavy fraction (HF), and the physically recombined fraction (RF). For the LF, 2.00 g (dry weight equivalent) of substrate was mixed with 18.00 g (dry weight) of acid-washed quartz sand (650-850 μm diameter) and placed in a glass scintillation vial. Similarly, for WS and HF, 10.00 g of substrate was mixed with 10.00 g sand. The RF was created by mixing LF and HF back together in the proportions from which they were recovered from the soil (Table 2.2), and combining 10.00 g of the mixture with 10.00 g of sand. Actual weights were tracked to the nearest 0.001 g. Sand was added to the C sources for

two reasons. The addition of sand yielded a larger sample that permitted more flexibility in the type and number of physical analyses that could be performed. In the case of the HF sand was added to create some degree of physical structure, possibly ameliorating any anoxic conditions that might occur during the incubation. In the use of the sand, we assume it was inert and underwent no appreciable weight change during the course of the incubation. The WS was produced and began incubating within 24 hr of the soil thawing.

Total initial C and N were measured on day 1 of substrate incubation (see below) and values were corrected for sand content. These analyses were carried out on a Leco CNS Analyzer (Leco, St. Joseph, MI) by the Central Analytical Lab (Corvallis, OR).

2.3.5 Incubation

Substrates were incubated for 300 d at 20 °C (± 1 °C). For LF, HF, and RF, substrates and sand were placed in open 20 mL glass scintillation vials, and six vials were placed in sealed 1 L mason jars (Stotzky 1965, Hart et al. 1994). All vials within a jar contained the same substrate from the same installation. To adequately contain the WS, three 60 mL vials were used in each of two mason jars. The multiple vials allowed periodic destructive sampling.

Each substrate was inoculated at the beginning of the incubation. Soil (0-5 cm) used as the source of inoculum was collected from the McDonald-Dunn Research Forest within a stand similar in age and composition to those from which the incubation soils were collected. The inoculum source was stored in a slightly open cooler at room temperature and humidity. The soil was kept moist by regularly adding tap water. Inoculum was collected by first adding the soil to distilled water in a 1 g:1.5 mL ratio of soil to water, followed by gentle shaking on a rotary shaker for 0.5 hr before being allowed to settle for another 0.5 hr. Finally, a 0.5 mL aliquot of supernatant from an inoculum mix was added to each vial. A fresh inoculum mix, from the original inoculum source, was created each time a new batch of substrates was ready to begin incubating.

The headspace of each jar was sampled through a septum installed in the cap of the jar. The septum in each jar was replaced frequently to maintain the integrity of the seal.

Before each sample was drawn, the air in the headspace was mixed by repeatedly and vigorously drawing air into a gas-tight syringe and plunging the air back into the jar. After mixing, a 500 µL sample was drawn and immediately injected into a GC-8A Series Shimadzu gas chromatograph with a 6 ft x 2 mm (ID) Porapak Q 80/100 column. If duplicate samples did not agree to within 3 %, a third sample was taken. Once the sampling of a jar was completed, the jar was flushed with CO_2-free air and resampled to obtain a baseline CO_2 concentration. Respiration was measured in the headspace of the jars at intervals (1-21 d) sufficient to maintain a CO_2 concentration of less than two percent.

Destructive sampling of the vials occurred at seven times: days 1, 10, 30, 60, 120, 210, and 300. On each sampling date, a single vial from each installation/substrate combination was removed from the jar. The substrate in the vial was removed and thoroughly mixed. The mixed substrate was then allocated to tests for extractable N, active biomass, and gravimetric water content.

Extractable N was measured by extracting moist substrate with 0.5 *M* K_2SO_4 at a 1 g:5 mL ratio of substrate to K_2SO_4. The extraction process consisted of shaking the substrates in the K_2SO_4 for 1 hr, allowing the slurries to settle for 0.5 hr, then pouring the supernatants through acid-rinsed Whatman no. 1 filters. Extracts were stored frozen at -20 °C until analysis. Once thawed, samples were shaken for 0.25 hr and then aliquots removed for total N determination and for ammonium and nitrate measurement. Total N was obtained using the persulfate oxidation method proposed by Cabrera and Beare (1993). Nitrate was measured on an Astoria Pacific Analyzer (Astoria Pacific Int'l, Astoria, OR) and ammonium on an Alpkem Flow Solution (OI Corporation, College Station, TX). Extractable-organic N was calculated by subtracting extract ammonium and nitrate from total N.

Active microbial biomass was obtained through direct microscopy (Soil Food Web, Inc., Corvallis, OR). Substrates were diluted and stained with fluorescein diacetate, allowing active bacteria and fungi to be viewed and measured with an epifluorescent microscope (Ingham and Klein, 1984; Stamatiadis et al., 1990).

2.3.6 Data Management and Analysis

In addition to the four physical substrates, a fifth (mathematical) substrate called the 'summed fraction' (SF) was derived using data collected from the LF and HF incubations. As with the RF, the SF was 'remixed' according to the proportions of light and HF recovered from specific soils (Table 2.2).

'Remaining C' was calculated at the time of each respiration measurement by subtracting the cumulative respiration to that point from the initial C. For each time point, the mg C respired g remaining^{-1} day^{-1} was calculated by subtracting mg C respired g remaining^{-1} of the previous time point from the present time point, then dividing by the number of days separating the time points.

For cumulative C respiration and net N mineralization, the statistical analysis was a randomized complete block design. The installations were blocks, while substrates were treatments. Although duplicates of all substrates from each installation were incubated, the duplicates were created to ensure against sample loss through accident. Prior to statistical analysis, the values for each set of duplicates were averaged, and those means treated as single replicates in statistical analyses.

Prior to all statistical tests, residuals of data were tested for constant variance. If necessary, natural log transformations were used to stabilize variance. When an analysis of variance (ANOVA) resulted in a p-value of less than 0.05, all possible comparisons between treatments were conducted using Fisher's Protected Least Significant Difference (FP LSD). Although not as 'conservative' as other multiple comparisons tests, FP LSD was considered appropriate in these cases because the comparisons were planned and there were never more than three treatments per test, thus minimizing compounded error (Einot and Gabriel, 1975).

Analysis of microscopy data and changes in rate of C mineralization involved comparisons of data both at a time point and between time points. Although different vials were measured each time, the same installation and substrate in each jar were being repeatedly measured through time, necessitating the use of a repeated measures ANOVA. Most data manipulation, and all statistical analyses, were conducted in SAS (SAS Institute, Inc., version 6.12).

2.4 Results and Discussion

2.4.1 Carbon mineralization

The 300 d cumulative respiration (mg C g^{-1} initial substrate) differed substantially among LF, HF, and RF ($F_{2, 12} = 142.3$, $p < 0.0001$). The LF produced nearly nine times the cumulative respiration of the HF ($t_{12} = 16.4$, $p < 0.001$), and nearly five times the cumulative respiration of the RF ($t_{12} = 11.7$, $p < 0.0001$) (Table 2.3). The RF was also more productive that the HF alone ($t_{12} = 4.7$, $p = 0.0005$). These findings corroborate the more direct chemical description of LF and HF provided by studies using solid state ^{13}C NMR. Gregorich et al. (1996) showed that LF is similar in chemistry to the above-ground plant materials from which it partly originated, with the exception that the LF showed decreased carbohydrate and increased sterol concentrations. These chemical shifts were considered to be the effects of partial microbial degradation. Similarly, Golchin et al. (1994a, 1994b) found LF to be more like plant litter, while HF was much more concentrated in alkyl and carbonyl. In a series of laboratory incubations of size and density fractions, Hassink (1995a, 1995b) demonstrated that light, intermediate, and heavy macroorganic matter (>150μm size fractions) were increasingly resistant to

Table 2.3. Multiple comparisons of cumulative respiration from LF, HF, and RF at 300 d. Fisher's protected LSD test was used to identify differences in natural log-transformed data. Data presented here have been back-transformed to median values and lower (CL_L) and upper (CL_U) 95 % confidence limits.

Substrate	mg C g substrate^{-1}			mg C g initial C^{-1}		
	median†	CL_L	CL_U	median†	CL_L	CL_U
LF	12.79a	8.65	18.92	52.77a	39.52	70.10
HF	1.44c	0.98	2.14	40.94ab	30.69	54.60
RF	2.71b	1.83	4.01	34.23b	25.64	45.60

†Values within a column followed by the same letter are not significantly different ($p > 0.05$); confidence intervals are provided as a general estimate of variability, but do not convey statistical significance.

degradation with increasing density. Respiration rates from the mineral-associated C fractions (< 150 μm size fractions) were less than one third those of the macroorganic matter.

It stands to reason that a substrate composed of 30-60 percent total C would degrade far more quickly than a substrate composed of only about six percent total C. But when the cumulative respiration of each fraction was adjusted for initial C (mg C g^{-1} initial C), the relative differences between the LF, HF, and RF were much smaller ($F_{2, 12} = 6.5$, $p = 0.01$) (Table 2.3). The large difference between LF and HF was reduced to only a marginal difference ($t_{12} = 2.1$, $p = 0.057$), while the difference between HF and RF was no longer significant ($t_{12} = 1.5$, $p = 0.16$). The statistical difference between the LF and RF was preserved ($t_{12} = 3.58$, $p = 0.004$), although the respiration of the LF was only 1.5 times that of the RF. Comparing substrates on the basis of the C that could potentially be mineralized, rather than the weight of the substrate, is a more effective way of considering the molecular recalcitrance of the substrates (Sollins et al. 1996). On this basis, the LF did not appear to be substantially more labile than the HF. This directly contradicts previous research by Alvarez and Alvarez (2000). They asserted that LF is the driving force of soil respiration, and that HF only contains a small fraction of active C. However, they did not directly incubate density fractions, but instead incubated whole soil and separated fractions after the incubation. Further, HF was not directly analyzed; HF-values were calculated from the difference between LF and whole soil. These methods, absent isotope tracer techniques, do not account for possible transfer of C and nutrients between LF and HF. This may lead to an overestimate of C and N 'mineralization' from the LF.

A comparison of respiration (mg C g^{-1} initial C) at 300 d from the WS, SF, and RF ($F_{2, 12} = 6.01$, $p = 0.01$) demonstrated that RF respiration was significantly lower than both the SF ($t_{12} = 2.49$, $p = 0.03$) and the WS ($t_{12} = 3.33$, $p = 0.006$) (Table 2.4). The WS and SF were not different from each other ($t_{12} = 0.84$, $p = 0.41$). This same pattern was reproduced when the data were analyzed on a substrate-basis. This peculiar pattern may be explained within the combined context of organic matter protection in soil (e.g.:

organo-mineral interaction and limited accessibility), the importance of dissolved organic C (DOC) to respiration, and the effects of residual NaPT on respiration.

Why is the cumulative respiration of the SF similar to that of the WS? Physical protection is typically considered to be a major factor in soil C cycling (Adu and Oades 1978, Ladd et al. 1993, Golchin 1994b, Kooistra and van Noordwijk 1996, Sollins et al. 1996). If physical protection of some organic matter is a significant factor in the soil, one might reasonably expect higher respiration from the organic matter once the physical protection is removed and the organic matter is exposed to microbial attack (Sørensen 1983, Franzluebbers 1999) . If removal of physical protection played a dominant role in the incubations, the SF should actually have had higher respiration than the WS.

Alternatively, the potential loss of DOC resulting from the fractionation process could be expected to depress respiration in the fractions. Cook and Allen (1992) observed a strong, positive relationship between DOC concentrations and respiration in the first 35 d of a long-term aerobic incubation, but not thereafter. In another laboratory incubation, Sato and Seto (1999) showed that respiration was highly correlated with amounts of DOC. Jandl and Sollins (1997) identified the forest floor as a source of DOC, noting that respiration rates were one third lower at sites where the forest floor was removed. Unlike the WS, the density fractions were subjected to extensive leaching by

Table 2.4. Multiple comparisons of cumulative respiration from WS, SF, and RF at 300 d. Fisher's protected LSD test was used to identify differences in natural log-transformed data. Data presented here have been back-transformed to median values and lower (CL_L) and upper (CL_U) 95 % confidence limits.

Substrate	mg C g substrate^{-1}			mg C g initial C^{-1}		
	median†	CL_L	CL_U	median†	CL_L	CL_U
WS	4.35a	3.11	6.10	54.60	41.60a	72.10
SF	3.61a	2.58	5.07	48.62	36.93a	64.01
RF	2.71b	1.93	3.80	34.23	26.00b	45.01

†Values within a column followed by the same letter are not significantly different ($p > 0.05$); confidence intervals are provided as a general estimate of variability, but do not convey statistical significance.

NaPT and water. If the resulting loss of DOC were a significant factor, it would likely result in lower respiration in the SF and RF.

A negative response in the fractions might also originate from residual NaPT contamination (Magid et al. 1996), in spite of multiple rinses of separated fractions with water during the fractionation process. The average recovery of density separates from the soil was 100.6 percent, but ranged from 98 to 104 percent (Table 2.2). While some error in recovery estimates may originate from the estimations of gravimetric water content of the whole soil and separated fractions, it is also possible that residual NaPT is responsible for 'exceptionally good' (i.e. > 100%) recovery of soil separates. These ideas are supported by the observation that the WS had a higher rate of respiration (mg C g^{-1} remaining C day^{-1}) than the LF and HF for the first 60 d of the incubation (Figure 2.1). Additionally, active microbial biomass values (μg biomass g C^{-1}) were not statistically different in the WS, LF, and HF, for the first 60 d (p-values > 0.1), implying a more actively respiring microbial population in the WS. This is especially interesting considering the relative heterogeneity of the WS. The fine, uniformly mixed fractions should have had more exposed surface area available to microbial attack than even a sieved (<2mm) soil. The WS would be much more likely to be composed of 'hot spots' as well as less productive areas (Killham 1994, Guggenberger et al. 1999). Yet, the entire matrix of a largely accessible, homogenized fraction should have been a veritable 'hot spot,' resulting in higher rates of respiration in the SF. It seems likely that there was some actively depressive effect on the fractions; but if loss of DOC, residual NaPT toxicity, or both, were dominant factors throughout the incubation, then WS should have exhibited higher cumulative respiration at 300 d than the SF.

It is possible that an increase in accessibility did, in fact, contribute to an increase in respiration even as the removal of DOC and addition of NaPT initially depressed respiration. A repeated measures ANOVA indicated that there was a significant time by substrate interaction in the rate of respiration ($F_{15, 70} = 5.23$, $p < 0.0001$). Although the WS produced higher rates of respiration during the first 60 d of the incubation, the difference did not persist throughout the 300 d (Figure 2.1). The net effect was a similar cumulative respiration measured in SF and WS at 300 d.

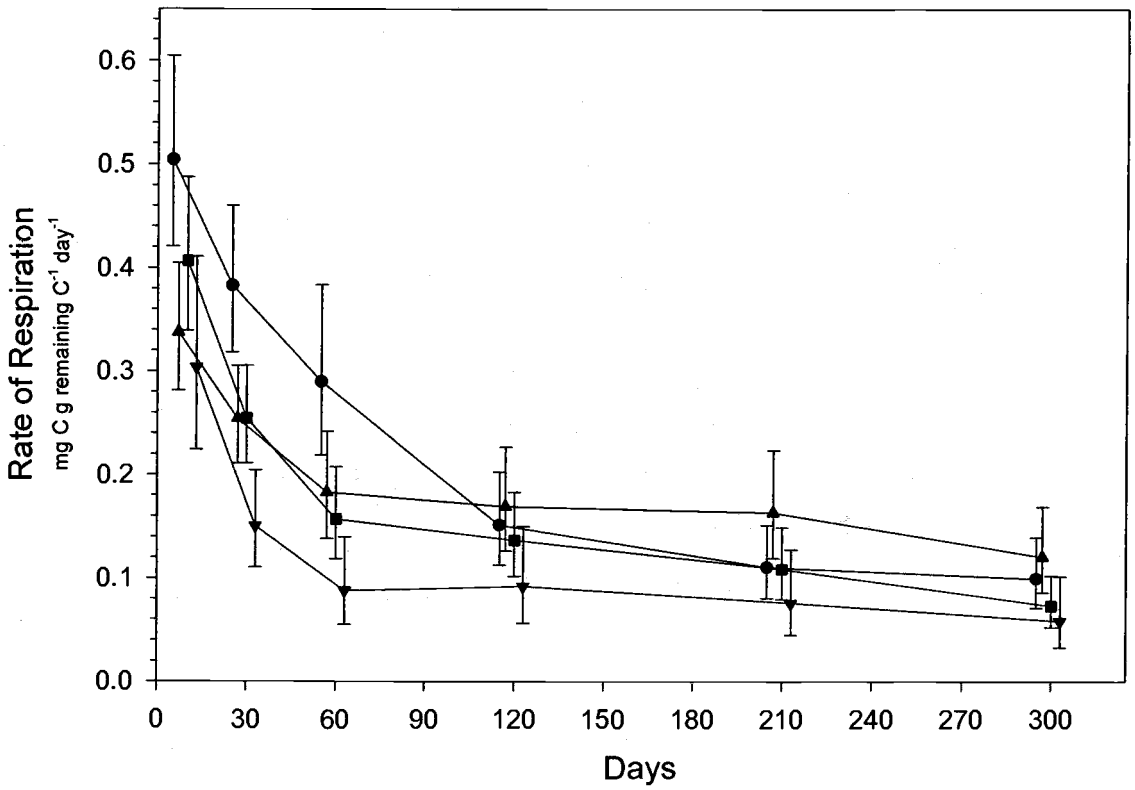

Figure 2.1. Rate of respiration through time in WS (●), RF (▼), LF (▲), and HF (■). Analyses were conducted on natural log-transformed data. The data presented here have been back transformed to median and 95 % confidence intervals. Symbols have been offset in time to avoid overlapping bars.

It is highly probable that the same factors contributing to depressed respiration in the separated fractions (LF and HF) also contributed to the depressed respiration observed in the RF. However, if these factors alone were at play the cumulative respiration of the RF should have been similar to that of the SF. The reduced respiration of the RF relative to the SF suggests that there may be one or more additional depressive factors in the RF. Since the RF was composed of the same components as the SF, but physically combined, the additional depressive factors were probably related to some aspect of the physical interaction of LF and HF.

A fundamental concept of both density and size-density fractionation is that the different fractions are spatially separated within and between soil aggregates of various es (Ladd et al. 1993). Golchin et al. (1994a, 1994b) described this spatial hierarchy as

sisting of "free" LF, residing interstitially between macroaggregates; the "occluded" LF, trapped between microaggregates; and the HF, clay- and silt- associated organic matter found mostly within microaggregates. The process of density fractionation removes this aggregation and structure.

The different chemical natures of LF and HF have been clearly established (Skjemstad et al. 1986, Golchin et al. 1994b). The present study revealed few statistical differences between substrates in active fungal and bacterial biomass estimates, partly as a result of high variability in biomass estimates. A literature search revealed no direct measures of the diversity or structure of the microbial populations degrading density or size fractions. Yet, considering the tremendous diversity of the soil microflora and the chemical dissimilarities of the LF and HF, it seems unlikely that the similar estimates of active fungi and bacteria in different substrates actually represent the same communities (Killham 1994). While many of the same species may exist in both communities, the relative proportions of the species are probably quite different. To the degree that these fractions are spatially separated, the microbial communities that degrade them are segregated (Chotte et al. 1998). Since the communities comprising the SF were completely segregated, and those comprising the RF were minimally segregated, the depressed respiration from the RF relative to the SF could have originated from some negative interaction between the different communities (Stahl and Christensen 1992, Weyman-Kaczmarkow and Wojcik-Wojtkowiak 1992, De Boer et al. 1996).

Alternatively, a purely physical interaction may have been responsible for the depressed RF respiration. The very fine particles of HF could have effectively coated fragments of the LF, inhibiting access and degradation by microorganisms. Larger fragments of LF were visible during destructive sampling, and upon casual inspection did not appear to be coated with HF. However, the small fragments were not discernable to the naked eye, and may have become trapped within flocculates upon initial wetting of the mix.

2.4.2 Net N mineralization

Net mineralization (mg N g^{-1} initial substrate) at 300 d, defined here as extractable nitrate plus extractable ammonium, followed similar patterns as C mineralization in the LF, HF, and RF (Table 2.5), but with fewer statistical differences between fractions. The F test was highly significant ($F_{2, 12} = 49.42$, $p < 0.0001$), driven by the 2.5-fold difference between the LF and the RF ($t_{12} = 8.55$, $p < 0.0001$) and the HF ($t_{12} = 9.36$, $p < 0.0001$) (Table 2.6). The RF and HF showed little evidence of a difference in net mineralization ($t_{12} = 1.79$, $p = 0.099$). When normalized by grams of initial N (mg N g^{-1} initial N), there were no significant differences between fractions ($F_{2, 12} = 0.83$, $p = 0.46$).

The high C:N ratio in LF may lead to a much higher rate of immobilization of inorganic N; thus, gross rates of N mineralization may be much higher in the LF (Sollins et al. 1984). To some extent, the results of the present study corroborate the findings of Sollins et al. (1984), who found net N mineralization from the HF to equal or exceed that of the LF. Sollins et al. (1984) directly incubated LF and HF and measured net N mineralization. They found that net N mineralization in the HF increased with increasing C:N ratio, but decreased in the LF.

Boone (1994) measured rates of potential net N mineralization from the LF and whole soil, and calculated HF values by difference. Although Boone (1994) concluded that the HF was the primary contributor to net N mineralization potential, the application

Table 2.5. Net N mineralization at 300 d in LF, HF, and RF. Fisher's protected LSD test was used to identify differences in log-transformed data. Data presented here have been back-transformed to median values and 95 % confidence limits.

Substrate	mg N g substrate^{-1}			mg N g initial N^{-1}		
	median†	CL_L	CL_U	median†	CL_L	CL_U
LF	0.24a	0.13	0.44	21.83a	14.88	31.19
HF	0.07b	0.04	0.13	21.50a	15.03	31.81
RF	0.09b	0.05	0.17	18.65a	12.81	27.11

†Values within a column followed by the same letter are not significantly different ($p > 0.05$).

of those conclusions to the present study are unclear. Analyses of variance of SF, RF, and WS on both a substrate ($F_{2, 12} = 47.10$, $p < 0.0001$) and a N ($F_{2, 12} = 70.75$, $p < 0.0001$) basis were highly significant (Table 2.6). Unlike C mineralization, SF did not differ from RF (per substrate: $t_{12} = 1.64$, $p = 0.13$; per N: $t_{12} = 1.80$, $p = 0.21$). In both cases, the WS exhibited twice the net mineralization of the fractions (per substrate: $t_{12} > 7$, $p < 0.0001$; per N: $t_{12} > 9$, $p < 0.0001$). If net N mineralization for the HF was calculated by difference from the WS and LF (Boone 1994), it would overestimate the net N mineralization of the HF in the present study by nearly three times.

Patterns of net nitrification through time varied widely by installation, but still allowed for qualitative grouping by substrate. The WS showed rapid increase in net nitrification in most installations by 30 d. Net nitrification was low in the LF at first, but increased rapidly in most installations after 60 d. The HF and RF appeared to follow similar trends to each other, in which nitrate was extractable in low quantities until 120 d, and began to increase slowly thereafter. Interpretation of these data is somewhat hampered by lack of gross nitrification data. If gross rates of nitrification are high, but rates of immobilization are also high, measures of low net nitrification could lead to the erroneous conclusion that nitrification is actually depressed (Davidson et al. 1992, Stark and Hart 1997). In the fractions, nitrification begins to increase long after the rate of respiration has stabilized at a low level (Figure 2.1). Considering the combination of low

Table 2.6. Net N mineralization at 300 d in WS, SF, and RF. Fisher's protected LSD test was used to identify differences between substrates. Tests of mg N substrate^{-1} were run on log-transformed data, and have been back-transformed to median values and 95 % confidence limits for presentation.

Substrate	mg N g substrate^{-1}			mg N g initial N^{-1}		
	median†	CL_L	CL_U	mean†	CL_L	CL_U
WS	0.23a	0.05	0.15	45.24a	38.60	51.87
SF	0.11b	0.07	0.18	23.85b	17.21	30.48
RF	0.09b	0.14	0.40	19.71b	13.07	26.34

†Values within a column followed by the same letter are not significantly different ($p > 0.05$).

respiration and increasing nitrification, it is possible that increased recalcitrance of the fractions with time allowed for greater competitiveness of autotrophic nitrifiers for ammonium (Hart et al. 1994). It is unclear why this would occur later in the HF than in the LF.

2.5 Conclusions

The density separation technique appears to be a viable method for isolating and studying different soil organic matter fractions, but these fractions should be considered more carefully in the context of microbial interaction and physical protection. In these forest soils, LF was somewhat less recalcitrant than HF, but not to the extent that chemical descriptions would suggest. Indeed, if the respiration of the SF at all approximates that of the WS, then HF is responsible for much more of the soil respiration than commonly expected. This is exlpainable in the context of the heterogeneity of soil structure and the soil microflora. A substance that is virtually unassailable to one set of organisms may be the ideal substrate to another group. It seems likely that there are different communities of microorganisms that are most efficient at degradation of the LF or HF. In well aggregated soils, these communities may be spatially segregated to the same extent that LF and HF are separated in the soil structure. If the communities are at all antagonistic or competitive, removing the spatial separation may lead to a net decrease in organic matter destabilization. Unfortunately, this scenario is little more than speculation. Future studies which incubate density or size fractions may wish to employ some type of fatty acid analysis to determine the structures of the communities degrading the fractions (Vestal and White 1989). Both the presence and abundance of different types of organisms could be compared between fractions and the whole soil. Differences between fractions and similarities to the whole soil may shed light on the relative importance and function of the fractions in the whole soil. Use of the same methodologies in a RF would yield important information on the interaction of these communities with each other and with soil structure.

2.6 Literature Cited

Adu, J.K., and J.M. Oades. 1978. Physical factors influencing decomposition of organic materials in soil aggregates. Soil Biol. Biochem. 10:109-115.

Alvarez, R., and C.R. Alvarez. 2000. Soil organic matter pools and their associations with carbon mineralization kinetics. Soil Sci. Soc. Am. J. 64:184-189.

Boone, R.D. 1994. Light-fraction soil organic matter: Origin and contribution to net nitrogen mineralization. Soil Biol. Biochem. 26:1459-1468.

Barrios, E., R.J. Buresh, and J.I. Sprent. 1996. Nitrogen mineralization in density fractions of soil organic matter from maize and legume cropping systems. Soil Biol. Biochem. 28:1459-1465.

Cabrera, M.L., and M.H. Beare. 1993. Alkaline persulfate oxidation for determining total nitrogen in microbial biomass extracts. Soil Sci. Soc. Am. J. 57:1007-1012.

Chotte, J.L., J.N. Ladd, and M. Amato. 1998. Sites of microbial assimilation, and turnover of soluble and particulate ^{14}C-Labelled substrates decomposing in a clay soil. Soil Biol. Biochem. 30:205-218.

Cook, B.D., and D.L. Allan. 1992. Dissolved organic carbon in old field soils: Total amounts as a measure of available resources for soil mineralization. Soil Biol. Biochem. 24:585-594.

Cambardella, C.A., and E.T. Elliot. 1993. Methods for physical separation and characterization of soil organic matter fractions. Geoderma 56:449-457.

Christensen, B.T. 1992. Physical fractionation of soil and organic matter in primary particle size and density separates. Adv. Soil Sci. 20:1-90.

Davidson, E.A. S.C. Hart, and M.K. Firestone. 1992. Internal cycling of nitrate in soils of a mature coniferous forest. Ecology. 73:1148-1156.

De Boer, W., P.J.A. Klein Gunnewiek, and D. Parkinson. 1996. Variability of N mineralization and nitrification in a simple, simulated microbial forest soil community. Soil Biol. Biochem. 28:203-211.

Franzluebbers, A.J. 1999. Potential C and N mineralization and microbial biomass from intact and increasingly disturbed soils of varying texture. Soil Biol. Biochem. 31:1083-1090.

Golchin, A., J.M. Oades, J.O. Skjemstad, and P. Clarke. 1994a. Study of free and occluded particulate organic matter in soils by solid-state ^{13}C NMR spectroscopy and scanning electron microscopy. Aust. J. Soil Res. 32:285-309.

Golchin, A., J.M. Oades, J.O. Skjemstad, and P. Clarke. 1994b. Soil structure and carbon cycling. Austr. J. Soil Res. 32:1043-1068.

Golchin, A., J.M. Oades, J.O. Skjemstad, and P. Clark. 1995. Structural and dynamic properties of soil organic matter as reflected by ^{13}C NMR spectroscopy in density fractions of an Oxisol under forest and pasture. Aust. J. Soil Res. 33:59-76.

Greenland, D.J., and W. Ford. 1964. Separation of partially humified organic materials by ultrasonic dispersion. Eighth Intern. Congress of Soil Sci. 3:137-148.

Gregorich, E.G., C.M. Monreal, M. Schnitzer, and H.R. Schulten. 1996. Transformation of plant residues into soil organic matter: chemical characterization of plant tissue, isolated soil fractions, and whole soils. Soil Science. 161:680-693.

Guggenberger, G., E.T. Elliott, S.D. Frey, J. Six, and K Paustian. 1999. Microbial contributions to the aggregation of a cultivated grassland soil amended with starch. Soil Biol. Biochem. 31:407-419.

Hart, S.C., G.E. Nason, D.D. Myrold, and D.A. Perry. 1994. Dynamics of gross nitrogen transformations in an old-growth forest soil: the carbon connection. Ecology 75:880-891.

Hassink, J. 1995a. Decomposition rate constants of size and density fractions of soil organic matter. Soil Sci. Soc. Am. J. 59:1631-1635.

Hassink, J. 1995b. Density fractions of soil macroorganic matter and microbial biomass as predictors of C and N mineralization. Soil Biol. Biochem. 27:1099-1108.

Hazard, J.W., and C.E. Peterson. 1984. Objectives and analytical methods of the Regional Forest Nutrition Project. Inst. Forest Resources Contribution no. 53. College of Forest Resources, Univ. of Washington, Seattle.

Ingham, E.R., and D.A. Klein. Soil fungi: measurement of hyphal length. Soil Biol. Biochem. 16:279-280.

Jandl, R., and P. Sollins. 1997. Water-extractable soil carbon in relation to the belowground carbon cycle. Biol. Fert. Soils 25:196-201.

Killham, K. 1994. Soil Ecology. p. 34-88. Cambridge University Press, Cambridge. 242 p.

Kooistra, M.J., and M. van Noordwijk. 1996. Soil architecture and distribution of organic matter. p. 15-53. *In* M.R. Carter and B.A. Steward (ed.) Structure and soil organic matter storage in agricultural soils. CRC Press Inc., Boca Raton, FL.

Ladd, J.N., R.C. Foster, and J.O. Skjemstad. 1993. Soil structure: carbon and nitrogen metabolism. Geoderma 56:401-434.

Ladd, J.N., M. van Gestel, L. Jocteur Monrozier, and M. Amato. 1996. Distribution of organic ^{14}C and ^{15}N in particle-size fractions of soils incubated with ^{14}C, ^{15}N-labelled glucose/NH_4, and legume and wheat straw residues. Soil Biol. Biochem. 28:893-905.

Magid, j., A. Gorissen, and K.E. Giller. 1996. In search of the elusive "active" fraction of soil organic matter: three size-density fractionation methods for tracing the fate of homogeneously ^{14}C-labelled plant materials. Soil Biol. Biochem. 28:89-99.

McGill, W.B., K.R. Cannon, J.A. Robertson, and F.D. Cook. 1986. Dynamics of soil microbial and water-soluble organic carbon in Brenton L after 50 years of cropping two rotations. Can. J. Soil Sci. 66:1-19.

Meijboom, F., J. Hassink, and M. van Noordwijk. 1995. Density fractionation of soil organic matter using silica suspensions. Soil Biol. Biochem. 27:1109-1111.

Sato, A., and M Seto. 1999. Relationship between rate of carbon dioxide evolution, microbial biomass carbon, and the amount of dissolved organic carbon as affected by temperature and water content of a forest and an arable soil. Communications in Soil Science and Plant Analysis. 30:2593-2605.

Skjemstad, J.O., R.C. Dalal, and P.F. Barron. 1986. Spectroscopic investigations of cultivation effects on organic matter of Vertisols. Soil Sci. Soc. Amer. J. 50:354-359.

Sollins, P., P. Homann, and B.A. Caldwell. 1996. Stabilization and destabilization of soil organic matter: mechanisms and controls. Geoderma. 74:65-105.

Sollins, P., C. Glassman, E.A. Paul, C. Swanston, K. Lajtha, J.W. Heil, and E.T. Elliott. 1999. Soil Carbon and Nitrogen: Pools and Fractions. In Standard Soil Methods for Long-Term Ecological Research. Robertson, G. P., C. S. Bledsoe, D. C. Coleman, and P. Sollins, eds. Oxford University Press, New York. pp. 89-105.

Sollins, P., G. Spycher, and C.A. Glassman. 1984. Net nitrogen mineralization from light- and heavy-fraction forest soil organic matter. Soil Biol. Biochem. 16:31-37.

Sørensen, L.H. 1983. The influence of stress treatments on the microbial biomass and the rate of decomposition of humified material in soils containing different amounts of clay. Plant Soil 75:107-119.

Spycher G., P. Sollins, and S. Rose. 1983. Carbon and nitrogen in the light fraction of a forest soil: vertical distribution and seasonal patterns. Soil Sci. 135:79-87.

Stahl, P.D., and M. Christensen. 1992. In vitro mycelial interactions among members of a soil microfungal community. Soil Biol. Biochem. 24:309-316.

Stamatiadis, S., J.W. Doran, and E.R. Ingham. 1990. Use of staining to separate fungal and bacterial activity in soil. Soil Biol. Biochem. 22:81-88.

Stark, J.M., and S.C. Hart. 1997. High rates of nitrification and nitrate turnover in undisturbed coniferous forests. Nature. 385:61-64.

Stevenson, F.J. 1994. Extraction, fractionation, and general chemical composition of soil organic matter. p. 1-58. *In* Humus Chemistry: Genesis, composition, reactions. Second Edition. John Wiley and Sons, Inc., New York, NY. 496 p.

Stotzky, G. 1965. Microbial respiration. p. 1550-1572. *In* C.A. Black (ed.) Methods of soil analysis. Part 2. Agron. Monogr. 9. ASA, Madison, WI.

Strickland, T.C. and P. Sollins. 1987. Improved method for separating light- and heavy-fraction organic material from soil. Soil Sci. Soc. Am. J. 51:1390-1393.

Van Veen, J.A. and P.J. Kuikman. 1990. Soil structural aspects of decomposition of soil organic matter by microorganisms. Biogeochem. 11:213-234.

Vestal, J.R., and D.C. White. 1989. Lipid analysis in microbial ecology: quantitative approaches to the study of microbial communities. Bioscience. 39:535-541.

Weyman-Kaczmarkowa, W., and D. Wojcik-Wojtkowiak. 1992. Microbial degradation of plant materials and allelochemicals formation in different soils. Developments in agricultural and managed-forest ecology. 25:127-136

CHAPTER 3

3. Influence of Long-Term Nitrogen Fertilization on Carbon and Nitrogen Dynamics in Forest Soils and Density Fractions

Christopher W. Swanston, Peter S. Homann, Phillip Sollins
Bruce Caldwell, David D. Myrold, Mark G. Johnson

3.1 Abstract

Numerous studies have indicated that increased C:N ratios may affect the decomposition of different types of organic matter in contrasting ways. A conceptual model is presented which proposes that labile organic matter degrades more quickly with decreasing C:N ratio, while recalcitrant organic matter degrades more slowly. Using a series of paired plots in which one of each pair had undergone long-term N fertilization, we sought to determine the effects of elevated N on the stability of C in forest soils. Soils and organic fractions were subjected to a 300 d incubation in which respiration, net N mineralization, and microbial biomass, were measured. We hypothesized that the O2 horizon and light fraction (LF) would exhibit increased respiration with decreased C:N, and that respiration in the whole soil (WS), heavy fraction (HF), and physically recombined fraction (RF), would be depressed with decreased C:N. In all substrates, however, elevated N resulted in lower cumulative respiration. Net N mineralization between N-treatments was similar. Additionally, LF showed only marginally higher respiration than HF, regardless of C:N ratio. The mechanisms most effective at degrading these substrates are negatively affected by elevated N, but may not be the same for each substrate. Laboratory incubations of fertilized forest soils have shown the potential for N to stabilize soil C. While laboratory results may not survive the variability of the natural environment, the potential for elevated N to stabilize C in soil suggests the need for more detailed field measurements.

3.2 Introduction

Fertilization with N is an increasingly important and widespread practice in forests of the Pacific Northwest (Allen 1987, Hermann and Lavender 1999). The effects of N fertilization on tree growth are generally positive in the first years following amendment (Hopmans and Chappell 1994, Weetman et al. 1997), but the effects on soil nutrient cycling are less well understood (Smolander et al. 1994). Berg (1986) hypothesized that fertilization with N would lead to greater organic matter (OM) stability, resulting in a depression of the rate of nutrient cycling and reduction the relative growth rate of fertilized conifers in the long-run. Fog (1988) addressed the first part of this hypothesis

with an exhaustive review covering the effects of increased N on organic matter decomposition in litter and soil. Citing dozens of seemingly contradictory papers, Fog (1988) noted that numerous studies had found a negative N effect, while others reported a neutral or positive effect of N on OM decomposition. Through careful consideration of the type of substrate being degraded, the C and N concentrations in the substrate, and the decomposition-strategies of organisms thought to be the primary degraders of the substrate, he was able to merge the disparate studies into an integrated empirical model. According to this model, addition of N increases the rate of degradation of labile OM with low C:N, but decreases the rate of decomposition of recalcitrant OM with high C:N ratios.

This empirical model has been broadened into a more general conceptual model, summarized in Figure 3.1. Organic matter destabilization is defined as increasing the potential for OM loss through respiration, leaching, or conversion into another OM component (Sollins et al. 1996). Although OM is often referred to as 'labile' or 'recalcitrant,' a discrete sample of OM may include both labile and recalcitrant components (McClaugherty and Berg 1988, Paul and Clark 1989, Zech and Guggenberger 1996). Measures of mass loss and respiration of a whole sample may be

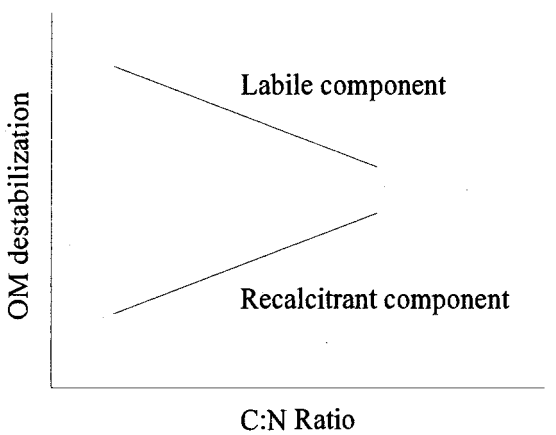

Figure 3.1. Conceptual model describing the destabilization of OM. Increasing C:N ratio affects the destabilization of 'Labile' and 'Recalcitrant' components of OM in an opposite manner.

dominated by the activity of one component of the OM at a specific point in time, but both components are probably degraded simultaneously (McGill and Cole 1981, Rayner and Boddy 1988, Harmon et al. 1990, Van Veen and Kuikman 1990, Killham 1994, Entry and Blackman 1995, Andrén and Kätterer 1997).

Densimetric methods are commonly used to physically separate two fractions of the soil organic matter considered to represent the labile and recalcitrant components (Christensen 1992, Hassink 1995a). Density fractionation involves disrupting soil aggregation within a dense liquid medium, allowing a 'light fraction' to float to the surface where it is collected, followed by collection of the sedimentary 'heavy fraction' (Sollins et al. 1999). The light fraction (LF) is chemically and visually similar to litter (Spycher et al. 1983, Skjemstad et al. 1986, Golchin 1994a). The heavy fraction (HF) is composed of organo-mineral complexes and humified C (Young and Spycher 1983, Golchin 1994b, Golchin 1995). LF is typically considered the "active" component and the primary source of heterotrophic respiration and N mineralization in soil (Spycher et al. 1983, Theng et a. 1989, Hassink 1995b). Conversely, the HF is considered to be the more recalcitrant component of soil organic matter. Although the HF may contain the bulk of the soil C and N, mineralization of the HF is thought to be severely curtailed by physical protection and chemical recalcitrance (McGill et al. 1986, Alvarez and Alvarez 2000).

In Chapter 2, the link between the concept and the methodology of density fractionation was considered. The general conclusion was that density fractionation is a viable method of separating two distinct soil OM components, but that the way in which the conceptual model for density fractionation describes the two OM components should be partially adjusted. As described by Golchin et al. (1994), LF may reside primarily in interstitial spaces, while microaggregates are primarily composed of HF. Light- and heavy-fractions are not only separated spatially in the soil structure, but probably also host different microbial community structures. Thus, in some soils, LF and HF may not differ in chemical recalcitrance, but instead owe differences in stability to their arrangement in soil structure and to their interaction with different microbial

communities. In the present study, the fit of the conceptual model is further tested by measuring the effect of increased N on the fractions.

To determine the effect of incorporated N on OM decomposition, litter and soils were collected from a series of paired N-fertilized and unfertilized plots in second growth Douglas-fir forests. The plots were amended with N periodically between 1969 and 1986, and the soil and litter collected in 1995. The soils were separated into light- and heavy-fractions. The litter, whole soil, and density fractions were incubated for 300 d to determine the effect of elevated N on C lability in the fractions and the whole soil. It was hypothesized that the O2 horizon and LF would exhibit increased decomposition with added N, while C in the HF would become more stable. Whole soil, composed primarily of HF, was expected to manifest reduced C destabilization.

3.3 Materials and Methods

3.3.1 Site Characteristics

Soils were collected from seven forested sites in western Washington and Oregon (Table 3.1). The installations were part of a large network established by the Stand Management Cooperative (SMC) Nutrition Project (Hazard and Peterson 1984) in 1969 to evaluate the response of these forests to fertilization. The overstory of each of the installations used in the present study was composed of 46 to 72-year old second-growth Douglas-fir (*Pseudotsuga menziesii* (Mirb). Franc) at the time of sampling. Each installation contained a N-fertilized and a non-fertilized paired plot.

Fertilized plots were amended with urea N at periodic times over a 16 yr period. The plots received 448 kg ha^{-1} at plot establishment in 1969, followed by an additional 224 kg ha^{-1} in years 8, 12, and 16, for a cumulative total of 1120 kg ha^{-1}. Two plots, at installations 1 and 43, did not receive the N addition in year 16, so had totals of only 896 kg ha^{-1}.

The criteria used to choose sites were designed to help focus the study on the effects of an increase in N concentration relative to C concentration. Installations were thus chosen on the basis of 1) lower C:N ratio in the whole soil in the fertilized plots

Table 3.1. Characteristics of seven Stand Management Cooperative installations. The installation number is in parentheses after the name.

Installation	Location	Great group	Parent Material	Textural class	Plot	Soil (0-5 cm)			O2 horizon		
						% C	% N	C:N	% C	% N	C:N
Cedar Falls (1)	47.410 N 121.815 W	Durochrept	Glacial till	gravelly loam	Ctrl	10.60	0.72	15	19.0	0.82	23
					Urea	11.69	0.83	14	23.9	1.19	20
Deep Creek (20)	45.962 N 123.307 W	Haplumbrept	Glacial sediments	loam	Ctrl	5.10	0.45	11	28.8	1.25	22
					Urea	5.07	0.57	10	20.0	1.02	20
Skykomish (43)	47.735 N 121.249 W	Haplorthod	Granite	fine sand	Ctrl	3.91	0.24	16	37.1	1.20	30
					Urea	3.69	0.27	13	35.7	1.62	22
Headquarter Camp (57)	46.219 N 122.732 W	Haplohumult	Igneous	loam	Ctrl	10.30	0.72	14	25.1	1.17	21
					Urea	9.30	0.73	13	23.1	1.00	23
Fourth Creek (65)	43.804 N 122.381 W	not classified	Pumice	loam	Ctrl	8.13	0.47	17	28.9	1.05	27
					Urea	9.44	0.65	14	31.3	1.21	26
Elk Creek (89)	43.365 N 123.868 W	Haplumbrept	Sandstone	loam	Ctrl	10.59	0.65	16	19.3	0.83	23
					Urea	7.71	0.65	12	18.6	0.98	19
Cristy Falls (103)	43.904 N 122.301 W	not classified	Pumice and ash	loam	Ctrl	10.81	0.60	18	31.5	1.36	23
					Urea	11.20	0.75	15	22.1	1.10	20

compared to control plots, and 2) the similarity in the C concentration of both plots at a specific installation. Variable C concentration between installations was considered acceptable, but similar C concentration between plots at an installation was considered necessary. To adequately test the hypothesis that elevated N stabilizes C, all conditions must be the same except the amount of N in the OM. If C also varies, especially if there is more C in the N-amended plots, the 'extra' C in one plot may not be as stable because of less interaction with mineral surfaces. Data on C, N and density fractions from Homann et al. (unpublished data, 2000) were initially used to decide which installations would be included in the present study. Data on soils and fractions used in the present study were collected as the incubations were set up and initiated.

3.3.2 Soil Sampling and Storage

Soils were collected in August and September, 1995. Plots were sampled using a 5 x 5 grid with a 4.5 m spacing, beginning 1.4 m from the corner of each 20 x 20 m plot. At each sampling point, the O1 and O2 horizons were removed separately, and a 10 x 10 cm section of the 0-5 cm mineral soil was sampled from the side of a mini soil pit. O2 and mineral soil samples from alternating grid points were immediately composited and sieved (< 2mm) into a homogeneous fine fraction. Samples from the remaining grid points were similarly composited and sieved, resulting in two composited samples per strata per plot. Moist subsamples of this fine fraction were removed and transported to the Forestry Sciences Laboratory in Corvallis, OR. Mineral soils were tightly sealed in plastic bags and stored frozen at -20°C (Meijboom et al. 1995, Magid et al. 1996, Sollins et al. 1999). O2 samples were oven dried (60 °C) and stored in sealed plastic bags.

3.3.3 Density Fractionation

The method of density fractionation is explained in depth in Chapter 2. The method was adapted from that described by Sollins et al. (1999). Field-moist mineral soils were placed in a beaker with sodium polytungstate (NaPT, Sometu-US, Van Nuys, CA; adjusted to 1.65 g mL^{-1}), mixed with a benchtop mixer, and allowed to settle for 48 hr. Light fraction was aspirated from the surface of the NaPT, and then the separation

Table 3.2. Percent recovery, C, and N in light fractions, heavy fractions, and physically recombined fractions.

Installation		Amount of soil fractionated (g dry wt)	Recovery %	Light Fraction				Heavy Fraction				Recombined Fraction		
				% of recovered	%C	% N	C:N	% of recovered	% C	% N	C:N	% C	% N	C:N
1	Cntrl	417.27	100.74	18.28	28.62	1.30	22	81.72	5.63	0.41	14	10.80	0.63	17
	Urea	415.83	98.71	21.44	28.22	1.43	20	78.56	5.59	0.47	12	12.60	0.78	16
20	Cntrl	691.57	100.13	8.68	25.83	1.11	23	91.32	3.28	0.38	9	4.70	0.40	12
	Urea	696.72	100.44	8.03	24.37	1.30	19	91.97	3.59	0.37	10	5.40	0.43	13
43	Cntrl	704.60	101.65	16.90	16.20	0.68	24	83.10	1.09	0.11	10	3.70	0.20	19
	Urea	704.55	100.39	17.04	16.24	0.84	19	82.96	0.89	0.10	9	6.70	0.24	15
57	Cntrl	423.09	98.69	17.99	28.00	1.50	19	82.01	5.23	0.51	10	9.72	0.70	14
	Urea	420.74	97.49	16.02	21.40	1.25	17	83.98	4.42	0.47	9	8.49	0.70	12
65	Cntrl	423.45	98.98	20.98	23.49	1.05	22	79.02	3.10	0.29	11	9.32	0.50	19
	Urea	429.50	100.69	18.82	28.00	1.55	18	81.18	2.80	0.28	10	8.80	0.58	15
89	Cntrl	500.11	99.03	17.12	26.41	1.24	21	82.88	5.29	0.46	12	10.36	0.61	17
	Urea	424.21	99.76	15.72	22.49	1.30	17	84.28	3.90	0.39	10	8.13	0.62	13
103	Cntrl	366.71	105.06	32.63	24.47	1.00	24	67.37	4.10	0.41	10	11.31	0.58	19
	Urea	424.35	103.40	24.41	30.52	1.70	18	75.59	4.30	0.42	10	9.91	0.64	15

procedure was repeated on the sediment. Light- and heavy fractions were rinsed repeatedly in distilled water to remove NaPT contamination, followed by oven-drying at 50 °C for 48 and 72 hr, respectively. Dry (50 °C) heavy fraction material was ground with a mortar and pestle to remove drying-related aggregation. Characteristics of the density fractions are provided in Table 3.2.

3.3.4 Substrates

Analysis of substrates are explained in more depth in Chapter 2. Five substrates were analyzed: the O2 horizon (O2), the whole soil (WS), the light fraction (LF), the heavy fraction (HF), and the physically recombined fraction (RF). For the LF and O2, 2.00 g (dry weight equivalent) of substrate was mixed with 18.00 g (dry weight) of acid-washed quartz sand and placed in a glass scintillation vial. Similarly, for WS and HF, 10.00 g of substrate was mixed with 10.00 g sand. The RF was created by mixing LF and HF back together in the proportions from which they were recovered from the soil (Table 3.2), and combining 10.00 g of mixture with 10.00 g of sand. Actual weights were tracked to the nearest 0.001 g. Sand was added to the C sources for two reasons. The addition of sand yielded a larger sample that permitted more flexibility in the type and number of physical analyses that could be performed. In the case of the HF sand was added to create some degree of physical structure, possibly ameliorating any anoxic conditions that might occur during the incubation. In the using the sand, we assume it was inert and underwent no appreciable weight change during the course of the incubation. The WS was produced and begun incubating within 24 hr of the soil thawing.

Total initial C and N were measured on day 1 of substrate incubation (see below) and values were corrected for content of the added sand. These analyses were carried out on a Leco CNS Analyzer (Leco, St. Joseph, MI) by the Central Analytical Lab (Corvallis, OR).

3.3.5 Incubation

Substrates were incubated for 300 d at 20 °C (± 1 °C). For LF, HF, and RF, substrates and sand were placed in open 20 mL glass scintillation vials, and six vials

were placed in sealed 1 L mason jars (Chapter 2, Stotzky 1965, Hart et al. 1994). All vials within a jar contained the same substrate from the same installation. To adequately contain the WS, three 60 mL vials were used in each of two mason jars. The multiple vials allowed periodic destructive sampling.

The headspace of each jar was sampled through a septum installed in the cap of the jar. Before each sample was drawn, the air in the headspace was mixed by repeatedly and vigorously drawing air into a gas-tight syringe and plunging the air back into the jar. After mixing, a 500 µL sample was drawn and immediately injected into a GC-8A Series Shimadzu gas chromatograph with a 6 ft x 2 mm (ID) Porapak Q 80/100 column. Once the sampling of a jar was completed, the jar was flushed with CO_2-free air and resampled to obtain a baseline CO_2 concentration. Respiration was measured in the headspace of the jars at intervals (1-21 d) sufficient to maintain a CO_2 concentration of less than two percent (Šantrůčková and Šimek 1994, Šantrůčková and Šimek 1997).

Destructive sampling of the vials occurred at seven times: days 1, 10, 30, 60, 120, 210, and 300. At each time, a single vial from each installation/substrate combination was removed from the jar. The substrate in the vial was removed, thoroughly mixed, then allocated to tests for N mineralization, active biomass, extra-cellular enzyme activity (not included in the present study), and gravimetric water content.

Extractable N was measured by extracting moist substrate with 0.5 *M* K_2SO_4 at a 1:5 (g:mL) ratio of substrate to K_2SO_4. The extraction process consisted of shaking the substrates in the K_2SO_4 for 1 hr, allowing the slurries to settle for 0.5 hr, then pouring the supernatants through acid-rinsed Whatman no. 1 filters. Extracts were stored frozen at -20 °C until analysis. Once thawed, samples were shaken for 0.25 hr and then aliquots removed for total N determination and for ammonium and nitrate measurement. Total N was obtained using the persulfate oxidation method proposed by Cabrera and Beare (1993). Nitrate was measured on an Astoria Pacific Analyzer (Astoria Pacific Int'l, Astoria, OR) and ammonium on an Alpkem Flow Solution (OI Corporation, College Station, TX). Extractable-organic N was calculated by subtracting extract ammonium and nitrate from total N.

Active microbial biomass was obtained through direct microscopy (Soil Food Web, Inc., Corvallis, OR). Substrates were diluted and stained with fluorescein diacetate, allowing active bacteria and fungi to be viewed and measured with an epifluorescent microscope (Ingham and Klein, 1984; Stamatiadis et al., 1990).

3.3.6 Data Management and Analysis

'Remaining C' was calculated at the time of each respiration measurement by subtracting the cumulative respiration to that point from the initial C. For each time point, the mg C respired g remaining^{-1} day^{-1} was calculated by subtracting mg C respired g remaining^{-1} of the previous time point from the present time point, then dividing by the number of days separating the time points.

For cumulative C respiration and net N mineralization and nitrification, the statistical analysis was a split plot design. The installations were blocks, the source of replication. Control and fertilized plots were 'whole plots,' the largest experimental units. Substrates could not be 'randomly applied,' and were subject to the treatment applied to the whole plot from which they were derived. Thus, the substrates from each whole plot were 'split-plots,' the smallest experimental unit. Although duplicates of all substrates from each installation were incubated, the duplicates were only created to ensure against sample loss through accident. Prior to statistical analysis, the values for each set of duplicates were averaged, and those means treated as single replicates in statistical analyses.

Prior to all statistical tests, plots of residuals were examined for constant variance. Most of the datasets required natural logarithm transformations to stabilize variance. When an analysis of variance (ANOVA) resulted in a p-value of less than 0.1, pre-planned comparisons of interest were made using contrasts. Analysis of microscopy data and changes in rate of C mineralization involved comparisons of data both at a time point and between time points. Although different vials were measured each time, the same installation and substrate in each jar were being repeatedly measured through time, necessitating the use of a repeated measures ANOVA. Means and confidence limits generated by the ANOVA's are back-transformed and provided in several figures and

tables to illustrate trends and variability in the data. The reader should be cautioned, however, that the 95 percent confidence intervals of the observations are not the same as the 95 percent confidence intervals of the differences between observations. These latter differences, calculated at the whole-plot and split-plot levels within blocks, adjust for blocking error that is not accounted for in the confidence intervals plotted in the figures. Most data manipulation, and all statistical analyses, were conducted in SAS (SAS Institute, Inc., version 6.12).

3.4 Results and Discussion

3.4.1 Pre-incubation

An ANOVA did not provide convincing evidence of an interaction between N-treatment and substrate, indicating that fertilization did not affect the C:N ratios differently in any of the substrates ($F_{4, 48} = 1.19$, $p = 0.12$). Any suggestion of interaction most likely arises from C:N ratios in the HF, which did not appear to be as divergent between treatments as those in the other fractions. The overall lack of interaction may be primarily related to the long-term nature of the N-amendments at the SMC installations. While N has been incorporated into the LF and HF in different amounts in 'short term' studies of a few days to a few years (Strickland et al. 1992, Swanston and Myrold 1998), the 26-yr length of the SMC experiments might be expected to result in patterns somewhat different from shorter studies. In the 10-15 yr between the last fertilization and the collection of soils for the present study, the added N has had the opportunity to cycle several times through some of the organic matter, and to become thoroughly incorporated into the various fractions. Additionally, blocks in the present study were not chosen randomly, but rather on the basis of similarity in C concentration between N-treated plots at an installation. This criterion may have introduced bias, and certainly limited the range of inference of the results. Thus the effects of fertilization on the C:N ratios of density fractions, as presented here, should be applied outside the context of this study with great caution. The goal of the present study was to investigate in effects of increased N on C stability, not to research the effects of fertilization on site

characteristics, such as C:N ratios in soil fractions (Homann et al. unpublished data 2000).

As expected, N fertilization resulted in about 20 percent lower C:N ratios in the substrates from the amended plots ($F_{1,6} = 29.85$, $p = 0.002$), and there were significant differences in the C:N ratios between the five different substrates at the split-plot level ($F_{4,48} = 115.38$, $p < 0.0001$). Because there was no interaction between N-treatment and substrate type, comparisons of substrates were made using average values of fertilized and non-fertilized substrates. A contrast of LF and HF produced the predictable result that LF had about double the C:N ratio of the HF ($t_{48} = 15.03$, $p < 0.0001$). Comparison of C:N ratios in the RF and WS produced marginal evidence that N was preferentially lost during the fractionation process, as the C:N ratio of the RF was about eight percent higher than the WS ($t_{48} = 1.87$, $p = 0.067$). This finding lends further support to the assertion that nutrient values in the WS, LF, and HF should be measured directly, and not calculated by difference (Swanston and Myrold, 1998). The C:N ratio in the O2 substrate was about 12 percent higher than that in the LF ($t_{48} = 2.74$, $p < 0.0001$). On first impression, this would seem to indicate that the LF has undergone greater microbial degradation, which might include higher degrees of N conservation and import. However, since C and N may have been lost at different rates during the density fractionation process, it is difficult to ascertain the extent to which the smaller C:N ratio indicates greater decomposition of the LF.

3.4.2 Carbon Mineralization

An analysis of variance of total cumulative respiration (mg C g initial C^{-1}) at 300 d revealed no significant interaction between N-treatment and substrate, indicating that all five substrates responded similarly to elevated N ($F_{4,48} = 0.23$, $p = 0.92$). The response to elevated N was negative in all substrates ($F_{1,6} = 5.42$, $p = 0.0588$). Although previous work had indicated that the LF and HF may exhibit similar recalcitrance (Chapter 2), it was expected that the hypothetically different populations degrading each would respond differently to elevated N. The O2, especially, was expected to respond positively to elevated N.

Prescott et al. (2000) studied dynamics of C, N, and mass in litterfall, litter and soil of nine Douglas-fir forests in western Oregon and Washington. They found that decreasing C:N ratio in the soil positively influenced litter decomposition. Four of their sites were in common with those of the present study (installations: 1, 20, 43, 57). Their study incorporated only non-fertilized plots at the installations, but the installations included a natural range of C:N ratios in soil and litter. As can be seen in Figure 3.2, respiration from the control O2 material collected from installations 1, 20, 43, and 57 respired more with decreasing C:N ratio (Table 3.1) of the O2 substrate. This trend is preserved in the elevated-N O2 substrates from fertilized plots, but the elevated N appeared to depress the respiration in those substrates. Installations 65, 89, and 103 do not appear to follow the trend of higher respiration with lower C:N in the substrate. Prescott et al. (2000) cautioned that differences between sites in the understory species may have contributed to the relationship between soil C:N ratio and litter decomposition. Several other potential site-related influences were also considered. Results from the present study, which allowed for direct comparisons of the effects of increased N at an installation, seem to indicate that site factors other than soil C:N may indeed have been largely responsible for the observed positive relationship between soil C:N ratio and litter decomposition. Specifically, Salal (*Gaultheria shallon* Pursh) and Oregon grape (*Mahonia nervosa* (Pursh) Nutt.) at the N-poor sites may have produced a more lignin-rich litter input to the forest floor than the sword fern (*Polystichum munitum* (Kaulf.) Presl.) at the N-rich sites.

While the O2 substrate was composed of degraded litter, and LF has been described as having both the appearance (Spycher et al. 1983) and chemical nature (Skjemstad et al. 1986) of degraded litter, it would seem that lignin concentration and the degree of previous decomposition of the OM may be of prime importance. Berg (1986) described Scots pine (*Pinus silvestris*) litter as undergoing at least two phases of decomposition. The first phase is characterized by the rapid destabilization of labile OM components such as non-lignified carbohydrates and soluble organic C, and is enhanced by high N concentrations. Following loss of most of the non-lignified C, the second phase is dominated by the degradation of the residual lignin. High nitrogen concentrations inhibit

decomposition of these more recalcitrant components (Berg & McClaugherty 1987, Melillo et al. 1989). Harmon et al. (1990) confirmed these trends in several species, and combined the two phases of decomposition into a second order kinetic equation. They observed that litter with high initial concentrations of N had the highest initial rates of decomposition. Species with the lower N concentrations, however, had higher rates of decomposition in later months, presumably when mass loss was dominated by lignin decomposition. Short-term studies researching the effect of added N on litter

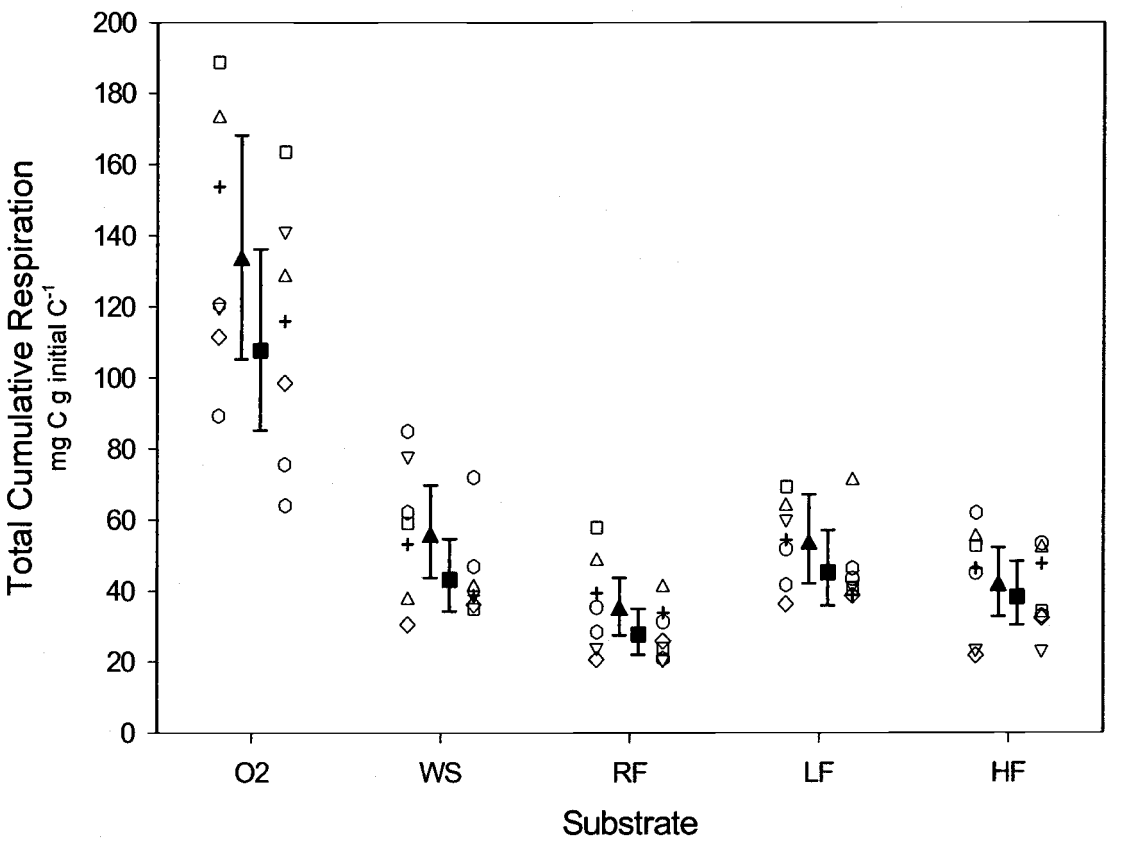

Figure 3.2. Total cumulative respiration (mg C g initial C^{-1}) from O2, WS, RF, LF, and HF substrates at 300 d. Data were transformed by natural logarithm, analyzed, then back-transformed for presentation here. Closed symbols represent median values for control (▲) and fertilized (■) substrates, and bars represent 95 percent confidence intervals. Open symbols to the left of each set of medians represent values from control plots, while symbols to the right represent values from fertilized plots. Specific installation designations are 1 (+), 20 (□), 43 (○), 57 (△), 65 (⬡), 89 (◇), and 103 (▽). Please note that confidence intervals are provided as a general estimate of variability, but do not convey statistical significance.

decomposition may be more likely to see an initial positive effect of N on mass loss, and even a positive cumulative effect, since the study may not out-last the decomposition of non-lignified C in the litter (Fog, 1988). Conversely, the O2 and LF in the present, long-term study, may have been rapidly deprived of the labile, high-N, non-lignified C in the first years after the last fertilization. At the time the litter and soils were collected for the incubation, the bulk of the remaining C in the O2 and LF may have been lignified. Berg (1988) traced the fate of ^{15}N in labeled litter in a Scots pine forest. He noted that ^{15}N was conserved in the litter in general, and appeared to concentrate in the Klason lignin fraction (KL) as mass was lost from the litter. In addition to an increase in the concentration of ^{15}N in the KL, and an increase in the concentration of KL in the residual litter, the total weight of KL increased. This observation supports the assertion that KL may include microbial products as well as cutin, lignin, and tannin (Berg 1988, Berg et al. 1995, Preston et al. 1997). The incorporation of N into KL was probably greater in the O2 and LF of the fertilized plots, resulting in slower decomposition of the elevated-N OM (Schulten et al. 1992).

Repeated measures ANOVA's revealed no significant N-effects on active fungal or bacterial biomass in any of the substrates (p-values > 0.1). It follows that if the populations were of similar size in control and elevated-N substrates, then the biomass in the control substrates must have been more active, possibly owing to less stabilized C. This reasoning also applies to the differences in respiration between the O2 and LF; active biomass was never higher in the O2 substrate in spite of much higher respiration (Table 3.3), implying higher activity in the O2. Although the LF may be similar in many respects to litter, and did not respond differently than O2 to elevated N (Figure 3.2), LF appears to be substantially more recalcitrant than even highly degraded litter at these sites.

The negative responses to elevated N in WS, HF, and RF supported the hypotheses proposed in the introduction. Insofar as the soils in the present study were composed of up to 95 percent HF, and HF contributes significantly to soil respiration (Chapter 2), it follows that respiration in WS and RF will drop if respiration in the HF is repressed. The HF is composed of complex, recalcitrant organic C that is often bound to soil minerals,

Table 3.3. Results of orthogonal contrasts of the cumulative respiration of O2, LF, and HF at 300 d. Data were transformed by natural logarithm, analyzed, then back-transformed for presentation here. CL is the 95 percent confidence limit.

Substrate	mg C g initial C^{-1}		
	Median†	Lower CL	Upper CL
O2	119.22a	99.50	142.98
LF	48.62b	40.58	58.30
HF	39.38c	32.85	47.20

†Values within a column followed by the same latter are not significantly different ($p > 0.05$).
Note: Confidence limits are provided as a general estimate of variability, but do not convey statistical signifcance.

and may be largely protected within soil microaggregates (Young and Spycher 1979, Preston and Newman 1992, Golchin 1994b). The HF may exhibit lability comparable to the LF when soil structure is removed as a barrier to microbial access (Figure 2.2). Yet, regardless of the location of HF, the mechanisms thought to be most effective at destabilizing the inherently complex molecules are often inhibited by elevated N (Fog 1988, Paul and Clark 1989, Bichat et al. 1999). Fog (1988) proposed that 'bombardment' with ·OH radicals is an important mechanism for degradation of recalcitrant organic matter, such as humic and fulvic acids, but that the formation of ·OH radicals is inhibited by elevated N (Forney et al. 1982, Kelley and Reddy 1982). Bichat et al. (1999) studied the degradation of ^{14}C- and ^{15}N-labeled atrazine, a recalcitrant heterocyclic, in soil and pure culture. With the addition of N, one of the three species of bacteria used in pure culture was severely inhibited. In soil, addition of N inhibited degradation of atrazine unless the soil was inoculated with one of the two species not influenced by added-N. It appears that the mechanisms involved in destabilizing C in both the LF and the HF were inhibited by elevated N, which readily explains the response of the WS and RF.

Less easily explained is the lower overall respiration of the RF, when compared to the WS (Table 3.4). The RF presumably contains the same OM components as the WS.

Table 3.4. Results of orthogonal contrasts of the cumulative respiration of WS and RF at 300 d. Data were transformed by natural logarithm, analyzed, then back-transformed for presentation here. CL is the 95 percent confidence limit.

Substrate	mg C g initial C^{-1}		
	Median†	Lower CL	Upper CL
WS	48.42a	40.40	58.04
RF	30.57b	25.50	36.65

†Values within a column followed by the same latter are not significantly different ($p > 0.05$).
Note: Confidence limits are provided as a general estimate of variability, but do not convey statistical signifcance.

The exception, of course, is NaPT- and water-extractable C and N. The percent C and N values for WS and RF listed in Tables 3.1 and 3.2 indicate that both C and N were lost in the fractionation process. While such losses might be expected to contribute to an initial depression of respiration in the RF, the greater accessibility of substrate should have compensated for the initial loss of C and N in the long run. Indeed, the WS does appear to have access to a more labile component of soil OM during the initial stages of the incubation (Figure 3.3a). However, although this OM component appears to have been largely exhausted by 120 d, the WS continued to maintain a higher rate of respiration at all time periods (p-values < 0.05). Swanston (Chapter 2) suggested that the depression in respiration may have been the result of a competitive interaction between different microbial communities that degrade LF and HF. These communities are probably separated spatially in the soil structure, ameliorating effects of competition in the WS. The lack of structure in the RF would have placed the communities in much closer contact, resulting in increased negative effects of competition and consequent depression of respiration.

Swanston (Chapter 2) also noted that although the cumulative respiration (mg C g initial C^{-1}) of LF was higher than that of HF, the magnitude of the difference did not adequately represent the conceptual model of a purely 'labile' and 'recalcitrant' fraction. Namely, factors such as microbial and spatial heterogeneity in soil might play more

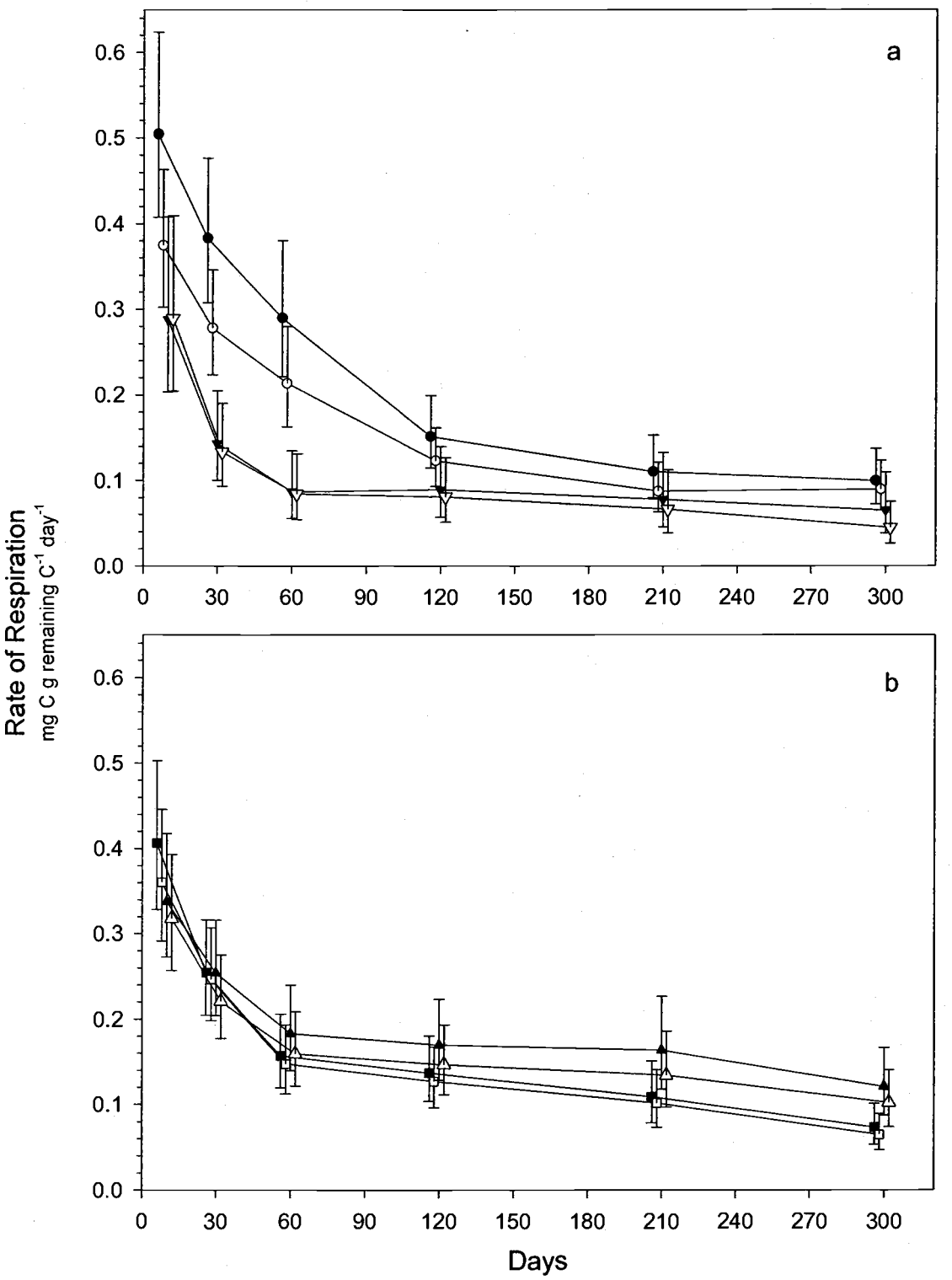

Figure 3.3. Rate of respiration through time in a) control WS (●) and RF (▼), and elevated-N WS (○) and RF (▽), and b) control LF (■) and HF (▲), and elevated-N LF (□) and HF (△). Data were transformed by natural logarithm, analyzed, then back-transformed for presentation here. Bars represent 95 % confidence intervals.

significant roles than previously considered. The addition of the elevated-N LF to the analysis increased the statistical significance of the difference, but not the magnitude (Table 3.3). Although the mechanisms contributing to the decrease in respiration in the LF and HF may have been different, the pattern of decreased cumulative respiration with elevated N was similar between the two fractions. A notable difference in the pattern of the rate of respiration through time, however, is that the LF maintains a higher rate of respiration later into the incubation (Figure 3.3b). The rate of respiration of the HF was not different than that of the LF during the first 120 d of the incubation (p-values > 0.1), but dropped below the LF by 210 d and was still lower at 300 d (p-values < 0.05). This could indicate that the LF does, in fact, have a greater proportion of less-recalcitrant C. Alternatively, it could mean that the residues of LF decomposition are less recalcitrant than the residues of HF decomposition, and are more easily recycled. Regardless of the mechanism, this separation towards the end of the incubation is largely responsible for the difference in 300 d cumulative respiration between LF and HF; the cumulative respiration of LF and HF were similar in the first 210 d of incubation (p-values > 0.1).

The timing of the differences between the LF and HF beg the question of biological significance. Soils in these forested sites can be expected to have frequent input of particulate and dissolved OM (Prescott et al. 2000, Jandl and Sollins 1997) that would contribute to the LF and HF (Chotte et al. 1998). This frequent replenishment of less recalcitrant C would probably obviate the separation of rates illustrated in Figure 3.3a. Surely the LF is the source of a disproportionate amount of heterotrophic soil respiration (Chapter 2), but that contribution is less a factor of chemical recalcitrance and more related to the high concentration of C in the LF and to the spatial arrangement of LF and HF in the soil structure.

Likewise, the biological significance of the effects of elevated N on C mineralization in these soils should be considered within the context of forest biology. The estimates of C mineralization are, of course, not directly applicable to *in situ* forest soils. Carbon dioxide evolution measured in the present study was dominated by heterotrophic respiration, while CO_2 evolution measured in the forest may include up to 55 percent root respiration (Andrews et al. 1999). Nevertheless, the Stotzky incubation method (Stotzky

1965) is adequate for comparing the relative effects of N addition on C stability in various substrates. The elevated N in the fertilized plots does appear to have a stabilizing effect on the soil C. If inputs and outputs were as limited in the forest as they were in the incubation, then the fertilized plots should be mineralizing several hundred kg C ha^{-1} yr^{-1} less than the C mineralized from the control plots. Studies of litter and soil on these plots have determined that there is no statistically significant difference in the C storage in soils and litter of the control and fertilized plots (Homann et al. unpublished data 2000), or of C input through litterfall (Chappell et al. 1999). If the fertilized plots contain more stable C, but control and fertilized plots receive similar above-ground C inputs and store similar amounts of C, then it is reasonable to conclude that different processes of C destabilization must dominate in control and fertilized plots. In considering the effects of elevated N on litter decomposition, Fog (1988) hypothesized that production of soluble organics would increase, largely at the expense of humus production. This would lead to an over-estimate of the rate of OM decomposition, especially in field experiments, where soluble compounds would be lost through leaching (Qualls and Haines 1992, Noguchi et al. 1999).

A more parsimonious set of explanations is that the difference in C stability between the litter of the control and fertilized plots is not biologically significant, but is instead overwhelmed by annual differences in litterfall and site quality. Homann et al. (unpublished data 2000) found more C in the O2 litter of the fertilized plots, but not a statistically greater amount. When those C estimates are coupled with the statistically different C mineralization estimates from the incubation in the present study, they result in annual C mineralization estimates in control and fertilized plots that are within five percent of each other. Although above-ground C inputs to soil may be similar between the plots, below-ground C inputs may not be similar. Beets and Whitehead (1996) determined that increased N availability in *Pinus radiata* plantations in New Zealand resulted in decreased fine root production. Greater C stability in fertilized soils in the SMC plots may have been offset by lower C input from fine root turnover.

3.4.3 Net N mineralization

Although net N mineralization was not different between the N-treatments at 300 d ($F_{1,6} = 1.91$, $p = 0.22$), net nitrification was two times higher in substrates from the fertilized plots ($F_{1,6} = 6.45$, $p = 0.044$). These findings support observations by Chappell et al. (1999), who investigated N cycling in litterfall and litter of several SMC installations. They found no significant differences in net N mineralization from composited Oi, Oe, and Oa litter. Additionally, they measured substantial nitrification in three plots, which were N-amended. They concluded that there was no long-term increase in N availability induced by repeated, long-term N fertilization.

Net N mineralization (mg N g initial N^{-1}) was about twice as high in the O2 as in the LF and HF, which were not different (Table 3.5). Light fraction does not initially appear to contain substantially more labile N than the HF. The story, however, becomes more complex when one considers net N mineralization of the LF and HF in relation to their C:N ratios. In perhaps the earliest incubation of LF, Greenland and Ford (1964) observed that net nitrification in LF was negatively related to the C:N ratio. Sollins et al. (1984) later determined that net N mineralization from the LF was also negatively correlated to the LF C:N ratio. Most recently, Barrios et al. (1996) confirmed this

Table 3.5. Results of orthogonal contrasts of the net N mineralization, and net nitrification, from O2, LF, and HF at 300 d. Data were transformed by natural logarithm, analyzed, then back-transformed for presentation here. CL_L is the lower 95 percent confidence limit, and CL_U is the upper 95 percent confidence limit.

Substrate	Net N Mineralization			Net Nitrification		
	mg N g initial N^{-1}					
	Median†	CL_L	CL_U	Median†	CL_L	CL_U
O2	48.08a	35.95	64.30	4.32b	1.63	11.45
LF	25.66b	19.18	34.30	19.70a	7.42	52.30
HF	21.63b	16.16	28.91	3.63b	1.36	9.53

†Values within a column followed by the same latter are not significantly different ($p > 0.05$).
Note: Confidence limits are provided as a general estimate of variability, but do not convey statistical signifcance.

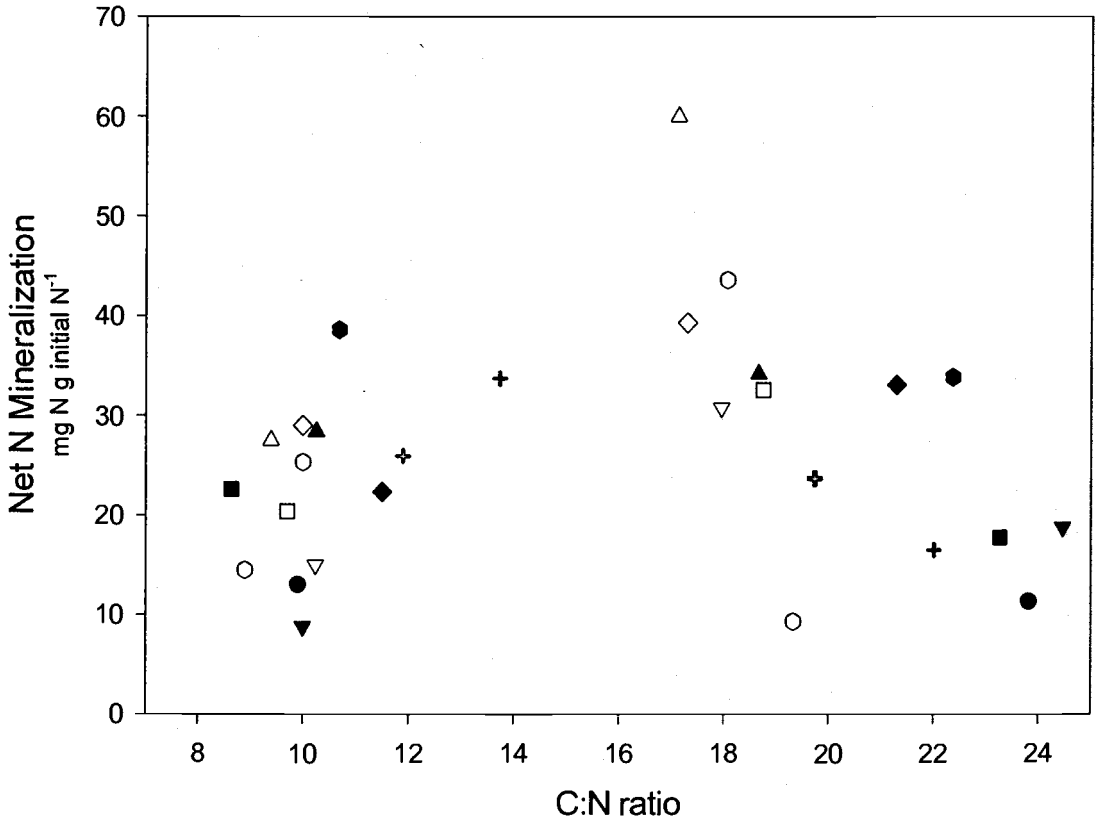

Figure 3.4. Net N mineralization of LF and HF plotted against the C:N ratios of those fractions. All values with a C:N ratio of less than 16 are HF; C:N values above 16 are LF. Solid symbols represent fractions from control plots, while open symbols represent fractions from fertilized plots. Specific installation designations are 1 (+), 20 (■), 43 (●), 57 (▲), 65 (⬢), 89 (◆), and 103 (▼).

negative relationship in incubations of LF. In the present study, net N mineralization in the LF also decreased with increasing C:N ratio of the LF (Figure 3.4). This is in contrast to the HF, which exhibited a positive trend with increasing C:N ratio.

Sollins et al. (1984), in what appears to be the only previously published report of net N mineralization from a HF incubation, reported a strong positive relationship between net N mineralization and C:N ratio in the HF. The trends are not as clear in the present study, partly as a result of smaller ranges of C:N ratios. Nevertheless, the general trends illustrated in Figure 3.4 add support to those reported by Sollins et al. (1984). They hypothesized that while low net N mineralization in the LF resulted from high N immobilization at high C:N ratios, low net N mineralization in HF may have resulted

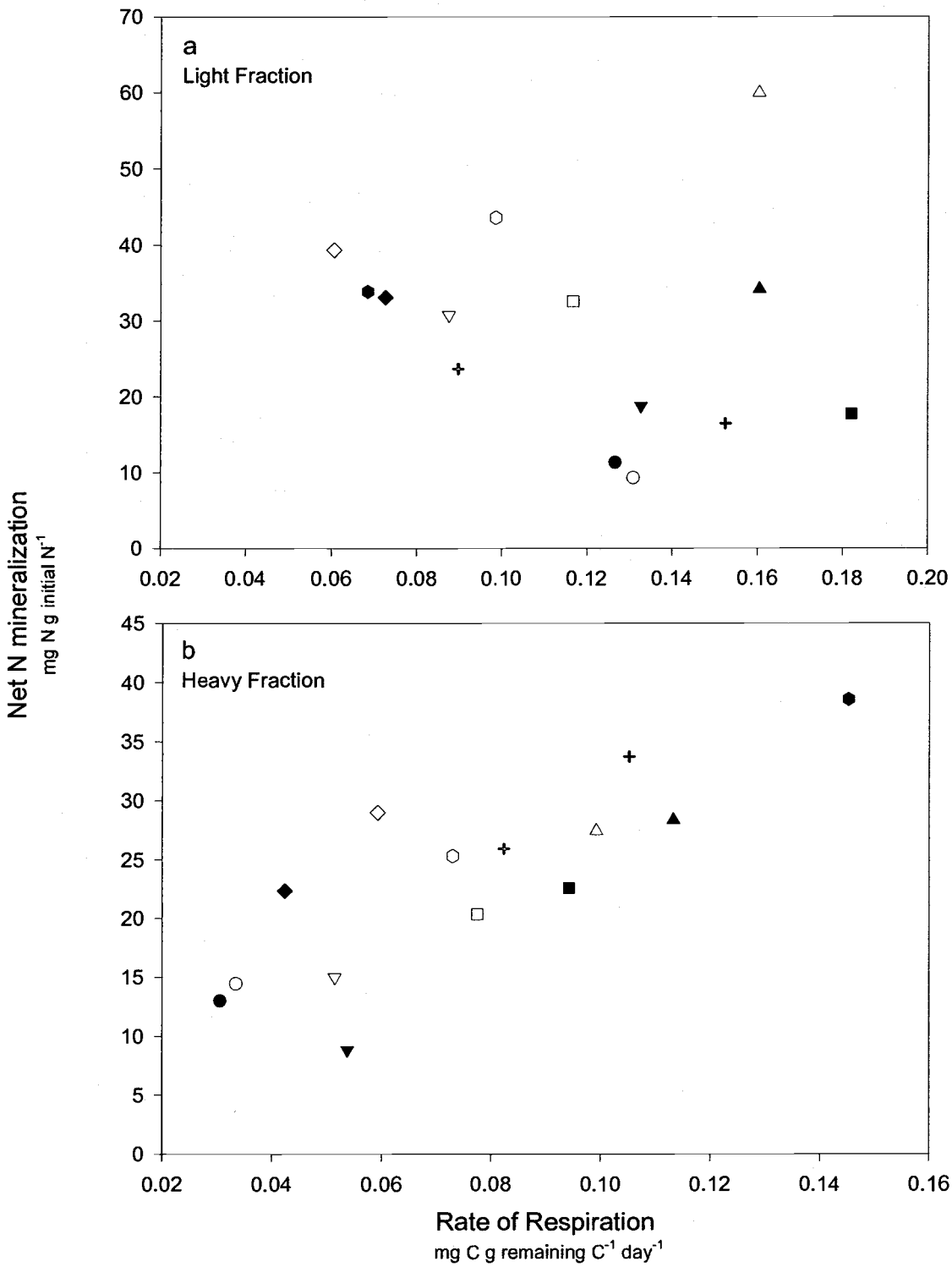

Figure 3.5. Net N mineralization plotted against rate of respiration in LF (a) and HF (b). Solid symbols represent fractions from control plots, while open symbols represent fractions from fertilized plots. Specific installation designations are 1 (+), 20 (■), 43 (●), 57 (▲), 65 (⬢), 89 (◆), and 103 (▼).

from a combination of chemical recalcitrance and physical protection at low C:N ratios. As a possible explanation for low net N mineralization from LF, Boone (1994) also hypothesized that more N might be immobilized in LF than HF during incubation, resulting in lower net rates of N mineralization in LF. If these hypotheses are accurate, one would expect to see a negative relationship between net N mineralization and rate of respiration in the LF, and a positive relationship between net N mineralization and rate of respiration in the HF. Indeed, these relationships can be seen in Figure 3.5.

Hart et al. (1994) and Hart (1999) have linked *increased* N mineralization to greater substrate recalcitrance, and a consequent decrease in N demand and immobilization. All substrates, regardless of C:N ratio, did exhibit higher net N mineralization as the incubation progressed. However, the contrasting patterns of the LF and HF as measured at 300 d, and illustrated in Figure 3.4, were similar when measured at 10 d. This suggests that these contrasting trends are not simply products of the incubation, but are instead related to the nature of the substrate.

Several trends are illustrated in Figures 3.4 and 3.5 that, when interpreted qualitatively, may reveal processes that are otherwise obscured in the greater split-plot analysis of variance. The ANOVA indicates that when separated from the confines of soil structure, net N mineralization from HF is comparable to that from LF. However, the lowest net N mineralization in HF appears to occur at both low C:N ratios (Figure 3.4) and low respiration (Figure 3.5b). The lowest net N mineralization in LF appears to occur at high C:N ratios (Figure 3.4) and high respiration (Figure 3.5a). Net N mineralization in HF, then, appears to be typified by release of N in excess of metabolic demands, while net N mineralization in LF appears to be typified by immobilization of N to meet metabolic demands.

Net nitrification of the LF, however, was about four times higher than the O2 and HF, which were not different. Using ^{15}N tracer techniques, Hart et al. (1994) concluded that nitrifiers are most competitive when heterotrophic populations are inactive or in decline. The active biomass was not different between the O2 and LF at 300 d (p-values > 0.1), although both were higher than the HF (p-values < 0.05). Since the rate of respiration was still nearly twice as high in the O2 as in the LF at 300 d, it seems likely

Table 3.6. Results of orthogonal contrasts of the net N mineralization, and net nitrification, from WS and RF at 300 d. Data were transformed by natural logarithm, analyzed, then back-transformed for presentation here. CL_L is the lower 95 percent confidence limit, and CL_U is the upper 95 percent confidence limit.

Substrate	Net N Mineralization			Net Nitrification		
	mg N g initial N^{-1}					
	Median†	CL_L	CL_U	Median†	CL_L	CL_U
WS	43.34a	32.40	25.34	35.98a	13.55	95.50
RF	18.95b	14.17	57.95	2.36b	0.89	6.27

†Values within a column followed by the same latter are not significantly different ($p > 0.05$).
Note: Confidence limits are provided as a general estimate of variability, but do not convey statistical signifcance.

that the low levels of extractable nitrate in the O2 were the result of higher rates of nitrate immobilization or inhibition of nitrifiers.

In spite of decreasing active biomass and respiration in the HF, it appears that nitrifiers were still inhibited at 300 d. Killham (1994) proposed that, if nitrification were directly inhibited, polyphenolic compounds would most likely be the source of allelopathy. Polyphenols may be synthesized by microorganisms (Stevenson 1994), and would likely abound in the humus-rich HF.

The trend of net N mineralization in the WS and RF (Table 3.6) also paralleled that of C mineralization (Table 3.4). Net N mineralization in the RF was substantially lower than that in the WS. The difference in the net nitrification, however, was striking. Considering that RF was composed of both LF and HF, it would seem that the contribution of LF to net nitrification in the RF was severely curtailed. The rate of respiration in the RF was almost half that in the WS ($p = 0.01$), but the active biomass of bacteria and fungi were not different in the two substrates (p-values > 0.1). It appears that the processes dominating net nitrification in the HF are also dominating net nitrification in the RF.

3.5 Conclusions

The hypothesis that the WS, RF, and HF would exhibit depressed respiration with elevated N was upheld in a 300 d aerobic incubation of those substrates. The hypothesis that elevated N would positively affect respiration in the O2 and LF was not upheld in a similar incubation. Two possible reasons are immediately apparent for the inaccuracy of the latter hypothesis: 1) the conceptual model (Figure 3.1) does not correctly predict the behavior of labile soil OM components, and 2) O2 and LF are degraded to the extent that they also respond to elevated N in a similar manner to more recalcitrant OM. Since the model is based largely on experiences with the short-term addition of urea or inorganic N to substrates, it is certainly possible that it does not correctly predict the behavior of OM that has had decades to incorporate N and develop high intrinsic concentrations of N. The organisms decomposing the OM may respond differently to high levels of inorganic N and dissolved organic N than to high concentrations of N within an OM molecule. Thus, if O2 and LF were directly amended with N, they may respond positively, as originally hypothesized. Alternatively, the O2 and LF are well-degraded, if not quite so much as the HF. As discussed in Chapter 2, the LF is not as labile as previously thought. It may be more aptly considered in terms of soil structure and microbial association, and less in terms of lability. Elevated N increased the stability of C in O2, WS, LF, HF, and RF substrates to a similar extent. It appears that the mechanisms most effective at degrading these substrates are negatively affected by elevated N, although the mechanisms may not be the same in each substrate. Carbon in the O2 was, nonetheless, substantially more labile than that in the LF, which was in turn only somewhat more labile than that in the HF. The LF and HF were typified by different patterns of N cycling. These patterns have received infrequent consideration in the literature, and should be investigated in more depth.

In spite of the statistical significance of the negative effects of N on C lability, there is some question as to the biological significance of these findings. Chappell et al. (1999) and Prescott et al. (2000) have studied C and N dynamics in litter and litterfall in both control and fertilized plots of the SMC. Their work indicates that site factors such as vegetation and soil texture may strongly influence the long-term C and N dynamics of

these sites. In contrast, even heavy fertilization over the course of several years may only yield a transitory effect on long-term C and N dynamics. Although a laboratory experiment may be able to detect differences between fertilized and unfertilized plots even ten years after the last fertilizer application, these differences may be overwhelmed by natural variability in the actual forest. Nevertheless, with the observation that elevated N has stabilized C in the major soil C pools, more focused field investigation into the (variable?) fate of C in fertilized and non-fertilized soils is warranted.

3.6 Literature Cited

Allen, H.L. 1987. Forest fertilizers. J. Forestry 85:37-46.

Alvarez, R., and C.R. Alvarez. 2000. Soil organic matter pools and their associations with carbon mineralization kinetics. Soil Sci. Soc. Am. J. 64:184-189.

Andrén, O., and T. Kätterer. 1997. ICBM: The introductory carbon balance model for exploration of soil carbon balances. Ecol. Appl. 7:1226-1236.

Andrews, J.A., K.G. Harrison, R. Matamala, and W.H. Schlesinger. Separation of root respiration from total soil respiration using carbon-13 labeling during free-air carbon dioxide enrichment (FACE). Soil Sci. Soc. Am. J. 63:1429-1435.

Barrios, E., R.J. Buresh, and J.I. Sprent. 1996b. Nitrogen mineralization in density fractions of soil organic matter from maize and legume cropping systems. Soil Biol. Biochem. 28:1459-1465.

Beets, P.N., and D. Whitehead. 1996. Carbon partitioning in *Pinus radiata* in relation to foliage nitrogen status. Tree Phys. 16:131-138.

Berg, B. 1986. Nutrient release from litter and hums in coniferous forest soils – a mini review. Scand. J. For. Res. 1:359-369.

Berg, B. 1988. Dynamics of nitrogen(^{15}N) in decomposing Scots pine (*Pinus sylvestris*) needle litter. Long-term decomposition in a Scots pine forest. VI. Can. J. Bot. 66:1539-1546.

Berg, B. C. McClaugherty, A. Virzo de Santo, M.B. Johansson, and G. Ekbohm. 1995. Decomposition of litter and soil organic matter: Can we distinguish a mechanism for soil organic matter buildup? Scand. J. For. Res. 10:108-119.

Bichat, F., G.K. Sims, and R.L. Mulvaney. 1999. Microbial utilization of heterocyclic nitrogen from atrazine. Soil Sci. Soc. Am. J. 63:100-110.

Chappell, H.N., C.E. Prescott, and L. Vesterdal. 1999. Long-term effects of nitrogen fertilization on nitrogen availability in coastal Douglas-fir forest floors. Soil Sci. Soc. Am. J. 63:1448-1454.

Chotte, J.L., J.N. Ladd, and M. Amato. 1998. Sites of microbial assimilation, and turnover of soluble and particulate ^{14}C-Labelled substrates decomposing in a clay soil. Soil Biol. Biochem. 30:205-218.

Christensen, B.T. 1992. Physical fractionation of soil and organic matter in primary particle size and density separates. Adv. Soil Sci. 20:1-90.

Entry, J.A., and C.B. Blackman. 1995. Influence of carbon and nitrogen on cellulose and lignin degradation in forest soils. Can. J. For. Soils 25:1231-1236.

Fog, K. 1988. The effect of added nitrogen on the rate of decomposition of organic matter. Biol. Rev. 63:433-462.

Forney, L.J., C.A. Reddy, M. Tien, and S.D. Aust. 1982. The involvement of hydroxyl radical derived from hydrogen peroxide in lignin degradation by the white-rot fungus *Phanerochaete chrysosporium.* J. Biol. Chem. 257:11355-11462.

Golchin, A., J.M. Oades, J.O. Skjemstad, and P. Clarke. 1994a. Study of free and occluded particulate organic matter in soils by solid-state ^{13}C NMR spectroscopy and scanning electron microscopy. Aust. J. Soil Res. 32:285-309.

Golchin, A., J.M. Oades, J.O. Skjemstad, and P. Clarke. 1994b. Soil structure and carbon cycling. Austr. J. Soil Res. 32:1043-1068.

Golchin, A., J.M. Oades, J.O. Skjemstad, and P. Clark. 1995. Structural and dynamic properties of soil organic matter as reflected by ^{13}C NMR spectroscopy in density fractions of an Oxisol under forest and pasture. Aust. J. Soil Res. 33:59-76.

Harmon, M.E., G.A. Baker, G. Spycher, and S.E. Greene. 1990. Leaf-litter decomposition in the *Picea/Tsuga* forests of Olympic National Park, Washington, U.S.A. For. Ecol. And Manage. 31:55-66.

Hart, S.C. 1999. Nitrogen transformations in fallen tree boles and mineral soil of an old-growth forest. Ecology 80:1385-1394.

Hart, S.C., G.E. Nason, D.D. Myrold, and D.A. Perry. 1994. Dynamics of gross nitrogen transformations in an old-growth forest soil: the carbon connection. Ecology 75:880-891.

Hassink, J. 1995a. Density fractions of soil macroorganic matter and microbial biomass as predictors of C and N mineralization. Soil Biol. Biochem. 27:1099-1108.

Hassink, J. 1995b. Decomposition rate constants of size and density fractions of soil organic matter. Soil Sci. Soc. Am. J. 59:1631-1635.

Hazard, J.W., and C.E. Peterson. 1984. Objectives and analytical methods of the Regional Forest Nutrition Project. Inst. Forest Resources Contribution no. 53. College of Forest Resources, Univ. of Washington, Seattle.

Hermann, R.K., and D.P. Lavender. 1999. Douglas-fir planted forests. New Forests 17/18:53-70.

Hopmans, P., and H.N. Chappell. 1994. Growth response of young, thinned Douglas-fir stands to nitrogen fertilizer in relation to soil properties and tree nutrition. Can J. For. Res. 24:1684-1688.

Ingham, E.R., and D.A. Klein. Soil fungi: measurement of hyphal length. Soil Biol. Biochem. 16:279-280.

Jandl, R., and P. Sollins. 1997. Water-extractable soil carbon in relation to the belowground carbon cycle. Biol. Fert. Soils 25:196-201.

Kelley, R.L. and C.A. Reddy. 1982. Ethylene production from alpha-OXO-gama-methylthiobutyric acid is a sensitive measure of lignolytic activity by *Phanerochaete chrysosporium*. Biochm. J. 206:423-425.

Killham, K. 1994. Soil Ecology. p. 89-108. Cambridge University Press, Cambridge. 242 p.

Magid, j., A. Gorissen, and K.E. Giller. 1996. In search of the elusive "active" fraction of soil organic matter: three size-density fractionation methods for tracing the fate of homogeneously ^{14}C-labelled plant materials. Soil Biol. Biochem. 28:89-99.

McClaugherty, C. and B. Berg. 1987. Cellulose, Lignin, and Nitrogen concentrations as rate regulating factors in late stages of forest litter decomposition. Pedobiologia 30:101-112.

McGill, W.B., and C.V. Cole. 1981. Comparative aspects of cycling of organic C, N, S and P through soil organic matter. Geoderma 26:267-286.

Meijboom, F., J. Hassink, and M. van Noordwijk. 1995. Density fractionation of soil organic matter using silica suspensions. Soil Biol. Biochem. 27:1109-1111.

Melillo, J.M., J.D. Aber, A.E. Linkins, A. Ricca, B. Fry, and K.J. Nadlehoffer. 1989. Carbon and nitrogen dynamics along the decay continuum: plant litter to soil organic matter. Plant Soil 115:189-198.

Noguchi, S., Y. Tsuboyama, R.C. Sidle, and I. Hosoda. Morphological characteristics of macropores and the distribution of preferential flow pathways in a forested slope segment. Soil Sci. Soc. Am. J. 63:1413-1423.

Paul, E.A., and F.E. Clark. 1989. Soil Microbiology and Biochemistry. Academic Press, Inc., New York, NY. pp. 91-144.

Prescott, C.E., H.N. Chappell, and L. Vesterdal. 2000. Nitrogen turnover in forest floors of coastal Douglas-fir at sites differing in soil nitrogen capital. Ecology 81:1878-1886.

Preston, C.M., and R.H. Newman. 1992. Demonstration of spatial heterogeneity in the organic matter of de-ashed humin samples by solid-state ^{13}C CPMAS NMR. Can. J. Soil Sci. 72:13-19.

Preston, C.M., J.A. Trofymow, B.G. Sayer, and J. Niu. 1997. ^{13}C nuclear magnetic resonance spectroscopy with cross-polarization and magic-angel spinning investigation of the proximate-analysis fractions used to assess litter quality in decomposition studies. Can. J. Bot. 75:1601-1613.

Qualls, R.G., and B.L. Haines. Biodegradability of dissolved organic matter in forest throughfall, soil solution, and stream water. Soil Sci. Soc. Am. J. 56:578-586.

Rayner, A.D.M., and L. Boddy. 1988. Fungal decomposition of wood: Its biology and ecology. p. 231-285. John Wiley & Sons, New York, NY. 587 p.

Šantrůčková, H, and M. Šimek. 1994. Soil microorganisms at different CO_2 and O_2 tensions. Folia Microbiol 39:225-230.

Šantrůčková, H, and M. Šimek. 1997. Effect of soil CO_2 concentration on microbial biomass. Biol. Fertil. Soils 25:269-273.

Schulten, H.-R., P. Leinweber, and G. Reuter. 1992. Initial formation of soil organic matter from grass residues in a long-term experiment. Biol. Fertil. Soils 14:237-245.

Skjemstad, J.O., R.C. Dalal, and P.F. Barron. 1986. Spectroscopic investigations of cultivation effects on organic matter of Vertisols. Soil Sci. Soc. Amer. J. 50:354-359.

Smolander, A., A. Kurka, V. Kitunen, and E. Malkonen. 1994. Microbial biomass C and N, and respiratory activity in soil of repeatedly limed and – and P-fertilized Norway spruce stands. Soil Biol. Biochem. 26:957-962.

Sollins, P., C. Glassman, E.A. Paul, C. Swanston, K. Lajtha, J.W. Heil, and E.T. Elliott. 1999. Soil Carbon and Nitrogen: Pools and Fractions. In Standard Soil Methods for Long-Term Ecological Research. Robertson, G. P., C. S. Bledsoe, D. C. Coleman, and P. Sollins, eds. Oxford University Press, New York. pp. 89-105.

Sollins, P., P. Homann, and B.A. Caldwell. 1996. Stabilization and destabilization of soil organic matter: mechanisms and controls. Geoderma. 74:65-105.

Sollins, P., G. Spycher, and C.A. Glassman. 1984. Net nitrogen mineralization from light- and heavy-fraction forest soil organic matter. Soil Biol. Biochem. 16:31-37.

Spycher G., P. Sollins, and S. Rose. 1983. Carbon and nitrogen in the light fraction of a forest soil: vertical distribution and seasonal patterns. Soil Sci. 135:79-87.

Stamatiadis, S., J.W. Doran, and E.R. Ingham. 1990. Use of staining to separate fungal and bacterial activity in soil. Soil Biol. Biochem. 22:81-88.

Stotzky, G. 1965. Microbial respiration. p. 1550-1572. *In* C.A. Black (ed.) Methods of soil analysis. Part 2. Agron. Monogr. 9. ASA, Madison, WI.

Strickland, T.C. and P. Sollins. 1987. Improved method for separating light- and heavy-fraction organic material from soil. Soil Sci. Soc. Am. J. 51:1390-1393.

Swanston, C.W., and D.D. Myrold. 1997. Incorporation of nitrogen from decomposing red alder leaves into plants and soil of a recent clearcut in Oregon. Can. J. For. Res. 27:1496-1502.

Theng, B.K., K.R. Tate, P. Sollins, *with* N. Moris, N. Nadkarni, and R.L. Tate III. 1989. Constituents of organic matter in temperate and tropical soils. p. 5-31. *In* D.C. Coleman et al. (ed.), Dynamics of Soil Organic Matter in Tropical Ecosystems. NIFTAL Project, Department of Agronomy and Soil Science, College of Tropical Agriculture and Human Resources, University of Hawaii, Hawaii.

Van Veen, J.A. and P.J. Kuikman. 1990. Soil structural aspects of decomposition of soil organic matter by micro-organisms. Biogeochem. 11:213-234.

Weetman, G.F., C.E. Prescott, F.L. Kohlberger, and R.M. Fournier. 1997. Ten-year growth response of coastal Douglas-fir on Vancouver Island to N and S fertilization in an optimum nutrition trial. Can. J. For. Res. 27:1478-1482.

Young, J.L., and G. Spycher. 1979. Water-dispersible soil orgaicpmineral particles: I. carbon and nitrogen distribution. Soil Sci. Soc. Am. J. 43:324-328.

Zeck, W., and G. Guggenberger. 1996. p. 101-170. Organic matter dynamics in forest soils of temperate and tropical ecosystems. *In* A.Piccolo (ed.) Humic substances in terrestrial ecosystems. Elsevier Science, New York, NY. 675 p.

4. Conclusions

4.1 Density Fractionation

The density separation technique appears to be a viable method for isolating and studying different soil organic matter fractions, but these fractions should be considered more carefully in the context of microbial interaction and physical protection. In these forest soils, light fraction was somewhat less recalcitrant than heavy fraction, but not to the extent that chemical descriptions would suggest. Indeed, if the respiration of the SF at all approximates that of the WS, then HF is responsible for much more of the soil respiration than commonly expected. This is understandable in the context of the heterogeneity of soil structure and the soil microflora. A substance that is virtually unassailable to one set of organisms may be the ideal substrate to another group. It seems likely that there are different communities of microorganisms that are most efficient at degradation of the light fraction or heavy fraction. In well aggregated soils, these communities may be spatially segregated to the same extent that LF and HF are separated in the soil structure. If the communities are at all antagonistic or competitive, removing the spatial separation may lead to a net decrease in organic matter destabilization. Unfortunately, this scenario is little more than speculation. Future studies which incubate density or size fractions may wish to employ some type of fatty acid analysis to determine the structures of the communities degrading the fractions (Vestal and White 1989). Both the presence and abundance of different types of organisms could be compared between fractions and the whole soil. Differences between fractions and similarities to the whole soil may shed light on the relative importance and function of the fractions in the whole soil. Use of the same methodologies in a RF would yield important information on the interaction of these communities with each other and with soil structure.

4.2 Influence of Nitrogen on Soil Carbon Stability

The hypothesis that the WS, RF, and HF would exhibit depressed respiration with elevated N was upheld in a 300 d aerobic incubation of those substrates. The hypothesis

that elevated N would positively affect respiration in the O2 and LF was not upheld in a similar incubation. Two possible reasons are immediately apparent for the inaccuracy of the latter hypothesis: 1) the conceptual model (Figure 3.1) does not correctly predict the behavior of labile soil OM components, and 2) O2 and LF are degraded to the extent that they also respond to elevated N in a similar manner to more recalcitrant OM. Since the model is based largely on experiences with the short-term addition of urea or inorganic N to substrates, it is certainly possible that it does not correctly predict the behavior of OM that has had decades to incorporate N and develop high intrinsic concentrations of N. The organisms decomposing the OM may respond differently to high levels of inorganic N and dissolved organic N than to high concentrations of N within an OM molecule. Thus, if O2 and LF were directly amended with N, they may respond positively, as originally hypothesized. Alternatively, the O2 and LF are well-degraded, if not quite so much as the HF. As discussed in Chapter 2, the LF is not as labile as previously thought. It may be more aptly considered in terms of soil structure and microbial association, and less in terms of lability. Elevated N increased the stability of C in O2, WS, LF, HF, and RF substrates to a similar extent. It appears that the mechanisms most effective at degrading these substrates are negatively affected by elevated N, although the mechanisms may not be the same in each substrate. Carbon in the O2 was, nonetheless, substantially more labile than that in the LF, which was in turn only somewhat more labile than that in the HF. The LF and HF were typified by different patterns of N cycling. These patterns have received infrequent consideration in the literature, and should be investigated in more depth.

In spite of the statistical significance of the negative effects of N on C lability, there is some question as to the biological significance of these findings. Chappell et al. (1999) and Prescott et al. (2000) have studied C and N dynamics in litter and litterfall in both control and fertilized plots of the SMC. Their work indicates that site factors such as vegetation and soil texture may strongly influence the long-term C and N dynamics of these sites. In contrast, even heavy fertilization over the course of several years may only yield a transitory effect on long-term C and N dynamics. Although a laboratory experiment may be able to detect differences between fertilized and unfertilized plots

even ten years after the last fertilizer application, these differences may be overwhelmed by natural variability in the actual forest. Nevertheless, with the observation that elevated N has stabilized C in the major soil C pools, more focused field investigation into the (variable?) fate of C in fertilized and non-fertilized soils is warranted.

4.3 Synthesis

The two major conclusions of this dissertation are: 1) the LF may not be substantially more labile than the HF, and 2) elevated N, persisting for nearly ten years after fertilization, results in increased C stabilization in litter and soil.

The first conclusion will hopefully spur thought and discussion on the conceptual model behind density fractionation. It may not be enough to simply fractionate a soil, analyze the LF and the HF, and conclude that the experimental treatment has short-term or long-term consequences because it affected the LF or the HF in a certain way. More thought needs to be given to the spatial heterogeneity of the soil. If different soils are used, their relative structures should be discussed and compared. Also, focus on microbial communities should be greatly increased. Not just the weight of bacterial or fungal biomass, but also the structure of the communities. More information needs to be generated on which communities preferentially degrade the LF and HF, and the relative abundance of these communities in the soil.

More thought should also be given to the 'bulk density' of LF. Light fraction is composed of porous materials that may actually provide habitat for microorganisms (Chotte et al. 1998, Guggenberger 1999). Evacuating the air from the LF, or centrifuging, presumably fills many of the pore spaces with densimetric solution, and effectively increases the density of the organic material. This may result in lower recovery of LF and 'contamination' of the HF. Is this what we want? Is it just the density of the C-structures that makes LF 'labile?' Or is it also that the overall structure of the LF organic matter provides a distinctly different habitat from that of the HF? This habitat could be expected to have different O_2 concentrations, nutrient and water input, and protection from predators. Gravity sedimentation (Strickland and Sollins 1987, Sollins et al. 1999) ideally minimizes saturation of the porous LF, but not perfectly.

Ludox, a silica-based densimetric solution with a high surface tension, supposedly further minimizes saturation (Meijboom et al. 1995). The maximum density of Ludox is only 1.4, however, which is troubling. Meijboom et al. (1995) claim that a low density of Ludox is equivalent to a high density of NaPT because of the high viscosity of Ludox. But Barrios et al. (1996a) found that less LF was separated in Ludox at <1.13 g mL^{-1} than in NaI at < 1.7 g mL^{-1}. There is no clear answer, but I think that Strickland and Sollins (1987) have proposed the best of the current alternatives.

As discussed in Chapter 1, greater effort should be directed to determining the appropriate density of the densimetric solution for each soil, and comparing fractions upon that basis. In this regard, I believe that my own work falls woefully short of the standard I've set. Two of the soils used in this study were of pyroclastic origin. At a density of 1.65 g mL^{-1} it is likely that the LF of the soils was contaminated with pumice and ash (Sollins and Spycher 1983). The concentration of C and N in the LF of these soils was probably underestimated to some extent.

In spite of this shortfall, the LF and HF were clearly different from each other structurally, and matched the classic operational definitions (Greenland and Ford 1964, Spycher et al. 1983). So, irregardless of the similar labilities of LF and HF (Chapter 2), I found it surprising that the LF and HF responded similarly to elevated N. Although I was able to speculate on some the possible N-induced changes in the chemical structures of the substrates, I was reminded of my own admonition in Chapter 1: the C was not simply cycling, it was being cycled. So why was less of it being cycled in the substrates with elevated N? The chemical changes were probably different in the LF and HF, but it is ultimately the microbiota that must adapt to those changes. Once again, the information on the microbiological communities was inadequate to elucidate the differences in respiration that I measured. This leads to one of the same conclusions as in Chapter 2, that more microbiological information and detailed analysis is needed. This is probably true for both the initial characterization of the field soils, as well as the more intensive characterization of the substrates.

Initial microbial characterization of the LF and HF is problematic, as some organisms could be expected to be washed away or lysed during the fractionation process. A

possible microbiologically-focused densimetric technique might employ sequentially greater dispersion energies to the soil slurry, but perhaps avoid sonication. After each dispersion, LF and supernatant could be collected. The sediment/aggregates could be gently washed, then resuspended for the next level of dispersion. The supernatant, wash, and LF from each step could be analyzed using fatty acid analysis techniques. To the extent that organisms are closely associated with clay, or residing within LF (Chotte et al. 1998), this method might successfully isolate communities associated with either the LF, occluded LF, or the HF.

It is true that site variability could ultimately have more effect of the C cycling than elevated N. An observation that microbial populations were initially different in the soils and fractions of the paired plots would lend some credence to the speculation that C was being cycled differently in the plots. The utility of the study in Chapter 3 is that it showed that the C is somewhat more stable in the N-amended plots, and thus has the *potential* to be cycled more slowly in the field. Analyses of microbial populations and DOC in field soils are the next step in the investigation of N effects on C stability.

Bibliography

Adu, J.K., and J.M. Oades. 1978. Physical factors influencing decomposition of organic materials in soil aggregates. Soil Biol. Biochem. 10:109-115.

Allen, H.L. 1987. Forest fertilizers. J. Forestry 85:37-46.

Alvarez, R., and C.R. Alvarez. 2000. Soil organic matter pools and their associations with carbon mineralization kinetics. Soil Sci. Soc. Am. J. 64:184-189.

Andrén, O., and T. Kätterer. 1997. ICBM: The introductory carbon balance model for exploration of soil carbon balances. Ecol. Appl. 7:1226-1236.

Andrews, J.A., K.G. Harrison, R. Matamala, and W.H. Schlesinger. Separation of root respiration from total soil respiration using carbon-13 labeling during free-air carbon dioxide enrichment (FACE). Soil Sci. Soc. Am. J. 63:1429-1435.

Barrios, E., R.J. Buresh, and J.I. Sprent. 1996a. Organic matter in soil particle size and density fractions from maize and legume cropping systems. Soil Biol. Biochem. 28:185-193.

Barrios, E., R.J. Buresh, and J.I. Sprent. 1996b. Nitrogen mineralization in density fractions of soil organic matter from maize and legume cropping systems. Soil Biol. Biochem. 28:1459-1465.

Beets, P.N., and D. Whitehead. 1996. Carbon partitioning in *Pinus radiata* in relation to foliage nitrogen status. Tree Phys. 16:131-138.

Berg, B. 1986. Nutrient release from litter and hums in coniferous forest soils – a mini review. Scand. J. For. Res. 1:359-369.

Berg, B. 1988. Dynamics of nitrogen(^{15}N) in decomposing Scots pine (*Pinus sylvestris*) needle litter. Long-term decomposition in a Scots pine forest. VI. Can. J. Bot. 66:1539-1546.

Berg, B. C. McClaugherty, A. Virzo de Santo, M.B. Johansson, and G. Ekbohm. 1995. Decomposition of litter and soil organic matter: Can we distinguish a mechanism for soil organic matter buildup? Scand. J. For. Res. 10:108-119.

Bichat, F., G.K. Sims, and R.L. Mulvaney. 1999. Microbial utilization of heterocyclic nitrogen from atrazine. Soil Sci. Soc. Am. J. 63:100-110.

Boone, R.D. 1994. Light-fraction soil organic matter: Origin and contribution to net nitrogen mineralization. Soil Biol. Biochem. 26:1459-1468.

Cabrera, M.L., and M.H. Beare. 1993. Alkaline persulfate oxidation for determining total nitrogen in microbial biomass extracts. Soil Sci. Soc. Am. J. 57:1007-1012.

Cambardella, C.A., and E.T. Elliot. 1992. Particulate soil organic-matter changes across a grassland cultivation sequence. Soil Sci. Soc. Am. J. 65:777-783.

Cambardella, C.A., and E.T. Elliot. 1993. Methods for physical separation and characterization of soil organic matter fractions. Geoderma 56:449-457.

Camberdella, C.A., and E.T. Elliot. 1994. Carbon and nitrogen dynamics of soil organic matter fractions from cultivated grassland soils. Soil Sci. Soc. Am. J. 58:123-130.

Chappell, H.N., C.E. Prescott, and L. Vesterdal. 1999. Long-term effects of nitrogen fertilization on nitrogen availability in coastal Douglas-fir forest floors. Soil Sci. Soc. Am. J. 63:1448-1454.

Chotte, J.L., J.N. Ladd, and M. Amato. 1998. Sites of microbial assimilation, and turnover of soluble and particulate ^{14}C-Labelled substrates decomposing in a clay soil. Soil Biol. Biochem. 30:205-218.

Christensen, B.T. 1992. Physical fractionation of soil and organic matter in primary particle size and density separates. Adv. Soil Sci. 20:1-90.

Cook, B.D., and D.L. Allan. 1992. Dissolved organic carbon in old field soils: Total amounts as a measure of available resources for soil mineralization. Soil Biol. Biochem. 24:585-594.

Davidson, E.A. S.C. Hart, and M.K. Firestone. 1992. Internal cycling of nitrate in soils of a mature coniferous forest. Ecology. 73:1148-1156.

De Boer, W., P.J.A. Klein Gunnewiek, and D. Parkinson. 1996. Variability of N mineralization and nitrification in a simple, simulated microbial forest soil community. Soil Biol. Biochem. 28:203-211.

Entry, J.A., and C.B. Blackman. 1995. Influence of carbon and nitrogen on cellulose and lignin degradation in forest soils. Can. J. For. Soils 25:1231-1236.

Fog, K. 1988. The effect of added nitrogen on the rate of decomposition of organic matter. Biol. Rev. 63:433-462.

Forney, L.J., C.A. Reddy, M. Tien, and S.D. Aust. 1982. The involvement of hydroxyl radical derived from hydrogen peroxide in lignin degradation by the white-rot fungus *Phanerochaete chrysosporium*. J. Biol. Chem. 257:11355-11462.

Franzluebbers, A.J. 1999. Potential C and N mineralization and microbial biomass from intact and increasingly disturbed soils of varying texture. Soil Biol. Biochem. 31:1083-1090.

Golchin, A., J.M. Oades, J.O. Skjemstad, and P. Clarke. 1994a. Study of free and occluded particulate organic matter in soils by solid-state ^{13}C NMR spectroscopy and scanning electron microscopy. Aust. J. Soil Res. 32:285-309.

Golchin, A., J.M. Oades, J.O. Skjemstad, and P. Clarke. 1994b. Soil structure and carbon cycling. Austr. J. Soil Res. 32:1043-1068.

Golchin, A., J.M. Oades, J.O. Skjemstad, and P. Clark. 1995. Structural and dynamic properties of soil organic matter as reflected by ^{13}C NMR spectroscopy in density fractions of an Oxisol under forest and pasture. Aust. J. Soil Res. 33:59-76.

Greenland, D.J., and W. Ford. 1964. Separation of partially humified organic materials by ultrasonic dispersion. Eighth Intern. Congress of Soil Sci. 3:137-148.

Gregorich, E.G., C.M. Monreal, M. Schnitzer, and H.R. Schulten. 1996. Transformation of plant residues into soil organic matter: chemical characterization of plant tissue, isolated soil fractions, and whole soils. Soil Science. 161:680-693.

Guggenberger, G., E.T. Elliott, S.D. Frey, J. Six, and K Paustian. 1999. Microbial contributions to the aggregation of a cultivated grassland soil amended with starch. Soil Biol. Biochem. 31:407-419.

Harmon, M.E., G.A. Baker, G. Spycher, and S.E. Greene. 1990. Leaf-litter decomposition in the *Picea/Tsuga* forests of Olympic National Park, Washington, U.S.A. For. Ecol. And Manage. 31:55-66.

Hart, S.C. 1999. Nitrogen transformations in fallen tree boles and mineral soil of an old-growth forest. Ecology 80:1385-1394.

Hart, S.C., G.E. Nason, D.D. Myrold, and D.A. Perry. 1994. Dynamics of gross nitrogen transformations in an old-growth forest soil: the carbon connection. Ecology 75:880-891.

Hassink, J. 1995a. Decomposition rate constants of size and density fractions of soil organic matter. Soil Sci. Soc. Am. J. 59:1631-1635.

Hassink, J. 1995b. Decomposition rate constants of size and density fractions of soil organic matter. Soil Sci. Soc. Am. J. 59:1631-1635.

Hazard, J.W., and C.E. Peterson. 1984. Objectives and analytical methods of the Regional Forest Nutrition Project. Inst. Forest Resources Contribution no. 53. College of Forest Resources, Univ. of Washington, Seattle.

Hermann, R.K., and D.P. Lavender. 1999. Douglas-fir planted forests. New Forests. 17/18:53-70.

Holland, J.H. 1998. Emergence: From Chaos to Order. Addison-Wesley Publishing Company, Inc. Reading, Mass. 258 p.

Hopmans, P., and H.N. Chappell. 1994. Growth response of young, thinned Douglas-fir stands to nitrogen fertilizer in relation to soil properties and tree nutrition. Can J. For. Res. 24:1684-1688.

Ingham, E.R., and D.A. Klein. Soil fungi: measurement of hyphal length. Soil Biol. Biochem. 16:279-280.

Jandl, R., and P. Sollins. 1997. Water-extractable soil carbon in relation to the belowground carbon cycle. Biol. Fert. Soils 25:196-201.

Kelley, R.L. and C.A. Reddy. 1982. Ethylene production from alpha-OXO-gama-methylthiobutyric acid is a sensitive measure of lignolytic activity by *Phanerochaete chrysosporium*. Biochem. J. 206:423-425.

Killham, K. 1994. Soil Ecology. p. 89-108. Cambridge University Press, Cambridge. 242 p.

Kogel-Knabner, I., P.G. Hatcher, and W. Zech. Chemical structural studies of forest soil humic acids: aromatic carbon fraction. Soil Sci. Soc. Am. J. 55:241-247.

Kooistra, M.J., and M. van Noordwijk. 1996. Soil architecture and distribution of organic matter. p. 15-53. *In* M.R. Carter and B.A. Steward (ed.) Structure and soil organic matter storage in agricultural soils. CRC Press Inc., Boca Raton, FL.

Ladd, J.N., R.C. Foster, and J.O. Skjemstad. 1993. Soil structure: carbon and nitrogen metabolism. Geoderma 56:401-434.

Ladd, J.N., M. van Gestel, L. Jocteur Monrozier, and M. Amato. 1996. Distribution of organic ^{14}C and ^{15}N in particle-size fractions of soils incubated with ^{14}C, ^{15}N-labelled glucose/NH_4, and legume and wheat straw residues. Soil Biol. Biochem. 28:893-905.

Magid, j., A. Gorissen, and K.E. Giller. 1996. In search of the elusive "active" fraction of soil organic matter: three size-density fractionation methods for tracing the fate of homogeneously ^{14}C-labelled plant materials. Soil Biol. Biochem. 28:89-99.

Mahieu, N., D.S. Powlson, and E.W. Randall. 1999. Statistical analysis of published carbon-13 CPMAS NMR spectra of soil organic matter. Soil Sci. Soc. Am. J. 63:307-319.

McClaugherty, C. and B. Berg. 1987. Cellulose, Lignin, and Nitrogen concentrations as rate regulating factors in late stages of forest litter decomposition. Pedobiologia 30:101-112.

McGill, W.B., K.R. Cannon, J.A. Robertson, and F.D. Cook. 1986. Dynamics of soil microbial and water-soluble organic carbon in Brenton L after 50 years of cropping two rotations. Can. J. Soil Sci. 66:1-19.

McGill, W.B., and C.V. Cole. 1981. Comparative aspects of cycling of organic C, N, S and P through soil organic matter. Geoderma 26:267-286.

Meijboom, F., J. Hassink, and M. van Noordwijk. 1995. Density fractionation of soil organic matter using silica suspensions. Soil Biol. Biochem. 27:1109-1111.

Melillo, J.M., J.D. Aber, A.E. Linkins, A. Ricca, B. Fry, and K.J. Nadlehoffer. 1989. Carbon and nitrogen dynamics along the decay continuum: plant litter to soil organic matter. Plant Soil 115:189-198.

Miglierina, A.M., and R.A. Rosell. 1995. Humus quantity and quality of an entic Haplustoll under different soil-crop management systems. Comm. Soil Sci. Plant Anal. 26:3343-3355.

Noguchi, S., Y. Tsuboyama, R.C. Sidle, and I. Hosoda. Morphological characteristics of macropores and the distribution of preferential flow pathways in a forested slope segment. Soil Sci. Soc. Am. J. 63:1413-1423.

Paul, E.A., and F.E. Clark. 1989. Soil Microbiology and Biochemistry. Academic Press, Inc., New York, NY. pp. 91-144.

Prescott, C.E., H.N. Chappell, and L. Vesterdal. 2000. Nitrogen turnover in forest floors of coastal Douglas-fir at sites differing in soil nitrogen capital. Ecology 81:1878-1886.

Preston, C.M., J.A. Trofymow, B.G. Sayer, and J. Niu. 1997. ^{13}C nuclear magnetic resonance spectroscopy with cross-polarization and magic-angel spinning investigation of the proximate-analysis fractions used to assess litter quality in decomposition studies. Can. J. Bot. 75:1601-1613.

Preston, C.M., and R.H. Newman. 1992. Demonstration of spatial heterogeneity in the organic matter of de-ashed humin samples by solid-state ^{13}C CPMAS NMR. Can. J. Soil Sci. 72:13-19.

Qualls, R.G., and B.L. Haines. Biodegradability of dissolved organic matter in forest throughfall, soil solution, and stream water. Soil Sci. Soc. Am. J. 56:578-586.

Rayner, A.D.M., and L. Boddy. 1988. Fungal decomposition of wood: Its biology and ecology. p. 231-285. John Wiley & Sons, New York, NY. 587 p.

Richter, M., I. Mizuno, S. Aranguez, and S. Uriarte. 1975. Densimetric fractionation of soil organo-mineral complexes. J. Soil Sci. 26:112-123.

Šantrůčková, H, and M. Šimek. 1994. Soil microorganisms at different CO_2 and O_2 tensions. Folia Microbiol 39:225-230.

Šantrůčková, H, and M. Šimek. 1997. Effect of soil CO_2 concentration on microbial biomass. Biol. Fertil. Soils 25:269-273.

Sato, A., and M Seto. 1999. Relationship between rate of carbon dioxide evolution, microbial biomass carbon, and the amount of dissolved organic carbon as affected by temperature and water content of a forest and an arable soil. Communications in Soil Science and Plant Analysis. 30:2593-2605.

Schulten, H.-R., P. Leinweber, and G. Reuter. 1992. Initial formation of soil organic matter from grass residues in a long-term experiment. Biol. Fertil. Soils 14:237-245.

Skjemstad, J.O., R.C. Dalal, and P.F. Barron. 1986. Spectroscopic investigations of cultivation effects on organic matter of Vertisols. Soil Sci. Soc. Amer. J. 50:354-359.

Smolander, A., A. Kurka, V. Kitunen, and E. Malkonen. 1994. Microbial biomass C and N, and respiratory activity in soil of repeatedly limed and – and P-fertilized Norway spruce stands. Soil Biol. Biochem. 26:957-962.

Sollins, P., G. Spycher, and C.A. Glassman. 1984. Net nitrogen mineralization from light- and heavy-fraction forest soil organic matter. Soil Biol. Biochem. 16:31-37.

Sollins, P., P. Homann, and B.A. Caldwell. 1996. Stabilization and destabilization of soil organic matter: mechanisms and controls. Geoderma. 74:65-105.

Sollins, P., C. Glassman, E.A. Paul, C. Swanston, K. Lajtha, J.W. Heil, and E.T. Elliott. 1999. Soil Carbon and Nitrogen: Pools and Fractions. In Standard Soil Methods for Long-Term Ecological Research. Robertson, G. P., C. S. Bledsoe, D. C. Coleman, and P. Sollins, eds. Oxford University Press, New York. pp. 89-105.

Sørensen, L.H. 1983. The influence of stress treatments on the microbial biomass and the rate of decomposition of humified material in soils containing different amounts of clay. Plant Soil 75:107-119.

Spycher, G., P. Sollins, and S. Rose. 1983. Carbon and nitrogen in the light fraction of a forest soil: vertical distribution and seasonal patterns. Soil Sci. 135:79-87.

Spycher, G., and J.L. Young. 1979. Water-dispersible soil organic-mineral particles: II. Inorganic amorphous and crystalline phases in density fractions of clay-size particles. Soil Sci. Soc. Am. J. 43:328-332.

Stahl, P.D., and M. Christensen. 1992. In vitro mycelial interactions among members of a soil microfungal community. Soil Biol. Biochem. 24:309-316.

Stamatiadis, S., J.W. Doran, and E.R. Ingham. 1990. Use of staining to separate fungal and bacterial activity in soil. Soil Biol. Biochem. 22:81-88.

Stark, J.M., and S.C. Hart. 1997. High rates of nitrification and nitrate turnover in undisturbed coniferous forests. Nature. 385:61-64.

Stevenson, F.J. 1994. Extraction, fractionation, and general chemical composition of soil organic matter. p. 1-58. *In* Humus Chemistry: Genesis, composition, reactions. Second Edition. John Wiley and Sons, Inc., New York, NY. 496 p.

Stotzky, G. 1965. Microbial respiration. p. 1550-1572. *In* C.A. Black (ed.) Methods of soil analysis. Part 2. Agron. Monogr. 9. ASA, Madison, WI.

Strickland, T.C. and P. Sollins. 1987. Improved method for separating light- and heavy-fraction organic material from soil. Soil Sci. Soc. Am. J. 51:1390-1393.

Strickland, T.C., P. Sollins, N. Rudd, and D.S. Schimel. 1992. Rapid stabilization and mobilization of ^{15}N in forest and range soils. Soil Biol. Biochem. 24:849-855.

Swanston, C.W., and D.D. Myrold. 1997. Incorporation of nitrogen from decomposing red alder leaves into plants and soil of a recent clearcut in Oregon. Can. J. For. Res. 27:1496-1502.

Theng, B.K., K.R. Tate, P. Sollins, *with* N. Moris, N. Nadkarni, and R.L. Tate III. 1989. Constituents of organic matter in temperate and tropical soils. p. 5-31. *In* D.C. Coleman et al. (ed.), Dynamics of Soil Organic Matter in Tropical Ecosystems. NIFTAL Project, Department of Agronomy and Soil Science, College of Tropical Agriculture and Human Resources, University of Hawaii, Hawaii.

Tiessen, H., and J.W.B. Stewart. 1983. Particle-size fractions and their use in studies of soil orgaic matter: II. Cultivation effects on organic matter composition in size fractions. Soil Sci. Soc. Am. J. 47:509-514.

Van Veen, J.A. and P.J. Kuikman. 1990. Soil structural aspects of decomposition of soil organic matter by microorganisms. Biogeochem. 11:213-234.

Vestal, J.R., and D.C. White. 1989. Lipid analysis in microbial ecology: quantitative approaches to the study of microbial communities. Bioscience. 39:535-541.

Weetman, G.F., C.E. Prescott, F.L. Kohlberger, and R.M. Fournier. 1997. Ten-year growth response of coastal Douglas-fir on Vancouver Island to N and S fertilization in an optimum nutrition trial. Can. J. For. Res. 27:1478-1482.

Weyman-Kaczmarkowa, W., and D. Wojcik-Wojtkowiak. 1992. Microbial degradation of plant materials and allelochemicals formation in different soils. Developments in agricultural and managed-forest ecology. 25:127-136

Young, J.L., and G. Spycher. 1979. Water-dispersible soil orgaicpmineral particles: I. carbon and nitrogen distribution. Soil Sci. Soc. Am. J. 43:324-328.

Zeck, W., and G. Guggenberger. 1996. p. 101-170. Organic matter dynamics in forest soils of temperate and tropical ecosystems. *In* A.Piccolo (ed.) Humic substances in terrestrial ecosystems. Elsevier Science, New York, NY. 675 p.