NOTE TO USERS

This reproduction is the best copy available.

UMI®

University of Alberta

Soil organic carbon and microbial community distribution at the
Breton Classical plots

by

Xin Zhang Ⓒ

A thesis submitted to the Faculty of Graduate Studies and Research in
partial fulfillment of the

requirements for the degree of *Master of Science*

in

Soil Science

Department of *Renewable Resources*

Edmonton, Alberta

Fall 2005

Library and
Archives Canada

Bibliothèque et
Archives Canada

Published Heritage
Branch

Direction du
Patrimoine de l'édition

395 Wellington Street
Ottawa ON K1A 0N4
Canada

395, rue Wellington
Ottawa ON K1A 0N4
Canada

NOTICE:
The author has granted a non-exclusive license allowing Library and Archives Canada to reproduce, publish, archive, preserve, conserve, communicate to the public by telecommunication or on the Internet, loan, distribute and sell theses worldwide, for commercial or non-commercial purposes, in microform, paper, electronic and/or any other formats.

The author retains copyright ownership and moral rights in this thesis. Neither the thesis nor substantial extracts from it may be printed or otherwise reproduced without the author's permission.

AVIS:
L'auteur a accordé une licence non exclusive permettant à la Bibliothèque et Archives Canada de reproduire, publier, archiver, sauvegarder, conserver, transmettre au public par télécommunication ou par l'Internet, prêter, distribuer et vendre des thèses partout dans le monde, à des fins commerciales ou autres, sur support microforme, papier, électronique et/ou autres formats.

L'auteur conserve la propriété du droit d'auteur et des droits moraux qui protège cette thèse. Ni la thèse ni des extraits substantiels de celle-ci ne doivent être imprimés ou autrement reproduits sans son autorisation.

In compliance with the Canadian Privacy Act some supporting forms may have been removed from this thesis.

While these forms may be included in the document page count, their removal does not represent any loss of content from the thesis.

Conformément à la loi canadienne sur la protection de la vie privée, quelques formulaires secondaires ont été enlevés de cette thèse.

Bien que ces formulaires aient inclus dans la pagination, il n'y aura aucun contenu manquant.

Canada

Abstract

This study assessed the influence of long-term fertilization and cropping management on organic carbon dynamics in the Gray Luvisolic soils of the Breton Classical Plots in central Alberta. Specific objectives were to characterize carbon distribution and mineralization fluxes in physical fraction pools, and to determine the size and composition of the soil microbial communities. Long-term manure addition resulted in an increase in carbon concentration in all fractions, which was greater in the limed than in the unlimed plots. When expressed on a gram of carbon basis, results from a laboratory incubation showed that clay had the lowest mineralization rate of all fractions. Manure significantly increased the sand-associated, but significantly decreased the clay-associated carbon mineralization. In the 5 year rotation plots, soil microbial carbon and nitrogen were significantly increased by manure and liming, and both showed a positive correlation to soil water content. NPKS fertilization and manure application increased the recovery of several individual microbial phospholipids fatty acids.

Acknowledgements

The completion of this thesis could not have been possible without the contribution and support of many individuals. First and foremost is my supervisor, Dr. Sylvie Quideau, for the encouragement she gave to me and her patience on improving my writing skills. Her enthusiasm for soil science and dedication for her work will be imprinted on my mind forever. I would like to thank Dr. Jim Robertson who is always willing to share his expertise and knowledge at anytime. I will never forget my 'special field assistant'. I would like to thank Dr. Noorallah Juma for his concern and valuable suggestion. It was an honour to work with the individuals who possessed not only exemplary professional capabilities, but also whose personal characters shone.

I would like to give special thanks to Chung Nguyen for the SAS analysis and encouragement and a great thanks to Monica Molina for her understanding, supporting and lab analysis. Thanks to Kirsten Hannam for the time and energy she spent on helping me improve my English skills as well as for her help with the PLFA analysis. Thanks to Clive Figueiredo and Allan Harms for the lab analysis. Thanks to Dick Puurveen for valuable technical assistance.

I would like to thank our lab group: Roxanne McMillan, Eartha Dupuis, Suzy Byron, Steve Clark, Maija Raudsepp, Chrie Frantik and Jennifer Lloyd for their lab and field assistance. Thanks to Xiao Tan, Zhili Feng, and Tinghui Jiang for their valuable suggestions.

The last and the biggest thank to my lovely daughter and my husband for their understanding and everlasting support.

Table of Contents

List of Tables

List of Figures

List of General Abbreviations

AMS	Accelerator Mass Spectrometry
C	Carbon
CFE	Chloroform Fumigation-Extraction
CFI	Chloroform Fumigation-Incubation
Ck	Control
Cl	Clay
CPMAS	Cross-Polarization Magic-Angle Spinning
CS	Coarse Silt
DOC	Dissolved Organic Carbon
DON	Dissolved Organic Nitrogen
Fl	Floatables
FS	Fine Silt
FYM	Farm Yard Manure
G-	Gram- negative Bacteria
G+	Gram- positive Bacteria
HC	Hydrolysable Carbon
LPS	Lipopolysaccharides
M	Manure treatment
MBC	Soil Microbial Biomass Carbon
MBN	Soil Microbial Biomass Nitrogen
MRT	Mean Residence Time
MS	Medium Silt
NHC	Non-hydrolysable Carbon
NMR	Nuclear Magnetic Resonance Spectroscopy
NPKS	Nitrogen, Phosphorus, Potassium and Sulfur fertilization
O.D.	Oven Dried
PLFAs	Phospholipids Fatty Acids
POM	Particulate Organic Matter
RDA	Redundancy Analysis
RR	Root Respiration
SA	Sand
SIC	Soil Inorganic Carbon
SIR	Substrate-Induced Respiration
SMB	Soil Microbial Biomass
SOC	Soil Organic Carbon
SOM	Soil Organic Matter
SWC	Soil Water Content
TC	Total Carbon
TN	Total Nitrogen

Chapter1

General Introduction

Literature review

1.1 Introduction

Soil pools constitute a major global carbon reservoir comprising both soil organic carbon (SOC) and soil inorganic carbon (SIC) components. The SOC pool consists of "a mixture of plant and animal residues at various stages of decomposition, of substances synthesized microbiologically and/or chemically from the breakdown products, and of the bodies of live microorganisms and small animals" (Schnitzer, 1991). The SIC pool includes elemental C and carbonate minerals of primary and secondary origin. Primary carbonates are inherited from the parent material while secondary carbonates are precipitated through the reaction of atmospheric CO_2 with Ca^{2+} and Mg^{2+} present in the soil solution (Lal and Kimble, 2000). Total global soil C stores including both SOC and SIC pools in the top 1m soil layer constitute about 2300 Pg, which is three times the atmospheric pool, estimated at 760 Pg, and 3.7 times the biotic pool estimated at 620 Pg (Lal, 2002). The reservoir of soil carbon has been considered as both a significant source and sink of atmospheric CO_2. Whether a soil will act as a C sink or a source is dependent upon both C fluxes into or out of the soil, and the residence time of C in the soil. A soil source results when net decomposition exceeds C inputs to the soil, either as a result of human activities such as clearing forests for agriculture (Houghton et al., 1983, Davidson and Ackerman, 1993) or because of increased decomposition rates due to an increase in temperature (Schimel et al. 1994). A soil sink may result from increases in plant C inputs to the soil from increases in photosynthesis either from elevated atmospheric CO_2 levels (i.e. C fertilization) or because of N fertilization (Melillo et al, 1996, Townsend et al. 1996). In this case, net accumulation of C in the soil is estimated from the difference between net ecosystem C uptake and trees growth rate (Wofsy et al. 1993).

It is estimated that approximately 1500 Pg of C is stored as organic matter in

1

the upper metre of soils (Lal, 2002). An astute management of soil organic matter (SOM) is pivotal to global environmental issues. It has been suggested that improved land management could result in sequestration of a substantial amount of soil C within a few decades and could be an option to reduce or at least mitigate the current increase in atmospheric CO_2 concentration (Metting et al., 1999; Post et al., 1998).

On a conceptual basis, SOM can be partitioned into the following forms or pools (Stevenson and Cole, 1999): (1) Litter: macroorganic matter (e.g., crop residues) that lies on the soil surface; (2) Light Fraction: plant residues and their partial decomposition products that reside within the soil proper; (3) Microbial biomass: cells of living microorganisms, notably bacteria, actinomycetes, and fungi; (4) Faunal biomass: tissues of animals (primarily invertebrates); (5) Belowground plant constituents: primarily roots with lesser amounts of dead roots and exudates; (6) Water-soluble organics: organic substances dissolved in the soil solution; (7) Stable humus: humified remains of plant and animal tissues that have become stabilized by microbial and chemical transformations and/or by association with inorganic soil components.

A major aim of research concerning soil organic matter has focused on simulation models whereby predictions can be made regarding nutrient cycling (N, P, S), soil organic matter storage, and the impact of residue management practices on environmental quality and soil productivity. A key component of most models is the distribution of organic matter, and nutrients contained within fractions (pools) that have distinct turnover times or mean residence times (MRT) (Stevenson and Cole, 1999). For this purpose, the soil organic matter has been partitioned into at least three identifiable C pools: root exudates and rapidly decomposed components of fresh plant litter ("active" pool); stabilized organic matter that persists in soils over several thousands of years ("passive" pool); and a poorly defined "intermediate" or "slow" C pool that has turnover times in the range of years to centuries (Trumbore, 1997).

1.2 Isotopic approaches to the study of SOM dynamics

Carbon has two stable isotopes ^{13}C and ^{12}C and a radioactive isotope ^{14}C.

Approximately 98.89% of all C in nature is the lighter isotope ^{12}C and 1.11% is ^{13}C. The stable C isotopic composition of a sample is generally expressed as the difference in parts per thousand (‰) from a standard because natural variation in the ratio of ^{13}C /^{12}C is small:

$$\delta^{13}C\ (‰) = \left[\frac{\left(^{13}C / _{12}C \right)_{sample}}{\left(^{13}C / _{12}C \right)_{PDB}} - 1 \right] \times 1000$$

where (^{13}C/ ^{12}C)$_{PDB}$ is the international PDB limestone standard (Craig, 1957).

1.2.1 Natural abundance ^{13}C and SOC turnover

The ^{13}C/ ^{12}C ratio of organic C found in terrestrial environments is determined largely by the C isotope fractionation that occurs during photosynthesis. Plants with the C_3 photosynthetic pathway exhibit greater discrimination against ^{13}C than plants with the C_4 pathway. The C_3 plants have δ^{13}C values ranging from approximately -40 to -23‰, with many occurring at about -26‰ while C_4 plants have δ^{13}C values ranging from -19 to -9 ‰ (Smith and Epstein, 1971; O'Leary, 1981). If a community dominated by C_3 plants has been compositionally stable for a relatively long time, then the soil organic matter (SOM) in that community should have similar δ^{13}C values as the C_3 plant. So these natural isotopic differences between plants can be used to study the dynamics of organic matter in soil. If that C_3 community is converted to a C_4 plant community, then the isotopic composition of the SOM will begin to shift towards that of the C_4 vegetation as the C_3 component decays out of the soil and is replaced by C_4 organic matter inputs; the rate at which the original mass of C_3-derived organic matter decays out of the system through time is a direct measure of the turn-over rate of organic matter. Changes in ^{13}C abundance in SOM after a shift in vegetation from C_3 to C_4 plants therefore can be used to define the size of the fast-cycling organic matter pool. For instance, Balesdent et al. (1987, 1988) proposed that after a given time of corn crop cultivation on a previous forest or prairie soil, the fraction f of soil carbon coming from the corn crop can be calculated from:

3

$$f = \frac{\delta - \delta_0}{\delta_1 - \delta_0} \quad \text{where}$$

δ: equal to the abundance ratio of soil organic C at that time;

δ_0:   equal to the value for the original soil;

δ_1:   equal to the value for the crop.

When the total soil C content, C, is known, the quantity, C_1, of C from the crop can be determined from $C_1 = C\,f$. The quantity, C_0, of residual C from the initial soil is $C_0 = C\,(1 - f)$. The decrease in C_0 as a function of time describes the decay of the whole pool of initial C from the prairie vegetation or a forest soil.

For instance, in a forest soil converted to continuous corn, 22% of the total organic C had turned over after 13 years (Balesdent et al., 1987 and 1988). On the other hand, the turnover time of the stable SOM pool was estimated to be >600 years.

The use of the ^{13}C natural abundance technique coupled with particle-size fractionation has further advanced SOM turnover studies, since these methods are well suited to study SOC dynamics over a time scale ranging from a few to several hundred years. Organic carbon in the top 0-5 cm layer of soils is transferred from standing biomass into the coarsest soil size fraction and then is degraded over time, with the residue progressively transferred into more resistant and finer particle sizes. Balesdent reported that particle size fractions coarser than 50μm and those finer than 2μm contained the youngest organic matter, and that the turn-over rate for the silt sized fraction was slower (Balesdent, 1987 and 1988). Solomon et al. (2002) further reported that after deforestation (C_3 forest) and conversion to continuous corn at Wushwush (25 years) and Munesa (30 years) (both in Ethiopia), forest-derived SOC declined by 48 and 61% respectively. Eighty and 96% of the initial forest-derived SOC (250-2000 μm) was lost from the sand, while 73 and 85% was lost from the silt fraction (2-250 μm). In contrast, 92 and 65% of the original SOC at Wushwush and Munesa was still present in the clay fraction (Solomon et al., 2002), demonstrating that SOM bound to clay (< 2μm) was more stable than SOM associated with sand and silt fractions. These results further suggested that SOC in sand is a very labile component of SOM and

4

is a more sensitive indicator of changes in soil C storage in response to land use changes than the other fractions.

Under similar climatic conditions, and comparable soil texture, carbon sequestration is primarily controlled by the amount and quality of plant residues and organic fertilizers. It is therefore essential to understand the dynamics of their decomposition in soils in order to predict the effects of soil and crop management on soil carbon content. Manure may be an important soil carbon pool source and at the same time organic fertilizer combined with the inorganic fertilizers can greatly influence the quality and quantity of the crop residues. Some studies on carbon dynamics following organic or inorganic fertilization were conducted. The Rothamsted classical field experiments on the effects of manuring on the amount SOM showed that the plots receiving FYM at 3.5t /ha annually over 20 years contain more SOC than the unmanured plot, but still did not achieve SOC steady state (Jenkinson and Rayner, 1977). From a long-term rye and maize monocultures experiment, SOC contents were higher in the surface soils of NPK fertilized plots than in the unfertilized plots (John et al., 2003). Gregorich et al. (1996) found that after 32 years of maize cultivation 22–30% of SOC was maize-derived under NPK treatment as compared to 15–20% in an unfertilized field.

Natural abundance ^{13}C analyses may be also successfully used with respiration measurements to quantify short-term crop residue decomposition rates under undisturbed field conditions. The combined use of natural abundance of ^{13}C and CO_2 emissions allows for (i) study of the decomposition of residues that are incorporated into the soil via natural root growth and typical agricultural practices and (ii) measurement of instantaneous decomposition rates, which provide information on the same time scale as changes of the variables that modulate decomposition of residues in soils (Rochette et al., 1999). The decomposition of maize residues during the year following their return to the soil significantly increased the ^{13}C isotope ratio of the soil CO_2 by 2 to 7 ‰ relative to the unamended bare control plots (Rochette et al., 1999).

1.2.2 Bomb ^{14}C and soil carbon turnover

5

Turnover rate of carbon in soils may be influenced by different factors: the chemical structure of the organic fraction; the diversity and activity of the soil biota; and the interaction between organic materials and the mineral matrix, such as the formation of organo-mineral complexes that are typically more resistant to microbial attack than uncomplexed organic materials (Quideau et al., 2000). Because active, slow and stable SOC play very different roles in SOC dynamics and nutrient cycling, it is desirable to define their pool sizes and respective turnover times.

The bombardment of ^{14}N by cosmic radiation in the atmosphere produces a low level of ^{14}C which when incorporated into plant tissue by photosynthesis provides a long-term tracer for SOM dating (^{14}C half-life is 5568 years). Radiocarbon dating using naturally occurring ^{14}C is particularly useful in characterizing the age of the more passive or resistant fractions of SOM, i.e.; with a turnover rate ranging from hundreds to thousands years, and in describing long-term SOM dynamics (O'Brien and Stout, 1978; O'Brien, 1984). Atmospheric testing of thermonuclear weapons in the 1950s and 1960s produced an input spike of $^{14}CO_2$ to the atmosphere, which subsequently entered terrestrial ecosystems through plants and was then recycled through animals, microorganisms, and soil organic matter to eventually enrich SOC with "bomb" ^{14}C. This increase can be regarded as an in situ labeling of soil organic C and permits estimates to be made for the MRTs (mean residence time) and turnover rates for both the older and younger soil organic carbon (Hsieh, 1992; O' Brien and Stout, 1978; Trumbore, 1993; Quideau et al., 2000).

Accelerator mass spectrometry (AMS) now allows ^{14}C measurements to be made on very small sample sizes, about 10,000 times smaller than those needed for decay counting (Trumbore and Zhang, 1996). Measurement of ^{14}C is used to determine the age of soil organic matter according to the following formula:

$$MRT = -(1/\lambda) \times \ln(F) \tag{1}$$

Where F is the fraction Modern carbon, and λ is the rate constant for the radioactive decay of ^{14}C ($\lambda = 0.000121$ per year). The fraction Modern carbon is calculated from the ^{14}C value corrected for the radioactive decay of the oxalate standard since 1950:

6

$$F = (1.0057 \times 10^{-3} \times {}^{14}C) + 1.0057 \qquad (2)$$

Carbon dating with ^{14}C, ^{13}C analyses, and extended incubation (more than 1000 days) of soils from historical plots can be combined to determine pool sizes and fluxes required in C balance calculations. Paul et al. (1997) found that 70% of the total soil C contained in 85-year cultivated wheat plots at Akron, CO., was derived from the native prairie vegetation. Carbon dating showed that the residue of acid hydrolysis comprised 56% of total C with a MRT over 2600 years (Paul et al., 1997). Collins et al. (2000) reported that the active pools represented 3-8% of the SOC with an average field MRT of 100 days by using an extended laboratory incubation followed by acid hydrolysis. The slow pools comprised 50% of SOC in the surface and up to 65% in the subsoils; they had MRTs ranging from 12 to 28 years for C_4-derived C and from 40 to 80 years for C_3-derived C. The resistant pool decreased from an average of 50% at the surface to 30% at depth (Collins et al., 2000)

Various physical, chemical and biological procedures for fractionating SOC have been developed to obtain pools with different turnover rates (Hsieh, 1992; Trumbore and Zheng, 1996; Quideau et al., 2000). For example, Quideau fractionated SOC into the floatable fraction (isolated from the sand fraction by floatation in water, density < 1.0 g/cm^3), sand fraction (>50 μm), coarse and medium silt (5-50 μm), fine silt fraction (2-5 μm), and clay fraction (<2 μm). Comparison of the ^{14}C analysis results in pre- and post-bomb soil samples showed that carbon associated with the fine silt fraction was relatively old, suggesting no or little recent carbon input to this fraction. The younger age for the clay-sized C, on the other hand, indicated more recent C input, possibly from dissolved organic carbon in percolating waters, or as a result of SOM mineralization processes from larger size fractions (Quideau et al., 2000).

Some researchers combined the density separation method and the chemical method to fractionate soil samples between low density (which is lighter than 2.0 g/cm^3 or 1.6 g/cm^3) and high density (which is heavier than 2.0 g/cm^3 or 1.6 g/cm^3). The dense fraction was hydrolyzed and further fractionated as hydrolysable (HC) and non-hydrolysable (NHC). Comparison of ^{14}C in pre- and post-bomb SOC acid-hydrolysis fractions(HC) and the positive δ ‰ ^{14}C values for

7

the hydrolysable fractions showed that most of this carbon was newly derived from ^{14}C $-CO_2$ (Martel and Paul, 1974; Trumbore and Zhang, 1996). The ^{14}C data of 0-20 cm soil collected in 1959 and 1992 further showed that the organic carbon associated with the dense fraction ($\rho > 2.0$ g/ cm^3) contains carbon of greater age than low-density fractions ($\rho < 2.0$ g/ cm^3), and non-hydrolyzable organic matter has the lowest turnover rate (Trumbore, 1993 and 1997).

After studying the dynamics of resistant soil carbon of several agricultural soils derived from forest and grassland soils, Paul et al. (2001) proposed that the MRTs of the total SOC were inversely correlated to sand content and directly related to clay content. Silt did not have a significant effect on the MRT of total SOC, but was positively correlated with the MRT of the NHC (Paul et al., 2001).

Using a combination of ^{14}C labeled plant material and radiocarbon dating, Jenkinson and Rayner (1977) followed the flux of C through five SOM pools (decomposable plant material, resistant plant material, soil microbial biomass, physically protected, and chemically stabilized organic matter) and constructed a model describing the turnover of SOM from soils under long-term management at the Rothamsted Experiment Station in Harpenden, U. K. (Jenkinson and Rayner, 1977). The ages of these pools ranged from several months for plant materials to several thousand years for chemically and physically stabilized SOM fractions (Jenkinson and Rayner, 1977). Hsieh proposed that the turnover of soil organic C could be described by just two major pools: an active pool with a turnover time of less than a few decades and a stable pool with a turnover time from hundreds to thousand years (Hsieh, 1992).

Martel and Paul (1974) measured the ^{14}C content of several agricultural soils and compared them to ^{14}C distribution in a soil labeled with ^{14}C-acetate. Carbon dating showed the MRTs for the surface horizon to be 350 years but varied from modern to 1910 years for different chemical fractions. Their work also indicated that SOM in surface horizons turned over more rapidly than in lower horizons (Martel and Paul, 1974).

1.3 ^{13}C and ^{14}C labeling and belowground C translocation

The inputs of plant organic matter to soil occur continuously through the

8

whole life of plants. During the growing season, organic substances are released into soil in the form of rhizodeposition such as water-soluble exudates, secretions, lysates, mucilages, sloughed-off cells and decaying roots. Of these substances, only soluble root exudates are readily available for microorganisms. When plants die, large, discrete inputs occur in the forms of root and shoot residues (Van Vuuren, 2000).

Currently three tracer methods are commonly used for the estimation of C input into the soil by plants: pulse labeling, continuous labeling, and ^{13}C natural abundance. The first two methods are based on the artificial labeling of plants: shoots are exposed to CO_2 in an atmosphere labeled with ^{14}C or ^{13}C. The shoots assimilate the labeled CO_2 and translocate a part of it to the soil. This allows for the calculation of C input by plants into the soil on the unlabeled background (Kuzyakov et al., 2000). Pulse labeling provides information on the recent photosynthate distribution at specific developmental stages of plants and can be used for kinetic investigations of CO_2 evolution from the soil. The limitation of pulse labeling is that the results of C allocation observed for a specific growth stage cannot be directly extrapolated to the whole growth period. Another shortcoming of studies employing ^{14}C or ^{13}C pulse labeling is that most of the time only organic matter fractions with relatively short turnover times (such as amino acids or carbohydrates) are labeled during the course of the experiment. The natural ^{13}C technique, by contrast, has the advantage of providing *in situ* labeling for thousands of years achieving labeling of all organic matter fractions, including those with extremely long turnover times (Balesdent, 1988).

The rhizodeposition of root exudates which represents an easily available carbon source greatly influences C turnover in soils and can lead to C accumulation or consumption due to its influence on the microbial activity in the rhizosphere. Unfortunately root derived substances have not been sufficiently investigated today because of several analytical challenges including: (1) their low concentration and (2) their fast decomposition rates. In this respect, the application of labeling experiments with C isotopes (^{14}C and ^{13}C) in rhizosphere studies has led to significant progress in the understanding of the C cycling within the rhizosphere (Kuzyakov et al., 2000).

9

A series of labeling pulses applied at regular intervals during plant growth (continuous labeling) has been found to provide a reasonable estimation of the amount of total C transferred by the plant into the soil and of the translocation of below-ground C (Wander and Yang, 2000; Kuzyakov et al., 2001). Most studies of the below-ground C translocation and partitioning have been conducted on cereals, mainly on wheat and barley (Gregoy and Atwell, 1991; Keith et al, 1986; Swinne et al, 1995a and b). Wheat transfered 26% of its total assimilated carbon below ground and barley 17% of the total. Carbon translocated below-ground was used for:

1). Root growth, around 7-13% of the total assimilated C was found in roots after the experiments;

2). Root respiration, around 7-14% of the total assimilated C was for root maintenance during growth and ion uptake;

3). Exudates, root hairs and fine roots, about 1-2% of the total assimilated C was decomposed by microorganisms to CO_2 shortly after release into the rhizosphere (Kuzyakov and Domananski, 2000).

The release of photosynthesized C from plant roots provides an important C source for biological activity in soils. The factors that control residue decomposition rate include temperature (Nyham, 1976; Lu et al., 2003), moisture (Schomberg et al., 1994), soil mineral nutrient supply (Cardon, 1996) and composition of the plant residues (Van Vuuren et al., 2000; Quideau et al., 2003). The incorporation of plant residues and organic manures significantly increased microbial biomass in rice soils in both pot and field experiments (Witt et al., 2000; Kuzyakov et al., 2000).

Much of the work on soil organic matter turnover has employed [14]C (Stevenson and Cole, 1999) or ^{13}C labeled soil and plant materials. The work has allowed the development of models for decomposition of crop residues in soil. These models recognize that individual consideration must be given to the different substrate components each of which may follow a different decomposition rate. A laboratory incubation experiment was conducted to investigate the fates of plant-derived C during a simulated fallow period in a rice soil (Lu et al., 2003). The results showed that the decomposition of the

10

incorporated residues could be divided into two phases: an initial rapid phase followed by a slower phase. The soil labile carbon pools (microbial biomass C and water-extractable organic C) were significantly increased by residue incorporation, especially during the rapid phase of residue decomposition.

Labeling with $^{14}CO_2$ allows separation of total soil CO_2 fluxes into autotrophic and heterotrophic respiration. Pulse labeling of shoots with $^{14}CO_2$ has been used in an attempt to further divide root-derived CO_2 into CO_2 originating from root respiration (RR) and that from microbial respiration of root-borne substances during plant growth on non-sterile soils. RR contributes about 40-50% of the root-derived CO_2 efflux, while the remaining 50-60% is derived from the microbial decomposition of root exudates and other rhizodeposits. The relative contribution of rhizomicrobial respiration to the total root-derived CO_2 efflux from soil increases with time following the pulse labeling (Kuzyakov et al., 2002 a and b).

1.4 Chemical characteristics of soil organic carbon as revealed by ^{13}C NMR

Nowadays major information on SOM composition comes from molecular-level chemical analyses of specific plant or microbial components in combination with ^{13}C NMR spectroscopy. Solid–state ^{13}C nuclear magnetic resonance spectroscopy (NMR) has become an important tool for examining the chemical structure of natural organic materials and the chemical changes associated with decomposition. Some of the first CPMAS ^{13}C NMR spectra of whole soil were reported by Wilson et al. (1981). Since then, solid-state ^{13}C NMR spectroscopy has become a major tool in investigations of soil organic matter structure (Wilson, 1987 and 1990). A major advantage of the solid-state cross-polarization magic-angle spinning (CPMAS) ^{13}C NMR technique is the possibility to obtain structural information on SOM in bulk soils or solid fractions without the necessity of extracting the organic material, as the extracts contain only part of the total SOM for many soils.

The ^{13}C NMR spectra of soil or humic substances are generally divided into four main chemical-shift regions, representing alkyl C (0-50 ppm), O-alkyl C (50-110 ppm), aromatic C (110-160 ppm), and carboxyl C (160-200 ppm) (Mahieu et al., 1999). Statistical analysis from NMR results in the published literature (76

11

papers) showed that there was a remarkable similarity among all soils with respect to the distribution of different forms of C despite the wide range of land use (arable, grassland, uncultivated, forest), climate (from tropical rainforest to tundra), cropping practice, fertilizer or manure application, and the different spectrometer characteristics and experimental conditions used. Functional groups in whole soils were always in the same abundance order despite the generally wide proportion range: O-alkyls (a mean of 45% of the spectrum, increasing with soil C content), followed by alkyls (mean 25%), aromatics (mean 20%), and finally carbonyls (mean 10%, decreasing with soil C content). Humic and fulvic acids contained much smaller proportions of O-alkyls than whole soils (means of 26%) (Mahieu et al., 1999).

Solid-state ^{13}C NMR can also be used to characterize the bulk chemical composition of plant residues entering the soil. The traditional view of decomposition and humification of organic residues in soils includes a loss of proteinaceous and carbohydrate materials and an accumulation of humic materials containing a high proportion of aromatic carbon. The more routine application of solid-state ^{13}C NMR spectroscopy and other analytical techniques to monitor changes in the chemical composition of carbon contained in decomposing natural organic materials has revealed that organic materials containing alkyl carbon accumulate, whereas aromatic carbon, presumably derived from lignin structures, accumulates as the carbohydrates cellulose and hemicellulose are utilized, but then disappears with further decomposition to leave a residue with high contents of alkyl carbon (Bracewell and Robertson, 1987; Baldock et al., 1992; Zech et al., 1992). The ratio of alkyl/O-alkyl carbon contents (A/O-A ratio) may provide an even more sensitive index of the extent of decomposition (Baldock and Preston, 1995; Baldock et al., 1997; Golchin et, al., 1994, 1995 and 1997). Generally, the relative amount of alkyl C increases during biodegradation, whereas the amount of O-alkyl C shows a relative decrease.

Mineral soil with low C contents has not been investigated as much as C-rich soils. This is due to the fact that spectra from solid samples low in organic carbon (< 5 g/ kg soil) are more difficult to obtain because of sensitivity problems (Mahieu et al., 1999). This can be overcome by selectively removing the mineral fractions

12

with 10 % HF and concentrating the organic carbon content (Skjemstad et al., 1994; Schmidt et al., 1997). Studies have shown that this treatment does not lead to major changes within the resolution of solid-state NMR spectra in bulk samples as well as in particle-size fractions (Preston and Newman, 1995; Preston, 1996; Skjemstad et al., 1994; Schmidt et al., 1997).

It was shown that physical fractionation yields SOC pools of different functions and the dynamic processes of litter decomposition and introduction into more stable pools can be followed easily, especially if isotopic methods are used in combination with NMR analysis (Stemmer et al., 1999). Particle-size fractionations are therefore an interesting tool for research related with soil SOC dynamics. The clay fraction is more different from the bulk soil in terms of OM composition than the coarse fractions. Clay-size fractions generally show a higher content of alkyl carbon than the whole soils (Baldock et al., 1992; Mahieu et al., 1999). The sand-sized fractions are dominated by high proportions of O-alkyl carbon similar to the whole soil. The intermediate size fractions contain the greatest content of aromatic carbon (Baldock et al., 1992).

Some analyses of the organic chemical composition of microbial biomass in soils with the use of ^{13}C NMR spectroscopy have been made (Baldock et al., 1990; Kögel-Knabner et al., 1992; Knicker and Liidemann, 1995; Golchin et al., 1996). Baldock et al. (1990) first demonstrated that the composition of bacterial biomass differed distinctly from fungal biomass. Both groups contain high proportions of alkyl C structures and small amounts of aromatic C, but fungi have more O-alkyl C and less alkyl C compared to bacteria (Baldock et al., 1990). Golchin et al. (1996) concluded that microbial materials synthesized from glucose by soil microorganisms in a laboratory incubation experiment were mostly O-alkyl, alkyl and carboxyl carbon. Phenolic and aromatic structures were only found in small amounts (Golchin et al., 1996).

1.5 Soil microbial biomass and microbial community structure

The soil microbial community is virtually responsible for all biochemical transformations occurring in soils. The potential influence of the soil microbes may be assessed by its size and structure. The measure of the size of the soil

13

microbial biomass (SMB) is of importance in studies of nutrient cycling in soils and has been used as an ecological marker. It has been found that there often is a close relationship between the size of the soil microbial biomass and the soil organic carbon content (Jenkinson and Ladd, 1981, Smith and Paul, 1990).

The methods of measuring SMB are numerous and include chloroform fumigation-incubation (CFI), chloroform fumigation-extraction (CFE), substrate-induced respiration (SIR), and analyses of phospholipids fatty acids (PLFAs) etc. Chloroform fumigation to lyse soil microbial cells followed by incubation has become a standard tool for determining total SMB in soils since it was first reported (Jenkinson and Powlson, 1976). The quicker and simpler procedure of CFE has become a widely used method of SMB determination (Vance et al., 1987). Estimates of SMB by CFE are not influenced by the presence of non-biomass materials in the soil, such as root fragments or freshly added substrates (Martens, 1995), nor are they sensitive to prolonged storage at 4° C (Ross, 1991).

The SIR procedure for measuring the SMB was first presented by Anderson and Domsch (1978). This method measures the response of the SMB to the addition of a readily available substrate.

Phospholipids are found in the membranes of all living cells, and rapidly decompose to diglycerides following cell death (White et al., 1979). The total phospholipid fatty acid (PLFA) content of the soil extracts has been used to estimate soil microbial biomass (Klamer and Bååth, 1998; Denton et al., 1999). It has been found to be at least as sensitive an index of SMB as soil respiration following perturbations (Frostegård et al., 1993 a and b). An advantage of this procedure is that the extracts are easily analyzed to identify the different extracted PLFAs yielding information about both the size of the SMB and its structural composition (Zelles et al., 1992; Frostegård et al., 1993b; White and Ringleberg, 1998). PLFA is also a biochemical method in that it can provide *in situ* detailed information about the structure of the live microbial community and detect subsequent changes in this community following various disturbances (Fedele, 1986; Bååth et al., 1992; Frostegård et al., 1993a and b; Zelles and Bai, 1994). A good linear relationship between total PLFA and CFE-MBC (R^2 = 0.77) (Bailey,

14

2002), as well as between total PLFA and SIR (r = - 0.96) was observed (Beyer, 1995).

PLFA profiles were found to be sensitive indicators of changes in microbial community structure due to differences in agricultural management regimes that included grassland, bare follow, and crop rotations, different organic matter inputs, liming and burning treatments (Zelles et al., 1992; Bååth et al., 1995). High organic matter inputs can increase the microbial biomass carbon, microbial nitrogen and significantly influence PLFA profiles (Bossio and Scow, 1998a; Peacock et al., 2001; Hopkins and Shiel, 1996). Cropped plots were higher in microbial biomass than their fallowed counter-parts. Under fallow, microbial biomass was greatest in no till and least in plowed plots (Drijber et al., 2000). The size of the microbial biomass (total PLFAs) was negatively affected by a low soil pH (Frostegard et al., 1993a) and clear-cutting (Bååth et al., 1995).

Several phospholipid fatty acids have been found to be biomarkers for specific microorganism groups, since different subsets of the community have different PLFA patterns. Bacteria characteristically contain 18:1ω7, i17:1ω7, 17:0cyc, 19:0 cyc, p10-17:0, i15:0, a15:0 and i16:0 PLFAs (Tunlid and White, 1992). The PLFA 10Me18:0 is mainly found in actinomycetes (Kroppensted, 1985) and 18:2ω6 is indicative of fungi although small quantities of 18:2ω6 also occur in some bacteria (Jantzen et al., 1987).

A clear change in the composition of the community structure at different sampling periods was observed (Bai, 2000; Peterson et al., 2002). Bossio, et al. (1998b) reported that a group of fatty acids (i14:0, a15:0, 16:1ω7c, 16:1ω5c, 14:0, and 18:2ω6c) was enriched in the organic farming plots and another group (10Me16:0, 2OH 16:1 and 10Me17:0) was consistently lower in relative abundance in the organic farming system.

Background and rationale

The Breton Classical Plots were established in 1929 near the village of Breton, 100 km southwest of Edmonton, by the Department of Soils, University of

15

Alberta. These plots were originally designed by Dr. F. A. Wyatt and Dr. J. D. Newton to find 'a system of farming suitable for the wooded soil belt', so the crop rotation studies started in 1930. The Breton Classical Plots are the only continuous, long-term plots on Gray Luvisols in Canada and possibly in the world.

Figure 1.1 Aerial view of the Breton Plots (from website: www.rr.ualberta.ca)

Over the past 70 years' research, the Breton Plots have provided some very important information on the agricultural management and dynamics of cultivated Luvisolic soils (Robertson, 1991, Chakraborty, 2001):

(1) Overcoming nutrient deficiencies and improving crop quality

Gray Luvisolic soils have a thin A horizon which has a slightly acidic reaction (pH 6.0 to 6.5), low organic C content (10 to 20 g kg^{-1}) and low nutrient supplying capacity. The A horizons often develop poor tilth when cultivated. Most soil related problems such as crusting, low water holding capacity, low fertility, and low buffering capacity against pH change arise from the low organic matter content of the A horizon. Impeded water transmission and restricted root growth may arise from the presence of a dense, very firm and acidic B horizon.

Several important practical conclusions had been drawn after more than 60

16

years of research (Robertson, 1991): first, that the addition of sulphur to legumes has resulted in marked yield increases; secondly, that the nitrogen needed to obtain satisfactory cereal crops can be obtained from legumes and manure as well as from commercial fertilizer; and thirdly, that manure has improved the soil tilth properties and has increased the macro- and micro- nutrient levels in the soils as compared to the control (Ck) plots.

(2) Dynamics of soil organic carbon

Gray Luvisolic soils are inherently low in organic matter, but careful management of these soils can result in increased soil carbon levels. Using longer crop rotations that include forages and manure applications was found to increase crop yields and SOC levels. Between 1938 and 1998, while SOC declined at the rate of 14 and 7 g C m^{-2} yr^{-1} in the upper 0.15 m depth of the control and NPKS treatments of the 2-yr rotation, it gained 7 g C m^{-2} yr^{-1} in the manure treatment of the same rotation, and 28 g C m^{-2} yr^{-1} in the manure treatment of the 5-yr rotation (Grant et al., 2001).

The overall objective of this study was to take advantage of the ongoing long-term studies at the Breton plots to reach a better understanding of the influence of manure addition and NPKS fertilization on soil organic matter processes in cultivated Luvisolic soils. Specific objectives were as follows:

1. To experimentally determine long-term changes in soil organic carbon (SOC) pools at the Classical Breton Plots by using a physical separation method on archived samples as well as on soils (0-15 cm depth) sampled in 2003. In particular, we were interested to test the hypothesis proposed by Izaurralde et al. (2001) concerning the redistribution of SOC from the active to the passive pool. Secondly, changes in SOC storage in these different pools were assessed as a function of cropping management (2-yr versus 5-yr rotation) and fertilization treatments: no added fertilizer (Ck), full chemical fertilization (NPKS), and farmyard manure application (M) (see Chapter 2).

2. To examine the influence of chemical fertilization and manure application on the size of the soil microbial biomass at the Breton Classical Plots, and to study the effects of manure and fertilizer on the microbial community

17

diversity using PLFA analysis (see Chapter 3).

3. To partition total soil respiration into the soil's different physical fractions, to compare carbon mineralization from the whole soil with that of selected soil carbon fractions, and to determine whether long-term manure application had significant effects on carbon mineralization fluxes at the Breton Plots (see Chapter 4).

18

References

Anderson, J.P.E., Domsch, K.H., 1978. A physiological method for the quantiative measurement of microbial biomass in soils. Soil Biology & Biochemistry 10, 215-221.

Bååth, E., Frostegård, A., Fritze, H., 1992. Soil bacterial biomass, activity, phospoholipid fatty acid pattern, and pH tolerance in an area polluted with alkaline dust decomposition. Applied and Environmental Microbiology 58, 4026-4031.

Bååth, E., Frostegård, A., Pennanen, T., Fritze, H., 1995. Microbial community structure and pH response in relation to soil organic matter quality in wood-ash fertilized, clear-cut or burned coniferous forest soil. Soil Biology & Biochemistry 27, 229-240.

Bai, Q., 2000. Characterization of microbial consortia in paddy rice soil by phospholipid analysis. Microbial Ecology 39, 273-281.

Bailey, V.L., 2002. Relationships between soil microbial biomass determined by chloroform fumigation-extraction, substrate-induced respiration, and phospholipids fatty acid analysis. Soil Biology & Biochemistry 34, 1385-1389.

Baldock, J.A., Oades, J.M., Vassallo, A.M., Wilson, M.A., 1990. Solid state CP/MAS ^{13}CNMR analysis of bacterial and fungal cultures isolated from a soil incubated with glucose. Australian Journal of Soil Research 28, 213-225.

Baldock, J.A., Oades, J.M., Water, A.G., Peng, X., Vassallo, A.M., Wilson, M.A., 1992. Aspects of the chemical structure of soil organic materials as revealed by solid-state ^{13}C NMR spectroscopy. Biochemistry 16, 1-42.

Baldock, J.A., Preston, C.M., 1995. Chemistry of carbon decomposition processes in forests as revealed by solid-state ^{13}C nuclear magnetic resonance. In: McFee, W. W., Kelly, J. M., (Eds.), Carbon Forms and Functions in Forest Soils, Soil Science Society of America Journal pp. 89-117.

Baldock, J.A., Oades, J.M., Nelson, P.N., Skene, T.M.,Golchin, A., Clarke, P. , 1997. Assessing the extent of decomposition of natural organic materials

using solid-state 13C NMR spectroscopy. Australian Journal of Soil Research 35, 1061-1083.

Balesdent, J., Mariotti, A., Guillet, B., 1987. Natural ^{13}C abundance as a tracer for studies of soil organic matter dynamics. Soil Biology & Biochemistry 19, 25-30.

Balesdent, J., Wagner, G.H., Mariotti, A., 1988. Soil organic matter turnover in long-term field experiments as revealed by carbon-13 natural abundance. Soil Science Society of America Journal 52, 118-124.

Beyer, L., 1995. Soil microbial biomass and organic matter composition in soils under cultivation. Biology and Fertility of Soils 19, 197-202.

Bossio, D.A., Scow K.W., 1998a. Impacts of carbon and flooding on soil microbial communities: phospholipid fatty acid profiles and substrate utilization patterns. Microbial Ecology 35, 265-278.

Bossio, D.A., Scow K.W., Gunapala, N., Graham, K.J., 1998b. Determinants of soil microbial communities: effects of agricultural management, season, and soil type on phospholipid fatty acid profiles, Microbial Ecology 36, 1-12.

Bracewell, J.M., Robertson, G.W., 1987. Characteristics of soil organic matter in temperate soil by soil by cuie-point pyrolysis-mass spectrometry, 3, Transformations occurring in surface organic horizons. Geoderma 40, 333-344.

Cardon, Z.G., 1996. Influence of rhizodeposition under elevated CO_2 on plant nutrition and soil organic matter. Plant Soil 187, 277-288.

Chakraborty, S. 2001. Impact of phosphorus on soil organic matter dynamics in the long-term Classical Plots at Breton, Alberta. M.Sc. Thesis, Renewable Resources Dept, Univ. of Alberta, Edmonton. pp. 1-104.

Collins, H.P., Elliott, E.T., Paustian, K., Bundy, L.G., Dick, W.A., Huggins, D.R., Smucker, A.J.M., Paul, E.A., 2000. Soil carbon pools and fluxes in long-term corn belt agroecosystems. Soil Biology & Biochemistry 32, 157-168.

Craig, H., 1957. Isotopic standards for carbon and oxygen and correction factors for mass spectrometric analysis of carbon dioxide. Geochimica et

Cosmochimica Acta 12, 133-149.

Davidson, E.A., Ackerman, I.L., 1993. Changes in soil carbon inventories following cultivation of previously untilled soils. Biogeochemistry 20, 161-193.

Denton, C.S., Bardgett, R.D., Cook, R., Hobbs, P.J., 1999. Low amount of root herbivory positively influence the rhizosphere microbial community in a temperate grassland soil. Soil Biology & Biochemistry 31, 155-165.

Drijber, R.A., Doran, J.W., Pankhurst, A.M., Lyon, D.J., 2000. Changes in soil microbial community structure with tillage under long-term wheat-fallow management. Soil Biology & Biochemistry 32, 1419-1430.

Fedele, T.W., 1986. Microbial distribution in soil-new techniques. In: Megusar F, Gantar M, (Eds.), Perspectives in Microbial Ecology. Slovene Society for Microbiology, Ljubjana, pp. 493-498.

Frostegård, A., Bååth, E., Tunlid, A., 1993a. Shifts in the structure of soil microbial communities in limited forests as revealed by phospholipid fatty acid analysis. Soil Biology & Biochemistry 25, 723-730.

Frostegård, A., Tunlid, A., Bååth, E., 1993b. Phospholipid fatty acid composition, biomass, and activity of microbial communities from two soil types experimentally exposed to different heavy metals. Applied and Environmental Microbiology 59, 3605-3617.

Golchin, A., Oades, J.M., Skjemstad, J.O., Clarke, P., 1994. Soil structure and carbon cycling. Australian Journal of Soil Research 32, 1043-1068.

Golchin, A., Clarke, P., Oades, J.M., Skjemstad, J.O., 1995. The effects of cultivation on the composition of organic matter and structural stability of soils. Australian Journal of Soil Research 33, 975-993.

Golchin, A., Clarke, P., Oades, J.M., 1996. The heterogeneous nature of microbial products as shown by solid-state ^{13}C CPMAS NMR spectroscopy. Biochemistry 34, 71-97.

Golchin, A., Baldock, J.A., Oades, J.M., 1997. A model linking organic matter decomposition, chemistry, and aggregate dynamics. In: Lal, R., Kimble, J.M., Follett, R.F. Stewart, B.A., (Eds.), Soil Processes and The Carbon Cycle, CRC Press, Boca Raton, pp. 245-266.

Grant, R.F., Juma, N.G., Robertson, J.A., Izaurralde, R.C., McGill, W.B., 2001. Long-term changes in soil carbon under different fertilizer, manure, and rotation: testing the mathematical model ecosys from the Breton Plots. Soil Science Society of America Journal 65, 205-214.

Gregorich, E.G., Ellert, B.H., Drury, C.F., Liang, B.C., 1996. Fertilization effects on soil organic matter turnover and corn residue C storage. Soil Science Society of America Journal 60, 472–476.

Gregoy, P.J., Atwell, B.J., 1991. The fate of carbon in pulse labeled crops of barley and wheat. Plant and Soil 136, 205-213.

Houghton, R.A., J.E. Hobbie, J.M. Melillo, B. Moore, B.J. Peterson, G.R. Shaver, G.M. Woodwell. 1983. Changes in the carbon content of terrestrial biota and soils between 1860 and 1980: A net release of CO_2 to the atmosphere. Ecological Monographs 53, 235-262.

Hopkins, D.W., Shiel, R.S., 1996. Size and activity of soil microbial communities in long-term experimental grassland plots treated with manure and inorganic fertilizers. Biology and Fertility of Soils 22, 66-70.

Hsieh, Y.P., 1992. Pool size and mean age of stable soil organic carbon in cropland. Soil Science Society of America Journal 56, 460-464.

Izaurralde, R.C., McGill, W.B., Robertson, J.A., Juma, N.G., Thurston, J.J., 2001. Carbon balance of the Breton Classical Plots over half a century. Soil Science Society of America Journal 65, 431-441.

Jenkinson, D.S., Powlson, D.S., 1976. The effects of biocidal treatments on metabolism in soil--I. Fumigation with chloroform. Soil Biology & Biochemistry 8,167-177.

Jenkinson, D.S., Rayner, J.H., 1977. The turnover of soil organic matter in some of the Rothamsted classical experiments. Soil Science 123, 298-305.

Jantzen, E., Kvalheim, O.M., Hauge, T.A., Hagen, N., Bövre, K., 1987. Grouping of bacteria by Simca pattern recognition on gas chromatographic lipid data: Patterns among Moraxella and rod-shaped Neisseria. Systematic and applied microbiology 9, 142-150.

Jenkinson, D.S., Ladd, J.N., 1981. Microbial biomass in soil: measurement

22

and turnover. In: Bollag, J. M., Stotsky, G., (Eds.), Soil Biochemistry, Dekker, New York, pp. 415-471.

John, B., Ludwig, B., Flessa, H., 2003. Carbon dynamics determined by natural ^{13}C abundance in microcosm experiments with soils from long-term maize and rye monocultures. Soil Biology & Biochemistry 35, 1193-1202.

Keith, H., Oades, J.M., Martin, J.K., 1986. Input of carbon to soil from wheat plants. Soil Biology & Biochemistry 18, 445-449.

Klamer, M., Bååth, E., 1998. Microbial community dynamics during composting of straw material studied using phospholipid fatty acid analysis. FEMS Microbiology Ecology 27, 9-20.

Knicker, H., Lüdemann, H.D., 1995. C-13 and N-15 NMR analysis of some fungal melanins in comparison with soil organic matter. Organic Geochemistry 23, 1023-1028.

Kögel-Knabner, I., de Leeuw, J.W., Hatcher, P.G., 1992, Nature and distribution of alkyl carbon in forest soil profiles: implications for the origin and humification of aliphatic biopolymers. The Science of the Total Environment, 117/118, 175-185.

Kroppenstedt, R. M. 1985. Fatty acid and menaquinone analysis of actinomycetes
and related organisms. Technical Series (Society for Applied Bacteriology) 20,173–199.

Kuzyakov, Y., Fridel, J.K., Stahr, K., 2000. Review of mechanisms and quantification of priming effects. Soil Biology & Biochemistry 32, 1485-1498.

Kuzyakov, Y., Domanski, G., 2000. Carbon input by plants into the soil. Journal of Plant Nutrient and Soil Science 163, 421-431.

Kuzyakov, Y., Ehrensberger, H., Stahr, K., 2001. Carbon partitioning and below-ground translocation by Lolium perenne. Soil Biology & Biochemistry 33, 61-74.

Kuzyakov, Y., 2002a. Review: factors effecting rhizosphere priming effects. Journal of Plant Nutrient and Soil Science 165, 382-396.

Kuzyakov, Y., 2002b. Separating microbial respiration of exudates from root respiration in non-sterile soils: a comparison of four methods. Soil Biology & Biochemistry 34, 1621-1631.

Lal, R., Kimble, J.M., 2000. Pedogenic carbonates and the global carbon cycle. In: Lal, R.,(Ed.), Global Climate Change and Pedogenic Carbonates, CRC/Lewis Publishers, Boca Raton, FL., pp.1-14.

Lal, R., 2002. Why carbon sequestration in agricultural soils? In: Kimble, J. M., Lal, R., Follett, R.F., (Ed.), Agricultural Practices and Policies for Carbon Sequestration in Soil, Lewis Publishers, pp. 21-30.

Lu, Y., Watanabe, A., Kimura, M., 2003. Carbon dynamics of rhizodeposits, root- and shoot- residues in a rice soil. Soil Biology & Biochemistry 35, 1223-1230.

Mahieu, N., Powlson, D.S., Randall, E.W., 1999. Statistical analysis of published C-13 CPMAS NMR spectra of soil organic matter. Soil Science Society of America Journal 63, 307-319.

Martel, Y.A., Paul, E.A., 1974. The use of radiocarbon dating of organic matter in the study of soil genesis. Soil Science Society of America Journal 38, 501-506.

Martens, R., 1995. Current methods for measuring microbial biomass C in soil: potentials and limitations. Biology and Fertility of Soils 19, 87-99.

Melillo, J.M., Houghton, R.A., Kicklighter, D.W., McGuire, A.D., 1996. Tropical deforestation and the global carbon budget. Annual Review of Energy and the Environment 21, 293-310.

Metting, F., Smith, J., Amthor, J., 1999. Science needs and new technology for soil carbon sequestration. In: Rosenberg, N., Izaurrade, R., Malone, E., (Eds.), Carbon Sequestration in Soils—SCIENCE, Monitoring and Beyond, Proceedings of the St. Mithaels workshop. Battelle Press, Columbus, OH., pp.1-34.

Nyham, J.W., 1976. Influences of soil temperature and water tension on the decomposition rate of carbon-14 labeled herbage. Soil Science 121, 288-293.

O'Brien B.J., Stout, J.D., 1978. Movement and turnover of soil organic matter

24

as indicated by carbon isotope measurements. Soil Biology & Biochemistry 10, 309-317

O'Brien B.J., 1984. Soil organic carbon fluxes and turnover rates estimated from radiocarbon enrichments. Soil Biology & Biochemistry 16, 115-120.

O'Leary, M., 1981. Carbon isotope fraction in plants. Phytochemistry 20, 553-557.

Paul, E.A., Paustian, K.H., Elloitt, E.T., Cole, C.V., 1997. Soil Organic Matter in Temperate Agroecosystems: Long-term Experiments in North America. CRC Press, Boca Raton, FL.

Paul, E.A., Collins, H.P., Leavitt, S.W., 2001. Dynamics of resistant soil carbon of Midwestern agricultural soils measured by naturally occurring ^{14}C abundance. Geoderma 104, 239-256.

Peacock, A.D., Mullen, M.D., Ringelberg, D.B., Tyler, D.D., Hedrick, D.B., Gale, P.M., White, D.C., 2001. Soil microbial community responses to dairy manure or ammonium nitrate applications. Soil Biology & Biochemistry 33, 1011-1019.

Peterson, S.O., Frohne, P.S., Kennedy, A.C., 2002. Dynamics of a soil microbial community under spring wheat. Soil Science Society of America Journal 66, 826-833.

Post, W., Izaurralde, R., Mann, L., Bliss, N., 1998. Monitoring and verifying soil organic carbon sequestration. In: Rosenberg, N., Izaurrade, R., Malone, E., (Eds.), Carbon Sequestration in Soils—SCIENCE, Monitoring and Beyond, Proceedings of the St. Mithaels workshop. Battelle Press, Columbus, OH., pp.41-66.

Preston, C.M., Newman, R.H., 1995. A long-term effect of N fertilization on the 13C CPMAS NMR of de-ashed humin in a second- growth Douglas-fir stand of coastal British Columbia. Geoderma 68, 229–241.

Preston, C.M., 1996. Applications of NMR to soil organic matter analysis: history and prospects. Soil Science 161, 144-166.

Quideau, S.A., Anderson, M.A., Graham, R.C., Chadwick, O.A., Trumbore, S.E., 2000. Soil organic matter processes: characterization by ^{13}C NMR and ^{14}C measurements. Forest Ecology and Management 138, 19-27.

25

Quideau, S.A., Graham, R.C., Feng, X., Chadwick, O.A., 2003. Natural isotopic distribution in soil surface horizons differentiated by vegetation. Soil Science Society of America Journal 67, 1544-1550.

Robertson, J.A., 1991. Long-term results from the Breton Plots, Robertson, J.A. (Eds.) 61st Annual Breton Plots: Field Day and Soils/Crops Clinic, University of Alberta, Edmonton. pp. 14-33.

Rochette, P., Angers, D.A., Flanagan, L.B., 1999. Maize residue decomposition measurement using soil carbon dioxide fluxes and natural abundance of carbon-13. Soil Science Society of America Journal 63, 1385-1396.

Ross, D.J., 1991. Microbial biomass in a stored soil: a comparison of different estimation procedures. Soil Biology & Biochemistry 23, 1005-1007.

Schimel, D.S., Braswell, B.H., Holland, E.A., McKeown, R., Ojima, D.S., Painter, T.H., Parton, W.J., Townsend, A.R., 1994. Climatic, edaphic and biotic controls over storage and turnover of carbon in soils. Global Biogeochemical Cycles 8, 279-293.

Schmidt, M.W.I., Knicker, H., Hatcher, P.G., Kogel-Knabner, I., 1997. Improvement of ^{13}C and ^{15}N NMR spectra of bulk soils, particle size fractions and organic material by treatment with hydrofluoric acid (10%). European Journal of Soil Science 48, 319-328

Schnitzer, M., 1991. Soil organic matter- the next 75 years. Soil Science 151, 41-58.

Schomberg, H.H., Steiner, J.L., Unger, P.W., 1994. Decomposition and nitrogen dynamics of crop residue quality and water effects. Soil Science Society of America Journal 58, 372-381.

Skjemstad, J.O., Clarke, P., Taylor, J.A., Oades, J.M., Newman, R.H., 1994. The removal of magnetic materials from surface soils - a solid state ^{13}C CPMAS NMR study. Australian Journal of Soil Research 1215-1229.

Smith, B.N., Epstein, S., 1971. Two categories of ^{13}C/^{12}C ratios for higher plants. Plant Physiology 47, 380.

Smith, J.L., Paul, E.A., 1990. The significance of soil biomass estimations. In: Bollag, J. M., Stotsky, G., (Eds.), Soil Biochemistry, Dekker, New York, pp.

357-396.

Solomon, D., Fritzsche, F., Lehmann, J., Tekalign, M., Zech, W., 2002. Soil organic matter dynamics in the subhumid agroecosystems of the Ethiopian Highland: evidence from natural ^{13}C abundance and particle-size fractionation. Soil Science Society of America Journal 66, 969-978.

Stemmer, M., von Lu¨tzow, M., Kandeler, E. Pichlmayer, F., Gerzabek, M.H., 1999. The effect of maize straw placement on mineraliza-tion of C and N in soil particle size fractions. European Journal of Soil Science 50,73–86.

Stevenson, F.J., Cole, M.A., 1999. Cycles of Soil –Carbon, Nitrogen, Phosphorus, Sulfur Micronutrients. Second Edition John Wiley & Sons Inc.

Swinne, J., Van Veen, J.A., Merckx, R., 1995a. Carbon fluxes in the rhizosphere of winter wheat and spring barley with conventional vs integrated farming. Soil Biology & Biochemistry 27, 811-820.

Swinne, J., Van Veen, J.A., Merckx, R., 1995b. Root decay and turnover of rhizodeposits estimated by ^{14}C pulse-labeling in field-grown winter wheat and spring barley. Soil Biology & Biochemistry 27, 211-217.

Townsend, A.R., Braswell, B.H., Holland, E.A., Penner, J.E., 1996. Spatial and temporal patterns in terrestrial carbon storage due to deposition of anthropogenic nitrogen. Ecological Applications 6, 806-814.

Trumbore, S. E., 1993. Comparison of carbon dynamics in tropical and temperate soil using radiocarbon measurements. Global Biogeochemical Cycles, 7, 275-290.

Trumbore, S. E., Zheng, S., 1996. Comparison of fractionation methods for soil organic matter ^{14}C analysis. Radiocarbon 38, 219-229.

Trumbore, S.E., 1997. Potential responses of soil organic carbon to global environmental change. Proc. National Academy of Sciences USA Vol 94, pp. 8284-8291.

Tunlid, A., White, D., 1992. Biochemical analysis of biomass, community structure, nutritional status, and metabolic activity of microbial communities in soil. In: Stotzky, G., Bollag, J., M., (Eds.), Soil Biochemistry, Dekker, New York, pp. 229-262.

Van Vuuren, M.M.I., Robinson, D., Scrimgeour, C.M., Raven, J.A., Fitter, A.H., 2000. Decomposition of ^{13}C-labeled wheat root systems following growth at different CO_2 concentrations. Soil Biology & Biochemistry 32, 403-413.

Vance, E.D., Brookes, P.C., Jenkinson, D.S., 1987. An extraction method for measuring soil microbial biomass C. Soil Biology & Biochemistry 19, 703-707.

Wander, M.M., Yang, X., 2000. Influence of tillage on the dynamics of loose- and occluded particulate and humified organic matter fractions. Soil Biology & Biochemistry 32, 1151-1160.

White, D., Davis, W.M., Nickels, J.S., King, J.D., Bobbie, R.J., 1979. Determination of the sedimentary microbial biomass by extractable lipid phosphate. Oecologia 40, 51-62.

White, D.C., Ringleberg, D.B., 1998. Signature lipid biomarker analysis. In: Burlage, R.S., Atlas, R., Stahl, D., Geesey, G., Sayler, G. (Eds.), Techniques in Microbial Ecology, Oxford University Press, New York, pp. 255-272.

Wilson, M.A., Pugmire, R.J., Zilm, K.W., Gob, K.M., Heng, S., Grant, D., 1981. Cross-polarization ^{13}C NMR spectroscopy with magic angle spinning characterizes organic matter in wholesoils. Nature 294, 648-650.

Wilson, M.A., 1987. NMR Techniques and Applications in Geochemistry and Soil Chemistry, Pergamon Press, Oxford, pp. 352.

Wilson, M.A., 1990. Application of nuclear magnetic resonance spectroscopy to organic matter in whole soils. In: Humic Substances in Soil and Crop Sciences, Selected Readings. American Society of Agronomy and Soil Science Society of America, Madison, WI, pp. 221-260.

Witt, C., Gaunt, J.L., Galicia, C.C., Ottow, J.C.G., Neue, H.U., 2000. A rapid chloroform-fumigation extraction method for measuring soil microbial biomass carbon and nitrogen in flooded rice soils. Biology and Fertility of Soils 30, 510-519.

Wofsy, S.C., Goulden, M.L., Munger, J.W., Fan, S-.M., Bakwin, P.S., Daube, B.C., Bassow, S.L., Bazzaz, F.A., 1993. Net exchange of CO2 in a mid-latitude forest. Science 260, 1314-1317.

Zech, W., Ziegler, F., Kogel-Knabner, I., Haumaier, L., 1992. Humic substances distribution and transformation in forest soil. The Science of the Total Environment 117/118, 155-174.

Zelles, L., Bai, Q.Y., Beck, T., Beese, F., 1992. Signature fatty acids in phospholipids and lipopolysaccharides as indicators of microbial biomass and community structure in agricultural soils. Soil Biology & Biochemistry 24, 317-323.

Zelles, L., Bai, Q.Y., 1994. Fatty acid patterns of phospholipids and lipopolysaccharides in environmental samples. Chemosphere 28, 391-411.

Chapter 2

Management effects on carbon sequestration in soil physical fractions at the Breton Classical Plots

Introduction

The soil organic carbon (SOC) pool encompasses a variety of constituents from particulate debris such as plant, animal, and microbial residues to highly amorphous organic matter substances including all stages of decay, which may be intimately associated with inorganic soil components to form organo-mineral complexes. The turnover of these different SOC components varies due to the complex interaction of biological, chemical, and physical processes in soil (Christensen, 1992). Several multiple-pool models describing SOC turnover have been proposed (Jenkinson and Rayner, 1977; Christensen, 1992; Paul et al., 1995; Cambardella and Elliott, 1992). From an experimental point of view, soil organic matter is often partitioned into different compartments or fractions meant to mimic the different pools identified in these models.

Paul et al., (2001) proposed a combination of biological and chemical approaches to analytically determine SOC pool sizes and turnover rates (Paul et al., 2001). Following dispersion with sodium hexametaphosphate, the light fraction was separated by floatation in sodium iodide (d=1.7g/cm^3); the NaI residue was acid hydrolyzed to obtain a residue that was called the "resistant pool". A drawback of this protocol is that some components of organic matter may be modified through solubilization, oxidation or repolymerization during fractionation (Collins et al., 1997).

The physical fractionation techniques are considered chemically less destructive than separation techniques that use chemical solvents, and the results obtained from physical soil fractions are anticipated to relate more directly to the structure and function of SOC in situ (Christensen, 1992). The physical protection of organic materials, which restricts their accessibility to microorganisms and enzymes, is believed to be an important mechanism controlling SOM turnover. Mechanical disruption of soil particles is commonly achieved using sonication or shaking (Christensen, 1992; Collins et al., 1997). Vibrating sound waves create

30

microscopic bubbles that upon collapse produce a high enough energy to disrupt soil aggregates. One of the problems with the use of sonication in SOC studies is the potential for organic matter redistribution among the different fractions. Increasing the intensity of sonication results in the recovery of increasing amounts of organic matter in the fine silt and clay fractions. However, low levels of sonication provide incomplete dispersion of soils, causing microaggregates of smaller size particles to be included in the silt and sand fractions (Collins et al., 1997). A good compromise is a 5-minute ultrasonic treatment. This allows the occluded organic materials that are in different stages of decomposition to be separated because they have formed different degrees of association with mineral particles and thus have different densities (Golchin et al., 1994).

Wet sieving is used more often than dry sieving to determine the particle size distribution and stability of soil aggregates. Specifically, soil fractions with a diameter > 53µm, can be isolated through wet sieving. Within the > 53µm particles, the floatable fraction, composed of noncomplexed SOC with a density lower than 1.0 g/cm^3 can be further separated from the mineral particles by using water floatation. This fraction is considered to be made of only partially decomposed plant residues and animal debris with a relatively high C: N ratio and a rapid turnover rate. The fraction including particles with a diameter ranging from 2 to 20 µm (i.e. the organo-silt complex) consists of humified plant and microbial debris associated with stable organo-mineral microaggregates that have not been destroyed during sonication. The fractionation including particles with a diameter < 2 µm (i.e. the organo-clay fraction) is dominated by amorphous organic matter acting as a cement for the clay particles. It may contain plant cells as well as bacterial cells or colonies at different stages of decomposition (Feller, et al., 2000).

The Breton Classical Plots long-term fertilization trials were established in 1929 by the Department of Soils, University of Alberta (Chakraborty, 2001). They provide a model of how diverse cropping practices affect typical Gray Luvisolic soils after 70 years of farming. Using longer crop rotations that include forages and manure applications was found to increase crop yields and SOC levels. Between 1938 and 1998, while SOC declined at the rate of 14 and 7 g C m^{-2} yr^{-1} in

the 0.15 m depth of the Check and NPKS treatments of the 2-yr rotation, it gained 7 g C m^{-2} yr^{-1} in the Manure treatment of the same rotation, and 28 g C m^{-2} yr^{-1} in the Manure treatment of the 5-yr rotation (Grant et al., 2001). Izaurralde et al. (2001) fitted a simple three-compartment model to these measured long-term changes in total SOC. Results from the modeling exercise suggested a more rapid SOC turnover for the 2-yr than for the 5-yr rotation plots, a loss of carbon from the active SOC compartment with a decomposition rate of 0.0037 yr^{-1}, but a gain by the passive SOC compartment with a decomposition rate of 0.0005 yr^{-1}. Finally, the Breton Plots receiving manure did not appear to have reached steady state by 1990, but instead were continuing to sequester carbon (Izaurralde et al., 2001).

The overall objective of this study was to experimentally determine long-term changes in SOC pools at the Classical Breton Plots by using a physical separation method on archived samples as well as on soils (0-15 cm depth) sampled in 2003. In particular, we were interested to test the hypothesis proposed by Izaurralde et al (2001) concerning the redistribution of SOC from the active to the passive pool. Secondly, changes in SOC storage in these different pools were assessed as a function of cropping management (2-yr versus 5-yr rotation) and fertilization treatments: no added fertilizer (Ck), full chemical fertilization (NPKS), and farmyard manure application (M).

Materials and methods
The Breton Classical Plots

The Breton Plots are located 110 km SW of Edmonton (latitude 53°06' N, longitude 114° 26' W, altitude 854 m above sea level). The long-term annual precipitation at the experimental site is 547 mm, while the air temperature averages 2.1°C annually. The soil is an Orthic Gray Luvisol developed on glacial till parent material under boreal forest vegetation (Izaurralde et al., 2001). The original experimental design included two cropping systems: (a) continuous wheat (*Triticum aestivum* L.) and (b) a 4-yr rotation with three years of cereal grains and one year of legumes. In 1938, the continuous wheat system was converted to a wheat-fallow rotation to help control weeds, and in 1939, the 4-yr rotation was

32

changed to a 5-yr rotation of wheat, oats (*Avena sativa* L.), barley (*Hordeum vulgare* L.), and two years of forage. The forage crop has varied over the years but has always included a legume (Robertson, 1991). From a practical point of view, a total of 66 plots accommodated the two nonreplicated crop rotations; i.e., six blocks of land (Series A-F) were established in factorial combination with 11 different fertilization plots (Figure 2.1.). Soil amendments have varied over the years and are shown in Table 1. Finally, starting in 1972, lime was added to the east half (E) of the plots in series A-D and F, and to the entire area of series E when the soil pH dropped below 6.0 (Chakraborty, 2001).

Soil sampling and analysis

Two out of the 6 experimental series (A-F) present at the Breton Classical Plots were selected for this study: Series E, corresponding to the 2-yr rotation, and Series C, corresponding to the 5-yr rotation. In 2003, the western (W) and eastern (E) halves of Plot 1 (Ck), Plot 2 (M), and Plot 3 (NPKS) of the two series were sampled by randomly taking 9 cores (0-15 cm depth) within each plot and compositing them into 3 replicate samples per plot. Following air-drying, samples were sieved to pass a 2-mm sieve.

Soil samples (0-15 cm) that had been taken and archived from the Ck, M, and NPKS treatments in 1936, 1938, 1972, 1983, 1985, 1990, and 1998 were also used in this study (Table 2). All soil samples were fractionated according to the following procedure:

1. Weigh 20 grams of the < 2 mm soil sample into a 250 ml plastic bottle.
2. Add 100 ml of distilled water to the bottle and sonicate the soil sample for 5 minutes at 60% of maximum power.
3. Wet sieve through a 53 μm sieve and wash with a coarse jet of water. This step separates the floatable fraction (diameter > 53 μm and density < 1.0 g/cm^3) and the sand fraction (diameter > 53 μm and density > 1.0 g/cm^3) from the other fractions.
4. Separate the clay fraction (diameter < 2 μm) from the mixed suspension (diameter < 53 μm) by centrifugation at 500 rpm for 6 minutes (25°C).
5. Separate the fine silt fraction (2 μm < diameter < 5 μm), medium silt (5 μm

33

< diameter < 20 μm) and coarse silt (20 μm < diameter < 53 μm) by sedimentation.

6. Subsample a known volume from the clay and fine silt dispersions, and record its oven-dried (105 °C) weight to calculate the total weight of each fraction.

7. Flocculate the clay and fine silt dispersions using 2N KCl. The flocculated clay and fine silt are centrifuged to get rid of the extra water. Then the KCl is completely washed out from the clay and fine silt by dialysis.

8. Freeze-dry the clay and fine silt fractions. Dry the sand, floatable, coarse silt and medium silt fractions in the oven at 50 °C, and record the dry weight of each fraction. All soil fractions are finely ground using a ball grinder.

The procedures are also described in the following flow chart:
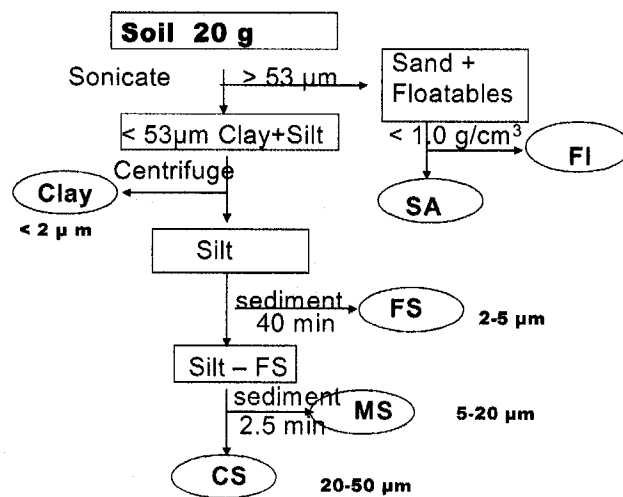
Total carbon and nitrogen in all size fractions were determined by dry combustion using a Carlo Erba NA1500 C and N Analyzer. Differences between physical fractions from soils sampled in 2003 as well as the influence of liming were analyzed using a paired t-test (n =12). On the other hand, the

34

experimental design did not allow for statistical analysis with regards to the influence of fertilization on soil carbon.

Results

2.1 Soil texture and recovery rates

The soil is a silt loam containing on average 19%wt clay and 40%wt sand. Recovery rate ranged from 94.4 to 99.4% (Figure 2.2). Average recovery rate following physical fractionation was 97.5% (stdev = 0.013) based on the weight of the individual particle size fractions. The largest fraction was the sand, based on the ratio of the individual fraction weight to the whole soil weight (%wt), while the floatable fraction was the smallest fraction (Figure 2.2). The proportion of each fraction decreased in the following order: sand > clay, coarse silt > medium silt > fine silt > floatables.

2.2 Carbon concentrations in the different soil physical fractions

Carbon and nitrogen concentrations for all soil fractions and collection dates are reported in the appendix (Table 2.2). Average carbon concentrations in the initial (1936 and 1938) samples were as follows: whole (unfractionated) soil: 1.52%; coarse silt: 0.50%; medium silt: 1.47%; fine silt: 2.31%; clay: 2.33%.

2.2.1. 5-yr rotation plots

Carbon concentration of the clay, fine silt and medium silt fractions as well as of the whole soils was higher in the manure plots than in the control (Ck) plots (Figures 2.3 and 2.4). The magnitude of carbon concentration for both the manured and Ck plots followed the following order: clay > fine silt > medium silt (Figure 2.3). In the manure treated plots (M) of the 5-yr rotation (C-Series), carbon concentrations in the clay, fine silt and medium silt fractions tended to increase with time. In the Ck plots, carbon concentration of the clay and medium silt fractions increased with time, whereas carbon concentration associated with the fine silt fraction tended to decrease over the same period. The whole soil carbon concentration of the manure treatment in the 5-yr rotation showed an increasing trend while Ck showed a slowly increasing trend with time even though the trend was not significant (Figure 2.4).

2.2.2. 2-yr rotation plots

In the manure treated plots of the 2-yr rotation (E-Series) carbon concentration of the medium silt, fine silt, and clay tended to increase with time, and the increase was faster for the fine silt than for the other two fractions (Figure 2.5). In the Ck plots, on the other hand, carbon concentrations of the clay, fine silt and medium silt soil fractions decreased with time, and the decrease was slower for the clay than for the fine and medium silt fractions. As was observed for the 5-yr rotation plots, the magnitude of carbon concentration followed the following order: clay > fine silt > medium silt. The whole soil carbon concentration of the manure treatment in the 2-yr rotation showed an increasing trend while the Ck showed a decreasing trend with time even though the trend was not significant (Figure 2.6).

2.3 Carbon distribution in different fractions

Carbon distribution in a given fraction (D_i) was calculated from the following formula:

$$D_i (\%) = (\frac{C_i \times f_i}{\sum C_i \times f_i}) \times 100\% \quad \text{where,}$$

C_i = carbon concentration in fraction i (g C kg^{-1} soil fraction i);

f_i = fraction distribution (%).

In the 2003 soil samples, different fractions contained very different amounts of carbon (Figure 2.7). The clay fraction contained the highest amount of carbon with about 37% of the total soil carbon (stdev = 0.02); the sand and floatable (> 53 μm) fraction contained the next highest amount, with about 22% (stdev = 0.03), while the coarse silt contained the least amount, with about 8% (stdev = 0.02). The medium and fine silt fractions contained intermediate amounts, with 17% (stdev = 0.03) and 15% (stdev = 0.01) respectively, of the total soil carbon.

2.4 Carbon recovery during physical fractionation

The carbon recovery (% total soil carbon) of 2003 soil samples was calculated by dividing the amount of carbon recovered in the different physical

36

fractions by the amount of carbon contained in the whole soil prior to fractionation according to the following formulae:

Carbon recovery (%) =

$$\frac{\sum \text{carbon in 5 soil fractions (g C/kg soil)}}{\text{total soil carbon (g C/kg soil)}} \times 100$$

where

$\sum$ carbon in 5 soil fractions (g C/kg soil) = $[cl_c$ (C/kg cl) $\times$ cl (%wt)] + $[fs_c$ (C/kg fs) $\times$ fs (%wt)] + $[ms_c$ (C/kg ms) $\times$ ms (%wt)] + $[cs_c$ (C/kg cs) $\times$ cs (%wt)] + $[sd_c$ (C/kg sd) $\times$ sd (%wt)], and clay : cl; fine silt: fs; medium silt: ms, coarse silt: cs; sd: sand + floatables.

For the 5-yr rotation, carbon recovery followed the same trend in the limed and unlimed plots and decreased in the following order: Ck > NPKS> M (Figure 2.8). For the 2-yr rotation, both east and west plots also showed a similar trend and decreased as follows: NPKS > Ck > M.

2.5 C: N ratios of the whole soils and different soil fractions

Nitrogen concentrations were significantly correlated to carbon concentrations in the soil physical fractions as well as in the unfractionated soil samples (p-value < 0.05). The C/N ratios were quite different among the different soil fractions (Figure 2.9). The floatable fraction exhibited the highest C/N ratio, while the clay, sand and coarse silt fractions showed the lowest ratio. The floatable fraction had a higher C/N ratio than the whole soil, while the opposite was true for the clay, sand, and coarse silt fractions. The medium and fine silt fractions displayed intermediate C/N ratios, which were similar to what was measured in the whole soil samples prior to physical fractionation. Finally, the whole soil C/N ratios (averaged over 3 treatments) in the 5-yr rotation plots (C-series) were significantly higher than those of the 2-yr rotation plots (E series) at α = 0.05 (Figure 2.10).

2.6 Changes in carbon concentrations since 1972

Changes in carbon concentration in the <53 μm soil fractions as well as in the unfractionated samples were calculated by comparison with the carbon

37

concentrations in the 1972 sample from the Check (Ck) plots (Figure 2.11). Carbon concentration in the <53 µm soil fractions is further defined as the sum of carbon contained in the clay, fine, medium, and coarse silt fractions:

$[cl_c$ (C/kg cl) × cl (%wt)] + [fs_c (C/kg fs) × fs (%wt)] + [ms_c (C/kg ms) × ms (%wt)] + [cs_c (C/kg cs) × cs (%wt)] + [sd_c (C/kg sd)

where clay : cl; fine silt: fs; medium silt: ms; and coarse silt: cs.

For the limed plots in the 5-yr rotation the increase in carbon concentration for the <53 µm soil fractions of the Ck, M and NPKS treatments ranged from 17 to 61% as compared to the concentrations recorded for the soils sampled in 1972 (Figure 2.11). In the unlimed plots, the increase in total carbon of the <53 µm soil fractions ranged from 18 to 45%. The limed and unlimed plots exhibited the same trend with regards to the fertilization treatment, in that the manured plot showed the highest increase in carbon concentration, while the check (Ck) plot increased by the lowest increment. For the 2-yr rotation, manure fertilization resulted in an increase of carbon concentration of 54 and 34% in the east and west plots, respectively. In contrast, in the Ck plots, carbon concentration decreased by 10 and 30% in the east and west plots, respectively. Carbon concentration followed yet another scenario in the NPKS plots, as it did not change in the east plot, yet increased by 18% in the west plot.

For the whole (unfractionated) samples from the 5-yr rotation, carbon in the Ck soils basically stayed constant in the limed and unlimed plots, i.e.; the change was ≤ ± 5% (Figure 2.11.). Carbon concentration increased in the NPKS plots, and the increase (15%) was similar for the limed and unlimed treatments. In the manured plots, carbon concentration increased by 49% and 34% in the limed and unlimed plot, respectively. For the 2-yr rotation, total carbon concentrations of the Ck treatment were decreased by 19 and 22% in the east and west plot, respectively. Total carbon concentrations also decreased in the NPKS plots, by 12 and 5% in the east and west plot, respectively. On the other hand, concentrations increased by 64% and 37% in the east and west plot of the long-term manure treatment.

The change in carbon concentration of the < 53µm soil fractions followed the same trend as the change in total soil carbon with regards to its response to

38

manure and chemical fertilization:

1. Both the 2-yr and 5-yr rotations responded positively to manure application, and carbon concentration increased in all of the manured plots.

2. As seen for the long-term manure application, both the 2-yr and 5-yr rotations responded positively to NPKS application, in that either the decrease in carbon concentration was smaller, or the increase was greater than was the case for the check (Ck) plots. Furthermore, the 5-yr rotation responded more positively to NPKS than the 2-yr rotation, in that all of the 5-yr rotation plots exhibited an increase in carbon concentration both in the unfractionated samples and the <53 µm fractions. On the other hand, and as opposed to what was observed for the manured plots, there was no clear response to liming in the NPKS plots.

Discussion and conclusions

Comparing carbon recovered in the five physically separated soil fractions to total soil carbon demonstrated that about 20% of the total carbon was lost during fractionation in samples from the manured plots (Figure 2.8). These results showed that there must be a water-soluble carbon present in the soil samples, which is lost during the fractionation process. Christensen el al., (1987) also reported that some soil organic matter dissolved during particle size separation procedure, which may account for 1 to 11% of the total soil carbon. In our study, this carbon loss was larger for the samples originating from the manured plots than for those from the NPKS or from the Ck plots in both the 2-yr and 5-yr rotation. These results should be related to results presented in Chapter 3, where we found that water-soluble carbon was higher for the manured and NPKS plots than for the check plots.

The clay (< 2 µm) fraction showed the highest C concentration in this study. The second highest concentration was found associated with the fine silt (2-5 µm), while the third highest one occurred in the medium silt (5-20 µm) fraction. Higher C concentrations are typically observed in <5 µm primary organomineral separates (Christensen, 1996). Gale and Cambardella's (2000) ^{14}C-labeling decomposition experiment results showed that after 1 year 66% of the ^{14}C contained in surface residues had been respired as [14]CO_2, while 11% remained as

39

residues on the soil surface, and 16% had been incorporated into the mineral soil. Most of the surface residue-derived carbon present in the soil was associated with the silt and clay fractions, and only a small amount was associated with the particulate (i.e.; > 53 μm) fractions. Hence carbon was being rapidly transferred from the coarse organic matter pools into the smaller sized fractions as decomposition proceeded (Gale and Cambardella, 2000). Carbon decomposition rate further depends on carbon stabilization processes. The protection of soil carbon by silt and clay particles is well established (Feller and Beare, 1997; Hassink, 1997). Hassink defined the capacity of soil to preserve carbon by its association with silt and clay particles. Studies investigating the retention of specific microbial products (e.g. amino sugars) corroborate the proposition of Hassink (1997) that carbon associated with primary organomineral complexes are chemically protected and that the amount of protection increases with an increasing proportion of silt and clay in the soil (Chantigny, 1997; Guggenberger and Fred, 1999). Hence, clay plays a crucial role in the retention of total soil organic carbon. In our study, clay content in the Breton soils was about 20%, while the clay fraction contained more than 37% of the total soil carbon, which was by far the largest pool. The long-term manure fertilization increased the clay carbon concentration even further.

The C/N ratios decreased in the following order: floatables > medium silt > fine silt > clay > sand > coarse silt (Figure 2.9). Fractions containing a higher proportion of microbial metabolites and more decomposed organic matter are expected to have lower C/N ratios. For instance, the floatable fraction had a higher C/N ratio than the whole soil because it likely contained some undecomposed plant residues. Similarly, in this study, the clay had a significantly lower C/N ratio than the fine and medium silt fractions, which in turn had a significantly lower C/N ratio than the floatables. These results can be explained on the basis of a progressively higher degree of decomposition as the organic matter is transferred from the floatables to the smaller sized fractions, from the medium silt to the fine silt and finally the clay. On the other hand, both the sand and coarse silt fractions had a C/N ratio lower than that of the clay, which was unexpected. A lot of charcoal could be visually observed in the sand fraction, which should have

40

resulted in an even wider C/N ratio. However, if the particulate organic matter (with a wide C/N ratio) originally present in the coarse silt and sand fractions was successfully recovered as part of the floatables by density separation, it is conceivable that the carbon left behind would have a more advanced degree of decomposition.

In the 5-yr rotation, carbon concentration of the clay fraction showed an increasing trend with time in all plots and the manured plots showed the highest increase (Figures 2.3 and 2.11). In the 2-yr rotation, similar results were obtained except that the Ck showed a decreasing trend with time (Figure 2.5). The negative effects of a fallow system on soil carbon levels also have been demonstrated in the Askov long-term experiments in Denmark (Christensen and Johnston, 1997) and the long-term Lethbridge crop rotation study in southern Alberta (Monreal and Janzen, 1993). After 80 years of cultivation, the average soil organic carbon concentration in the top 0- 15 cm decreased significantly by 23 and 21 % in the fallow-wheat and fallow-wheat-wheat rotation, respectively (Monreal and Janzen, 1993). At the Breton Plots, higher soil carbon concentrations in the 5-yr rotation plots as compared to the 2-yr rotation plots were due to differences in carbon inputs. From 1939 to 1990 the average carbon addition to the soil from plant residues has been estimated to be much lower at 205 kg ha^{-1} yr^{-1} in the 2-yr rotation than the 470 kg ha^{-1} yr^{-1} that was estimated for the 5-yr rotation (Izaurralde et al., 2001). Alternatively, differences between the two rotation types could be linked to differences in decomposition rates. The 5-yr rotation had a significantly higher C/N ratio than the 2-yr rotation (Figure 2.10). Izaurralde et al., 2001 similarly reported that the 2-yr rotation narrowed the C/N ratio from 11 to 10, while the 5-yr rotation maintained the C/N ratio closer to the original ratio of 11. Consequently, a lower C/N ratio in the 2-yr rotation plots could indicate a faster decomposition than in the 5-yr rotation, which, in addition to reduced carbon inputs, would contribute to the decrease in soil carbon concentration.

Izaurralde's model suggested that carbon was lost from the active compartment and accumulated in the passive compartment (Izaurralde et al, 2001). Results from my study, at least for the 5-yr rotation, seem to agree with this proposed scenario, if we assume that the <53 μm fractions as depicted on Figure

41

2.11 correspond to the passive pool from Izaurralde's model. Indeed for both the limed and unlimed plots of the manure, NPKS and Ck treatments, there was an increase in carbon concentrations in the <53 μm fractions that was greater than the increase in the unfractionated soils.

42

References

Camberdella, C. A., and Elliott, E. T. 1992. Particulate soil organic matter changes across a grassland cultivation sequence. Soil Sci. Soc. Am. J. 56: 777-783.

Chakraborty, S. 2001. Impact of phosphorus on soil organic matter dynamics in the long-term Classical Plots at Breton, Alberta. M.Sc. Thesis, Renewable Resources Dept, Univ. of Alberta, Edmonton. pp. 1-104.

Chantigny, M. H. 1997. Soil aggregation and fungal and bacterial biomass under annual and perennial cropping systems. Soil Sci. Soc. Am. J. 61: 262-267.

Christensen, E. T. 1987. Decomposability of organic matter in particle size fractions from field soils with straw incorporation. Soil Biol. Biochem. 19: 429-435.

Christensen, B. T. 1992. Physical fractionation of soil and organic matter in primary particle size and density separates. Pages 2-90 In B.A. Stewart, ed. Advances in Soil Science. Springer-Verlag New York Inc.

Christensen, E. T. 1996. Matching measurable soil organic matter fractions with conceptual pools in simulation models of carbon turnover: revision of model structure. Pages 143-179 in D.S. Powlson et al. ed. Evaluation of Soil Organic Matter Models. Spriger-Verlag berlin Heidelberg.

Christensen, E. T., and Johnston, A. E. 1997. Soil organic matter and soil quality-lesson learned from long-term experiments at Askov and Rothamsted. Pages 399-428 In E. G. Gregorich and M. R. Carter, ed. Soil Quality for Crop Production and Ecosystem Health. Elsevier publisher.

Collins, H. P., Paul, E. A., Paustian, K., and Elliott, E. T. 1997. Characterization of soil organic carbon relative to its stablility and turnover. Pages 51-72 In Soil Organic Matter in Temperate Agroecosystems: Long-term Experiments in North America. CRC Press, Boca Raton, FL.

Feller, C. and M.H. Beare. 1997. Physical control of soil organic matter dynamics in the tropics. Geoderma 79: 69-116.

Feller, C., Balesdent, J., Nicolardot, B., and Cerri, C. 2000. Approaching "functional" soil organic matter pools through particle-size fractionation: examples for tropical soils. Pages 53-67 In R. Lal, J. M. Kimble, R. F. Follett, and B. A. Stewart, ed. Assessment Methods for Soil Carbon. Lewis Publ., Boca Raton, FL.

Gale, W. J., and Cambardella, C. A. 2000. Carbon dynamics of surface residue-and root-derived organic matter under simulated no-till. Soil Sci. Soc. Am. J. 64: 190-195.

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

Grant, R. F., Juma, N. G., Robertson, J. A., Izaurralde, R. C., and McGill, W. B. 2001. Long-term changes in soil carbon under different fertilizer, manure, and rotation: testing the mathematical model ecosys from data from the Breton Plots. Soil Sci. Soc. Am. J. 65:205-214.

Guggenberger, G., and Fred, S. D. 1999. Bacterial and fungal cell-wall residues in conventional and no-tillage agroecosystems. Soil Sci. Soc. Am. J. 63: 1188-1198.

Hassink, J. 1997. The capacity of soils to preserve organic C and N by their association with clay and silt particles. Plant Soil 191: 77-87.

Izaurralde, R. C., McGill, W. B., Robertson, J. A., Juma, N. G., and Thurston, J. J. 2001. Carbon balance of the Breton Classical Plots over half a centry. Soil Sci. Soc. Am. J. 65: 431-441.

Jenkinson, D.S., Rayner, J.H., 1977. The turnover of soil organic matter in some of the Rothamsted classical experiments. Soil Science 123, 298-305.

Monreal, C. M., and Janzen, H. H. 1993. Soil organic-carbon dynamics after 80 years of cropping in Dark Brown Chernozem. Can. J. Soil Sci. 73: 133-136.

Paul, E. A., Horwath, W. R., Harris, D., Follett, R., Leavitt, S. W., Kimball, B. A., and Pregitzer, K. 1995. Establishing the pool sizes and fluxes in CO_2 emissions from soil organic matter turnover. Pages 297-305 In Advances in Soil Science and Global Change, R. Lal, T. Kimble, E. Levine, and B. A. Stewart, ed. CRC Press, Boca Raton, FL.

Paul, E.A., Collins, H.P., Leavitt, S.W., 2001. Dynamics of resistant soil carbon of Midwestern agricultural soils measured by naturally occurring ^{14}C abundance. Geoderma 104, 239-256.

Robertson, J.A., 1991. Long-term results from the Breton Plots, Robertson, J.A. (Eds.) 61st Annual Breton Plots: Field Day and Soils/Crops Clinic, University of Alberta, Edmonton. pp. 14-33.

45

Tables and Figures

Table 2.1. Approximate fertilizer, manure and lime applications at the Breton Classical Plots (from Robertson, 1991).

Amendment Code	Plot No.	Amendment	N P K S (1930-1979)	N P K S (1980- now)
M	2	Manure (cattle)	76-42-91-20 Annual equivalents	Amount depends on rotation and N application. The wheat-fallow rotation corresponds to 90 kg N ha^{-1} for each wheat crop. The cereal crops-forage rotation receives 175 kg N ha^{-1} every five years, applied in two equal applications.
NPKS	3	16-20-0+ 0-0-60	10-6-16-10 From 1944 to 1963, fertilizer was applied every second year at approximate rates of N9 P5 K14 S8 kg/ha	N amounts depend on the crop and its place in the rotation: wheat on fallow corresponds to 90 kg N ha^{-1}, wheat after forage to 50 kg N ha^{-1}, oats after wheat to 75 kg N ha^{-1}, barley as nurse crop to 50 kg N ha^{-1}. The legume-grass forage crop did not receive any N fertilization.
Ck	1	none	0-0-0-0	0-0-0-0
East (E) of A-D and F Series		lime	CaCO$_3$	From 1972, pH was adjusted to 6.5.
West (W) of A-D and F Series		unlime	No lime	

46

Table 2.2. List of soil samples used for fractionation in this study.

Sampling year	Series	Plot	Sampling year	Series	Plot
1936	C	1	1998	C	2W
1936	C	3	1998	C	3W
1938	E	1	1998	E	1W
1938	E	1	1998	E	2W
1938	E	2	1998	E	3W
1938	E	3	1998	C	1E
1938	E	3	1998	C	2E
1938	E	3	1998	C	3E
1972	C	1	1998	E	1E
1972	C	2	1998	E	2E
1972	C	3	1998	E	3E
1972	E	1	2003	C	1W
1972	E	2	2003	C	2W
1972	E	3	2003	C	3W
1983	C	1W	2003	C	1E
1983	C	2W	2003	C	2E
1983	C	3W	2003	C	3E
1985	E	1W	2003	E	1W
1985	E	2W	2003	E	2W
1985	E	3W	2003	E	3W
1990	C	1W	2003	E	1E
1990	C	2W	2003	E	2E
1990	C	3W	2003	E	3E
1998	C	1W			

Note: In the column Plot: W: west; E: east.

47

Figure 2.1. Layout of the Breton Classical Plots (from Izaurralde et al., 2001).

1-Ck	**W**	**E**					
2-M							
3-NPKS							
4-NKS							
5-Ck							
6-Lime							
7-NPK							
8-PKS							
9-NPKS							
10-NPS							
11-Ck							

F   E   D   C   B   A

48

Figure 2.2. Physical fraction distribution in the Breton Plots soil samples. Error bars represent one standard deviation from the mean, and different letters indicate significant differences at α = 0.05 using a Duncan's New Multiple Range test (n=41).
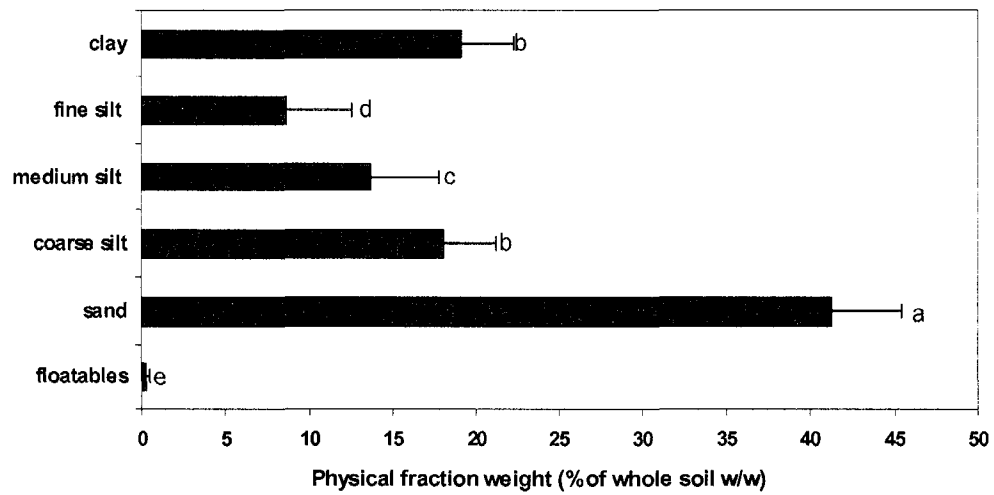
Figure 2.3. Changes in carbon concentrations of the clay, fine silt and medium silt fractions for the manured (M) and control (Ck) plots of the 5-yr rotation C series (Archived soil samples in 1972, 1983, 1990, 1998 and soil sampled in 2003).

C concentration (C g/kg soil)
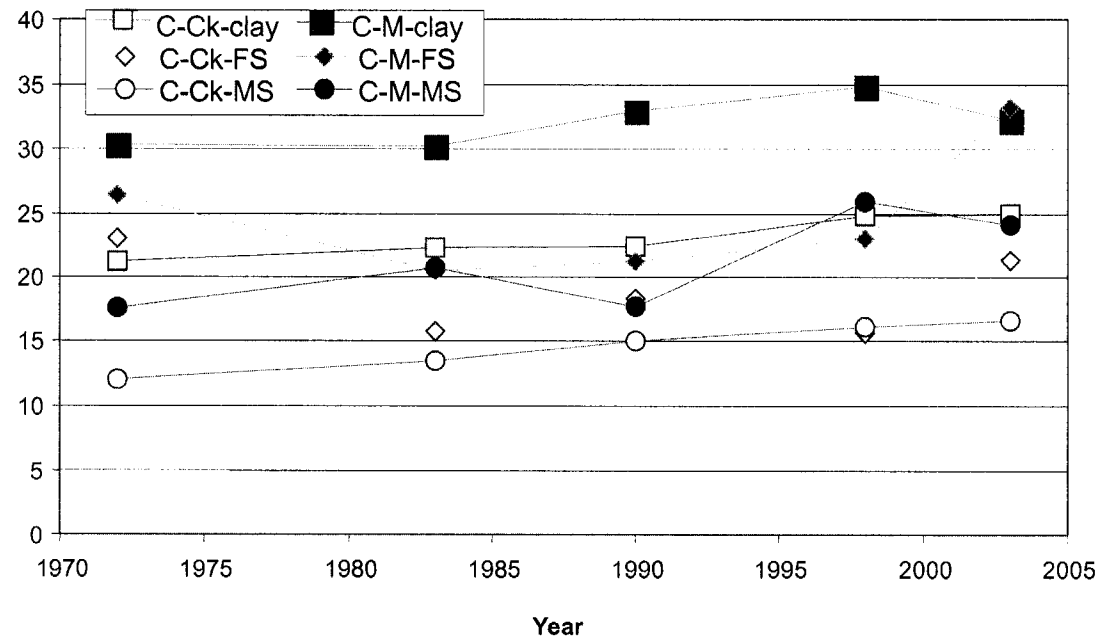
Clay: < 2µm; FS: fine silt (2-5 µm); MS: medium silt (5-20 µm).

Figure 2.4. Changes in carbon concentrations in the whole soil for the manured (M) and control (Ck) plots of the 5-yr rotation C series (Archived soil samples in 1972, 1979, 1990, 1998 and soil sampled in 2003).

C concentration (C g/ kg soil)
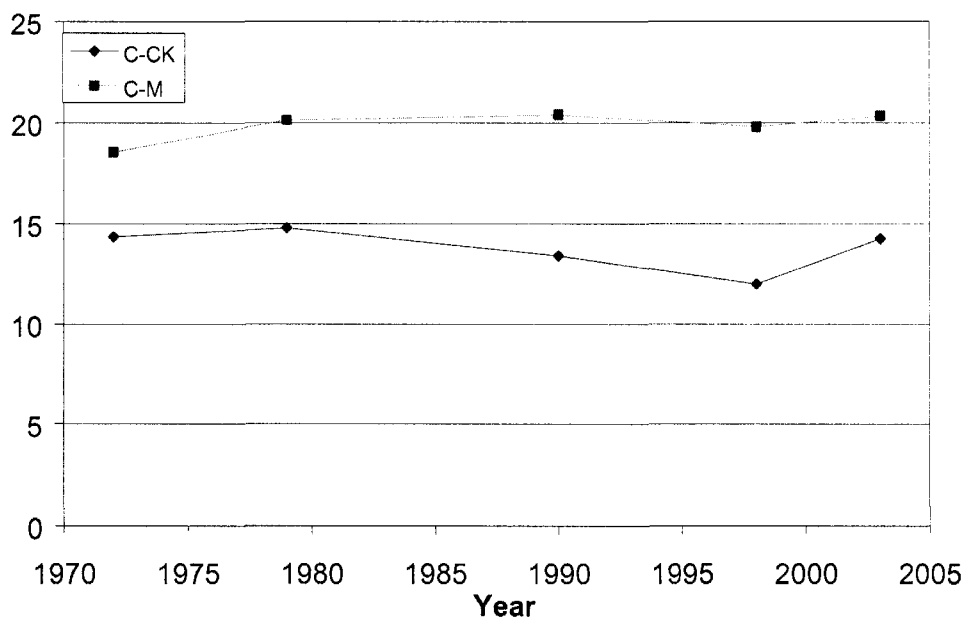
Figure 2.5. Changes in carbon concentrations of the clay, fine silt and medium silt fractions for the manured (M) and control (Ck) plots of the 2-yr rotation E series (Archived soil samples in 1972, 1990, 1998 and soil sampled in 2003).

C concentration (C g/kg soil fraction)
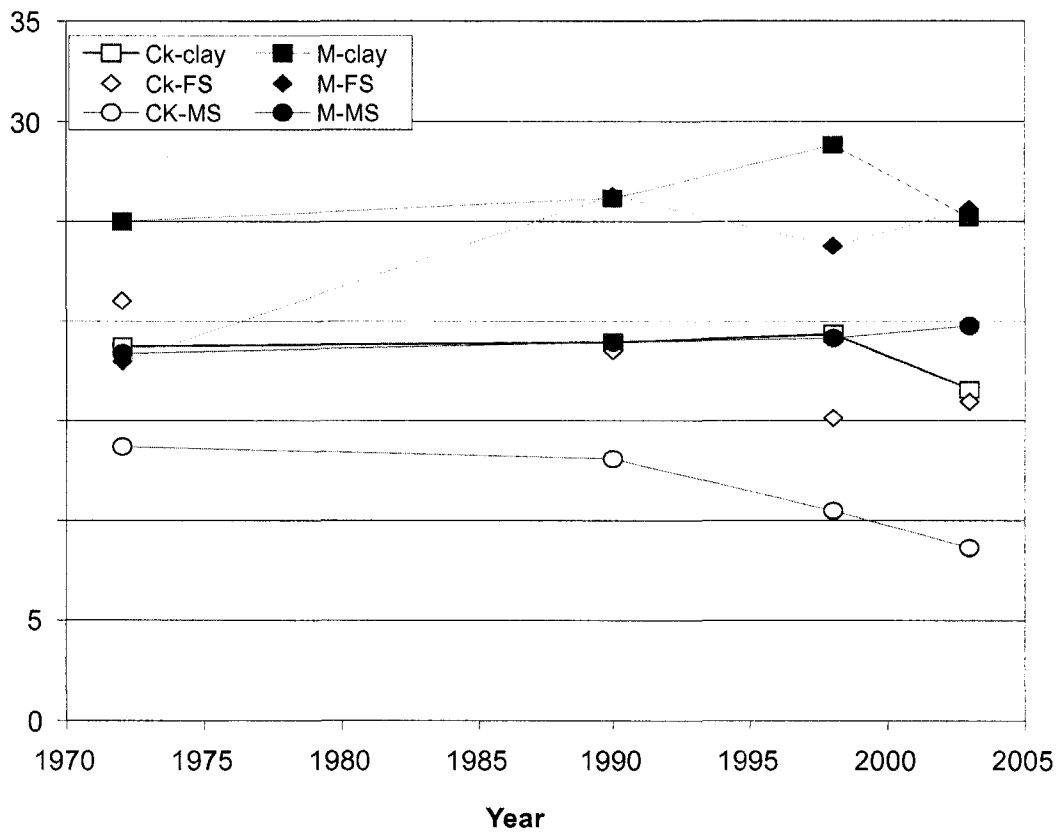
Figure 2.6. Changes in carbon concentrations of the unfractionated soil for the manured (M) and control (Ck) plots of the 2-yr rotation E series (Archived soil samples in 1972, 1979, 1990, 1998 and soil sampled in 2003).

C concentration (C g/kg soil)

53
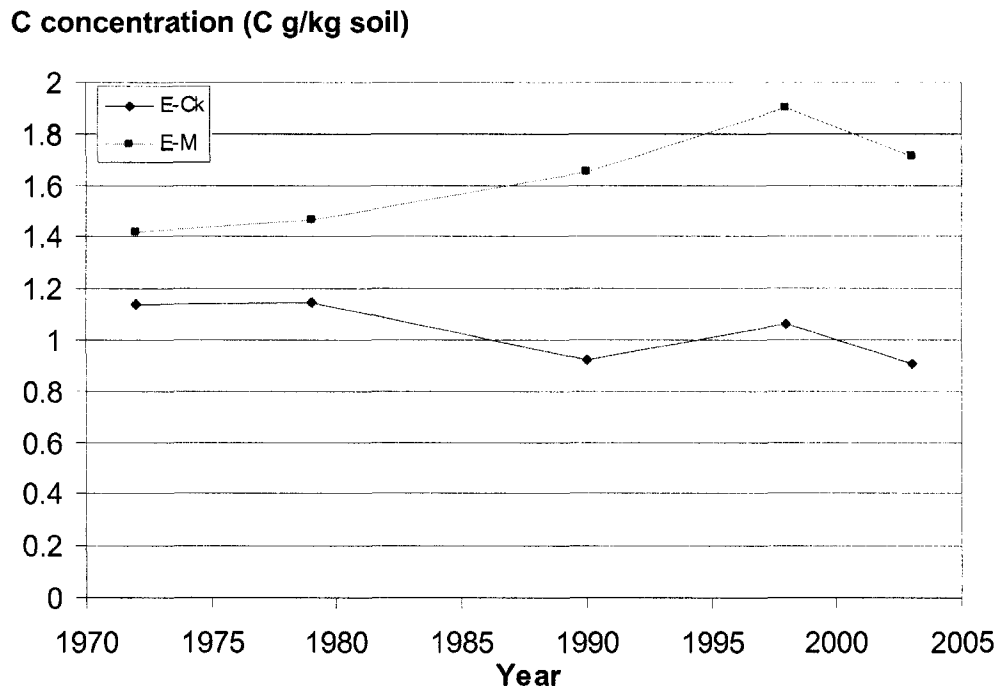
Figure 2.7. Carbon distribution (% of total soil carbon) for the 2003 soil samples. Error bars represent one standard deviation from the mean and different letters indicate significant differences at $\alpha = 0.05$ using a paired t-test (n=12).

Carbon distribution (%)
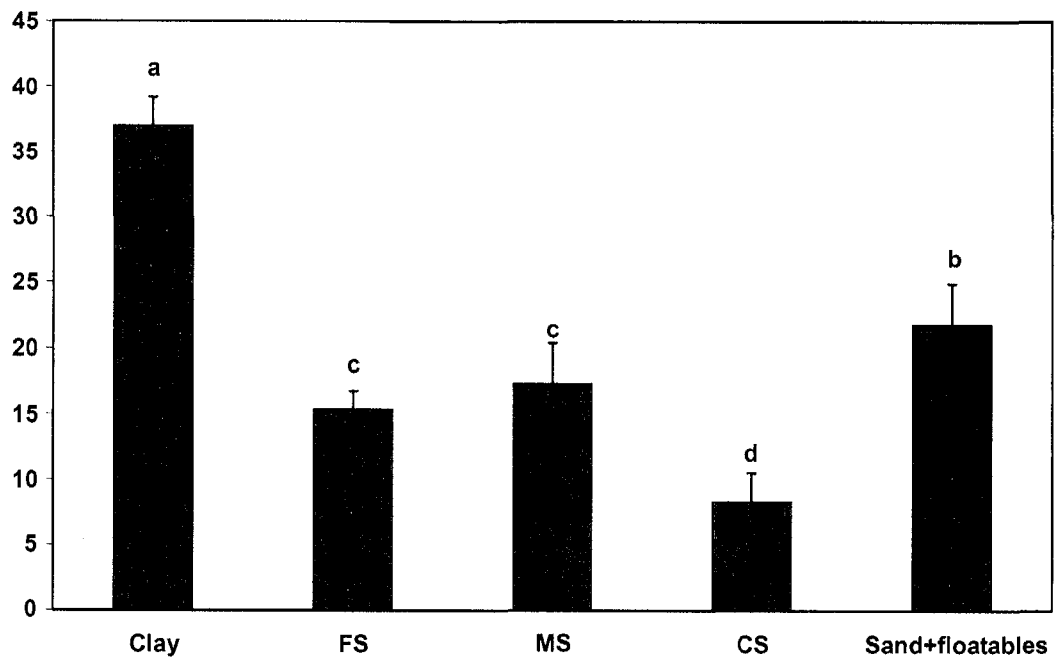
Clay: < 2μm; FS: fine silt (2-5 μm); MS: medium silt (5-20 μm); CS: coarse silt (20-53 μm); sand + floatables: >53 μm.

54

Figure 2.8. Carbon recovery (% total soil carbon) in the soil physical fractions for the 2003 samples

Carbon recovery (% total soil carbon)
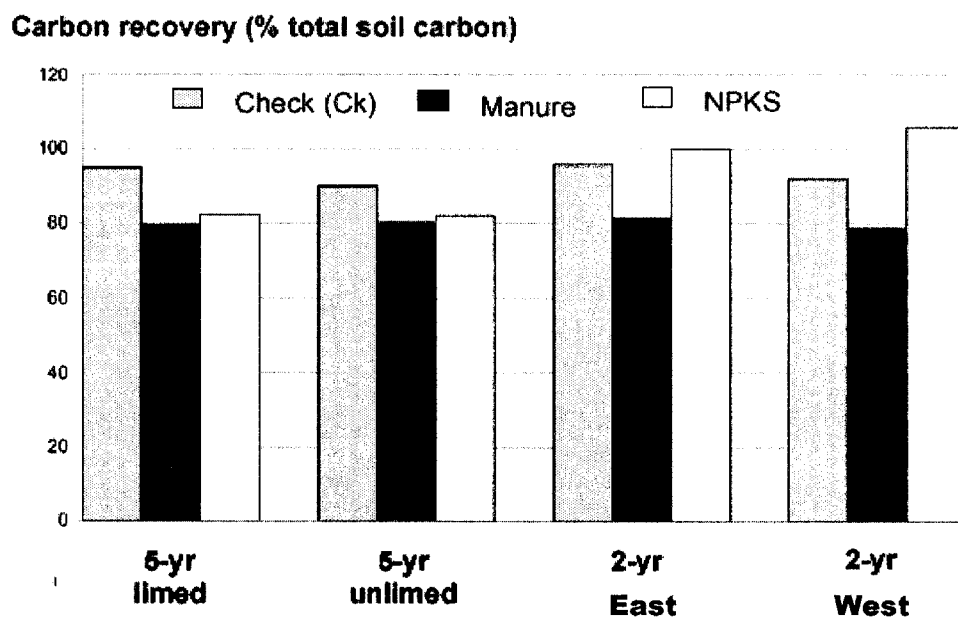
Figure 2.9. C/N ratios in the physical fractions and the whole soils for the 2003 soil samples. Error bars represent one standard deviation from the mean and different letters indicate significant differences at $\alpha = 0.05$ using a paired t-test (n=12).
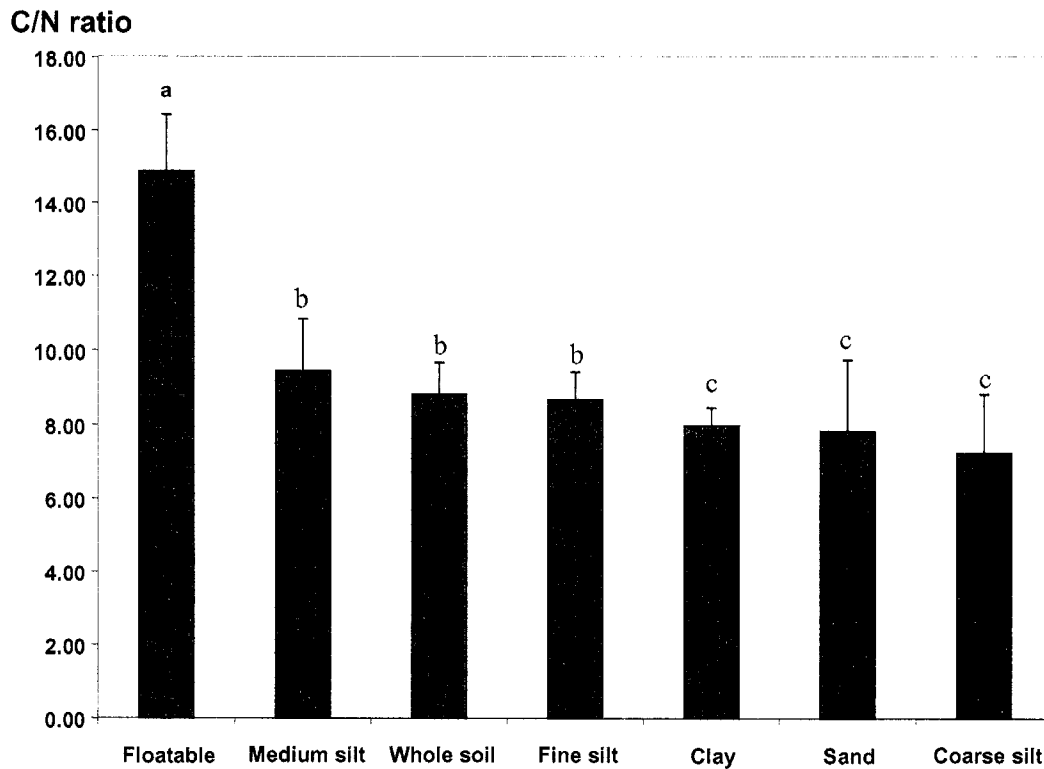
C/N ratio

Figure 2.10. C/N ratios (averaged over 3 treatments) for the 2003 samples of the 5-yr rotation (C series) and 2-yr rotation (E-series) plots. Error bars represent one standard deviation from the mean and different letters indicate significant differences at $\alpha = 0.05$ using a paired t-test (n=18).

C/N ratio
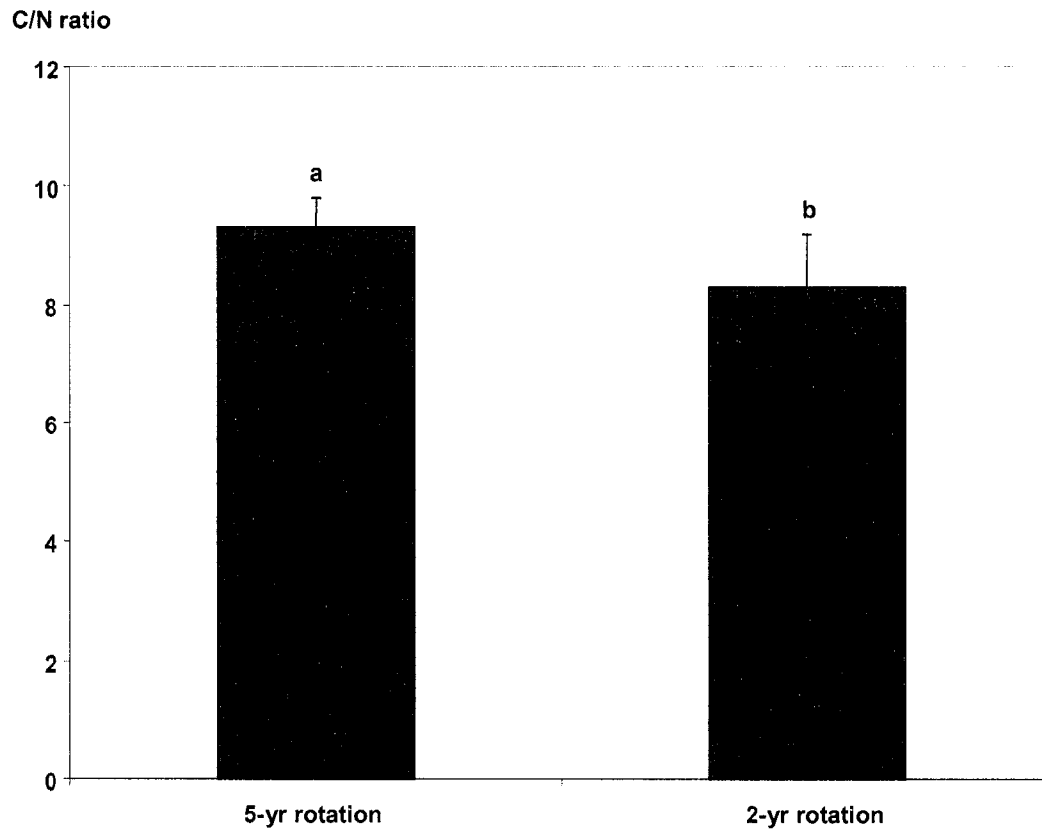
Figure 2.11. Changes in carbon concentration from 1972 to 2003 (expressed as % of carbon concentrations in the 1972 Ck) in the < 53 μm soil fractions and in the whole (unfractionated) soils.

< 53 (m fraction

whole soil

| 5-yr limed | 5-yr unlimed | 2-yr east | 2-yr west |
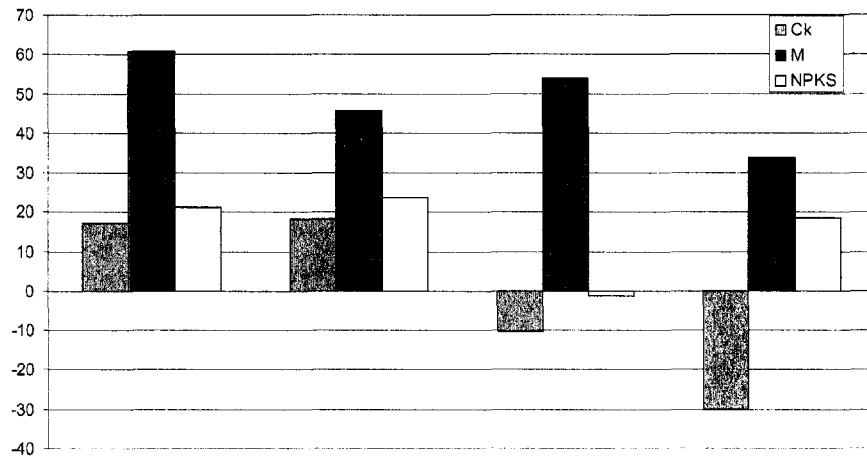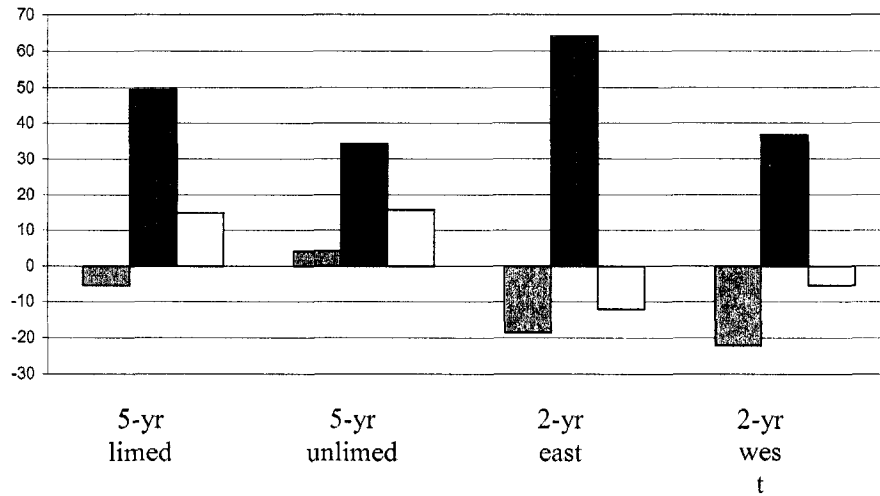
Chapter 3

Soil Microbial Biomass and Microbial Community Structure

Introduction

Soil microbial biomass is an important component of the soil organic matter that regulates the transformation and storage of nutrients. It is also the living part of the soil organic matter. Since microorganisms are more responsive to changes in the soil environment than the soil organic matter as a whole, measures of the size of the soil microbial biomass have been used for assessing changes in soil organic matter and nutrient cycling caused by changes in soil management and distinct agricultural practices. In particular, agricultural management practices such as the application of manure and chemical fertilizers have been shown to impact the size and activity of soil microbial communities (Bolton et al., 1985; Powlson et al., 1987; Fraser et al., 1988; Kirchner et al., 1993; Bossio et al., 1998a).

Methods of measuring the size of the soil microbial biomass include the chloroform fumigation incubation (CFI), chloroform fumigation extraction (CFE), and substrate induced respiration (SIR) techniques. Chloroform fumigation to lyse microbial cells in soil has become a standard tool for determining the total soil microbial biomass since it was first reported (Jenkinson and Powlson, 1976). Estimates of soil microbial biomass carbon (MBC) by the CFE method are not influenced by the presence of other organic materials in the soil, such as root fragments or freshly added substrates (Martens, 1995), nor are they sensitive to prolonged storage at 4°C (Ross, 1991). Hence, the CFE method was adopted in this study.

Phospholipids are found in all living cell membranes but not in storage structures and are rapidly turned over on cell death; therefore they may be used for characterizing the living biomass in soils (Tunlid and White, 1992, Paul and Clark, 1996). Phospholipid fatty acid (PLFA) analysis is a biochemical method that can provide information both about the size and the structure of the active soil microbial community (Vestal and White, 1989), as well as detect changes due to

59

environmental changes or differences in agricultural management practices. One of the advantages of the PLFA approach is that it is free of the limitations inherent to culturing approaches. As a fingerprint of the soil microbial community, the PLFA profiles provide a more sensitive measure of the change at the community level than do the traditional methods.

Results from PLFA analysis can be used to interpret effects at several levels. Using the entire profile of the PLFA spectrum as a fingerprint permits a detailed assessment of changes at the community level. With appropriate statistics, the degree of similarity between communities, or environmental effects on a community can be quantified and tested for significance (Bossio et al., 1998a and b). Another level involves differentiating between the major taxonomic groups, such as fungi versus bacteria. A finer level of resolution can be achieved by focusing on specific fatty acids that act as biomarkers of certain functional groups or species of microorganisms, and on the ratios of particular fatty acids through which the physiological state of microbial communities can be assessed (Bossio and Scow, 1998b). However, interpretation of PLFA profiles from entire soil communities is difficult because many fatty acids are common to different microorganisms, and there may be hundreds of different fatty acids in environmental samples (Zelles et al., 1992). Hence PLFA profile analysis so far has been largely limited to qualitative and univariate descriptions (Michael and Klug, 1995).

One of the objectives of this study was to examine the influence of chemical fertilization and manure application on the size of the soil microbial biomass at the Breton Classical Plots. A second objective was to study the effects of manure and fertilizer on the microbial community diversity using PLFA analysis.

Materials and methods

Sampling

Soil sampling for microbial biomass determination occurred at triplicate dates, i.e., on August 26, 2003, September 21, 2003 and, May 4, 2004. Samples were collected from the top 5 cm at both the limed and unlimed halves of Plot 5 (Control plot or Check), Plot 2 (Manure treatment), and Plot 3 (NPKS treatment) in

series A, C and F of the 5-yr rotation. An Oakfield soil sampler was used for collection. Nine points were randomly chosen for sampling within each half plot. Three of these samples were composited to yield a total of three samples for each half-plot. Before moving to the next half-plot, the sampler core was cleaned with ethanol and gloves were changed. During the September 2003 sampling, soil samples were divided into two parts: the first subsample was saved for CFE analysis, and the second subsample was for PLFA analysis. The CFE samples were kept refrigerated and analyzed within 48 hours of collection. The PLFA samples were stored in a super freezer at -86°C until analysis.

Microbial Biomass

Microbial biomass carbon and nitrogen were estimated on sieved (< 4 mm) soil samples using the CFE method as described by Vance et al. (1987). About 12 g of each soil sample was fumigated with chloroform for 24 hours while a second sample was left as control. Both the fumigated and unfumigated (control) soil samples were then extracted with 0.25 M K_2SO_4 (soil: extractant ratio = 1:4) by shaking 30 minutes at room temperature, followed by filtering through a 0.45 μm filter (Witter, 1993, Trinsoutrot et al., 2000, Jenkinson et al., 2004). The filtered extracts were kept frozen at -40 °C until analysis. Soluble (dissolved) organic carbon (DOC) concentration in the extracts was determined using a Shimadzu TOC-VCSH/CSN total Organic Carbon Analyzer. Soluble (dissolved) organic nitrogen (DON) for the soil samples collected in August and September 2003 was determined using the alkaline persulphate oxidization method, where the organic nitrogen and ammonium in a sample are oxidized to nitrate, and compared to an initial non-oxidized sample. Ammonium and nitrate concentrations were analyzed using a Technicon Auto Analyzer II, and DON was calculated as

$$DON = NO_3^-{}_{after\ oxidation} - (NO_3^- + NH_4^+)_{initial}$$

For the samples collected in May 2004, DON was directly measured using the Shimadzu TOC-VTN instrument. Microbial biomass C and N were then estimated from DOC and DON concentrations where

Microbial biomass C = $(DOC_{fumigated} - DOC_{unfumigated})/K_c$ and

Microbial biomass N = $(DON_{fumigated} - DON_{unfumigated})/K_n$ with a correction

61

factor of K_c and K_n=0.45 (Jenkinson et al. 2004).

Lipid extraction and PLFA analysis

Following freeze-drying, PLFA extraction and analysis was conducted as described by Frostergård et al. (1993a). Lipids were extracted from 1.50 ± 0.05 g of freeze-dried soil placed in a glass centrifuge tube by adding 9 ml of a chloroform: methanol: citrate buffer (1:2:0.8) according to the Bligh and Dyer (1959) technique. The mixture was vortexed every 30 minutes for 2 hours, and centrifuged at 1500 rpm for 15 minutes. The supernatant was decanted into a separation funnel, and another 2.5 ml of the chloroform: methanol: citrate buffer was added. The vortex and centrifuge, and decantation steps were repeated. Following decantation, 3.1 ml chloroform and 3.1 ml citrate buffer were added to the same separation funnel, shaken vigorously, and left in the fume hood overnight to separate. The lower organic phase was transferred to a 7 ml vial and chloroform was evaporated under nitrogen.

The PLFAs were separated from neutral and glycolipids on a solid phase extraction column. Specifically, the samples were dissolved in chloroform and slowly passed through a SPE silicic column. Neutral fatty acids were eluted from the column by adding chloroform, and glycolipids were washed out by adding acetone. The PLFAs were then eluted from the column using methanol, which was evaporated under nitrogen. Internal standards (19:0) were added to the samples then the phospholipids were subjected to mild alkaline methanolysis by adding 1 ml methanol: toluene (1:1) and 1 ml 0.2 M KOH to each sample and incubating them at 37°C for 15 minutes.

The fatty acid methyl esters were analyzed using a Hewlett-Packard 6890N Gas Chromatograph unit equipped with a 25 m × 0.2 mm inside diameter × 0.33 μm film thickness Ultra 2 column (Agilent). Gas chromatograph conditions were set, and peaks named using the Microbial Identification (MIDI) Sherlock protocol (MIDI Inc., Newark, DE). PLFAs were quantified by comparison of the peak areas with those of the internal standard (19:0) peak.

Fatty acids are designated in terms of the total number of carbon atoms: the number of double bonds, followed by the position of the double bond from the

62

methyl end of the molecule, indicated by ω and a number. The prefixes 'a', 'i' and 'br' indicate anteiso, iso and unknown branching, respectively. The prefix 'cy' indicates a cyclopropane fatty acid and methyl branching 'Me' is indicated as the position of the methyl group from the carboxyl end of the chain. The prefix 'C' (e.g.; C15:1) indicates that the PLFA has 15 carbon atoms and one double bond, but the arrangement of the carbon atoms (e.g. branching position) is not confirmed. The abbreviations 't' and 'c' indicate *trans* and *cis* configuration of the double bonds.

The following 11 PLFAs, i.e.; i15:0, a15:0, 15:0, i16:0, 16:1ω5c, i17:0, a17:0, 17:0cy, 17:0, 18:1ω7c, and 19:0c were considered to be mainly of bacterial origin and the sum of these PLFAs was chosen to represent bacterial biomass (Table 3.1). The linoleic acid 18:2ω6 (or 18:2ω6, 9) was used as an indicator of the fungal biomass (Frostegård et al., 1996a and b, Priha, et al., 1999). Since the outer cell membrane of Gram negative bacteria is mostly composed of lipopolysaccharides (LPS), which themselves consist largely of β-hydroxy fatty acids, it has been proposed that the β-hydroxy fatty acids can be used as an indicator of Gram negative bacteria (Parker, et al., 1982; Zelles, 1999). Finally, branched PLFAs were chosen as an indicator of Gram positive bacteria (Table 3.1).

Statistical Analysis

PLFA profiles were analyzed using CANOCO version 4.5 (Microcomputer Power, Inc., Ithaca, NY). Redundancy Analysis (RDA) was used to analyze the data. RDA allows direct assessment of the relation between known environmental variables and the multivariate data. The significance of the relationship can be tested with the Monte Carlo permutation test. Environmental variables analyzed in this study included fertilization, soil water content, soil microbial biomass carbon and soil microbial biomass nitrogen. Field series (A, C and F) was treated as a covariate. The RDA results were displayed on biplots in which relationships among environmental variables and individual PLFA are shown. PLFA cluster analysis was done using SAS (V8).

Results

3.1 Influence of Manure and NPKS fertilization on soil microbial biomass

Both the Manure and NPKS treatments significantly increased the soil microbial biomass carbon (MBC) at $\alpha = 0.05$ for all three dates of sampling as compared to the Control (Ck) plots (Tables 3.2 and 3.3). The Manure plots exhibited significantly higher MBC than the NPKS plots in September 2003 and May 2004, however no significant difference was found in August 2003. Lime application significantly ($\alpha = 0.05$) increased the MBC for all three sampling dates.

Manure significantly increased the soil microbial biomass nitrogen (MBN) at $\alpha=0.05$ for the three sampling dates compared to Ck. In a fashion similar to the MBC response, manure significantly increased MBN as compared to NPKS fertilization in September 2003 and May 2004, but there was no significant difference between the Manure and NPKS plots for soils sampled in August 2003. Liming significantly increased MBN ($\alpha=0.05$) for all three sampling dates.

Manure significantly decreased the measured MBC/MBN ratios in May 2004, but had significant effects neither in August nor in September 2003 (Figures 3.1). Lime application significantly decreased the MBC/MBN ratios in August 2003 and May 2004, but did not in September 2003 (Figures 3.2).

3.2 Influence of Manure and NPKS fertilization on soil water content

The soil moisture content of the Manure plots was significantly ($\alpha=0.05$) higher than that of the Ck plots for all three sampling dates, and significantly higher than that of the NPKS plots in September 2003 (Tables 3.2 and 3.3). The NPKS plots had significantly higher soil water content than the Ck plots in September 2003 and May 2004, but there was no significant difference between the two plot types in August 2003. Lime had no significant ($\alpha=0.05$) effect on soil moisture content in May 2004, but it significantly increased soil water content in August and September 2003 as compared to the un-limed plots.

3.3 Correlation between MBN, MBC and soil water content

Microbial biomass carbon was highest in September 2003, intermediate in August 2003, and lowest in May 2004. While MBN also was highest in September 2003, it reached its lowest values in August 2003 (Table 3.2). As a result, the

64

MBC/MBN ratios were higher in August 2003 than in May 2004 or September 2003 (Figures 3.1 and 3.2). For all three sampling dates, MBN and MBC were significantly (α=0.05) correlated (Table 3.4).

The correlation coefficients between MBN, MBC and soil water content (SWC) varied among sampling dates (Table 3.4). There was a significant correlation between MBN and SWC in September 2003 and May 2004, while MBC and SWC were only significantly correlated in May 2004.

3.4 Influence of Manure and NPKS fertilization on soil microbial community structure

PLFA analysis identified a total of 48 fatty acids. However, only the PLFAs that comprised an area greater than 1% of the total PLFA area in any given treatment were kept for analysis, yielding a total of 22 different PLFAs (Fig 3.3).

3.4.1. Redundancy analysis (RDA) ordination

Circles on t-value biplots, which are also often named the Van Dobben circles, show correlation between species and environmental variables (Ter Braak, 1990). For each variable, a circle can be drawn with as middle line, the line segment joining its coordinates on the biplot with the origin. The species with coordinates that lie inside the circle present a significant, positive regression coefficient with that particular environmental variable. A similar circle can be drawn in the opposite direction by mirroring the circle in the tangent at the origin, which indicates a significant negative regression coefficient between species and the environmental variable. In the biplot diagram presented on Figure 3.3, the two drawn circles correspond to the fertilization environmental variable (i.e.; chemical fertilization and manure). Almost all species (PLFAs) lines fell within that positive circle, indicating that chemical fertilizer and manure application positively affected all of these individual PLFAs in a similar fashion (Figure 3.3).

3.4.2. Univariate analysis

Statistical comparisons of individual measurements of PLFAs demonstrated a significant increase in the majority of individual PLFAs in response to manure or NPKS fertilization (Figure 3.4). For example, based on this analysis, the 16:0 10methyl PLFA was significantly enriched in the manure

65

treatment plots as compared to the NPKS treatment and the Ck plots. A group of 10 fatty acids, (including 16:0, 17:0, 20:0, 15:0 A, 16:0 ISO, 17:0 10 methyl, 17:0 A, 17:0 cyc, 17:0 ISO and 19:0 cyc ω8c) was significantly (α = 0.05) enriched in both the manure and NPKS treatments as compared to the Ck. Another group of fatty acids (including 14:0, 18:0, 14:0 ISO, 15:0 2OH, 16:1 2OH, 18:1 ω9c and TBSA 10Me 18:0) was also significantly enriched in the manure treatments as compared to the Ck, but in this case did not show any significant difference between the NPKS plots and either the manure or the Ck plots. Finally, for a third group (including 15:0, 15:1 ISOG, 18:1 ω7c and 16:1 ω7c) there was no significant difference between the manure, NPKS and Ck plots (Figure 3.4).

Similarly to what was observed for the individual PLFAs, fertilization and manure treatments significantly increased the microbial biomass of some of the fatty acid function groups (Figure 3.5). The biomarker groups representative of total bacteria, eubacterial anaerobes, actinomycetes, and total monounsaturated PLFAs were significantly (α = 0.05) increased in both the manure and the NPKS plots compared to the Ck. The PLFA groups indicative of fungal, Gram negative, and Gram positive bacterial biomass were significantly (α = 0.05) increased in the manure plots compared to the Ck, but there was no significant difference between the Ck and NPKS plots. Manure and NPKS had no significant effects on the group composed of saturated PLFAs (Figure 3.5). Finally, liming did not result in any significant differences in terms of either individual PLFA biomass or functional groups, and results are not presented here.

To identify potential effects of manure and NPKS fertilization on the soil microbial community composition, changes in percent distribution of various groups and subgroups of PLFA were considered. There was no significant (α = 0.05) difference between manure, NPKS and Ck for any of the functional groups presented on Figure 3.6. However, the NPKS fertilization tended to decrease the relative abundance of bacteria (including both Gram positive and Gram negative) when results were expressed as percentage of total PLFA area.

The fungal / bacterial biomass ratio was further calculated as:

$$= \frac{PLFA \ of \ bacterial \ group \ / \ total \ PLFA}{PLFA \ of \ fungal \ group \ / \ total \ PLFA} \qquad \text{(Formula 3.1)}$$

66

These ratios were extremely low (i.e.; < 0.2), suggesting that the PLFA method underestimated fungal biomass by using a single biomarker (18:2ω6). Compared to the Ck plots, the NPKS fertilization significantly (α = 0.05) increased the fungal / bacterial biomass ratio, but the manure treatment did not have any significant effect (Figure 3.7). The application of manure and chemical fertilizers (NPKS) had no significant effects on the ratio of Gram positive to Gram negative bacteria either. Finally, there was no significant (α = 0.05) fertilization effects on the ratio of monounsaturated to saturated PLFAs, the ratio of 17:0 cyc to16:1ω7, or the ratio of 19:0 cyc to 18:1ω7 (Figure 3.8).

Discussion and Conclusions

Influence of Manure and NPKS fertilization on MBC and MBN

In this study both the manure and NPKS fertilizer application significantly increased the soil MBC and MBN. Previous studies at the Breton plots also reported a significantly higher microbial biomass in the manured plots than in the Ck plots, although at that time there was no statistical difference between samples of the manure and mineral-fertilizer plots, or between samples of the fertilized plots and the Ck plots (McGill et al., 1986). While manure application can increase soluble carbon concentrations in soils, which in turn may increase MBC on a short-term basis, over a longer period, manure can also provide a more regular yet readily available stream of substrates to the microbial community because manure addition to soils contributes large amounts of fresh organic matter (Gregorich et al., 1998; Liang et al., 1998). Long term NPKS application may significantly increase MBC and MBN as well because chemical fertilization increases plant growth, which in turn increases soil carbon content through an increase in the amount of crop residues as well as through greater root biomass and exudates.

Microbial biomass carbon as measured in this study was highest in September, intermediate in August, and lowest in May 2004. Previous studies at the Breton Plots also reported seasonal variations in microbial biomass. In particular McGill et al. (1986) found that MBC typically increased from early spring to mid-summer, then diminished during September and October. In a similar

67

fashion, in the growing season of 1994, Carcamo studied MBC seasonal trends by sampling at the Breton Plots every three weeks. Carcamo reported that in September, MBC from the plots supporting oats was higher than in August, but that MBC stayed constant in hay plots (Carcamo, 1997). In our study, the September sampling occurred just following harvest, while in May 2004 it was just before cultivation. This may explain why MBC was highest in September, as the input of crop residues to the soil would result in a pulse of available organic substrates for the soil microbial community.

Microbial biomass carbon and nitrogen showed very good correlation with SWC in May 2004, as opposed to that observed in August and September (Table 3.4). This is likely due to differences in SWC at these different sampling dates. In May 2004, the average SWC was 20% (ranging from 14.7 to 27.3 %), while in August and September, 2003 it was much lower with an average of 7% (and a range of 5.3 to 10.7 %). So the SWC in May was close to the 60% field capacity water content, which is considered optimal for both crops and microbes. The SWC in August and September was much lower and closer to the permanent wilting point, and the crops and microbes were likely under stress conditions at this time. Hence in August and September soil conditions were likely outside the SWC dynamic range where MBC would be expected to respond.

Influence of Manure and NPKS fertilization on soil microbial community

A recent concern of researchers is the effect of agricultural management practices on the diversity of soil microbial communities. Measurement of soil PLFA profiles reflects the composition of *in situ* microbial communities and as such PLFAs have been used by some researchers to provide a useful fingerprint of microbial diversity in soils (Wander et al., 1995; Bossio et al., 1998 a and b). PLFA profiles have indicated differences in community composition that are consistent with differences in organic carbon inputs. For instance, typical Gram-negative bacteria PLFA biomarkers were observed to increase as a result of manuring (Peacock et al., 2001). Bossio et al. (1998a) reported that the large input of carbon substrates associated with the incorporation of poultry manure and some starter fertilizers such as fish powder and seaweed resulted in an enrichment of

68

specific groups of fatty acids (i14:0, a15:0, 16:1 ω7c, 16:1 ω5c, 14:0 and 18.2 ω6c). Liming has also been shown to cause a shift in the bacterial community to more abundant Gram negative but fewer Gram positive bacteria, although an increase in actinomycetes was observed (Frostegård et al., 1993a and b).

Cyclopropyl fatty acids have been proposed as indicators of stress conditions, including conditions of low carbon availability, low pH, low O_2, osmotic stress, and high temperature (Law, et al., 1963; Knivett and Cullen, 1965; Thomas and Batt, 1969; Guckert et al., 1985; Chang and Cronan, 1999; Mazumder et al., 2000; Boumahdi et al., 2001). For instance, increases in cyclopropyl fatty acids were consistently associated with decreases in substrate availability in rice soils (Bossio et al., 1998 a and b). Steenwerth et al. (2005) also reported that the ratio of 17 cy: pre (16:1 ω7c) declined after rewetting in two soils. A higher relative abundance of monounsaturated fatty acids has further been reported to occur with higher carbon inputs and greater availability of organic substrates (Zelles, et al., 1992; Bååth et al., 1995; Bossio and Scow, 1998b).

The present research clearly showed that chemical fertilization and manure application increased the recovery (as expressed per gram of soil) of several individual PLFAs, as well as of some specific groups of PLFAs (Figures 3.5 and 3.6). However, no statistically significant effects of fertilization were apparent from results of the univariate analysis. This was confirmed by results from the multivariate RDA, which indicated that fertilization basically increased the biomass of all individual PLFAs in a similar fashion (Figure 3.3). One notable exception was the ratio representative of the fungal/bacterial biomass, which was significantly higher in the NPKS plots than in the manure or Ck plots (Figure 3.7). These results should be related to the MBC/MBN ratios, which also provide a general index of the type of microorganisms present. That is, soil bacteria generally have a C: N ratio of 4:1 to 6:1 while fungi have a higher C: N ratio of 10:1 to 12:1 (Tate 1995). Consequently, a decrease in MBC/MBN ratios as observed with liming is indicative of a decrease in the fungal/bacterial biomass ratio. These results follow the increase in pH caused by liming as fungi are more prevalent than bacteria in soils of pH lower than about 5.5, whereas bacteria tend to predominate in soils with higher pH (Tate 1995).

69

Failure to detect differences in the composition of the soil microbial communities may be linked to the time of sampling for the PLFA analysis, which occurred in September 2003. It is possible that greater differences may be detected if the PLFA analysis were to be conducted on soils sampled in May 2004, as this is the only date where there was a significant difference in MBC/MBN ratios between fertilization treatments as well as a positive correlation between MBC and SWC.

References

Bååth, E., Frostegard, A., Pennanen, T., Fritze, H., 1995. Microbial community structure and pH response in relation to soil organic matter quality in wood-ash fertilized, clear-cut or burned coniferous forest soils. Soil biology and Biochemistry 2, 229-240.

Bligh, E.G., Dyer, W.J., 1959. A rapid method of total lipid extraction and purification. Canadian Journal of Biochemistry and Physiology 37, 911-917.

Bolton, J., Elliot, L.F., Papendick, P.R., Bezdicek, D.F., 1985. Soil microbial biomass and selected soil enzyme activities: effect of fertilization and cropping practices. Soil Biology & Biochemistry 17, 297-302.

Bossio, D.A., Scow, K.M., Gunapala, N., Graham, K.J., 1998a. Determinants of soil microbial communities: Effects of agricultural management, season and soil type on phospholipids fatty acid profiles. Microbial Ecology 36, 1-12.

Bossio, D.A., Scow, K.M., 1998b. Impacts of carbon and flooding on soil microbial communities: phospholipids fatty acid profiles and substrate utilization patterns, Microbial Ecology 35, 265-278.

Boumahdi, M., Mary, P., Hornez, J.P., 2001. Changes in fatty acid composition and degree of unsaturation of (brady)rhizobia as a response to phases of growth, reduced water activities and mild dessication, A. van Leeuwenhoek Int. The Journal of general microbiology 79, 73-79.

Carcamo, A.A., 1997. Carbon cycling in the Classical Breton Plots. MSc. Thesis. University of Alberta, Edmonton, Alberta. pp.1-117.

Cavigelli, M.A., Robertson, G.P., Klug, M.J., 1995. Fatty acid methyl ester (FAME) profiles as measures of soil microbial community structure. Plant and Soil 170, 99-113.

Chang, Y.Y., Cronan, J.E., 1999. Membrane cyclopropane fatty acid content is a major factor in acid resistance of Escherichia coli.. Molecular Microbiology 33, 249-259.

Fraser, D.G., Doran, J.W., Sahs, W.W., Lesoing, G.W., 1988. Soil microbial populations and activities under conventional and organic management. Journal of Environmental Quality 17, 585-590.

Frostegård, A., Bååth, E., Tunlid, A., 1993a. Shifts in the structure of soil microbial

71

communities in limed forests as revealed by phospholipid fatty acid analysis. Soil Biology & Biochemistry 25, 723-730.

Frostegård, A., Tunlid, A., Bååth, E., 1993b. Phospholipid fatty acid composition, biomass, and activity of microbial communities from two soil types experimentally exposed to different heavy metals. Applied and Environmental Microbiology 59, 3605-3617.

Gregorich, E.G., Rochetter, P., McGuire, S., Liang, B.C., Lessard, R., 1998. Soluble organic carbon and carbon dioxide fluxes in maize fields receiving spring-applied manure. Journal of Environmental Quality 27, 209-214.

Guckert J.B., Antworth CP., Nichols, P.D., White, D.C., 1985. Phospholipid, ester-linked fatty acid profiles as reproducible assays for changes in prokaryotic community structure of estuarine sediments. FEMS Microbiology Ecology 31, 147-158.

Jenkinson, D.S., Powlson, D.S., 1976. The effects of biocidal treatments on metabolism in soil--I. Fumigation with chloroform. Soil Biology & Biochemistry 8,167-177.

Jenkinson, D.S., Brookes, P.C., Powlson, D.S., 2004. Measuring soil microbial biomass. Soil Biology & Biochemistry 36, 5-7.

Kirchner, M.J., Wollum II, A.F., King, L.D., 1993. Soil microbial populations and activities in reduced chemical input agroecosystems. Soil Science Society of America Journal 57, 1289-1295.

Knivett, V.A., Cullen, J., 1965. Some factors affecting cyclopropane acid formation in Escherichia coli. Biochemical Journal 96, 771-776.

Law, J.H., Zalkin, H., Kaneshiro, T., 1963. Transmethylation reactions in bacterial lipids. Biochimica et Biophysica Acta 70, 143-151.

Liang, B.C., Mackenzie, A.F., Schnitzer, M., Monreal, C.M., Voroney, P.R., Beyaert, R.P., 1998. Management-induced change in labile soil organic matter under continuous corn in eastern Canadian soils. Biology and Fertility of Soils 26, 88-94.

Martens, R., 1995. Current methods for measuring microbial biomass C in soil: potentials and limitations. Biology and Fertility of Soils 19, 87-99.

Mazumder, R., Pinkart, H.C., Alban, P.S., Phelps, T.J., Benoit. R.E., 2000.

Low-substrate regulated microaerophilic behavior as a stress response of aquatic and soil bacteria. Current Microbiology., 41, 79-83.

McGill, W.B., Cannon, K.R., Robertson, J.A., Cook, F.D., 1986. Dynamics of soil microbial biomass and water-soluble organic C in Breton L after 50 years of cropping to two rotations. Canadian Journal of Soil Science 66, 1-19.

Michael, A., Klug, J., 1995. Fatty acid methyl ester (FAME) profiles as measures of soil microbial community structure. Plant and Soil 170, 99-113.

Parker, H.H., Smith, G.A., Fredrickson, H.L., Vestal, J.R., White, D.C., 1982. Sensitive assay, based on hydroxyl fatty acids from lipopolysaccharide lipid A, for Gram-negative bacteria in sediments. Applied and Environmental Microbiology 44, 1170-1177.

Paul, E.A., Clark, F.E., 1996. Soil Microbiology and Biochemistry (second edition). Acedemic Press, pp47-48.

Peacock, A.D., Mullen, M.D., Ringelberg, D.B., Tyler, D.D., Hedrick, D.B., Gale, P.M., White, D.C., 2001. Soil microbial community responses to dairy manure or ammonium nitrate applications. Soil Biology & Biochemistry 33, 1011-1019.

Peterson, S.O., Frohne, P.S., Kennedy, A.C., 2002. Dynamics of a soil microbial community under spring wheat. Soil Science Society of America Journal 66, 826-833.

Powlson, D.S., Brookes, P.C., Christensen, B.T., 1987. Measurement of soil microbial biomass provides a early indication of changes in total soil organic matter due to straw incorporation. Soil Biology & Biochemistry 19, 159-164.

Priha, O., Grayston, S.J., Pennanen, T., Smolander, A., 1999. Microbial activities related to C and N cycling and microbial community structure in the rhizospheres of Pinus sylvestris, Picea abies and Betula pendula seedlings in an organic and mineral soil, FEMS Microbiology Ecology, 30, 187-199.

Ross, D.J., 1991. Microbial biomass in a stored soil: a comparison of different estimation procedures. Soil Biology & Biochemistry 23, 1005-1007.

Steenwerth, K.L., Jackson, L.E., Calderon, F.J., Scow, K.M., Rolston, D.E., 2005. Response of microbial community composition and activity in agricultural land and grassland soils after a simulated rainfall. Soil Biology & Biochemistry (in press).

ter Braak C.J.F., 1990. CANOCO: A fortran program for canonical community ordination: update notes. Agricultural Mathematics Group, Wageningen, the Netherlands.

Tate, R.L., 1995. Soil Microbiology. John Wiley & Sons Inc.

Thomas, T.D., Batt, R.D., 1969. Degradation of cell constituents by starved Streptococcus lactis in relation to survival. The Journal of General Microbiology 58, 347-362.

Trinsoutrot, I., Recous, S., Mary, B., Nicolardota, B., 2000. C and N fluxes of decomposing 13C and 15N Brassica napus L.: effects of residue composition and N content. Soil Biology & Biochemistry 32, 1717-1730.

Tunlid, A., White, D.C., 1992. Biochemical analysis of biomass, community structure, nutritional status, and metabolic activity of microbial communities in soil. In: Soil Biochemistry, ed. G. Stotzky, J.M., Bollag, Vol 7. pp. 229-262. marcel Dekker, New York.

Vance, E.D., Brookes, P.C., Jenkinson, D.S., 1987. An extraction method for measuring soil microbial biomass C. Soil Biology & Biochemistry 19, 703-707.

Vestal, J.R., White, D.C., 1989. Lipid analysis in microbial ecology; Quantitytive approached to the study of microbial communities. BioScience 39, 535-541.

Wander, M.M., Hedrick, D.S., Kaufman, D., Traina, S.J., Stinner, B.R., Kehrmeyer, S.R., White, D.C., 1995. The functional significance of the microbial biomass in organic and conventionally managed soils. Plant and Soil 170, 87-97.

Witter, E., 1993. Size of the soil microbial biomass in a long-term field experiment as affected by different N-fertilizers and organic manures. Soil Biology & Biochemistry 25, 659-669.

Zelles, L., Bai, Q.Y., Beck, T., Beese, F., 1992. Signiture fatty acids in phospholipids and lipopolysaccharides as indicators of microbial biomass and community structure in agricultural soils. Soil Biology & Biochemistry 24, 317-323.

Zelles, L., Bai, Q.Y., 1994. Fatty acid patterns of phospholipids and lipopolysaccharides in environmental samples. Chemosphere 28, 391-411.

Zelles, Rackwitz, R., Bai, Q.Y., Beck, T., Beese, F., 1995. Discrimination of microbial diversity by fatty acid profiles of phospholipids and

lipopolysaccharides in different cultivated soils. Plant and Soil 170, 115-122.

Zelles, L., 1999. Fatty acid patterns of phospholipids and lipopolysaccharides in the characterization of microbial communities in soil: a review. Biology and Fertility of Soils 29, 111-129.

Tables and Figures

Table 3.1. Common phospholipid biomarkers (from Frostegård et al., 1993a; Zelles et al., 1994 and 1995; Cavigelli et al., 1995; Bossio et al., 1998a; Petersen et al., 2002).

Microbial group	PLFAs	Environmental indicator
Fungi	18:2 ω 6c	
Actinomycetes	10Me18:0	
Mycorrhizae	18:3, 20:3, 20:4, 16:1 ω 5c	
Gram-positive bacteria	Branched (i, a, Me)	
Eubacterial anaerobes	Cyclopropyl 17 & 19	
Gram negative bacteria (total hydroxy)	15:0 3OH + 16:1 2OH + 16:0 2OH + 18:0 2OH	
Total monounsaturated	i14:1ω5c + 15:1ω6c + 16:1ω7c + 16:1ω5c + 17:1ω9c + 18:1ω9c + 18:1ω7c	
Total saturated	12:0 + 13:0 + 14:0 + 15:0 + 16:0 + 17:0 + 18:0+ 19:0 + 20:0	
Total monounsaturated/ total saturated		Aerobic indicator
Cyclopropyl 17/ precursor (cy17/pre)	17:0cy / 16:1 ω7c	Physiological stress (slow growth)
Cyclopropyl 19/ precursor (cy19/pre)	19:0cy / 18:1 ω7c	Physiological stress (slow growth)
Total bacterial biomass	i15:0 + a15:0 + 15:0 + i16:0 + 16:1ω 5c + i17:0 + a17:0 + 17:0cy + 17:0 + 18:1ω7c + 19:0cy	

76

Table 3.2. Soil microbial biomass carbon (MBC in µg C /g oven dried soil) and nitrogen (MBN in µg N /g oven dried soil), and gravimetric soil water content (WC).

Series	Lime	Treatment	August 2003 MBC Mean	August 2003 MBC stdev	August 2003 MBN Mean	August 2003 MBN stdev	August 2003 WC(%) Mean	August 2003 WC(%) stdev	September 2003 MBC Mean	September 2003 MBC stdev	September 2003 MBN Mean	September 2003 MBN stdev	September 2003 WC(%) Mean	September 2003 WC(%) stdev	May 2004 MBC Mean	May 2004 MBC stdev	May 2004 MBN Mean	May 2004 MBN stdev	May 2004 WC(%) Mean	May 2004 WC(%) stdev
A	Limed	manure	636	50.4	41.8	0.0	8.9	1.7	940	158	129	10.1	9.8	2.1	576	44.8	76.0	11.5	18.2	1.5
		NPKS	673	47.8	42.1	1.5	6.8	0.3	771	127	105	38.9	8.1	0.3	502	26.1	65.1	5.9	17.0	0.5
		Ck	381	48.0	18.5	4.8	7.5	0.3	544	55.1	60.3	12.4	6.4	0.5	427	17.8	48.6	4.7	14.7	0.8
	Unlimed	manure	691	268	34.8	4.0	5.9	1.3	930	278	97.6	6.7	7.3	0.3	489	54.3	56.0	3.9	16.7	1.4
		NPKS	623	70.9	37.5	2.1	6.0	0.4	705	117	69.4	22.6	6.2	1.4	472	29.9	47.3	5.8	18.2	0.7
		Ck	343	61.8	17.5	0.4	5.3	0.3	553	25.9	50.8	22.3	5.6	0.3	394	16.2	42.0	0.5	15.2	0.6
C	Limed	manure	807	57.0	72.1	10.5	9.7	1.3	881	104	143	1.3	10.7	1.6	627	32.2	80.4	9.0	26.6	1.8
		NPKS	522	41.2	42.2	6.1	7.8	1.1	605	57.1	91.1	25.2	8.8	0.5	465	49.8	59.4	4.7	22.2	0.5
		Ck	412	150	35.9	3.9	8.1	0.4	586	21.8	66.8	27.0	8.2	0.4	443	110	51.9	16.5	18.2	1.1
	Unlimed	manure	625	59.9	37.0	8.2	6.1	0.4	811	112	151	36.3	8.5	1.3	486	82.9	51.4	9.2	21.8	2.6
		NPKS	469	25.8	31.7	5.5	7.6	0.3	575	95.6	65.0	4.3	6.5	0.4	378	10.9	31.6	14.5	21.8	1.4
		Ck	360	30.0	39.0	1.7	6.3	0.7	503	21.9	45.8	1.6	6.7	0.8	286	26.5	25.9	2.2	15.1	2.1
F	Limed	manure	1067	178	90.9	30.7	7.9	0.9	1343	583	239	104	8.6	1.0	868	154	118.3	27.1	27.3	0.9
		NPKS	919	293	91.5	53.1	8.4	0.4	1207	371	155	74.6	7.6	0.2	711	36.9	96.9	5.5	23.0	0.8
		Ck	915	60.7	70.0	8.9	7.1	0.2	1018	265	101	0.0	6.0	0.8	593	86.4	83.2	15.5	18.1	0.9
	Unlimed	manure	862	125	65.3	16.2	6.2	0.2	1196	189	92.6	28.3	9.2	0.4	725	77.4	83.5	14.2	24.9	1.2
		NPKS	876	102	47.5	6.6	5.6	0.8	875	12.0	79.6	33.0	6.7	0.2	558	79.0	62.2	22.5	22.8	1.7
		Ck	627	82.5	46.9	10.3	5.4	0.2	810	20.1	95.6	1.8	5.8	0.2	500	33.8	55.4	4.4	19.1	1.0

Note—Ck: control plots with no fertilizer; M: Manure treatment; NPKS: NPKS fertilizer treatment.

77

Table 3.3. Summary of paired-t test results for Table 3.2. Different letters indicate significant differences (a>b>c) as a result of fertilization or liming as analyzed with a paired t-test (α = 0.05 and n = 3).

Treatment	MBC Aug-03	Sep-03	May-04	MBN Aug-03	Sep-03	May-04	Water content (%) Aug-03	Sep-03	May-04
Manure	a	a	a	a	a	a	a	a	a
NPKS	a	b	b	ab	b	b	ab	b	a
Ck	b	c	c	b	b	c	b	c	b
limed	a	a	a	a	a	a	a	a	a
unlimed	b	b	b	b	b	b	b	b	a

Table 3.4. Pearson's correlation coefficients (r) between MBC, MBN, and SWC. The star indicates significance at α = 0.05.

		MBC	MBN	SWC
Aug 2003	MBC	1	0.81*	0.18
	MBN	0.81*	1	0.51
Sept 2003	MBC	1	0.78*	0.37
	MBN	0.78*	1	0.54*
May 2004	MBC	1	0.97*	0.74*
	MBN	0.97*	1	0.65*

Figure 3.1. Effects of manure and NPKS fertilization on microbial C/N ratios. Error bars represent standard deviations from the mean, and different letters correspond to differences at $\alpha = 0.05$ using a paired t-test.

MBC/MBN
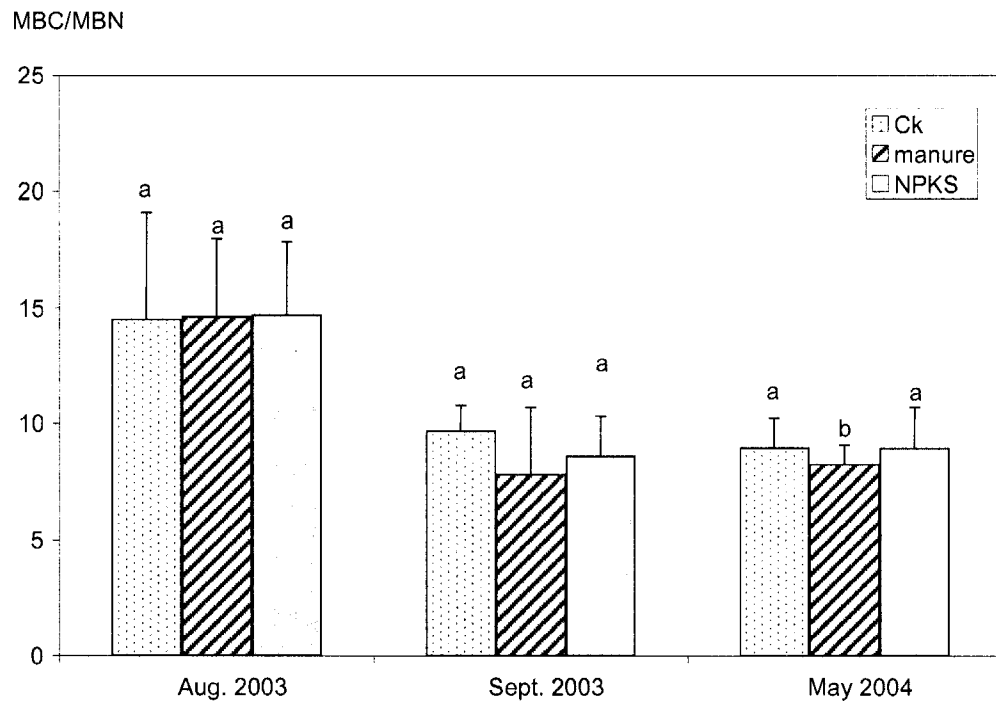
Figure 3.2. Effects of liming on microbial C/N ratios. Error bars represent standard deviations from the mean, and different letters correspond to differences at α = 0.05 using a paired t-test.

MBC/MBN
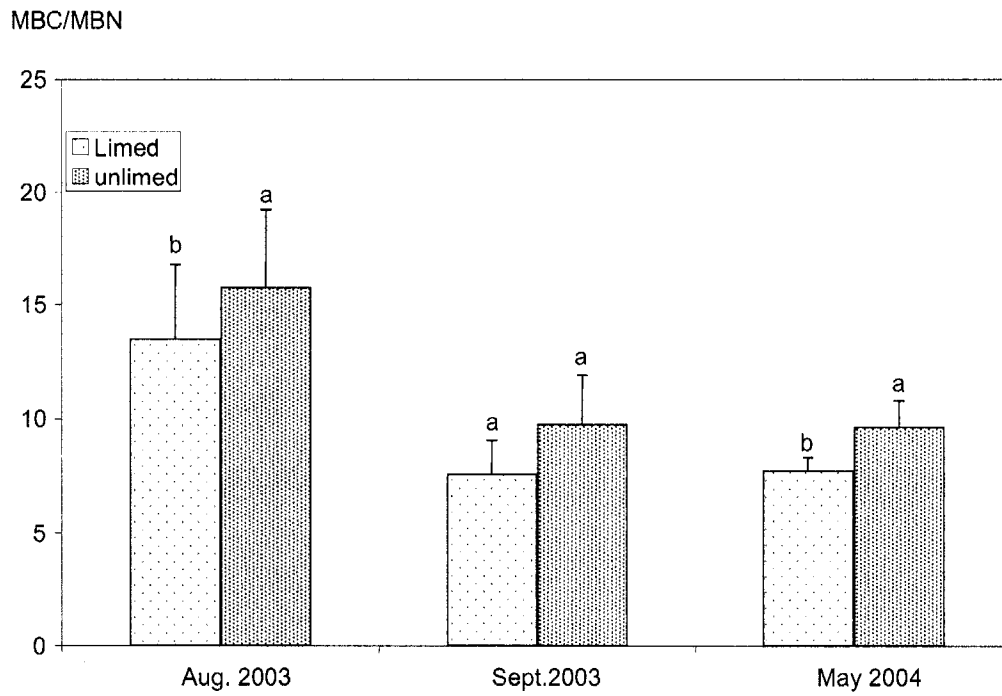
Figure 3.3. Biplot diagram from the redundancy analysis (RDA), summarizing the effects of fertilization (FERT) (NPKS and Manure), lime (LIMED), soil water content (water co.), microbial biomass carbon (MBC) and microbial biomass nitrogen (MBN) on PLFA profiles.
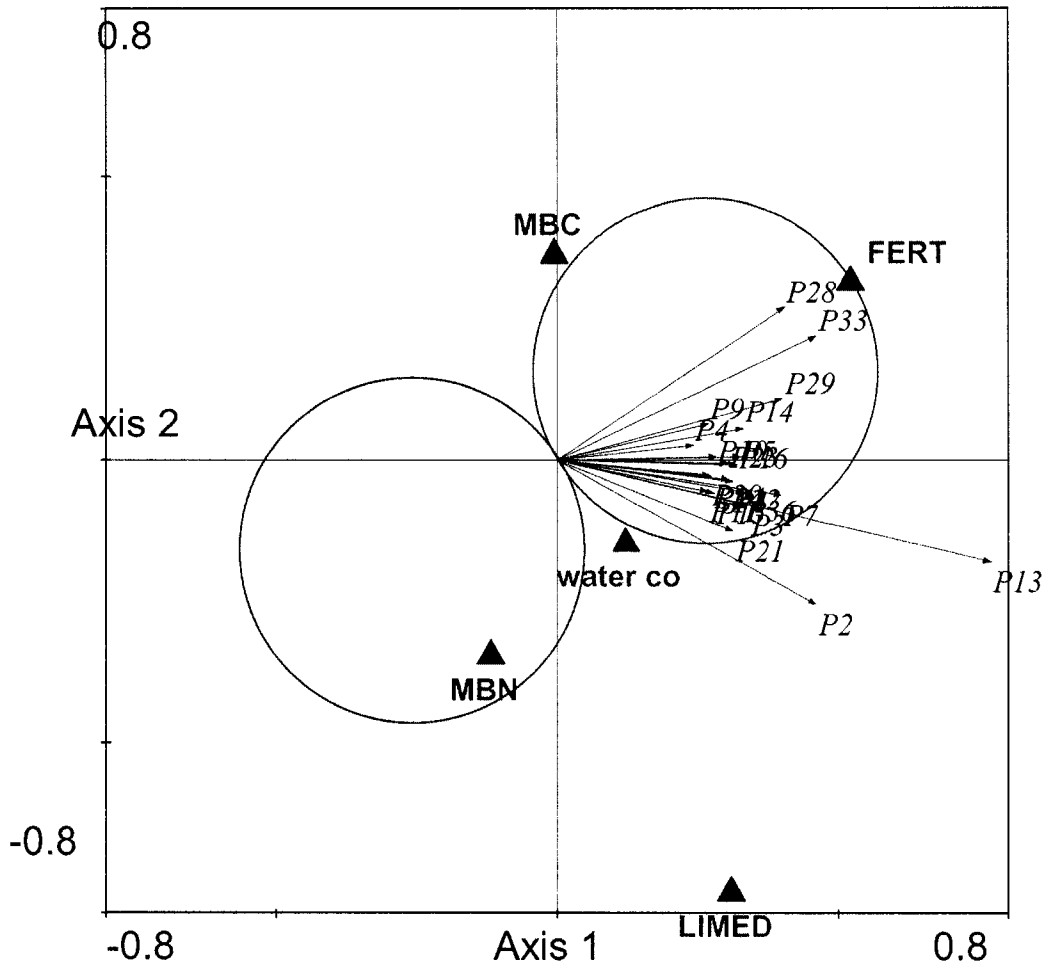
Numbers correspond to the following PLFAs:
1 = 14:0; 2 = 15:0; 3 = 16:0; 4 = 17:0; 5 = 18:0; 7 = 20:0; 9 = 14:0 ISO; 10 = 15:0 2OH; 11= 15:0 A; 13 = 15:1 ISO G; 14 = 16:0 10 methyl; 15 = 16:0 ISO;16 = 16:1 2OH; 21 = 17:0 10 methyl; 22 = 17:0A; 23 = 17:0 cyc; 24 = 17:0 ISO; 28 = 18:1ω7c; 29 = 18:1 ω9c; 30 = 19:0 cyc ω8c; 33 = 16:1 ω7c; 36 = TBSA10 Me 18:0.)

81

Figure 3.4 The effects of fertilization upon PLFAs(1 = 14:0; 2 = 15:0; 3 = 16:0; 4 = 17:0; 5 = 18:0; 7 = 20:0; 9 = 14:0 ISO; 10 = 15:0 2OH; 11 = 15:0 A; 13 = 15:1 ISO G; 14 = 16:0 10 methyl; 15 = 16:0 ISO; 16 = 16:1 2OH; 21 = 17:0 10 methyl; 22 = 17:0A; 23 = 17:0 cyc; 24 = 17:0 ISO; 28 = 18:1ω7c; 29 = 18:1 ω9c; 30 = 19:0 cyc ω8c; 33 = 16:1 ω7c; 36 = TBSA10 Me 18:0.) Error bars represent standard deviations and different letters indicate statistical differences at α = 0.05 using a paired t-test (n = 3) (Soil sampled in September, 2003).
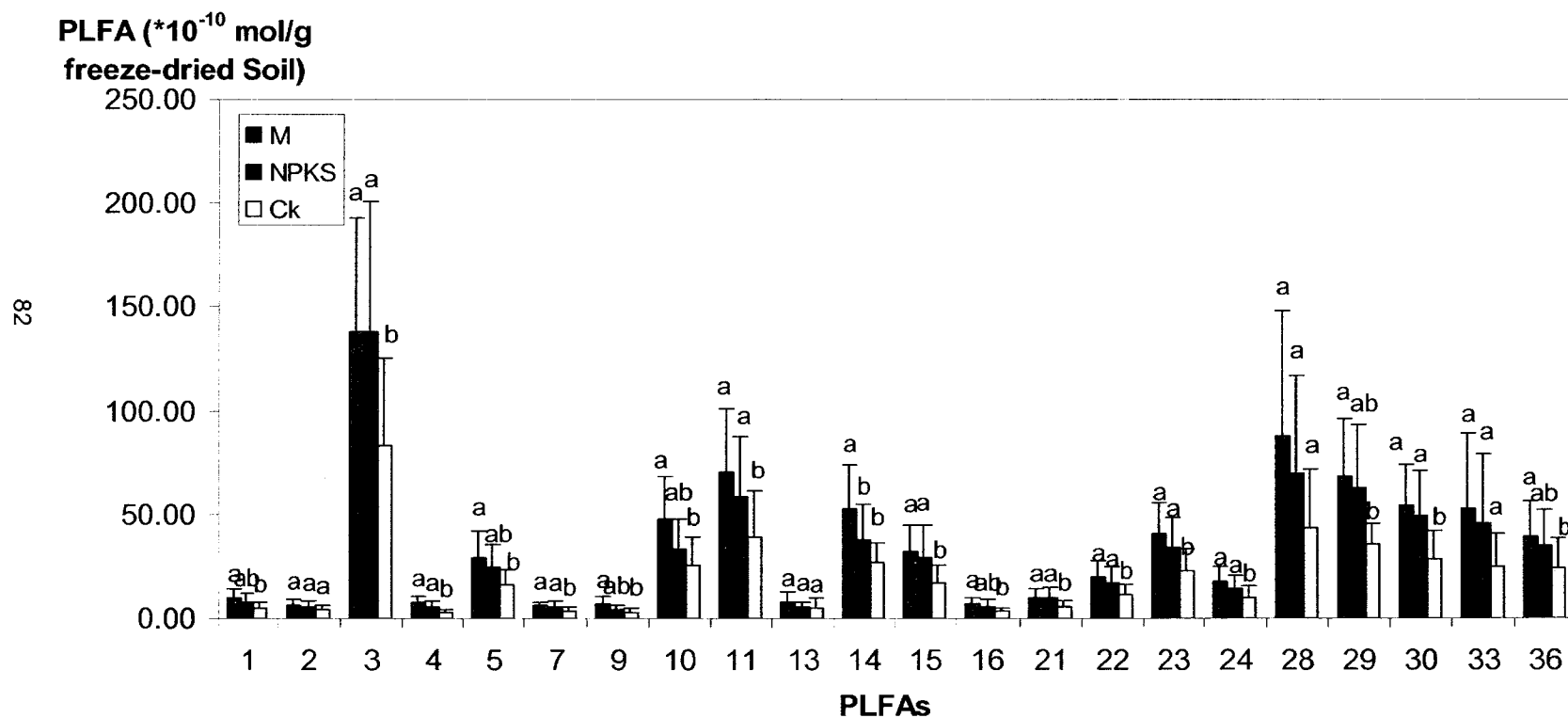
Figure 3.5. Fertilization influence on PLFAs indicative of microbial groups (see Table 3.1. for list of indicator PLFAs). Error bars represent standard deviations and different letters indicate statistical differences at α = 0.05 using a paired t-test (n = 3) (Soil sampled in September, 2003).
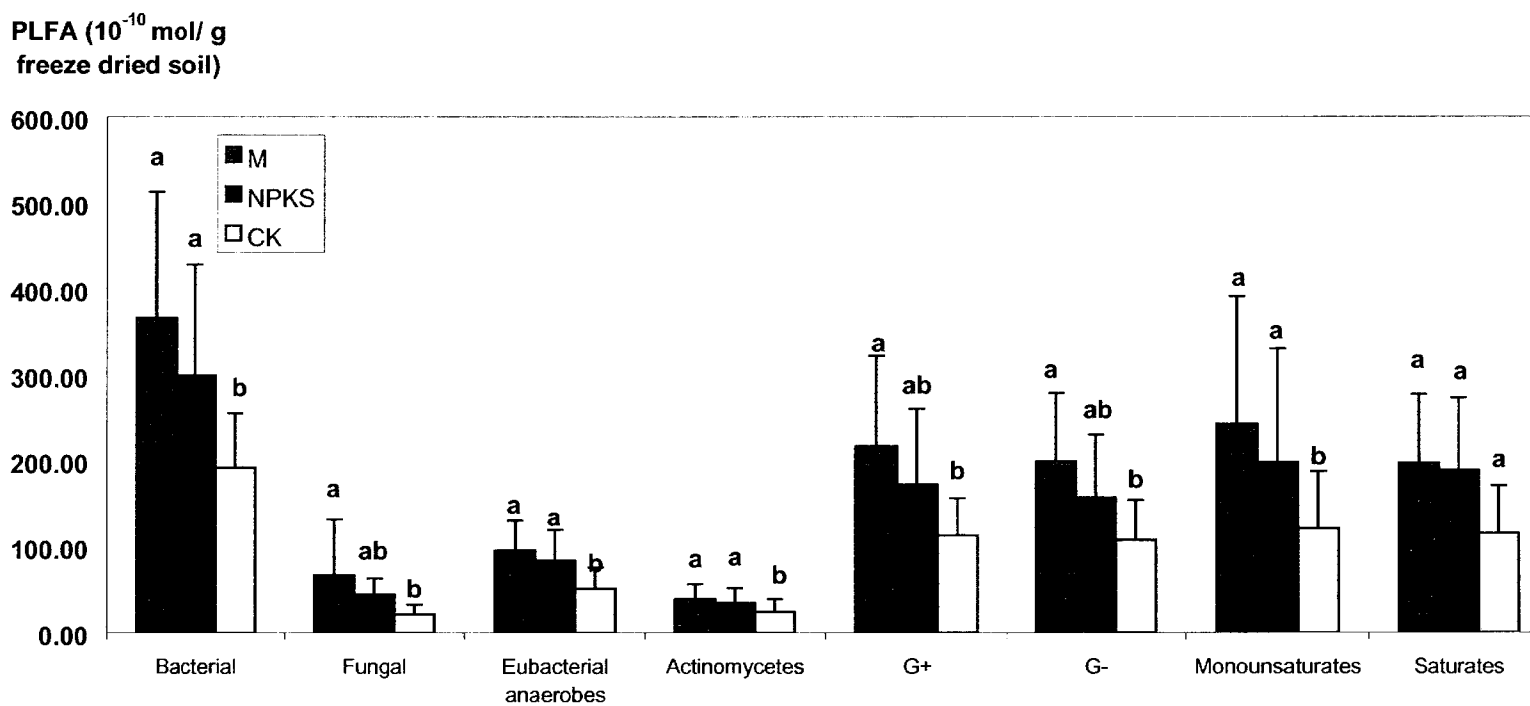
Figure 3.6. Fertilization influence on PLFAs distribution (expressed as % of total PLFA). Error bars represent standard deviations. See Table 3.1. for list of indicator PLFAs) (Soil sampled in September, 2003).
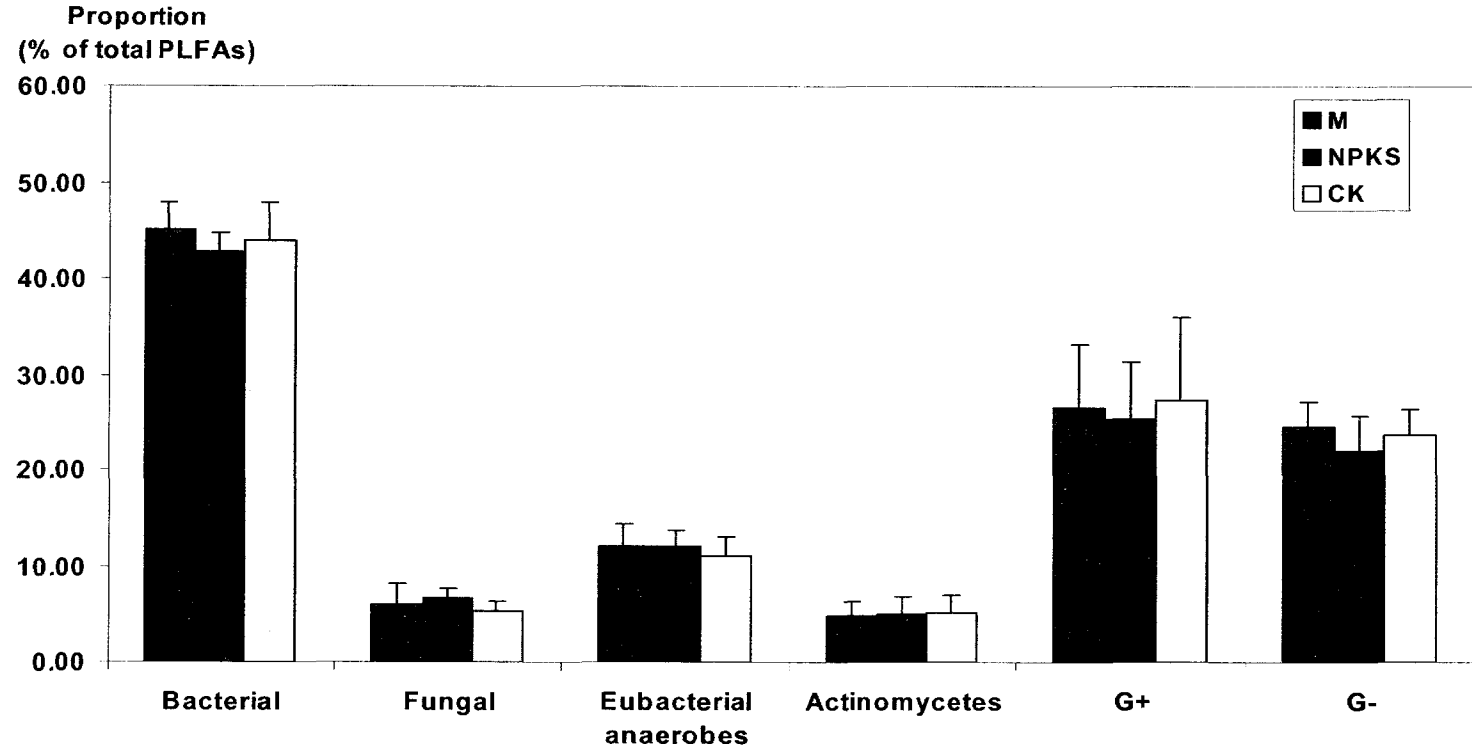
Figure 3.7. Effects of fertilization on PLFA ratios indicative of Gram positive/ Gram negative (G+/G-), and fungal/bacterial biomass. Error bars represent standard deviations and different letters indicate differences at α = 0.05 using a paired t-test (n = 3). Indicator PLFAs are listed in Table 3.1 (Soil sampled in September, 2003).
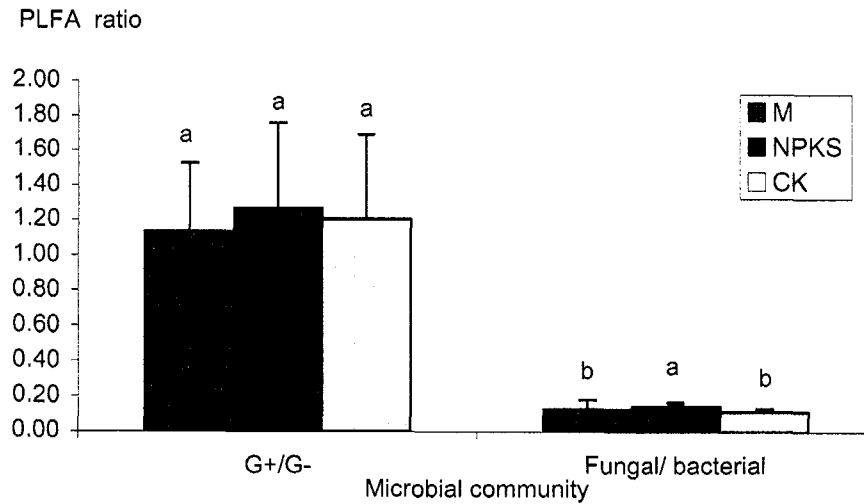
Figure 3.8. Effects of fertilization on PLFA ratios indicative of aerobic conditions (Monounsaturated/Saturated) or physiological stress (17:0cy / 16:1 ω7c and 19:0cy / 18:1 ω7c). Error bars represent standard deviations and different letters indicate differences at α = 0.05 using a paired t-test (n = 3). Indicator PLFAs are listed in Table 3.1 (Soil sampled in September, 2003).

PLFA ratio
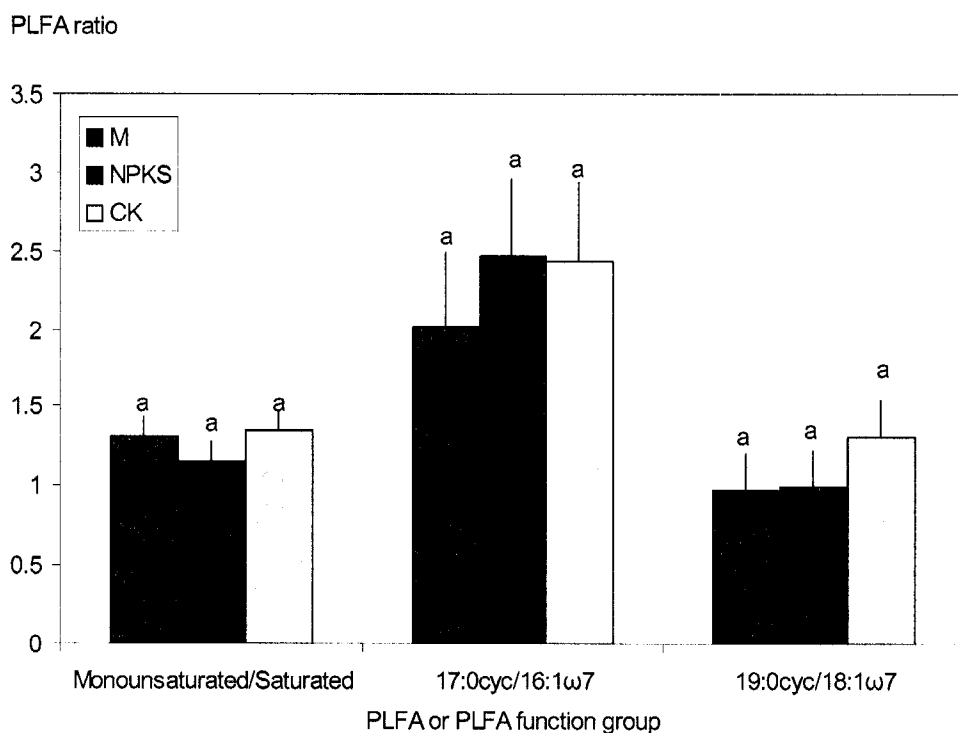
Chapter 4

Soil carbon mineralization at the Breton Classical Plots

Introduction

Loss of soil organic carbon (SOC) as CO_2 is a serious environmental problem. It is estimated that approximately 1500 Gt of C (1 Gt $=10^{12}$ kg) is stored as SOC in the upper meter of soils (Lal and Kimble, 2000). Land conversion (cultivation and deforestation) during the early 1990s resulted in an average SOC loss of 1.6 ± 0.7 Gt of C year^{-1} (Houghton, 1995). Long-term field studies can provide the foundation to better understand SOC dynamics and the underlying processes controlling SOC loss over decadal time scales. The Breton Classical Plots, started in 1929 in central Alberta, hold the longest agronomic and soil record for Gray Luvisolic soils in North America.

Mineralization is defined as the conversion of an element from an organic to an inorganic form (Paul and Clark, 1989), and soil carbon mineralization is the release of CO_2 from SOC and plant residues during decomposition and humification processes. SOC is protected against mineralization by various mechanisms. Three main mechanisms of SOC stabilization have been proposed: (1) physical protection, (2) chemical stabilization and (3) biochemical stabilization (Christensen, 1996).

The physical protection exerted by macro- and/or micro-aggregates on particulate organic matter (POM) is attributed to the compartmentalization of substrates and microbes, and to the reduced diffusion of O_2 into aggregates, which slows down microbial activity in the aggregates, especially in the micro-aggregates (Six et al., 2002). Recent studies indicate that the macroaggregate (>250 µm) structure exerts a minimal amount of physical protection (Beare et al., 1994; Pulleman and Marinissien, 2004) as compared to the free micro-aggregates or to the micro-aggregates contained within macro-aggregates (Skjemstad et al., 1996; Balesdent et al., 2000; Six et al., 2002; Denef et al., 2001).

Chemical stabilization of SOC arises from the chemical or physicochemical binding between SOC and mineral clay and silt particles (Six et al., 2002). A simple relationship exists between the clay (or clay + silt) content of a soil and total SOC storage, although this relationship may vary

87

between different types of land use and different clay types (Feller and Beare, 1997). Furthermore, the clay + silt content of a soil has been reported to be correlated to its maximum SOC storage capacity, indicating a saturation level for silt and clay associated carbon (Hassink and Whitmore, 1997).

Biochemical stabilization is defined as the stabilization of SOC due to its intrinsic chemical composition and through biochemical reactions (e.g. condensation) occurring in the soil (Six et al., 2002). SOC chemical composition can be an inherent property of the plant residues or can be attained during decomposition of these residues, rendering them more resistant to subsequent decomposition. This biochemically-stabilized pool is also referred to as the passive SOM pool (Parton et al., 1987) or non-hydrolysable fraction (Paul et al., 1995).

Measurement of CO_2 evolution or O_2 uptake in the field or in the laboratory is the well-accepted approach to study carbon mineralization. Results from field experiments are expected to be more reflective of the natural conditions under which soil organic substrates are mineralized while laboratory incubations have been used for comparing C and N mineralization potential from different soils and different crops (Christensen, 1987; Hassink, 1995; Parfitt et al., 2001a and b, 2002; Plante and McGill, 2002). The laboratory-based incubation method isolates CO_2 efflux due to SOC mineralization from the potential interference of root respiration fluxes.

Various fractionation schemes have been proposed to experimentally isolate the different SOC pools used in conceptual SOC models. Soil physical fractionation techniques are considered less destructive than chemical fractionation approaches, and results are anticipated to relate more directly to the structure and function of SOC *in situ* (Christensen, 1992). A 5 minutes ultrasonic treatment permits separation of the occluded organic materials that are in different stages of decomposition because these have formed different degrees of association with mineral particles and thus have different densities (Golchin, et al., 1994). Wet sieving is used more often than dry sieving to determine particle size distribution and stability of soil aggregates. Specifically, soil fractions with a diameter greater than 53μm can be isolated through wet sieving. The floatable fraction, which has a density lower than 1.0 g / cm^3 can be further separated from the mineral particles using water floatation. This floatable fraction, considered to be an unprotected SOM pool, consists of

partially decomposed plant residues, which are not closely associated with the soil minerals. Finally, the mineral particles can be separated into distinct size fractions using a combination of density and centrifugation techniques. The fraction including particles with a diameter ranging from 2 to 20 μm (i.e.; the organo-silt complex), consists of humified plant and microbial debris associated with stable microaggregates that have not been destroyed during sonication. The fraction including particles with a diameter less than 2 μm (i.e.; the organo-clay complex) is dominated by amorphous organic matter acting as cement for the clay particles. It may contain plant cells as well as bacterial cells or colonies at different stages of decomposition (Feller et al., 2000).

One of the objectives of this study was to partition total soil respiration into its different physical fractions and to compare carbon mineralization from the whole soil with that of selected SOC fractions. A second objective was to determine whether long-term manure application had significant effects on carbon mineralization fluxes at the Breton Classical Plots.

Materials and Methods

Field sampling and laboratory incubation

On September 21, 2003, 9 soil samples (0-15 cm depth) were taken from the control (Ck) and manure plots of the A, C, and F series of the 5 year rotation at Breton, Alberta. Three of these samples were mixed and composited to yield a total of 3 samples per plot, and brought back to the laboratory for analysis. After air-drying and sieving to pass a 2-mm-sieve, these samples were physically fractionated as described in Chapter 2. Whole soils and soil fractions were incubated at 24°C for 70 days from June 21, 2004 to August 31, 2004. The following five fractions were incubated: floatables, sand, coarse silt, medium + fine silt fraction (medium silt: fine silt =50:50 on a weight basis), and clay. Incubations were run in duplicates for the physical fractions, corresponding to a total of 180 incubated samples = 5 fractions * 2 incubation duplicates * 3 samples per plot * 3 plots * 2 treatments (Ck and manure). Whole soil samples were incubated only once, yielding an additional 18 samples for incubation = 3 samples per plot * 3 plots * 2 treatments.

For the whole soil samples, 10 g of fresh soil was used and the soil

moisture content was adjusted to 60% field capacity. For the soil mineral fractions, 2 g from each fraction was mixed with 8 g of purified quartz sand (40-70 mesh washed Ottawa Sand, Fisher) to improve aeration in these samples. For the floatable fraction, 0.2 g was mixed with 8 g of sand. A soil inoculum was obtained by shaking 100 g of fresh soil with 1000 ml water for 10 minutes and leaving the suspension to rest overnight (Christensen, 1987). This inoculum was buffered to pH =7, and 2 mg $Ca(NO_3)_2$, 5.23mg KH_2PO_4, 1.23 mg $Mg(NO_3)_2$, 1.24 mg $(NH_4)_2SO_4$, and 3.6 mg NH_4NO_3 were added to yield a solution containing N 2.0 mg /L , P 1.2 mg/L, K 1.5 mg/L, Ca 0.5 mg/L, Mg 0.2 mg/L and S 0.3mg/L. One ml of this solution was added to each sample before incubation.

The cumulative amount of CO_2 evolution from each incubation flask was measured every 14 days using the base trap method. Specifically, the amount of CO_2 absorbed by a NaOH solution (0.25 N) was determined using a back-titration with HCl (around 0.01N) after addition of excess $BaCl_2$ (Anderson, 1982).

Data analysis

The respiration rates from the laboratory experiment were initially analyzed by calculating the cumulative CO_2 efflux for a given incubation period, i.e., μgCO_2-C per g of oven-dried (O.D.) weight for the whole soil or for a given fraction. Results were then expressed on a per gram of carbon basis ($mgCO_2$-C per g of C in fraction) by dividing the CO_2 efflux by the carbon concentration in the whole soil sample or in each corresponding fraction (carbon concentration data are presented in Chapter 2). Finally, respiration rates from each fraction were calculated on a total soil weight basis (μg CO_2-C per g of O.D. soil) using the following formula $= A \times B$

Where, A is the cumulative efflux CO_2 ($\mu g\,CO_2$ - C / g fraction);

B is the fraction concentration (g fraction / g soil) .

Different regression models, including linear, logarithmic, and polynomial, were used to describe the relationship between cumulative CO_2 production and incubation time. The simple linear regression models systematically had the highest R^2 of the different models, hence fitted the data best. Simple linear regression equations were calculated:

90

$y = a + kx$

where y is the cumulative CO_2 production (μgCO_2-C per g C in fraction);

x is the incubation period (day);

a is a constant;

and k is the mineralization rate constant (μgCO_2-C per g C in fraction per day).

A multiple linear regression model was used to test for relationships between carbon mineralized from the whole soil and carbon mineralized from the five soil fractions. In this model, whole soil mineralized carbon (i.e.; cumulative CO_2 evolution from the whole soil during the entire incubation period) was the dependent variable and carbon mineralized from the five soil fractions (i.e.; cumulative CO_2 evolution from each soil fraction during the incubation period) were independent variables. The variables were selected by using the SAS STEPWISE procedure with a significance level of 0.1.

Cumulative CO_2 efflux data for the entire (70 days) incubation period were used to examine differences between fractions. A completely random design and the PROC GLM procedure of SAS were used to carry out a one-way Analysis of Variance followed by a Duncan's New Multiple Range test to determine significance between fractions at $\alpha = 0.05$.

A paired t test was used to check for the effects of manure. In this case, averages of cumulative CO_2 efflux data for the 70 days incubation period were calculated for each series (A, C, and F) and employed during the statistical analysis (i.e.; n=3).

Results

4.1 Soil carbon mineralization on a carbon basis

When results were expressed on a per gram of carbon basis, the sand and coarse silt fractions showed the highest mineralization rates (i.e.; highest cumulative CO_2 evolution) and the whole soil samples the lowest mineralization rate (Figure 4.1). Mineralization rate decreased in the following order: sand > coarse silt > medium + fine silt $\geq$ floatables $\geq$ clay $\geq$ whole soil (Table 4.1). The mineralization rate values (k) of the different fractions similarly decreased in the following order: sand > coarse silt > fine + medium silt $\cong$ floatables $\cong$ clay> whole soil (Table 4.3).

The manure treatment significantly increased the sand-associated C

91

mineralization and significantly decreased the clay-associated C (Figure 4.1 and Table 4.2). The other fractions and whole soil of the Ck had higher mineralization rates than those of the manure treatment but the differences were not significant. The k value also showed that the manure treatment decreased the C mineralization rate of the whole soil, clay fraction, medium + fine silt fraction, and floatable fraction (Table 4.3). Taken together, these results indicate that for the Breton Plots soils, the sand and clay-associated C was more sensitive to manure application than the silt- associated C.

4.2 Soil carbon mineralization on a soil weight basis

Cumulative CO_2 production was highest from the whole soil, intermediate from the sand and clay fractions, and least from the silt and floatable fractions (Figure 4.2 and Table 4.1). The whole soil had a higher CO_2 evolution (on a weight basis) than any of the fractions, yet CO_2 evolved from the whole soil was much less than the sum amount of the CO_2 evolved from all the soil fractions (Table 4.2). Specifically, CO_2 evolved from the whole soil was 684 μgCO_2-C/ g soil, while the sum from the five soil fractions corresponded to 1788 μgCO_2-C/ g soil over 70 days.

Although the sand and coarse silt fractions had a higher C mineralization (on a carbon basis) than the clay, for a given weight of soil, the clay evolved as much CO_2 as the sand, and significantly more than the coarse silt (Table 4.1). This is because the clay fraction contained 37.0% of the total soil carbon (see chapter 2), hence for a unit weight of soil it had the greatest amount of CO_2 evolution, corresponding to 30.1% of the total respiration from all fractions (i.e.; 1788 μgCO_2-C/ g soil). In contrast, the coarse silt fraction only contained 8.4% of the total soil carbon, and CO_2 efflux from the coarse silt represented 14.7% of the total respiration. In a similar fashion, the floatables exhibited a CO_2 production comparable to that of clay when results were expressed on a carbon basis, yet as the floatables contained a very small fraction (i.e., less than 1%) of the total soil carbon, they showed less CO_2 evolution than the other fractions for a unit weight of soil, corresponding to 11.0% of the total respiration flux. The medium and fine silt contained 17.4 and 15.4 % of the total soil carbon, respectively. They had a relatively low CO_2 evolution on a carbon basis, and similarly had a lower mineralization rate than

92

the sand and clay on a weight basis, which corresponded to 14.1% of the total CO_2 efflux.

The manure treatment significantly increased the CO_2 evolution from the clay fraction but decreased it from the floatables for a unit weight of soil compared with the Ck treatment (Figure 4.2 and Table 4.2). The manure treatment had no significant effects on the soil CO_2 evolution of the other soil fractions or on the whole soils in this study.

4.3 Step wise correlation analysis

Results from the SAS analysis showed that for the Ck treatment only the sand fraction was significantly ($\alpha = 0.1$) related to whole soil respiration and the equation was:

$Y = 1814.5 - 2.02\, X$ ($R^2 = 0.795$)

where X is the cumulative CO_2 production from the sand fraction;

and Y is the cumulative CO_2 production from the whole soil.

For the manure treatment both the clay and sand fractions were significantly related to whole soil respiration and the equation was:

$Y = -193.10 + 0.67\, X_1 + 0.77\, X_2$ ($R^2 = 0.897$)

where:

Y: cumulative CO_2 production from the whole soil;

X_1:   cumulative CO_2 production from the clay fraction;

X_2: cumulative CO_2 production from the sand fraction.

Discussion and Conclusions

Organic matter stability in soil fractions

Differences in organic matter distribution had been found when the soil was fractionated into floatables, sand, coarse, medium and fine silt, and clay (see chapter 2). The floatable pool had the highest C concentration, clay and fine silt were next, and sand and coarse silt had the lowest concentration. The 70-day incubation results showed that the sand and coarse silt fractions had the highest mineralization rates when results were expressed on a carbon

93

basis. These results are consistent with other reports in the literature. For instance, Gregorich et al. (1989) reported that the proportion of C mineralized during a 20-day laboratory incubation period decreased in the order (mg C/ g C): sand> whole soil> silt> clay. The large amount of CO_2 evolved from the sand and coarse silt is indicative of the rapid decomposability of the organic matter associated with these fractions, which has been reported to mostly contain fragments of plant residues (Parfitt and Salt, 2001; Hassink, 1995).

In this study, the clay had the lowest mineralization rate on a carbon basis. Hassink and Whitmore (1997), and Christensen (1987) also reported lower mineralization rates for the clay and silt fractions (< 20 μm), although silt-associated C is often found to be more resistant to microbial attack than that of clay (Gregorich et al., 1989; Parfitt and Salt, 2001). Christensen (1996) proposed that following the addition of fresh substrates, newly formed organic matter can be found in all fractions, although the clay sized organo-mineral complexes often show greater accumulations. In the longer term, the proportion of substrate-derived C in clay declines more rapidly than that in silt, thereby indicating a higher stability of silt-associated C (Christensen, 1996). Parfitt and Salt (2001) further speculated that fresh substrates absorbed on clay external surfaces are readily accessible to microbial attack, while substrates may be trapped in small pores and become physically protected from microbial attack when they associate with the silt fractions. A lower mineralization rate from the silt than from the clay was not observed in our study (Table 4.1). This may be related to the sonication procedure employed in our study that would have more efficiently disrupted micro-aggregates in the silt fractions. Alternatively, this may be linked to the inherent physical properties of the sampled Luvisolic soils, which have a very friable to friable consistence in their upper mineral horizons, and may contain relatively low amounts of silt-sized microaggregates.

Mineralization from the whole soil and sum of fractions

At the end of the 70-day incubation, the cumulative sum of CO_2 evolved from the five soil fractions was 2.6 times higher than that from the whole soil (Table 4.1). The CO_2 evolution measured during laboratory incubation of soil fractions reflects the chemical stability of the organic matter,

94

while the difference between the sum of CO_2 from the different size fractions and CO_2 from the whole soil may correspond to mineralization of the physically stabilized organic matter that is released during the fractionation procedure (Christensen, 1987). In our study, the whole soil was given lower disruptive energies than the individual fractions to minimize disruption of soil aggregation hence the greater soil C mineralization observed from the soil fractions was likely increased due to a greater disruption of the soil structure. Gregorich et al. (1989) also observed a substantially higher C mineralization when aggregates within the soil were disrupted than when they had not been broken up. A high proportion of organic matter associated with microaggregates consists of highly processed, humified "recalcitrant" moieties, while organic matter associated with macroaggregates is more readily mineralized (Christensen, 1987). Thus, it is probable that the increase in CO_2 evolution observed in our study was due to C released from macroaggregates rather than from microaggregates. In all cases, our results clearly demonstrate the significance of physical stabilization as a mechanism of C sequestration at the Breton Plots, since C mineralization was observed to more than double following disruption of the soil aggregates during the physical fractionation process.

Manure influence on soil mineralization

On a soil weight basis, the manure treatment significantly increased the CO_2 evolution from the clay fraction when compared with the Ck treatment. Furthermore, for the Ck samples, the whole soil C mineralization rate was only correlated to the C mineralization from the sand, whereas for the manure treatment, the whole soil mineralization was significantly correlated to both the sand and clay fractions. These results may be due to the fact that the soils under manure contained proportionally more carbon in the clay-size fraction as compared to the control soils, which in turn would contribute to a greater portion of the total soil respiration originating from this clay fraction. Unfortunately, our experimental design did not allow for statistical testing of this potential explanation.

On a carbon basis, the manure treatment significantly increased the sand-associated C mineralization and significantly decreased the clay-associated C mineralization (Table 4.2). Manure treatment had no

95

significant effects on CO_2 evolution from the other soil fractions. As reported in chapter 2, long-term manure addition at the Breton Plots has resulted in increased carbon sequestration in all soil fractions. Results from the laboratory incubation experiment further indicate that this increase in total soil C may be associated with differences in terms of C decomposability. Specifically, the manure-derived sand-sized C was more easily mineralized than that found in the control soils, while manure might have increased the recalcitrance of the organic matter associated with the clay fraction. Whether this increase in recalcitrance is linked to changes in the chemical structure of the clay-sized organic matter or to an increase in the amount of physically protected C still remains to be studied.

References

Anderson, J.P.E., 1982. Soil respiration. In: Page, A.L., Miller, R.H., Keeney, D.R. (Eds.), Methods of Soil Analysis. Part 2. Chemical and Microbiological Properties. ASA and SSSA, Madison, Wisconsin, pp. 831-871.

Balesdent, J., Chenu, C., Balabane, M., 2000. Relationship of soil organic matter dynamics to physical protection and tillage. Soil & Tillage Research 53, 215-230.

Beare, M.H., Cabrera, M.L., Hendrix, P.F., Coleman, D.C., 1994. Aggregate-protected and unprotected organic matter pools in conventional- and no-tillage soils. Soil Science Society of America Journal 58, 787-795.

Christensen, E.T., 1987. Decomposability of organic matter in particle size fractions from field soils with straw incorporation. Soil Biology & Biochemistry 19,429-435.

Christensen, E.T., 1992. Physical fractionation of soil and organic matter in primary particle size and density separates. Advances in Soil Science 20, 1-90.

Christensen, E.T., 1996. Matching measurable soil organic matter fractions with conceptual pools in simulation models of carbon turnover: revision of model structure. In NATO ASI Series, Vol. 138, Evaluation of soil organic matter models, David, S., (Eds) Springer-Verlag Berlin Heideberg, pp143-159.

Denef, K., Six, J., Bossuyt, H., Frey, S.D., Elliott, E.T., Merckx, R., Paustian, K., 2001. Influence of wet-dry cycles on the interrelationship between aggregate, particulate organic matter, and microbial community dynamics. Soil Biology & Biochemistry 33, 1599-1611.

Feller, C., Beare, M.H., 1997. Physical control of soil organic matter dynamics in the tropics. Geoderma 779, 69-116.

Feller, C., Balesdent, J., Nicolardot, B., and Cerri, C. 2000. Approaching "functional" soil organic matter pools through particle-size fractionation: examples for tropical soils. Pages 53-67 In R. Lal, J. M. Kimble, R. F. Follett, and B. A. Stewart, ed. Assessment Methods for Soil Carbon. Lewis Publ., Boca Raton, FL.

Golchin, A., Oades, J.M., Skjemstad, J.O., Clarke, P., 1994. Soil structure and carbon cycling. Australian Journal of Soil Research 32, 1043-1068.

Gregorich, E.G., Kachanoski, R.G., Voroney, R.P., 1989. Carbon mineralization in soil size fractions after various amounts of aggregate disruption. Journal of Soil Science, 40, 649-659.

Hassink, J., 1995. Decomposition rate constants of size and density fractions of soil organic matter. Soil Science Society of America Journal 59, 1631-1635.

Hassink, J., Whitmore, A.P., 1997. A model of the physical protection of organic matter in soils. Soil Science Society of America Journal 61, 131-139.

Houghton, R.A., 1995. Land use change and the carbon cycle. Global Change Biology 1, 275-87.

Lal, R., Kimble, J.M., 2000. Pedogenic carbonates and the global carbon cycle. In: Lal, R.,(Eds.), Global Climate Change and Pedogenic Carbonates, CRC/Lewis Publishers, Boca Raton, FL., pp.317-319.

Parfitt, R.L., Salt, G.J. 2001a. Carbon and nitrogen mineralization in sand, silt, and clay fractions of soils under maize and pasture. Australian Journal of Soil Research 39, 361-371.

Parfitt, R.L., Salt, G.J., Saggar, S., 2001b. Effect of leaching and clay content on carbon and nitrogen mineralization in maize and pasture soils. Australian Journal of Soil Research 39, 535-542.

Parfitt, R.L., Parshotam, A., Salt, G.J., 2002. Carbon turnover in two soils with constrasting mineralogy under long-term maize and pasture. Australian Journal of Soil Research 40, 127-136.

Parton, W.J., Schimel, D.S., Cole, C.V., Ojima, D.S., 1987. Analysis of factors controlling soil organic matter levels in Great Plains grasslands. Soil Science Society of America Journal 51, 1173-1179.

Paul, E.A., Horwath, W.R., Harris, D., Follett, R., Leavitt, S.W., Kimball, B.A. Pregitzer, K., 1995. Establishing the pool sizes and fluxes in CO_2 emissions from soil organic matter turnover. In: Lal, R., Kimble, T., Levine, E. Stewart, B. A., (Eds.), Advances in Soil Science of Soils and Global Change, CRC Press, Boca Raton, FL., pp. 297-305.

Paul E. A., Clark F.E., 1989. Soil microbiology and biochemistry, Academic Press, San Diego.

98

Plante, A.F., McGill, W.B., 2002. Soil aggregate dynamics and the retention of organic matter in laboratory-incubated soil with differing simulated tillage frequencies. Soil & Tillage Research 66, 79-92.

Pulleman M. M., Marinissen, J.C.Y., 2004. Carbon mineralization as affected by natural aggregation in pasture versus arable soil. Geoderma 120, 273-282.

Six, J., Conant, R.T., Paul, E.A., Paustian, K., 2002. Stabilization mechanisms of soil organic matter: Implications for C-saturation of soils. Plant and Soil 241, 155-176.

Skjemstad, J.O., Clarke, P., Taylor, J.A., Oades, J.M., McClure, S.G., 1996. The chemistry and nature of protected carbon in soil. Australian Journal of Soil Research 34, 251-271.

Tables and Figures

Table 4.1. Cumulative CO_2 efflux during the 70- day incubation for the soil fractions averaged across treatments (n =36) and the whole soils (n= 18). Numbers in parentheses represent one standard deviation from the mean, and different letters indicate differences between fractions at α = 0.05 using a Duncan's New Multiple Range test (Soil sampled in September, 2003).

Fraction	Cumulative CO_2 efflux (mgCO_2-C/ g C in fraction)	Cumulative CO_2 efflux (μgCO_2-C/ g soil)
Sand	399 (151)**a**	539 (171)**b**
Coarse silt	227 (57)**b**	262 (65)**c**
Medium + fine silt	114 (40)**c**	253 (58)**cd**
Clay	96 (21)**c**	538 (131)**b**
Floatables	100 (33)**c**	196 (61)**d**
Whole soil	40 (13)**c**	684 (163)**a**

100

Table 4.2. Cumulative CO_2 efflux during the 70- day incubation for the control (Ck) and manure (M) treatments. Numbers in parentheses represent one standard deviation from the mean, and different letters indicate differences between treatments (M vs Ck) at $\alpha = 0.05$ using a paired t-test (n = 3) (Soil sampled in September, 2003).

Fraction	Cumulative CO_2 efflux ($mgCO_2$-C/ gC in fraction)		Cumulative CO_2 efflux (μgCO_2-C/ g soil)	
	Ck	M	Ck	M
Sand	266 (47)b	439 (105)a	514 (91)a	559 (134)a
Coarse silt	235 (18)a	218 (9)a	264 (21)a	260 (11)a
Medium + fine silt	114 (12)a	102 (9)a	236 (25)a	270 (25)a
Clay	99 (3)a	94 (6)b	492 (16)b	585 (34)a
Floatables	117 (20)a	85 (8)a	222 (38)a	174 (17)b
Whole soil	47 (9)a	33 (6)a	742 (201)a	633 (119)a

Table 4.3. Mineralization rate constants (k in $\mu g CO_2\text{-}C \cdot gC^{-1}$ in fraction· day^{-1}) for the entire incubation period (70 days) and correlation coefficients (R^2) using a simple linear regression (df =3) (Soil sampled in September, 2003).

Fraction	Control (Ck)		Manure	
	k	R^2	k	R^2
Sand	3.809	0.963*	6.593	0.965*
Coarse silt	3.435	0.973*	3.043	0.965*
Medium + fine silt	1.485	0.982*	1.311	0.992*
Clay	1.187	0.995*	1.159	0.995*
Floatables	1.520	0.989*	1.059	0.992*
Whole soil	0.622	0.998*	0.475	0.991*

Figure 4.1. Cumulative CO_2 evolution, expressed as mg of CO_2-C per g of initial C during the 70-day incubation for the fractions and the whole soils (Soil sampled in September, 2003).

103
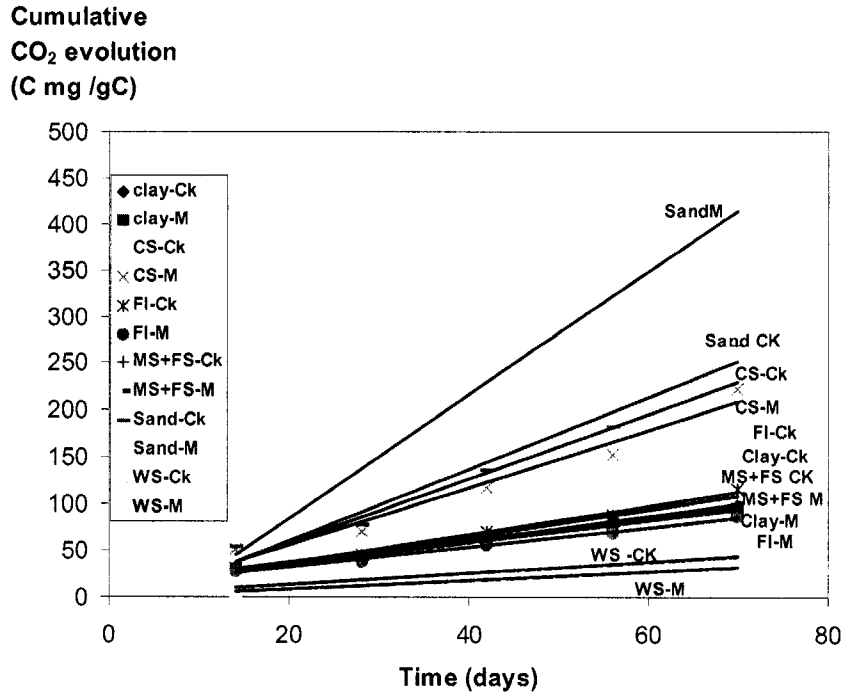
Figure 4.2. Cumulative CO₂ evolution, expressed as µg of CO₂-C per g of soil during the 70-day incubation for the fractions and the whole soils (Soil sampled in September, 2003).
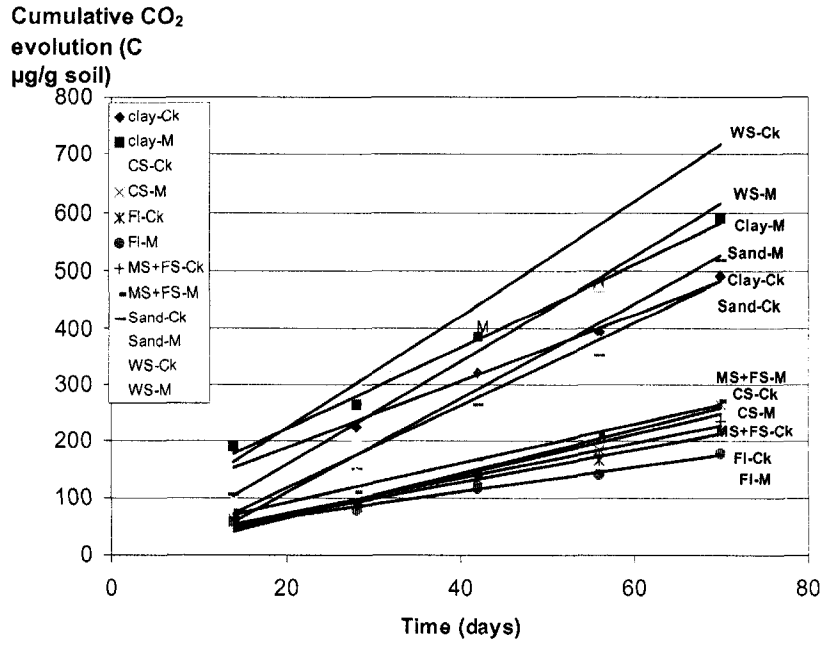
Chapter 5

Synthesis

The soil organic carbon (SOC) pool encompasses a variety of constituents from particulate debris such as plant, animal, and microbial residues to highly amorphous organic matter substances including all stages of decay, which may be intimately associated with inorganic soil components to form organo-mineral complexes. The turnover of these different SOC components varies due to the complex interaction of biological, chemical, and physical processes in soil (Christensen, 1992). Several multiple-pool models describing SOC turnover have been proposed (Jenkinson and Rayner, 1977; Christensen, 1992; Paul et al., 1995; Cambardella and Elliott, 1992). From an experimental point of view, soil organic matter is often partitioned into different compartments or fractions meant to mimic the different pools identified in these models.

Long-term field studies can provide the foundation to better understand SOC dynamics and the underlying processes controlling SOC loss over decadal time scales. The Breton Classical Plots long-term fertilization trials were established in 1929 by the Department of Soils, University of Alberta (Robertson, 1991). Izaurralde et al. (2001) fitted a simple three-compartment model to these measured long-term changes in total SOC. Results from the modeling exercise suggested a more rapid SOC turnover for the 2-yr than for the 5-yr rotation plots, a loss of carbon from the active SOC compartment with a decomposition rate of 0.0037 yr^{-1}, but a gain by the passive SOC compartment with a decomposition rate of 0.0005 yr^{-1}. Finally, the Breton Plots receiving manure did not appear to have reached steady state by 1990, but instead were continuing to sequester carbon (Izaurralde et al., 2001).

Measurement of CO_2 evolution or O_2 uptake in the field or in the laboratory is the well-accepted approach to study carbon mineralization. Results from field experiments are expected to be more reflective of the natural conditions under which soil organic substrates are mineralized while laboratory incubations have been used for comparing C and N mineralization potential from different soils and different crops (Christensen, 1987; Hassink, 1995; Parfitt et al., 2001 and 2002; Plante and McGill, 2002). The

105

laboratory-based incubation method isolates CO_2 efflux due to SOC mineralization from the potential interference of root respiration fluxes.

Soil microbial biomass is an important component of the soil organic matter that regulates the transformation and storage of nutrients. It also can be regarded as the living part of the soil organic matter. Since microorganisms are more responsive to changes in the soil environment than the soil organic matter as a whole, measures of the size of the soil microbial biomass have been used for assessing changes in soil organic matter and nutrient cycling caused by changes in soil management and distinct agricultural practices. In particular, agricultural management practices such as the application of manure and chemical fertilizers have been shown to impact the size and activity of soil microbial communities (Bolton et al., 1985; Powlson et al., 1987; Fraser et al., 1988; Kirchner et al., 1993; Bossio et al., 1998).

Phospholipids are found in all living cell membranes but not in storage structures and are rapidly turned over on cell death; therefore they may be used for characterizing the living biomass in soils (Tunlid and White, 1992, Paul and Clark, 1996). Phospholipid fatty acid (PLFA) analysis is a biochemical method that can provide information both about the size and the structure of the active soil microbial community (Vestal and White, 1989), as well as detect changes due to environmental changes or differences in agricultural management practices.

Main findings

Differences in organic matter distribution were found when the soil was fractionated into floatables, sand, coarse, medium, fine silt, and clay. The floatable pool had the highest C concentration, the clay and fine silt fractions were next, and the sand and coarse silt had the lowest concentration. The clay fraction contained the greatest amount of total carbon (37%), followed by the sand and floatable (> 53 μm) fraction (22%), the medium silt (17%), and the fine silt (15%). The coarse silt contained the least amount (8%). Compared to 1972, the long-term manure application had increased total soil carbon content and, as well, increased carbon concentration in the < 53 μm fraction. The increase was greater in the 5-yr than in the 2-yr rotation plots.

Carbon and N concentrations were significantly correlated in all soil fractions as well as in the unfractionated soils. The C/N ratio decreased in the

106

following order: floatable > medium silt > whole soil > clay and fine silt > sand and coarse silt. The whole soil C/N ratio in the 2-yr rotation was significantly lower than that of the 5-yr rotation, suggesting that carbon decomposition rate was faster in the 2-yr rotation than the 5-yr rotation.

A 70-day laboratory incubation showed that the sand and coarse silt fractions had the highest mineralization rate of all fractions when results were expressed on a carbon basis. These results are consistent with other reports in the literature. For instance, Gregorich et al. (1989) reported that the proportion of C mineralized during a 20-day laboratory incubation period decreased in the order (mg C/ g C): sand> whole soil> silt> clay. The large amount of CO_2 evolved from the sand and coarse silt is indicative of the rapid decomposability of the organic matter associated with these fractions, which has been reported to mostly contain fragments of plant residues (Parfitt and Salt, 2001; Hassink, 1995).

The clay had the lowest mineralization rate on a carbon basis. Hassink and Whitmore (1997), and Christensen (1987) also reported lower mineralization rates for clay and silt fractions (< 20 µm), although silt-associated carbon is often found to be more resistant to microbial attack than that of clay (Gregorich et al., 1989; Parfitt and Salt, 2001). Christensen (1996) proposed that following the addition of fresh substrates, newly formed organic matter can be found in all fractions, although the clay sized organo-mineral complexes often show greater accumulations. In the longer term, the proportion of substrate-derived carbon in clay declines more rapidly than that in silt, thereby indicating a higher stability of silt-associated carbon (Christensen, 1996). Parfitt and Salt (2001) further speculated that fresh substrates absorbed on clay external surfaces are readily accessible to microbial attack, while substrates may be trapped in small pores and become physically protected from microbial attack when they associate with the silt fractions. A lower mineralization rate from the silt than from the clay was not observed in our study. This may be related to the sonication procedure employed in our study that would have more efficiently disrupted micro-aggregates in the silt fractions. Alternatively, this may be linked to the inherent physical properties of the sampled Luvisolic soils, which have a very friable to friable consistence in their upper mineral horizons, and may contain relatively low amounts of silt-sized microaggregates

107

The cumulative sum of CO_2 evolved from the five isolated soil physical fractions was 2.6 times higher than that from the whole soil at the end of the 70-day incubation. The CO_2 evolution measured during laboratory incubation of soil fractions reflects the chemical stability of the organic matter, while the difference between the sum of CO_2 from the different size fractions and CO_2 from the whole soil may correspond to mineralization of the physically stabilized organic matter that is released during the fractionation procedure (Christensen, 1987). In our study, the whole soil was given lower disruptive energies than the individual fractions to minimize disruption of soil aggregation, hence the greater soil C mineralization observed from the soil fractions was likely due to a greater disruption of the soil structure in these fractions. A high proportion of organic matter associated with microaggregates consists of highly processed, humified "recalcitrant" moieties, while organic matter associated with macroaggregates is more readily mineralized (Christensen, 1987). Thus, it is probable that the increase in CO_2 evolution observed in our study was due to C released from macroaggregates rather than from microaggregates. In all cases, our results clearly demonstrate the significance of physical stabilization as a mechanism of C sequestration at the Breton Plots, since C mineralization was observed to more than double following disruption of the soil aggregates during the physical fractionation process.

On a soil weight basis, the manure treatment significantly increased the CO_2 evolution from the clay fraction when compared with the control (Ck) treatment. Furthermore, for the Ck samples, the whole soil C mineralization rate was only correlated to the C mineralization from the sand, whereas for the manure treatment, the whole soil mineralization was significantly correlated to both the sand and clay fractions. These results may be due to the fact that the soils under manure contained proportionally more carbon in the clay-size fraction as compared to the control soils, which in turn would contribute to a greater portion of the total soil respiration originating from this clay fraction. Unfortunately, our experimental design did not allow for statistical testing of this potential explanation.

On a carbon basis, the manure treatment significantly increased the sand-associated carbon mineralization and significantly decreased the clay-associated carbon mineralization. The manure treatment had no significant effects on CO_2 evolution from the other soil fractions. Hence

108

results from the laboratory incubation experiment further indicate that the increase in total soil carbon due to long term manure application may be associated with differences in carbon decomposability.

Microbial biomass carbon as measured in this study was highest in September, intermediate in August, and lowest in May. Previous studies at the Breton Plots also reported seasonal variations in microbial biomass (McGill et al., 1986). Microbial biomass carbon and nitrogen showed very good correlation with soil water content (SWC) in May, as opposed to what was observed in August and September. This is likely due to differences in SWC at these different sampling dates. In May of 2004, the average SWC was 20% (ranging from 14.7 to 27.3 %), while in August and September 2003 it was much lower with an average of 7% (and a range of 5.3 to 10.7 %). So the SWC in May was close to the 60% field capacity water content, which is considered optimal for both plants and microbes. The SWC in August and September was much lower and closer to the permanent wilting point, and the crops and microbes were likely under stress conditions at this time. Hence in August and September soil conditions were likely outside the SWC dynamic range where microbial biomass would be expected to respond to small differences in SWC.

Both the manure and NPKS fertilizer application significantly increased the soil microbial biomass carbon (MBC) and nitrogen (MBN). Previous studies at the Breton plots also reported a significantly higher microbial biomass in the manured plots than in the Ck plots, although at that time there was no statistical difference between samples of the manure and mineral-fertilizer plots, or between samples of the fertilized plots and the Ck plots (McGill et al., 1986). While manure application can increase soluble carbon concentrations in soils, which in turn may increase MBC on a short-term basis, over a longer period, manure can also provide a more regular yet readily available stream of substrates to the microbial community because manure addition to soils can lead to fairly large amounts of fresh organic matter (Gregorich et al., 1998; Liang et al., 1998). Long term NPKS application may significantly increase MBC and MBN as well because chemical fertilization increases plant growth, which in turn increases soil carbon content through an increase in the amount of crop residues as well as through greater root biomass and exudates.

A recent concern of researchers is the effect of agricultural

109

management practices on the diversity of soil microbial communities. Measurement of soil PLFA profiles reflects the composition of *in situ* microbial communities and as such PLFAs have been used by some researchers to provide a useful fingerprint of microbial diversity in soils (e.g.; Wander et al., 1995; Bossio et al., 1998). PLFA profiles have indicated differences in community composition that are consistent with differences in organic carbon inputs. The present research clearly showed that chemical fertilization and manure application increased the recovery (as expressed per gram of soil) of several individual PLFAs, as well as of some specific groups of PLFAs. This was confirmed by results from the multivariate RDA, which indicated that fertilization basically increased the biomass of all individual PLFAs in a similar fashion. One notable exception was the ratio representative of the fungal/bacterial biomass, which was significantly higher in the NPKS plots than in the manure or Ck plots. These results should be related to the MBC/MBN ratios, which also provide a general index of the type of microorganisms present. That is, soil bacteria generally have a C: N ratio of 4:1 to 6:1 while fungi have a higher C: N ratio of 10:1 to 12:1 (Tate 1995). Consequently, a decrease in MBC/MBN ratios as observed with liming is indicative of a decrease in the fungal/bacterial biomass ratio. These results follow the increase in pH caused by liming as fungi are more prevalent than bacteria in soils of pH lower than about 5.5, whereas bacteria tend to predominate in soils with higher pH (Tate 1995).

Future research

To better understand soil carbon decomposition processes and the underlying mechanisms responsible for carbon redistribution between the different carbon pools, it may be useful to characterize the chemical composition of the organic matter associated with the different soil fractions. In this regard, one useful analysis would be solid-state ^{13}C NMR analysis of the different soil fractions.

To better understand the relationships between chemical protection of the organic matter and mineralization rates from the different soil fractions, the carbon chemical properties should be studied before as well as after the laboratory incubation in future studies.

Due to some limitations associated with the physical fractionation

procedure, the results from this study could not reflect completely the physical protection. The relationship between carbon turnover within soil aggregates and physical protection of organic matter yet could be, in the future, studied through a combined incubation/ fractionation method, in which the whole soil incubation should be conducted first under controlled conditions, to be followed by the physical fractionation.

References

Bolton, J., Elliot, L.F., Papendick, P.R., Bezdicek, D.F., 1985. Soil microbial biomass and selected soil enzyme activities: effect of fertilization and cropping practices. Soil Biology & Biochemistry 17, 297-302.

Bossio, D.A., Scow, K.M., Gunapala, N., Graham, K.J., 1998. Determinants of soil microbial communities: Effects of agricultural management, season and soil type on phospholipids fatty acid profiles. Microbial Ecology 36, 1-12.

Camberdella, C. A., Elliott, E. T. 1992. Particulate soil organic matter changes across a grassland cultivation sequence. Soil Sci. Soc. Am. J. 56: 777-783.

Christensen, E. T. 1987. Decomposability of organic matter in particle size fractions from field soils with straw incorporation. Soil Biol. Biochem. 19: 429-435.

Christensen, B. T. 1992. Physical fractionation of soil and organic matter in primary particle size and density separates. Pages 2-90 In B.A. Stewart, ed. Advances in Soil Science. Springer-Verlag New York Inc.

Christensen, E.T., 1996. Matching measurable soil organic matter fractions with conceptual pools in simulation models of carbon turnover: revision of model structure. In NATO ASI Series, Vol. 138, Evaluation of soil organic matter models, David, S., (Eds) Springer-Verlag Berlin Heideberg, pp143-159.

Fraser, D.G., Doran, J.W., Sahs, W.W., Lesoing, G.W., 1988. Soil microbial populations and activities under conventional and organic management. Journal of Environmental Quality 17, 585-590.

Gregorich, E.G., Kachanoski, R.G., Voroney, R.P., 1989. Carbon mineralization in soil size fractions after various amounts of aggregate disruption. Journal of Soil Science, 40, 649-659.

Gregorich, E.G., Rochetter, P., McGuire, S., Liang, B.C., Lessard, R., 1998. Soluble organic carbon and carbon dioxide fluxes in maize fields receiving spring-applied manure. Journal of Environmental Quality 27, 209-214.

Hassink, J., 1995. Decomposition rate constants of size and density fractions of soil organic matter. Soil Science Society of America Journal 59,

1631-1635.

Hassink, J., Whitmore, A.P., 1997. A model of the physical protection of organic matter in soils. Soil Science Society of America Journal 61, 131-139.

Izaurralde, R. C., McGill, W. B., Robertson, J. A., Juma, N. G., Thurston, J. J. 2001. Carbon balance of the Breton Classical Plots over half a centry. Soil Sci. Soc. Am. J. 65: 431-441.

Jenkinson, D.S., Rayner, J.H., 1977. The turnover of soil organic matter in some of the Rothamsted classical experiments. Soil Science 123, 298-305.

Kirchner, M.J., Wollum II, A.F., King, L.D., 1993. Soil microbial populations and activities in reduced chemical input agroecosystems. Soil Science Society of America Journal 57, 1289-1295.

Liang, B.C., Mackenzie, A.F., Schnitzer, M., Monreal, C.M., Voroney, P.R., Beyaert, R.P., 1998. Management-induced change in liable soil organic matter under continuous corn in eastern Canadian soils. Biology and Fertility of Soils 26, 88-94.

McGill, W.B., Canon, K.R., Robertson, J.A., Cook, F.D., 1986. Dynamics of soil microbial biomass and water-soluble organic C in Breton after 50 years of cropping two rotations. Canadian journal of Soil Science 66, 1-19.

Parfitt, R.L., Salt, G.J. 2001. Carbon and nitrogen mineralization in sand, silt, and clay fractions of soils under maize and pasture. Australian Journal of Soil Research 39, 361-371.

Parfitt, R.L., Salt, G.J., Saggar, S., 2001. Effect of leaching and clay content on carbon and nitrogen mineralization in maize and pasture soils. Australian Journal of Soil Research 39, 535-542.

Parfitt, R.L., Parshotam, A., Salt, G.J., 2002. Carbon turnover in two soils with constrasting mineralogy under long-term maize and pasture. Australian Journal of Soil Research 39, 535-542.

Paul, E. A., Horwath, W. R., Harris, D., Follett, R., Leavitt, S. W., Kimball, B. A. and Pregitzer, K. 1995. Establishing the pool sizes and fluxes in CO_2 emissions from soil organic matter turnover. Pages 297-305 In Advances in Soil Science of Soils and Global Change, R. Lal, T. Kimble, E. Levine, and B. A. Stewart, ed. CRC Press, Boca Raton, FL.

Paul, E.A., Clark, F.E., 1996. Soil Microbiology and Biochemistry (second

edition). Acedemic Press, pp47-48.

Plante, A.F., McGill, W.B., 2002. Soil aggregate dynamics and the retention of organic matter in laboratory-incubated soil with differing simulated tillage frequencies. Soil & Tillage Research 66, 79-92.

Powlson, D.S., Brookes, P.C., Christensen, B.T., 1987. Measurement of soil microbial biomass provides a early indication of changes in total soil organic matter due to straw incorporation. Soil Biology & Biochemistry 19, 159-164.

Robertson, J.A., 1991. Long-term results from the Breton Plots, Robertson, J.A. (Eds.) 61st Annual Breton Plots: Field Day and Soils/Crops Clinic, University of Alberta, Edmonton. pp. 14-33.

Tate, R.L., 1995. Soil Microbiology. John Wiley & Sons Inc.

Tunlid, A., White, D.C., 1992. Biochemical analysis of biomass, community structure, nutritional status, and metabolic activity of microbial communities in soil. In: Soil Biochemistry, ed. G. Stotzky, J.M., Bollag, Vol 7. pp. 229-262. marcel Dekker, New York.

Vestal, J.R., White, D.C., 1989. Lipid analysis in microbial ecology; Quantitive approached to the study of microbial communities. BioScience 39, 535-541.

Wander, M.M., Hedrick, D.S., Kaufman, D., Traina, S.J., Stinner, B.R., Kehrmeyer, S.R., White, D.C., 1995. The functional significance of the microbial biomass in organic and conventionally managed soils. Plant and Soil 170, 87-97.

Appendix A TN and TC of soil fractions and whole soil

Year	Series	Plot	Half	fractions	Nitrogen wt (%)	Carbon wt (%)
1938	E	1		C	0.04	0.49
1938	E	3		C	0.05	0.50
1972	C	1		C	0.08	0.71
1972	E	1		C	0.07	0.29
1972	E	2		C	0.07	0.45
1972	E	3		C	0.08	0.40
1983	C	1	E	C	0.23	0.82
1983	C	2	E	C	0.10	0.81
1983	C	3	E	C	0.11	0.98
1990	C	1	E	C	0.08	0.63
1990	E	1	E	C	0.05	0.32
1990	E	2	E	C	0.11	0.50
1990	E	3	E	C	0.06	0.27
1990	C	2	E	C	0.08	0.68
1990	C	3	E	C	0.07	0.60
1998	C	1	E	C	0.10	0.88
1998	C	2	E	C	0.16	1.56
1998	C	3	E	C	0.13	1.24
1998	E	1	E	C	0.06	0.56
1998	E	2	E	C	0.12	1.16
1998	E	3	E	C	0.06	0.60
2003	C	1	E	C	0.10	0.67
2003	C	2	E	C	0.10	0.84
2003	C	3	E	C	0.08	0.62
2003	E	1	E	C	0.07	0.44
2003	E	2	E	C	0.11	0.85
2003	E	3	E	C	0.06	0.31
1983	C	1	W	C	0.07	0.28
1983	C	2	W	C	0.08	0.46
1983	C	3	W	C	0.06	0.30
1985	E	1	W	C	0.05	0.21
1985	E	2	W	C	0.03	0.23
1985	E	3	W	C	0.03	0.25
1990	C	1	W	C	0.04	0.30
1990	C	2	W	C	0.06	0.43
1990	C	3	W	C	0.06	0.41
1990	E	1	W	C	0.05	0.25
1990	E	2	W	C	0.07	0.28
1990	E	3	W	C	0.06	0.29
1998	C	1	W	C	0.03	0.28
1998	C	2	W	C	0.04	0.38
1998	C	3	W	C	0.04	0.32
1998	E	1	W	C	0.02	0.18
1998	E	2	W	C	0.01	0.16
1998	E	3	W	C	0.02	0.25
2003	C	1	W	C	0.08	0.62
2003	C	2	W	C	0.08	0.66

115

2003	C	3	W	C	0.08	0.63
2003	E	1	W	C	0.04	0.13
2003	E	2	W	C	0.11	0.81
2003	E	3	W	C	0.08	0.62
1938	E			C	0.04	0.40
1938	E	1		CL	0.24	2.24
1938	E	3		CL	0.27	2.42
1972	C	1		CL	0.29	2.12
1972	C	2		CL	0.34	3.03
1972	C	3		CL	0.44	2.84
1972	E	1		CL	0.22	1.88
1972	E	2		CL	0.29	2.50
1972	E	3		CL	0.28	2.31
1983	C	1	E	CL	0.25	2.22
1983	C	2	E	CL	0.35	3.02
1983	C	3	E	CL	0.35	2.60
1990	C	1	E	CL	0.25	2.23
1990	C	2	E	CL	0.33	3.29
1990	C	3	E	CL	0.32	2.69
1990	E	1	E	CL	0.23	2.05
1990	E	2	E	CL	0.32	2.94
1990	E	3	E	CL	0.30	2.56
1998	C	1	E	CL	0.29	2.47
1998	C	2	E	CL	0.40	3.49
1998	C	3	E	CL	0.34	3.02
1998	E	1	E	CL	0.23	2.28
1998	E	2	E	CL	0.32	3.12
1998	E	3	E	CL	0.21	1.86
2003	C	1	E	CL	0.31	2.49
2003	C	2	E	CL	0.38	3.23
2003	C	3	E	CL	0.32	2.65
2003	E	1	E	CL	0.22	1.66
2003	E	2	E	CL	0.33	2.81
2003	E	3	E	CL	0.25	1.96
1983	C	1	W	CL	0.28	2.36
1983	C	2	W	CL	0.33	2.89
1983	C	3	W	CL	0.30	2.72
1985	E	1	W	CL	0.18	1.44
1985	E	2	W	CL	0.25	2.22
1985	E	3	W	CL	0.27	2.34
1990	C	1	W	CL	0.31	2.53
1990	C	2	W	CL	0.32	2.87
1990	C	3	W	CL	0.34	3.04
1990	E	1	W	CL	0.22	1.74
1990	E	2	W	CL	0.26	2.30
1990	E	3	W	CL	0.23	2.21
1998	C	1	W	CL	0.30	2.64
1998	C	2	W	CL	0.40	3.53
1998	C	3	W	CL	0.37	3.14
1998	E	1	W	CL	0.18	1.59
1998	E	2	W	CL	0.29	2.65

1998	E	3	W	CL	0.24	2.14
2003	C	1	W	CL	0.34	2.61
2003	C	2	W	CL	0.38	3.27
2003	C	3	W	CL	0.33	2.72
2003	E	1	W	CL	0.23	1.66
2003	E	2	W	CL	0.29	2.24
2003	E	3	W	CL	0.31	2.26
2003	C	1	E	F	1.43	20.29
2003	C	2	E	F	1.96	28.50
2003	C	3	E	F	1.62	24.18
2003	E	1	E	F	0.94	16.26
2003	E	2	E	F	1.37	19.72
2003	E	3	E	F	1.46	19.39
2003	C	1	W	F	1.56	23.64
2003	C	2	W	F	1.77	25.62
2003	C	3	W	F	1.58	26.95
2003	E	1	W	F	0.82	12.43
2003	E	2	W	F	1.49	20.73
2003	E	3	W	F	1.09	17.72
1938	E	1		FL	1.26	37.43
1938	E	3		FL	1.02	38.25
1938	E			FL	0.99	33.93
1936	C	1		FS	0.20	1.53
1938	E	1		FS	0.25	2.06
1938	E	3		FS	0.34	2.96
1972	C	1		FS	0.36	2.29
1972	C	2		FS	0.30	2.64
1972	C	3		FS	0.29	2.59
1972	E	1		FS	0.22	2.11
1972	E	2		FS	0.21	1.80
1972	E	3		FS	0.27	2.57
1983	C	1	E	FS	0.20	1.58
1983	C	2	E	FS	0.24	2.04
1983	C	3	E	FS	0.26	2.23
1990	C	1	E	FS	0.21	1.83
1990	C	2	E	FS	0.23	2.11
1990	C	3	E	FS	0.22	1.96
1990	E	1	E	FS	0.22	2.02
1990	E	2	E	FS	0.30	2.88
1990	E	3	E	FS	0.23	2.20
1998	C	1	E	FS	0.18	1.57
1998	C	2	E	FS	0.26	2.29
1998	C	3	E	FS	0.23	2.05
1998	E	1	E	FS	0.16	1.43
1998	E	2	E	FS	0.22	1.99
1998	E	3	E	FS	0.21	2.29
2003	C	1	E	FS	0.26	2.12
2003	C	2	E	FS	0.35	3.32
2003	C	3	E	FS	0.24	2.31
2003	E	1	E	FS	0.23	1.58
2003	E	2	E	FS	0.30	2.85

117

2003	E	3	E	FS	0.23	1.94
1983	C	1	W	FS	0.24	1.97
1983	C	2	W	FS	0.29	2.46
1983	C	3	W	FS	0.24	2.21
1985	E	1	W	FS	0.17	1.34
1985	E	2	W	FS	0.20	1.68
1985	E	3	W	FS	0.22	1.84
1990	C	1	W	FS	0.25	2.17
1990	C	2	W	FS	0.28	2.57
1990	C	3	W	FS	0.31	2.73
1990	E	1	W	FS	0.20	1.69
1990	E	2	W	FS	0.27	2.37
1990	E	3	W	FS	0.21	2.10
1998	C	1	W	FS	0.24	2.20
1998	C	2	W	FS	0.34	3.01
1998	C	3	W	FS	0.30	2.74
1998	E	1	W	FS	0.18	1.60
1998	E	2	W	FS	0.29	2.77
1998	E	3	W	FS	0.23	1.94
2003	C	1	W	FS	0.23	2.02
2003	C	2	W	FS	0.30	2.82
2003	C	3	W	FS	0.27	2.34
2003	E	1	W	FS	0.20	1.62
2003	E	2	W	FS	0.26	2.27
2003	E	3	W	FS	0.24	1.96
1938	E			FS	0.23	2.10
1990	C	1	E	FSAND	0.09	1.17
1990	C	2	E	FSAND	0.17	2.07
1990	C	3	E	FSAND	0.12	1.66
1990	E	1	W	FSAND	0.02	0.34
1990	E	2	W	FSAND	0.08	0.71
1990	E	3	W	FSAND	0.05	0.40
1938	E	1		M	0.09	1.04
1938	E	3		M	0.20	2.59
1972	C	1		M	0.11	1.20
1972	C	2		M	0.16	1.75
1972	C	3		M	0.14	1.51
1972	E	1		M	0.13	1.37
1972	E	2		M	0.16	1.85
1972	E	3		M	0.16	1.69
1983	C	1	E	M	0.15	1.35
1983	C	2	E	M	0.21	2.06
1983	C	3	E	M	0.20	1.92
1990	C	1	E	M	0.16	1.50
1990	C	2	E	M	0.20	1.77
1990	C	3	E	M	0.18	1.63
1990	E	1	E	M	0.12	1.38
1990	E	2	E	M	0.17	1.94
1990	E	3	E	M	0.11	1.30
1998	C	1	E	M	0.17	1.62
1998	C	2	E	M	0.26	2.59

118

1998	C	3	E	M	0.20	1.98
1998	E	1	E	M	0.11	1.08
1998	E	2	E	M	0.18	1.84
1998	E	3	E	M	0.13	1.25
2003	C	1	E	M	0.16	1.66
2003	C	2	E	M	0.22	2.40
2003	C	3	E	M	0.16	1.64
2003	E	1	E	M	0.13	1.23
2003	E	2	E	M	0.10	0.95
2003	E	3	E	M	0.08	0.55
1983	C	1	W	M	0.13	1.36
1983	C	2	W	M	0.18	1.94
1983	C	3	W	M	0.21	1.55
1985	E	1	W	M	0.09	1.04
1985	E	2	W	M	0.12	1.10
1985	E	3	W	M	0.14	1.10
1990	C	1	W	M	0.13	1.52
1990	C	2	W	M	0.19	2.05
1990	C	3	W	M	0.15	1.71
1990	E	1	W	M	0.11	1.24
1990	E	2	W	M	0.16	1.86
1990	E	3	W	M	0.11	1.32
1998	C	1	W	M	0.12	1.49
1998	C	2	W	M	0.21	2.36
1998	C	3	W	M	0.06	0.75
1998	E	1	W	M	0.08	1.02
1998	E	2	W	M	0.17	2.00
1998	E	3	W	M	0.11	1.25
2003	C	1	W	M	0.16	1.69
2003	C	2	W	M	0.19	1.95
2003	C	3	W	M	0.18	1.67
2003	E	1	W	M	0.08	0.50
2003	E	2	W	M	0.20	2.00
2003	E	3	W	M	0.16	1.55
1998	C	1	E	S	0.05	0.77
1998	C	2	E	S	0.11	1.47
1998	C	3	E	S	0.09	1.35
1998	E	1	E	S	0.03	0.48
1998	E	2	E	S	0.11	1.57
1998	E	3	E	S	0.02	0.32
2003	C	1	E	S	0.05	0.40
2003	C	2	E	S	0.05	0.30
2003	C	3	E	S	0.05	0.40
2003	E	1	E	S	0.02	0.19
2003	E	2	E	S	0.08	0.72
2003	E	3	E	S	0.02	0.56
1998	C	1	W	S	0.04	0.69
1998	C	2	W	S	0.11	1.62
1998	C	3	W	S	0.09	1.43
1998	E	1	W	S	0.02	0.33
1998	E	2	W	S	0.08	1.15

119

1998	E	3	W	S	0.03	0.44
2003	C	1	W	S	0.05	0.47
2003	C	2	W	S	0.05	0.31
2003	C	3	W	S	0.05	0.28
2003	E	1	W	S	0.14	1.34
2003	E	2	W	S	0.04	0.18
2003	E	3	W	S	0.04	0.25
1983	C	1	E	S	0.09	1.08
1983	C	2	E	S	0.13	1.54
1983	C	3	E	S	0.12	1.67
1972	E	1		WS	0.13	1.14
1972	E	2		WS	0.15	1.42
1972	E	3		WS	0.14	1.27
1983	C	1	E	WS	0.11	0.88
1983	C	2	E	WS	0.13	1.20
1983	C	3	E	WS	0.12	1.14
1990	E	1	E	WS	0.10	0.92
1990	E	2	E	WS	0.16	1.65
1990	E	3	E	WS	0.11	1.08
1972	C	1		WS	0.13	1.44
1972	C	2		WS	0.17	1.86
1972	C	3		WS	0.15	1.63
1983	C	1	W	WS	0.11	0.97
1983	C	2	W	WS	0.13	1.21
1983	C	3	W	WS	0.11	1.13
1985	E	1	W	WS	0.08	0.62
1985	E	2	W	WS	0.09	0.77
1985	E	3	W	WS	0.10	0.74
1990	C	1	W	WS	0.13	1.34
1990	C	2	W	WS	0.18	2.04
1990	C	3	W	WS	0.18	1.84
1936	CK			WS	0.13	1.61
1936	C3			WS	0.13	1.39
1938	E3			WS	0.14	1.42
1938	E3			WS	0.14	1.70
1938	E3			WS	0.15	1.48
1998	C	1	E	WS	0.13	1.20
1998	C	2	E	WS	0.19	1.98
1998	C	3	E	WS	0.17	1.76
1998	E	1	E	WS	0.11	1.06
1998	E	2	E	WS	0.18	1.90
1998	E	3	E	WS	0.11	0.97
2003	C	1	E	WS	0.16	1.36
2003	C	2	E	WS	0.21	2.14
2003	C	3	E	WS	0.17	1.65
2003	E	1	E	WS	0.12	0.93
2003	E	2	E	WS	0.19	1.88
2003	E	3	E	WS	0.13	1.00
2003	C	1	W	WS	0.16	1.50
2003	C	2	W	WS	0.21	1.93
2003	C	3	W	WS	0.18	1.66

120

2003	E	1	W	WS	0.12	0.89
2003	E	2	W	WS	0.17	1.56
2003	E	3	W	WS	0.13	1.08

Note: 1 Plot: 1=Ck, 2=Manure; 3=NPKS; 2 half: E = limed; W = unlimed; 3. C: coarse silt; CL: clay; M: medium silt; F: floatable; FS: fine silt; WS: whole soil; Fsand: floatable and sand mixture.

Appendix B-1 Alkaline Persulfate Oxidation for Determining Total Nitrogen in Microbial Biomass Extracts

INTRODUCTION

Koroleff (1983) proposed an alkaline persulfate oxidation method for determining total N in natural water as an alternative to the acid Kjeldahl digestion. This digest uses persulfate ($K_2S_2O_8$) to oxidize N to NO_3^- at elevated temperature in an alkaline environment:

$$K_2S_2O_8 + H_2O \rightarrow 2\ KHSO_4 + 1/2O_2$$
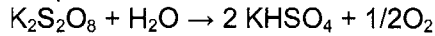
Gallardo and Schlesinger (1990) used the procedure described by D'Elia (1977) to measure total N in 0.5 M K_2SO_4 soil extracts. Ross (1992) adopted a nonalkaline persulfate oxidation procedure to obtain complete recovery of inorganic N in 0.5 K_2SO_4 extracts of soil with high initial NH_4^+ concentrations, but acknowledged the potential for incomplete oxidation of organic N forms. Cabrera (1992) reported that complete N recovery can be obtained with reagent / sample ratio of 1 and an autoclave time of 30 minutes in extracts containing as much as 250 mg glucose-C L-1 at a C/N ratio of 10:1.

The objective of this study is to evaluate and adapt the persulfate method to measure DON in 0.25M or 0.5M K_2SO_4 soil extracts for determination of soil microbial biomass N.

MATERIALS AND METHODS

1. Equipment:
   - Autoclave;
   - 2 500ml volumetric flasks;
   - 6 100ml volumetric flasks;
   - 6 150ml beakers;
   - 200 50 ml glass screw cap culture tubes with Teflon-lined caps;
   - 2 glass pipette 5ml each and 10ml each;
   - All the glass wares need to be acid cleaned: soaking in 10%HCl (v/v) more than 2 hours.

2. Reagents
   - Oxidizing reagent: First dissolve 15g NaOH (Fisher, Low N NaOH) in 500-600ml diH₂O approximately, then add 52.0 g persulfate, boric acid 31.2 g and volume to 1L. This is the oxidization reagent (0.375M NaOH).
   - Acetanilide Standards (N1):
     - Acetanilide Standards (accuracy): 0.4828g acetanilide dissolved in diH₂O volume to 500ml. This is 100ppm N1 standard.
     - 5ppm standard: take 5ml 100ppm into a 100ml volumetric flask and add 0.25M K_2SO_4 to 100ml.
     - 10ppm standard: take 10ml 100ppm into a 100ml volumetric flask and add 0.25M K_2SO_4 to 100ml.
     - 15ppm standard: take 15ml 100ppm into a 100ml volumetric flask and add 0.25M K_2SO_4 to 100ml.
   - Urea Standards (N2): 0.108g urea dissolved in diH2O volume to 500ml. It is 100ppm N2 standard. Then make 5ppm, 10ppm, 15ppm standards using 0.25M K2SO4 as above.
   - Sample J and R :

122

> J: 42.04 g soil (from Breton C3-E1-2) into 150ml 0.25M K_2SO_4, shaking 1.0hr. @ high speed.
> R: 5.0 g soil from forest into 50ml 0.5M K_2SO_4 shaking 1 hour at high speed.

3. Optimization studies

Take 5ml standards or samples into the acid cleaned, labeled tube and add oxidization reagent 2.5ml or 5.0 ml or 10 ml, tight the lid at once. Put the tubes into the steel sink. Using the autoclave bag wrap the sink completely. Put the sink wrapped with autoclave bag into the autoclave oven and autoclave 45minutes (@121℃). (drying time 15 minutes) After autoclave, open the oven door a little bit and wait for 10 minutes then, take the sink out.

Reagent volume	N1 standard 5ml			N2 standard 5ml		
	5ppm	10ppm	15ppm	5ppm	10ppm	15ppm
2.5ml						
5.0ml						
10.0ml						

All treatments were replicated 4 times.

RESULTS AND CONCLUSIONS

1. The Variable analysis results show: the two standards are significant different. Acetanilide has significant higher nitrogen recovery comparing to the Urea.

Fig. 1 T-test results for nitrogen recovery

Standards	Numbers of data	Mean N recovery (%)	Std. Dev.	t-Grouping
Acetanilide	36	92.28	4.05	A
Urea	36	89.84	3.63	B

2. The concentration of the standards is different, but not significantly.

Fig. 2 T-test results for nitrogen recovery

Standards concentration	Numbers of data	Mean N recovery (%)	Std. Dev.	t-Grouping
5ppm	24	91.675	2.69	A
10ppm	24	91.3488	4.34	AB
15ppm	24	90.1500	4.72	B

Lower nitrogen concentrations (5ppm, 10ppm) have higher recovery than 15ppm.

3. The volumes of the reagent are significant different.

The nitrogen recoveries are significant higher when adding 5ml or 10ml oxidization reagent than that of adding 2.5ml. The nitrogen can't be oxidized completely when adding 2.5ppm oxidization reagent.

Fig. 3 T-test results for nitrogen recovery

Oxidization reagent volume	Numbers of data	Mean N recovery (%)	Std. Dev.	t-Grouping

123

10ml	24	93.34	2.53	A
5ml	24	92.82	2.47	A
2.5ml	24	87.01	3.37	B

4. Acetanilide nitrogen standard:

The treatments are significant different. The combinations 10ppm with 10ml oxidization reagent and 15ppm nitrogen acetanilide with 10ml oxidization reagent have significant higher recoveries than the other combinations. For 5ppmN acetanilide, there are some difference between adding 3 different volumes reagent, but not significant. The treatments of 5ml and 10 ml reagent have higher N recoveries. For 10ppm N acetanilide, there is significant difference between 10ml reagent treatment and 5ml or 2.5ml reagent treatments, the later two are significant lower than the former. For 15ppm N acetanilide, there is significant difference between 2.5ml reagent treatment and 5ml or 10ml reagent treatments, the later two are significant higher than the former.

Fig. 4 Comparation of acetanilide combination

Oxidization reagent volume	Nitrogen concentration	LSMean of N recovery	t-grouping
2.5	5	90.00	C
2.5	10	87.525	CD
2.5	15	85.600	D
5	5	95.100	AB
5	10	91.950	BC
5	15	94.258	AB
10	5	93.150	ABC
10	10	96.700	A
10	15	96.200	A

5. Urea nitrogen standards:

For 5ppmN urea, there are some difference between adding 3 different volumes reagent, but not significant. The treatments of 5ml and 10 ml reagent have higher N recoveries. For 10ppm N urea, there is significant difference between 2.5ml reagent treatment and 5ml or 10ml reagent treatments, the later two are significant higher than the former. For 15ppm N urea, there is significant difference between 2.5ml reagent treatment and 5ml or 10ml reagent treatments, the later two are significant higher than the former.

Fig. 5 Comparation of urea combination

Oxidization reagent volume	Nitrogen concentration	LSMean of N recovery	t-grouping
2.5	5	88.350	BC
2.5	10	86.775	CD
2.5	15	83.825	D
5	5	93.000	A
5	10	93.550	A
5	15	89.068	BC
10	5	90.450	AB

124

| 10 | 10 | 91.593 | AB |
| 10 | 15 | 91.950 | AB |

6. Soil K_2SO_4 extrats

The 5ml and 10ml oxidization reagent have the better N recovery than 2.5 ml.
The 5ml and 10ml oxidization reagent have no significant difference.

Fig. 6. 0.25 M K_2SO_4 soil extracts NO_3^-

		Reagent volume(ml)	NO_3^-concentration in solution(ppm)	Average NO_3^- concentration in solution (ppm)	Calculated NO_3^-in extracts (ppm)
J	5	2.5			
J	5	2.5	4.06		
J	5	2.5	4.03		
J	5	2.5	4.01	4.058	6.086
J	5	5	3.22		
J	5	5	3.14		
J	5	5	3.12		
J	5	5	3.15	3.158	6.315
J	5	10	2.14		
J	5	10	2.07		
J	5	10	2.02		
J	5	10	2.16	2.098	6.293
R	5	2.5	2.58		
R	5	2.5	2.63		
R	5	2.5	2.64		
R	5	2.5	2.62	2.618	3.926
R	5	5	2.14		
R	5	5	2.12		
R	5	5	2.07		
R	5	5	2.05	2.095	4.19
R	5	10	1.41		
R	5	10	1.38		
R	5	10	1.43		
R	5	10	1.3	1.38	4.14

7. conclusion:

The persulfate method can be used to measure DON in 0.25M or 0.5M K_2SO_4 soil extracts (NO_3-N lower than 15ppm) for determination of soil microbial biomass N. For the samples which have lower than 5ppm NO_3-N, adding 5 ml oxidation reagent is enough; for the samples which have higher

125

than 10 ppm NO_3-N, adding 10 ml oxidization reagent is better.

REFERENCES

1. Koroleff, F. 1983, Determination of nutrients, In K., Grasshoff et al. (eds.) Methods of seawater analysis. Verlag Chemine, Weinheim, Germany, 168-169.

2. Gallardo, A., and Schlesinger, W., H., 1990, Estimating microbial biomass nitrogen using the fumigation-incubation and fumigation-extraction methods in a warm temperature forest soil. Soil Biol. Biochem., 22: 927-932.

3. D'Elia, C., F., et al., 1977, Determination of total nitrogen in aqueous samples using persulfate digestion. Limnol. Oceanogr., 22: 760-764.

4. Cabrera, M. L., et al., 1992, Alkaline persulfate oxidation for determining total nitrogen in microbial biomass extracts, Soil Sci., Soc. Am., J., 57: 1007-1012.

5. Ross, D., J., 1992, Influence of sieve mesh size on estimates of microbial carbon and nitrogen by fumigation extraction procedures in soils under pasture., Soil, Biol., Biochem., 20: 281-286.

Appendix B-2 Cluster analysis on all PLFAs
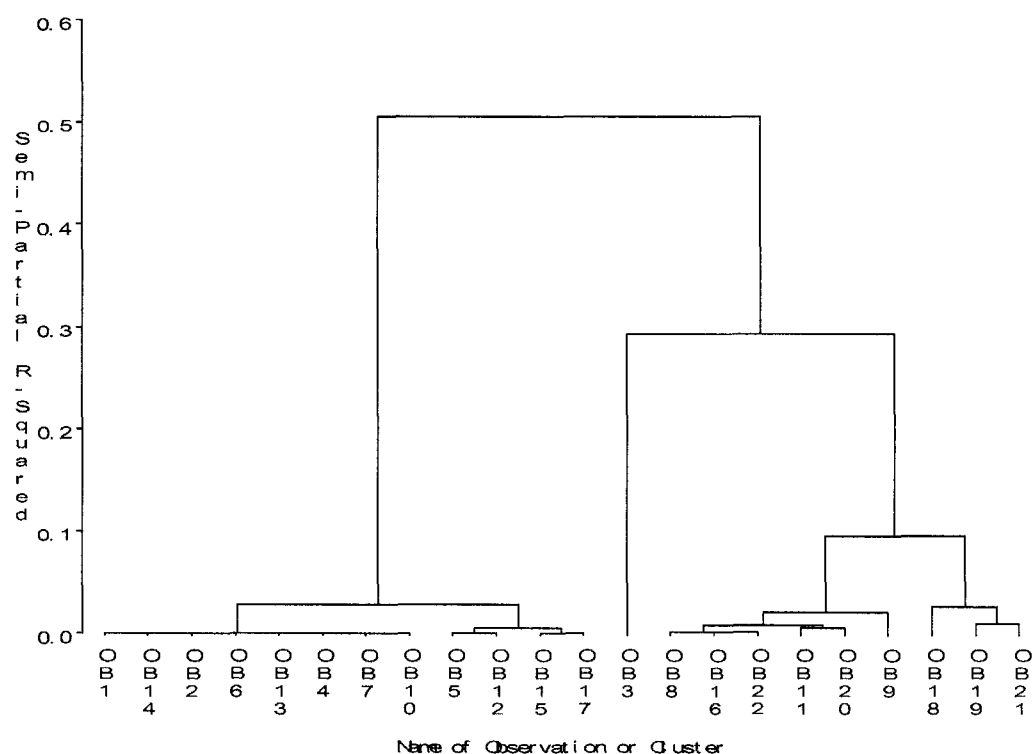
Figure B-2 Results of cluster analysis of PLFA mol percentages

Ob1	14:00	Ob9	15:0 A	Ob17	17:0 iso
Ob2	15:00	Ob10	15:1 ISOG	Ob18	18:1 ω7c
Ob3	16:00	Ob11	16:0 10methyl	Ob19	18:1ω 9 c
Ob4	17:00	Ob12	16:0 iso	Ob20	19:0 cycω8c
Ob5	18:00	Ob13	16:1 2 OH	Ob21	16:1 ω 7c
Ob6	20:00	Ob14	17:0 10methyl	Ob22	TBSA 10 Me18:0
Ob7	14:0 iso	Ob15	17:0 A		
Ob8	15:0 2 OH	Ob16	17:0cyc		

The PLFAs were separated into 4 clusters: One cluster contains 18:1 ω9c, 18:1 ω7c and 16:1 ω7c, which belongs to the monounsaturated PLFA group; one cluster contains 18:0, 16:0 iso, 17:0 a and 17:0 iso, and three of those PLFAs are Gram- negative specific PLFAs; one cluster contains 15:0 2OH, 15: 0 a, 16: 0 10 Methyl, 17:0 cyc, 19:0 cyc and TBSA 10Me 18:0; the last cluster contains 14:0, 15:0, 17:0, 20:0, 14:0 iso, 15:1 isoG, 16:1 2OH and 17:0 10 methyl, which are positively response to the fertilization.

127

Appendix B-3 Dissolved organic carbon (µg C/g O.D. soil)

Series	plot	half	Aug. 03	Sept.03	May 04
A	2	E	189.6	155.1	69.3
A	2	W	149.3	125.9	41.3
A	3	E	252.0	164.3	71.1
A	3	W	215.7	156.3	49.3
A	5	E	143.2	115.0	63.3
A	5	W	117.9	77.8	31.2
C	2	E	224.5	172.1	108.1
C	2	W	171.7	124.9	56.6
C	3	E	227.3	169.2	108.9
C	3	W	245.5	212.2	82.5
C	5	E	228.8	138.8	70.8
C	5	W	243.1	103.4	37.9
F	2	E	269.1	182.7	111.2
F	2	W	159.4	157.0	69.5
F	3	E	208.8	172.2	87.6
F	3	W	188.4	165.3	55.5
F	5	E	185.8	118.0	60.3
F	5	W	126.2	56.4	39.5

Appendix B-4 2003 Fall Soil pH (from Dr.Robertson)

Series	Treatment	Limed	Unlimed
A	Ck	7.91	6.48
A	manure	7.28	6.56
A	NPKS	7.37	5.46
C	Ck	7.14	6.08
C	manure	7.41	6.22
C	NPKS	7.04	4.95
F	Ck	7.6	6.57
F	manure	7.44	6.46
F	NPKS	7.41	5.68

128

Appendix C SAS procedure example

```
/*fractions mineralized C on soil base*/
Options formdlim='-';
DATA minC;
INPUT treatment$ C@@;
CARDS;
```

clay	544	cs	246	flo	216	f+m	312	sand	526	ws	510
clay	529	cs	273	flo	185	f+m	327	sand	762	ws	660
clay	470	cs	239	flo	201	f+m	253	sand	418	ws	480
clay	639	cs	343	flo	230	f+m	191	sand	613	ws	876
clay	336	cs	201	flo	195	f+m	212	sand	752	ws	745
clay	359	cs	209	flo	195	f+m	287	sand	571	ws	769
clay	524	cs	269	flo	232	f+m	256	sand	431	ws	859
clay	532	cs	187	flo	185	f+m	259	sand	509	ws	1037
clay	503	cs	410	flo	347	f+m	248	sand	510	ws	824
clay	463	cs	235	flo	.	f+m	214	sand	529	ws	609
clay	435	cs	194	flo	301	f+m	220	sand	711	ws	837
clay	463	cs	213	flo	264	f+m	171	sand	358	ws	484
clay	549	cs	339	flo	336	f+m	185	sand	415	ws	544
clay	436	cs	177	flo	203	f+m	238	sand	442	ws	532
clay	486	cs	379	flo	210	f+m	252	sand	300	ws	580
clay	469	cs	354	flo	160	f+m	175	sand	461	ws	671
clay	463	cs	242	flo	160	f+m	253	sand	385	ws	612
clay	653	cs	233	flo	107	f+m	188	sand	551		
clay	793	cs	198	flo	237	f+m	274	sand	522		
clay	433	cs	178	flo	133	f+m	214	sand	967		
clay	607	cs	226	flo	158	f+m	258	sand	434		
clay	401	cs	229	flo	200	f+m	269	sand	678		
clay	674	cs	348	flo	207	f+m	309	sand	998		
clay	495	cs	418	flo	210	f+m	321	sand	574		
clay	474	cs	214	flo	314	f+m	145	sand	.		
clay	670	cs	230	flo	148	f+m	272	sand	436		
clay	455	cs	290	flo	105	f+m	247	sand	511		
clay	500	cs	260	flo	107	f+m	273	sand	472		
clay	788	cs	259	flo	125	f+m	288	sand	407		
clay	495	cs	234	flo	141	f+m	236	sand	311		
clay	727	cs	300	flo	183	f+m	455	sand	402		
clay	607	cs	178	flo	172	f+m	314	sand	421		
clay	937	cs	243	flo	184	f+m	313	sand	578		
clay	609	cs	323	flo	168	f+m	236	sand	405		
clay	518	cs	265	flo	219	f+m	238	sand	902		
clay	351	cs	289	flo	120	f+m	205	sand	608		

```
;
run;

proc print;
run;

PROC glm data=minC;
class treatment;
model C=treatment;
```

```
means treatment/duncan;
means treatment/lsd cldiff;
means treatment/stderr;
run;
```

SAS results:

- -

                        The SAS System   9
                       The GLM Procedure
                    Class Level Information
           Class   Levels   Values
           treatment   6   clay cs f+m flo sand ws

                  Number of observations   197
NOTE: Due to missing values, only 195 observations can be used in this
      analysis.

- -
-                      The SAS System   10
                      The GLM Procedure
Dependent Variable: C
                           Sum of
Source   DF   Squares   Mean Square   F Value   Pr > F
Model   5   5625587.957   1125117.591   87.79   <.0001
Error   189   2422124.786   12815.475
Corrected Total   194   8047712.743

           R-Square   Coeff Var   Root MSE   C Mean
           0.699029   29.32578   113.2055   386.0270
Source   DF   Type I SS   Mean Square   F Value   Pr > F
treatment   5   5625587.957   1125117.591   87.79   <.0001
Source   DF   Type III SS   Mean Square   F Value   Pr > F
treatment   5   5625587.957   1125117.591   87.79   <.0001

- -
-                      The SAS System   11
                      The GLM Procedure
                Duncan's Multiple Range Test for C
   NOTE: This test controls the Type I comparisonwise error rate, not the
                    experimentwise error rate.

                Alpha   0.05
                Error Degrees of Freedom   189
                Error Mean Square   12815.48
                Harmonic Mean of Cell Sizes 30.10541
                   NOTE: Cell sizes are not equal.
        Number of Means   2   3   4   5   6
        Critical Range   57.56   60.59   62.61   64.09   65.25
           Means with the same letter are not significantly different.
           Duncan Grouping   Mean   N   treatment
                        A   684.10   17   ws
                        B   539.15   35   sand
                        B
                        B   538.49   36   clay
                        C   261.80   36   cs
                        C
                   D   C   253.00   36   f+m
                   D
                   D   195.91   35   flo

- -

                                130
```

The GLM Procedure
t Tests (LSD) for C
NOTE: This test controls the Type I comparisonwise error rate, not the
experimentwise error rate.


Alpha                          0.05
Error Degrees of Freedom        189
Error Mean Square          12815.48
Critical Value of t         1.97260


Comparisons significant at the 0.05 level are indicated by ***.

| treatment Comparison | Difference Between Means | 95% Confidence Limits | |
|---|---|---|---|
| ws   - sand | 144.94 | 78.93   210.96 | *** |
| ws   - clay | 145.61 | 79.89   211.32 | *** |
| ws   - cs | 422.30 | 356.59   488.02 | *** |
| ws   - f+m | 431.10 | 365.38   496.82 | *** |
| ws   - flo | 488.19 | 422.17   554.20 | *** |
| sand - ws | -144.94 | -210.96   -78.93 | *** |
| sand - clay | 0.66 | -52.34   53.67 | |
| sand - cs | 277.36 | 224.35   330.37 | *** |

------------------------------------------------------------------------
The SAS System                                        14
The GLM Procedure
t Tests (LSD) for C
Comparisons significant at the 0.05 level are indicated by ***.

| treatment Comparison | Difference Between Means | 95% Confidence Limits | |
|---|---|---|---|
| sand - f+m | 286.16 | 233.15   339.16 | *** |
| sand - flo | 343.24 | 289.86   396.62 | *** |
| clay - ws | -145.61 | -211.32   -79.89 | *** |
| clay - sand | -0.66 | -53.67   52.34 | |
| clay - cs | 276.69 | 224.06   329.33 | *** |
| clay - f+m | 285.49 | 232.86   338.13 | *** |
| clay - flo | 342.58 | 289.57   395.59 | *** |
| cs   - ws | -422.30 | -488.02   -356.59 | *** |
| cs   - sand | -277.36 | -330.37   -224.35 | *** |
| cs   - clay | -276.69 | -329.33   -224.06 | *** |
| cs   - f+m | 8.80 | -43.84   61.43 | |
| cs   - flo | 65.88 | 12.88   118.89 | *** |
| f+m  - ws | -431.10 | -496.82   -365.38 | *** |
| f+m  - sand | -286.16 | -339.16   -233.15 | *** |
| f+m  - clay | -285.49 | -338.13   -232.86 | *** |
| f+m  - cs | -8.80 | -61.43   43.84 | |
| f+m  - flo | 57.09 | 4.08   110.10 | *** |
| flo  - ws | -488.19 | -554.20   -422.17 | *** |

------------------------------------------------------------------------
The SAS System                                        15
The GLM Procedure
t Tests (LSD) for C
Comparisons significant at the 0.05 level are indicated by ***.

| treatment Comparison | Difference Between Means | 95% Confidence Limits |
|---|---|---|

```
flo  -  sand      -343.24     -396.62  -289.86  ***
flo  -  clay      -342.58     -395.59  -289.57  ***
flo  -  cs         -65.88     -118.89   -12.88  ***
flo  -  f+m        -57.09     -110.10    -4.08  ***
```