THE ROLE OF TEMPERATURE IN C AND N MINERALIZATION FROM SELECTED ARABLE NOVA SCOTIA SOILS

by

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This thesis is dedicated to my father, Paul Cooper, who was always an enthusiastic, loving and unconditional supporter of me in all of my endeavours. He provided me with an idyllic rural upbringing, and instilled in me a love of agriculture and farming that remains to this day.

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Abstract

This thesis focuses on the effects of soil management history and temperature on C and N mineralization. A preliminary field study indicated that N uptake by a crop was related to variability in substrate quantity and quality, and environmental factors. Further experiments in soil microcosms investigated the effects of soil temperature and management history on net C and N mineralization using soils from a fertility experiment with and without a history of manure application. Microcosms were incubated at 5, 15, 25 or 35°C, with and without the addition of ^{14}C-labelled wheat. For native C and N, and ^{14}C, the size of the substrate pool estimated using the first-order model of decomposition changed with temperature, contradicting one of the key assumptions of the first-order approach to modelling net C and N mineralization in soils. The temperature response of native C and N mineralization differed in the non-amended microcosms, with a substantial increase in the rate of N mineralization relative to C mineralization between 5 and 15°C. Microbial community structure changed with temperature, with distinct fungal communities present at 5°C. The size of the microbial biomass declined with increasing temperature, and metabolic quotients were also highest at 35°C. A further study using ^{13}C-labelled wheat indicated some differences in the accessibility of the wheat C due to management history at the coldest incubation temperature. The use of DNA-SIP along with density gradient centrifugation was used to separate wheat C from native C metabolizing communities, with a trend towards declining diversity with increasing density within the fungal population.

Current soil C and N models that include empirically-derived temperature response functions, already implicitly include temperature effects on biological parameters. In most cases these effects were not shown to interact with soil management history in these experiments, providing no evidence to support the more explicit inclusion of biological parameters, such as microbial community structure and size, in improved models. While measurement of biological parameters provided useful insights into the mechanisms behind variations in estimates of substrate pool size at different temperatures, evidence was not provided for the inclusion of biological parameters explicitly within soil decomposition models.

List of Abbreviations and Symbols Used

at.% ^{13}C - atom percent ^{13}C – calculated as $[R_{sample}/(R_{sample} + 1)] \times 100$; where R_{sample} is the $^{13}C/^{12}C$ ratio determined by IRMS

$\%^{14}C_i$ - percentage of ^{14}C initially added

$\%C_w$ - percentage of wheat C initially added

A - treatment with ^{14}C-labeled wheat

bp - base pair

CEC - cation exchange capacity

CFE - chloroform fumigation extraction

CHU - corn heat unit

CI - confidence interval

C_0 - potentially mineralizable C

CLSU - community level substrate utilization

CV - coefficient of variation

dpm - disintegrations per minute

E_a - activation energy of a substrate

e_{ass} - assimilation efficiency

e_{prod} - production efficiency

EOC -extractable organic carbon

H' - Shannon's diversity index

IRGA - infrared gas analyzer

IRMS - isotope ratio mass spectrometry

IRT - infrared transmitting

ISNT - Illinois soil nitrogen test

JD - Julian day

k - the relative rate constant used in the single pool exponential model of decay, in units per day (d^{-1}) ; the exponential decay constant

Mgt - management

N_e - easily mineralizable N i.e. the N mineralized in the first 14 day period of a leaching tube experiment

N_0 - potentially mineralizable N

NA - treatments not receiving any ^{14}C-labeled wheat

NLWR - non-limiting water range

OTU - operational taxonomic unit

p - probability

PAN - plant-available nitrogen

PC - principal component

PCR - polymerase chain reaction

PLFA - phospholipid fatty acid

qCO_2 - metabolic quotient

rcf - relative centrifugal force

SDS - sodium dodecyl sulfate

SE - standard error

SIP - stable isotope probing

SOM - soil organic matter

SPE - single pool exponential

SS - sum of squares

STU - soil thermal unit

SUE - substrate use efficiency

TDR - time domain reflectometry

Temp - temperature

TPAN – total potentially mineralizable N

TRF - temperature response function

T-RFLP - terminal-restriction fragment length polymorphism

TSR - two simultaneous reactions

WFPS - water filled pore space

z - the zero-order mineralization rate constant in units of mg per day (mg d^{-1})

Glossary

Ammonification is the conversion of organic N to ammonium N (Harmsen and van Schreven, 1955).

Assimilation is the conversion of metabolized nutrients into biomass.

Available N is the soil nitrogen that is available for crop uptake during a given interval of time. N availability in the field is a function of the net mineralization of N from soil pools of substrate, as well as the climatic, biotic, and soil physical properties that result in the removal of N from the system before it can be taken up by the plant.

Bulk soil is the soil located outside of the rhizosphere. Primary saprotrophs living in the bulk soil utilize native pools of soil organic C as their prime source of energy.

Decomposer community refers to the organisms present in the soil that convert litter into humus and carbon dioxide. This community includes both the primary and secondary saprotrophs (see definitions below). Since the decomposer community relies on organic carbon as a source of energy, all members of this community are heterotrophs.

Decomposition is the process of litter chemical decay caused by trophic interactions among living organisms. This is in contrast with simple degradation of litter by chemical reactions not caused by living organisms (Adl, 2003).

DNA-SIP is a technique in molecular biology in which microbial DNA is labelled with ^{13}C by providing living organisms with a source of ^{13}C-labelled substrate. The DNA is then extracted from the organisms and ^{13}C-labelled DNA is separated from ^{12}C-DNA by ultra-high speed centrifugation in a salt solution (usually caesium chloride or caesium trifluoroacetate). Fractionation of the resulting density gradient allows separation of the DNA by density, with the most highly labelled, ^{13}C-DNA migrating to regions of higher density than the ^{12}C-DNA (Radajewski et al., 2000).

First-order model of decay is the mathematical model most commonly fit to C and N mineralization data from soil decomposition studies. It is also sometimes referred to as the exponential decay model. In first-order reactions, the rate of transformation of a substrate is proportional to the substrate concentration (Paul and Clark, 1989).

Gross mineralization of N specifically refers to the gross amount of N converted from organic forms to inorganic forms by heterotrophic organisms in the soil. It does not account for the removal of N from inorganic pools for assimilation by soil organisms (immoblization).

Heterotrophs are organisms that cannot manufacture organic compounds and so must feed on organic materials that have originated in other plants and animals (Raven et al., 1999).

Immobilization is the assimilation of inorganic N into soil biomass.

Isopycnic refers to a salt solution of equal or constant density, with respect to either space or time.

Litter can be broadly defined as all that was recently living. This includes fallen leaves, woody debris and fallen bark, dead roots, cadavers, animal dung, insect frass and cuticles (Adl, 2003). Litter is essentially the raw material that fuels the soil food web.

Mesophilic describes organisms with midrange temperature optima. For example, *Esherichia coli*, a typical mesophile, has an optimum temperature of 39°C (Madigan, Martinko and Parker 2000).

Metabolic quotient (qCO_2) is a measure of the efficiency of microbial activity calculated by dividing the respiration rate on a given day by the microbial biomass measured at the same time. Metabolic quotient is also sometimes referred to as the biomass specific respiration (Fliessach et al., 2000).

Microbial biomass in this thesis refers to all microflora and microfauna present in the soil, including the decomposer community, plant symbionts, and plant pathogens.

Microfauna are soil animals not visible with the naked eye, including protozoa and nematodes (Jensen and Magid, 2002).

Microflora refers to bacteria and fungi in the soil (Jensen and Magid, 2002). Most primary saprotrophs are microflora.

Mineralization of N or C refers to the biologically-mediated conversion of organic forms of these nutrients to inorganic forms. In the case of N, it is the process by which organic N is transformed to ammonium N (Rees, 1989). This process is sometimes also called *ammonification*. In the case of C, mineralization refers to the conversion of organic C to CO_2 gas. This process is also sometimes called respiration.

Native C and N is the pool of organic C and N in the soil originating from humus and litter which has already undergone primary decomposition.

Net N mineralization is the change in inorganic N resulting from gross mineralization minus gross immobilization.

Osmotrophy is the transfer of nutrient molecules into cells through the cell membrane (Adl, 2003).

Plant-available nitrogen refers to an estimate of available N determined by measuring the uptake of N by a growing crop between planting and harvest. The inorganic N present in the soil at crop planting is subtracted from the final estimate, based on the assumption that this N was made available before the planting date. Inorganic N present in the soil at harvest is added to the final estimate, since this N was available for crop uptake, even though it was not measured as part of the crop N.

Potentially mineralizable C or N (C_0 or N_0) is an empirical parameter estimated by fitting net N mineralization or net C respiration data to a first-order model of mineralization or respiration. It represents the maximum amount of C or N in the soil litter pool available for respiration or mineralization.

Primary decomposition refers to the initial processes that modify the dead tissues and excreta from primary production, which constitute the litter. This includes lysis of cells in the dead tissues, release of soluble molecules, physical fragmentation of the tissues, and colonization by primary saprotrophs which further dissolve and partially ingest the organic matter. (Adl, 2003)

Primary saprotrophs are organisms involved in primary decomposition, which release digestive enzymes into the environment to solubilize a source of nutrient or substrate; the resulting smaller molecules are then absorbed by the organism through its cell membrane. Most primary saprotrophs are prokaryotes and fungal organisms. (Adl 2003)

R^2 is the coefficient of determination used to quantify the goodness of fit of experimental data to a model. It is a fraction between 0.0 and 1.0 with higher values indicating that the curve comes closer to the data. By convention, statisticians use uppercase (R^2) for the results of nonlinear and multiple regressions and lowercase (r^2) for the results of linear regression. (Motulsky and Christopoulos, 2004)

Relative rate constant is the first-order decay constant, usually symbolized by k, of the first-order model of decomposition.

Rhizosphere is the region of soil directly affected by the exudation of easily available C substrates from plant roots (Kuzyakov, 2006). Primary saprotrophs living in the rhizosphere utilize the C compounds from root exudates and sloughed off root cells as their primary source of energy.

Rhizosphere priming effect is the interaction between growing roots and soil organic matter decomposition resulting from increased microbial activity in the rhizosphere (Kuzyakov, 2006).

Secondary decomposition is the breakdown of the by-products of primary decomposition, including organic matter excreted by primary saprotrophs, microdetritus in varying stages of decay, colloids and amorphous organic matter. (Adl, 2003)

Secondary saprotrophs (also known as consumers) consume primary saprotrophs or litter which has been partially decomposed by primary saprotrophs. (Adl, 2003)

Shannon's diversity index (or Shannon's index of general diversity, H') is an overall index of diversity that combines the species richness and evenness components of diversity. It is calculated as: $H' = -\Sigma(n_i/N)\log(n_i/N)$ or $-\Sigma(P_i)\log(P_i)$, where n_i = importance value for each species, N = total importance values, P_i = importance probability for each species = n_i/N (Odum 1971).

Soil fauna are those soil organisms larger than fungi and bacteria, including protozoa, nematodes, microarthropods and earthworms. Soil fauna, particularly protozoa and nematodes, play an important role in the soil food web as grazers of fungi and bacteria.

Soil food web refers to the complex system of interactions among different functional groups and trophic levels of organisms in the soil.

Soil thermal unit is an alternative unit of time calculated by summing daily average temperatures above $0^{o}C$.

Substrate quality describes the availability of an organic carbon source for metabolism by heterotrophic microorganisms. An organic carbon source with high substrate quality is easily metabolized by microorganisms while substrates of low quality, sometimes described as recalcitrant, are only slowly metabolized by microorganisms.

Substrate utilization is the metabolism of organic C for biomass production and maintenance.

Temperature coefficient refers to the relative increase in the rate of C respiration or N mineralization for every $10^{o}C$ increase in temperature.

Trophic level is a step in the movement of energy through an ecosystem, represented by a particular set of organisms (Raven et al., 1999).

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Chapter 1: Introduction

This thesis examines temperature effects on net C and N mineralization in arable soils. It specifically examines the use of first-order kinetics to describe these processes, and investigates temperature effects on substrate utilization, and characteristics of the microbial biomass. The potential to make net C and N mineralization models more mechanistic, by incorporating temperature-driven changes in the size, species composition, and substrate use efficiency of the microbial biomass is examined.

1.1 Current Approaches to Modelling Net C and N Mineralization

The factors controlling the release of C and N from soil organic matter have been the subject of study for many years (Harmsen and van Schreven, 1955; Parton et al., 1987; Hofman, 1988; Jensen and Magid, 2002). Carbon respiration (or mineralization) and nitrogen mineralization are often modelled simultaneously because they are inextricably linked in organic molecules. Heterotrophic soil organisms metabolize organic matter to obtain C-precursors for biosynthesis and C-rich substrates for respiration and the subsequent release of energy. The demand for N is determined by the relative demands of biosynthesis and the N content of the substrates being metabolized. Nitrogen in excess of biosynthetic requirements is released to the soil solution (net mineralization). In the case where the N content of the substrate is insufficient to meet biosynthetic demands, inorganic N, if available, is obtained from the soil solution (net immobilization).

Recently, there has been heightened interest in C and N dynamics in soils, particularly the potential for increased microbial decomposition and the release of C from soils as a result of increases in global temperatures (Jenkinson et al., 1991; Kirschbaum, 1995; Giardina and Ryan, 2000; Rustad et al., 2000; Thornley and Cannell, 2001). Most

studies of soil C mineralization have been conducted on a relatively long time scale (years-to-centuries) (e.g. Jenkinson, 1990). The release of organic N from soils is of interest on both a long-term time scale as it relates to soil fertility and the productivity of natural and managed ecosystems, and on a shorter time scale (days-to-years) as it relates to the release of mineral N to agricultural crops and forests. Also of concern are the potential impacts on surrounding ecosystems through nitrate leaching to groundwater or emissions of nitrous oxide to the atmosphere.

Most models of C and N mineralization are based on first-order or exponential decay theory. According to this theory, a given pool of substrate breaks down to simpler products at a rate that is proportional to the size of the available pool of substrate. This is described by the mathematical expression:

$$- dS/dt = f(S_t) \qquad (1)$$

where S_t is the mass of the substrate pool at time t. Upon parameterization the proportionality is expressed as the first order rate constant k, yielding:

$$- dS/dt = kS \qquad (2)$$

The term "first-order" comes from this expression, since the quantity of substrate, S, is raised to the power of 1. First-order decay is sometimes called substrate-limited decay, because the instantaneous rate of decay (dS/dt) decreases as the pool of available substrate decreases in size, or becomes limiting. In zero-order decay, the substrate quantity, S, is raised to the power of zero i.e. the rate of decay is determined only by the rate constant, regardless of the quantity of S remaining.

First-order principles have been incorporated into most of the current models of C and N mineralization from soils, whether they are simple, single-pool models that explain

mineralization kinetics in laboratory incubations (Stanford and Smith, 1972; Smith et al., 1980; Bonde and Rosswall, 1987; Campbell et al., 1988; Ellert and Bettany, 1992; Cabrera, 1993; Macdonald et al., 1995; Thomsen et al., 2001; Dalias et al., 2002; Springob and Kirchmann, 2003) or more complex multi-pool models used to predict C and N dynamics in the field (e.g. the CENTURY model (Parton et al., 1987) or the Rothamsted turnover model (Jenkinson, 1990)). First-order approaches have been called rate constant approaches because environmental and soil effects on the rate of mineralization are incorporated via modifications to the first-order rate constant, k.

The first-order theory of decay was designed by chemists to explain chemical reaction rates among relatively simple compounds. When applying this theory to soil C and N mineralization, it is important to keep in mind the limitations of this approach. The substrate undergoing decomposition or decay in the soil is complex and heterogenous. In single-pool approaches, it is assumed that the quality and availability of the substrate for decomposition is uniform, while in multi-pool models some attempt is made to partition the substrate pool into different fractions with different degrees of susceptibility to decay. This is reflected in differences in the first-order rate constants for each pool; however, this is still a gross oversimplification of the situation in the soil, which consists of a large number of pools with a continuum of qualities ranging from readily degradable to extremely physically or chemically recalcitrant (Davidson and Jannsens, 2006; McGill et al., 1975; McGill and Paul, 1976).

Another limitation of the first-order approach is that it ignores the fact that net C and N mineralization are the result of two opposing processes: gross mineralization and gross assimilation (referred to as immobilization for N). The conversion of organic forms

of C and N to mineral forms (gross mineralization) is not a simple, one-step process, but rather involves intermediate steps mediated by different enzymes produced by a variety of organisms. In some cases, net C and N mineralization follows ingestion of a complex substrate by soil organisms, and subsequent excretion of waste C and N (e.g. for microfauna). In other cases (e.g. for microflora) digestion takes place extracellularly (Adl, 2003). In both cases, different net C and N mineralization can result from the same substrate, depending on the efficiency of the decomposer organisms' metabolism, and the composition of its biomass (particularly C:N ratio).

The effects of substrate quality and microbial biomass characteristics on the rate of net C and N mineralization are integral to the generalized rate constant (k) found in first-order models. The first-order approach is therefore an empirical method in which net C and N mineralization are observed and the first-order model fit to the data. In this approach, soil to soil variations due to substrate quality and microbial community effects are reflected in the value of k. This approach has worked well for laboratory incubations of soil at constant temperature. Stanford and Smith (1972) used this approach in a landmark study of the N mineralization potential of soils incubated under standardized conditions and concluded that for most agricultural soils (29 of the 39 in their study), an average value for the first-order rate constant of 0.054 wk^{-1} could be used. This implied that the substrate quality and microbial community differences that should result in variations in the value of k among different soils were not significant when the soils were incubated under standardized conditions and had similar management histories.

Another level of complexity is introduced into the prediction of net C and N mineralization when variations in temperature are incorporated into the model. In first-

order approaches, a temperature response function (TRF) is used to modify the rate constant. These response functions are usually empirical relationships derived from measured changes in net N or C mineralization at different temperatures. The rate constants of each pool in the model are modified and, in almost all cases it is assumed that the temperature response function of each relative rate constant in the model is the same (Davidson and Janssens, 2006; Fierer et al., 2005). In most models, the processes of soil C and N mineralization are directly linked and the same temperature response function is used to modify the rates of both processes.

A variety of temperature response functions have been used to describe the effects of temperature on the first-order rate constant. These can be classed broadly into those that assume a constant temperature coefficient (sometimes referred to as Q_{10}) regardless of the temperature range, and those that incorporate temperature coefficients that vary depending on the temperature range. The Arrhenius function, based on Arrhenius laws of chemistry, and the Q_{10} function, derived from Van't Hoff's law (1898), are the two functions with constant temperature coefficients that are most commonly used. Stanford et al. (1973) first proposed the Arrhenius function for modifying the first-order rate constant for net N mineralization in soils. They suggested that a temperature coefficient of 2 could be used to describe net N mineralization between 5 and 35°C; however, they also reported that the function did not provide reliable estimates of k at low temperatures (5°C), and opted to omit these data when fitting their Arrhenius function.

There are some fundamental flaws in the assumption that the temperature response of decomposition processes can be adequately predicted using Arrhenius and Q_{10} functions. These functions were derived from studies of simple chemical reactions,

and they can effectively model the effects of temperature on the individual reactions that make up the decomposition process (e.g. enzyme-mediated depolymerization reactions); however, decomposition is the result of a series of depolymerization reactions, each of which potentially has a different temperature response. In addition, Davidson and Jannsen (2006) explained in a recent review how Arrhenius laws are only valid when substrate is non-limiting, yet soil microbial communities are generally assumed to be operating under substrate-limited conditions. The reactions involved in decomposition are mediated by soil organisms that excrete enzymes to catalyze these processes. Such organisms are likely to respond to temperature differently as well (Kirschbaum, 1995; Rodrigo et al, 1997), so that not only the chemical reactions themselves, but the sources of catalysts for these reactions, are affected by temperature. Arrhenius and Q_{10} functions also do not explicitly include temperature-related changes in substrate quality and microbial community characteristics that are an integral part of the first-order rate constant.

Ratkowsky et al. (1982) proposed a quadratic function that describes the effects of temperature on bacterial growth. This function has a non-constant temperature coefficient, with higher coefficients at lower temperatures. This function has been used to model temperature effects on soil bacterial and fungal communities (Díaz-Raviña et al., 1994; Pietikäinen et al., 2005). Both the Rothamsted turnover model (Jenkinson, 1990) and the CENTURY model (Parton et al., 1987) use temperature response functions that include an increase in temperature response as temperatures decrease. This is also the case for the temperature response function proposed by Lloyd and Taylor (1994).

The biology behind the processes that result in net C and N mineralization in soils

is not explicit in the first-order model of decay, or the various temperature response functions used to modify the first-order rate constant. As pointed out by Grenon et al. (2004), while metabolic rates of individual microbial populations increase predictably with temperature, the effects of temperature on the soil functions resulting from microbial interactions are less predictable. It has been suggested that modellers need to include more biology (parameters relating to biomass size, community structure and metabolism) in their models of net C and N mineralization in order to more accurately describe soil functions, especially under changing temperatures (Schimel, 2001). This is particularly relevant if predictions about the effects of global warming on the release of CO_2 from soils, and the positive feedback mechanisms that this is expected to enhance, are to be relied upon (Jenkinson et al., 1991; Raich and Schlesinger, 1992; Townsend et al., 1992; Schimel et al., 1994; Kirschbaum, 1995). Fang et al. (2005a) suggested that at least the size of the microbial biomass should be included in models of decomposition, because this is the driving force behind the process. Zogg et al. (1997) observed changes in the structure of fungal and bacterial communities at different temperatures, and suggested that these differences result in changes in substrate pools accessed at different temperatures. Various researchers have observed a temperature-related change in the size of the potentially mineralizable pool of C and N estimated using the first-order model, and have hypothesized that differences in estimates of substrate pool size are related to a change in metabolic function of the microbial community with temperature (the functional shift hypothesis) (Ellert and Bettany 1992; Macdonald et al. 1995; Zogg et al. 1997; Zak et al. 1999; Dalias et al. 2002; Dalias et al. 2003). Recently, Davidson et al. (2006) encouraged the study of temperature effects on the various processes that

contribute to soil respiration, including substrate diffusion, O_2 supply, and microbial population growth. Results of such studies could help us to move beyond Q_{10} approaches to modelling respiration in terrestrial ecosystems. It is still not clear whether current models of net C and N mineralization could be improved by the inclusion of more biological parameters in defining metabolic rate constants.

1.2 Thesis Objectives and Findings

This thesis investigates the use of first-order kinetics to describe net mineralization of C and N from agricultural soils in Nova Scotia. In particular, the effects of temperature on microbial community characteristics are examined, in an attempt to determine how temperature-driven changes in these characteristics impact on predictions of net C and N mineralization.

In Chapter 2, the results of a field study into temperature effects on N availability to a crop during the growing season are reported. The availability of N for crop uptake in the field was found to be related to soil temperature, but short-term effects of growing season temperature could not be separated from the higher values of potentially mineralizable N estimated for the soils from the warmer sites, reflecting the longer-term impact of climate and management. The estimated first-order rate constants for four of the soils in the study were not significantly different; however values for potentially mineralizable N, and various laboratory indices of N availability, did vary among the sites. This study provided the impetus for the more in-depth experiments that formed the rest of the thesis work.

Chapter 3 reports the results of a microcosm study into the effects of temperature on the mineralization of C and N from native pools of soil organic matter. Carbon and N mineralization from two soils from the same site, one which had a history of manure

application, the other which had received no manure or fertilizers for the previous ten years, were monitored at a range of temperatures. Neither net C nor net N mineralization followed the principles of first-order kinetics, with temperature-dependent changes in substrate pool size observed. There was also a difference in the temperature response of net C mineralization compared with net N mineralization. Rates of net N mineralization increased more rapidly with rising temperature than rates of net C mineralization over the 5 to 15°C temperature range. Biomass characteristics including the biomass specific respiration (i.e. rate of respiration per unit biomass, or qCO_2), chloroform fumigation extraction (CFE) flush C, and fungal and bacterial species composition, were all shown to change with temperature. These effects were most evident at the extremes of temperatures, i.e. 5 and 35°C. Although management history had some effects on bacterial and fungal species composition, it did not affect the C and N mineralization function of the community. The results of this study indicated a need to readdress the modelling of net mineralization of native pools of C and N, considering that the temperature response of these two processes was not equivalent. Biological mechanisms for the differences in temperature effects on net C and N mineralization are also discussed.

Chapter 4 reports the effects of changing temperature on net C mineralization from ^{14}C-labeled plant litter. This study complements the work reported in Chapter 3 in which two soils with different management histories are compared. The mineralization of ^{14}C from the added litter could be fit to a first-order model at each of the temperatures in the study (5, 15, 25 and 35°C), but temperature-dependent changes in both k and the potentially mineralizable C (C_0) estimates were observed. There was an inverse

correlation between the two parameters. Total quantities of ^{14}C respired by the end of the experiment were not significantly affected by temperature, and management history had no effect on the utilization of the added substrate. Microbial utilization of C at different temperatures, estimated as the sum of the ^{14}C metabolized (^{14}C respired plus ^{14}C in the biomass) after 55 days, was highest at 25°C. Microbial communities with differences in microbial community structure and metabolic parameters (e.g. specific substrate use efficiency and total ^{14}C utilization) developed at the two temperature extremes in the study (5 and 35°C). The potential to make current plant litter decomposition models more mechanistic by including temperature effects on the biology of the process is discussed.

Chapter 5 reports the results of a second plant litter study in which an innovative new molecular technique, stable isotope probing, was applied to the study of C dynamics at three temperatures. The same soils as used in the previous two chapters were amended with ^{13}C-labelled wheat and incubated at 4, 22 or 35°C. Respiration of ^{12}C and ^{13}C was measured and biomass C and DNA were extracted from the soils periodically. The partitioning of ^{13}C into the biomass, respired C, and unmetabolized/stabilized ^{13}C was affected by temperature. The ^{13}C that was traced into the DNA of the metabolizing communities was shown to be part of a community that was less diverse than the wider, ^{12}C metabolizing community. Temperature-related differences in community structure, as observed in Chapter 4, were also reported in this study.

In Chapter 6, the implications of the results of this study for modelling net C and N mineralization are discussed. A simple spreadsheet model was used to simulate net C and N mineralization from a substrate, with microbial biomass characteristics (size, C:N

ratio, efficiency) included as a function of temperature. The potential to make current models more mechanistic by including biological parameters is discussed.

Chapter 2: Estimating Net Nitrogen Mineralization in the Field and Laboratory

2.1 Introduction

Nitrogen is the major plant nutrient most likely to limit crop yield. For this reason, in conventional farming systems, N is usually supplied to the crop in a synthetic form to ensure that N supply does not limit crop yield. However, the N supply from soil organic matter (SOM) can also contribute significantly to crop N needs (Hofman, 1988). Our current understanding of the process of N mineralization i.e. the conversion of organic forms of N to ammonia, and then nitrate via nitrite, was understood as long ago as the end of the 19th century (Harmsen and van Schreven, 1955). Since that time, innumerable studies have been published on the subject of predicting N supply to crops from soil organic matter. The subject is extremely challenging, because the N cycle is closely associated with the C cycle, and N availability to crops is therefore tied to general patterns of decomposition in the agroecosystem (Groffman et al., 1987). These rates of decomposition are affected by the interactions between climate, tillage, past management practices, and soil type, all of which play a role in the prediction of net N mineralization in the field.

Predicting the availability of soil N for crop production in a given year requires an understanding of both the sources and sinks of plant-available N. N sources include net N mineralization from organic N pools (SOM, crop residues, manure), synthetic N supply, biological N fixation, and atmospheric deposition. Sinks, or losses of N, include leaching to groundwater, denitrification, net immobilization and volatilization of ammonia. It has been traditionally assumed that ammonium and nitrate are the main sources of N available to plants. Recently this assumption has been challenged, as more

evidence has emerged that plants can take up organic N monomers (Schimel and Bennett, 2004); however, this phenomenon seems to be common only in N-limited natural ecosystems. For the purposes of this study of arable soils with a history of manure application, I will assume that inorganic N is the primary source of plant-available N in the field.

2.1.1 Factors Controlling N Mineralization in the Field

The mineralization of N from soil organic matter and litter is a key process affecting inorganic N concentration in agricultural soils during the growing season. While leaching of N may occur outside of the crop growth period in humid climates, it is assumed that the crop takes up all inorganic N that is available to it while actively growing. Predicting the magnitude of net N mineralization in a given growing season requires information about soil N mineralization potential, and the environmental factors (temperature, moisture) that control net N mineralization in the field.

N mineralization potential

The N mineralization potential is a function of several key factors. First, the quantity of organic N present in the soil partly determines the amount of N that will be mineralized (Fox and Piekielek, 1984); however, the form or composition of this organic N is also important. Nitrogen that is bound in complex, stable compounds (e.g. structural components of humic substances) is much less available for mineralization than is the N bound in relatively simple compounds like amino acids and amino sugars (Stevenson, 1982).

Another key factor may be the capacity of the soil microbial community to mineralize N. Soil microbial biomass size is not usually considered to be a factor limiting decomposition of organic matter; however, some studies have indicated that

management history can affect the capacity of the soil microbial biomass to breakdown added organic matter. Fliessbach et al. (2000) found that a soil with a long history of biodynamic management was able to mineralize more of an added ^{14}C-labelled wheat straw than a conventionally managed soil from the same experiment. This was attributed to differences in microbial biomass characteristics, in particular substrate use efficiency, between the two soils.

Finally, soil type (texture and mineralogy) can impact net N mineralization. It is widely understood that soils that are high in clay can form organo-mineral complexes in which soil organic matter is protected from degradation by enzymes (Loll and Bollag, 1983; El-Shinnawi et al., 1988). In addition, soils with a high cation exchange capacity (CEC) have a larger potential to bind enzymes and reduce their activity. These factors contribute to reductions in decomposition rates in soils that are high in clay and/or have a high CEC.

Within a climatic region, routine prediction of N availability in the field requires a laboratory-based measure of N mineralization potential. Potentially mineralizable N (N_0) estimated using the Stanford and Smith (1972) aerobic incubation method is commonly accepted as the most definitive measure of nitrogen mineralization potential (Carter and Macleod, 1987; Curtin and Wen, 1999). Since the estimate is the result of an incubation during which conditions for biological activity are optimized, it can be assumed to reflect differences in the soil microbial biomass characteristics and soil type that may affect decomposition rates. Griffin and Laine (1983) argued that because of the high degree of correlation between the estimate of pool size (N_0) and the decay rate constant (k), the product of these two parameters (N_0k) is a better indicator of N availability to crops,

representing both the size and availability of the organic N pool. Regardless of whether N_0 or N_0k is considered the best estimate of soil N mineralization potential, both of these estimates require a relatively long (at least 6 week) incubation period, and are labour intensive

The ideal laboratory index of soil N mineralization potential should be relatively quick and inexpensive, and effective for use with air-dried, sieved soils (the most common method of preparing and storing soils for routine nutrient analysis). The method should also have a high degree of reproducibility in the lab (low variability for measurements of the same sample). Finally, the laboratory index should be well correlated with more rigorous measures of N mineralization potential. Currently there is no consensus on the best laboratory index of soil N mineralization potential. Laboratory indices are usually related to N released after an aerobic or anaerobic incubation. Gianello and Bremner (1986a) recommended both the phosphate-borate NH_4^+-N and hot KCl-extractable NH_4^+-N methods as good predictors of N released by three different incubation methods. Carter and Macleod (1987) found that mineral N flush (using the chloroform fumigation incubation method) was a good predictor of N_0 ($r^2 = 0.94$) from a range of agricultural soils in Prince Edward Island. Finally, Khan et al. (2001) proposed a rapid amino sugar-N test (now known as the Illinois Soil Nitrogen Test, ISNT) which can accurately predict hydolyzable amino sugar-N, the pool of soil N that they believe accounts for differences in N response among corn fields in Illinois.

Environmental factors

Soils with similar N mineralization potentials may release different quantities of N, due to differences in temperature and moisture which determine the *in situ* rates of N

mineralization. In the first stage of organic matter decomposition, primary saprotrophs (bacteria and fungi) excrete enzymes into their environment to break down complex substrates into simpler compounds which can be absorbed by osmotrophy (Adl, 2003). A by-product of this process of extracellular digestion can be surplus inorganic N. The enzymes involved in decomposition react to changes in temperature according to Arrhenius principles. This was confirmed by landmark studies in the early 1970s that established the relationship between temperature and net N mineralizaton in laboratory microcosms (Stanford et al., 1973; Stanford et al., 1975). In these studies temperature effects on net N mineralization were modeled using an Arrhenius function, with a doubling of net N mineralization rates for every 10°C increase in temperature. Since these studies, Arrhenius equations have frequently been used as temperature response functions in models of decomposition (Addiscott, 1983; Ellert and Bettany, 1992; Van Schöll et al., 1997; Reichstein et al., 2000).

Honeycutt et al. (1988) proposed an empirical approach to incorporating temperature effects into field estimates of N availability. In this approach, time is expressed as soil thermal units (STU), calculated as the sum of the average daily soil temperature greater than 0°C. In the soil thermal unit approach it is assumed that N mineralization occurs at the same rate per STU regardless of the actual temperature (the rate constant k in the first-order equation is expressed in units of STU^{-1}). This eliminates the need to determine the relationship between temperature and the rate of N mineralization. The soil thermal unit method has proven to be effective for predicting N mineralization from a variety of organic amendments in the field (Honeycutt and Potaro, 1990; Griffin and Honeycutt, 2000; Griffin et al., 2002). The STU approach could be

useful for predicting the seasonal dynamics of N release. Historic data on temperature variability during the growing season could be combined with information about the rate of N release from a soil or added organic amendment, per STU, to predict the timing of N supply in a typical growing season.

Soil moisture is another key parameter controlling N mineralization. The soil organisms involved in decomposition require water to function. Bacteria are essentially aquatic organisms living in water films on the surface of soil particles or in the soil solution, while it is generally recognized that fungi can function at lower water potentials (Griffin, 1981; Kilham, 1994). Protozoa and nematodes are dependent on the existence of free water or water films to survive. When soil moisture drops below optimum levels, the activity of the soil food web is reduced. Drury et al. (2003) defined the non-limiting water range (NLWR) as the range of water contents where biological processes are independent of water content. This range can be quite broad: Drury et al. (2003) reported a NLWR that spanned 40 percentage points of water filled pore space (WFPS,42 to 82%) in a compacted loamy sand. In the same study, a clay loam soil amended with red clover had a NLWR that spanned only 5% WFPS (89 to 94% WFPS). While decomposition processes are not limited within the NLWR, oxygen may become limiting at the upper end of the range so that denitrification predominates. Consequently for some soils, optimum conditions for N mineralization may overlap with conditions ideal for denitrification. If water contents fluctuate around the upper range of the NLWR, then repeated cycles of ammonification (the conversion of organic N to ammonia) followed by nitrification (the conversion of ammonia to nitrate) and then denitrification (the conversion of nitrate to different forms of N gas), can lead to losses of available N from

the system. Matric potentials between -0.01 and -0.03 MPa are considered optimal for estimating mineral N accumulation (Campbell et al., 1988) because they are at the lower range of optimal N mineralization moisture contents, and therefore losses from denitrification may be minimized in this range. At the drier end of the spectrum, fluctuations in soil moisture i.e. the drying-rewetting effect, can cause higher net N mineralization than maintenance of soil moisture at a constant level, due to the so-called "flush" in mineral N that results from the die off of the microbial biomass under dry conditions (Cabrera, 1993).

Which factor predominates?

Studying N dynamics in the field is challenging because it is difficult to determine how nitrogen mineralization potential and environmental factors are interacting to affect plant-available N. Geographical scale is an important factor to consider. Litter decomposition studies have shown that climate exerts the strongest influence on decay rates between geographic regions (Insam, 1990), but locally, litter chemistry (substrate quality and quantity) becomes a more important driver of decay rates (Swift et al., 1979; Adl, 2003). This implies that within a given climatic region, an estimate of N mineralization potential alone should provide a good indication of relative differences in plant-available N between sites in a growing season.

The effect of climatic variation on plant-available N was demonstrated in a study by Fox and Piekielek (1984) in which they failed to find a statistically significant correlation between N availability determined by an anaerobic incubation method, and crop N uptake at 67 sites throughout Pennsylvania ($r = 0.31$). A suite of other chemical indices of N mineralization potential were also not well correlated with crop N uptake,

although some (e.g. total soil N) were weakly correlated with N estimated by the anaerobic incubation method ($r = 0.79$). Although experimental sites of Fox and Piekielek's were all within one state and involved similar soil types (silt loam), environmental and topographical variation had a greater effect on crop N uptake than N mineralization potential. The same researchers had better results in a later study that included a number of heavily manured sites and thus had a greater range of N mineralization potential (Hong et al., 1990). In this later study, both soil NO_3 and the 200 nm absorbance of a $NaHCO_3$ soil extract, were moderately well correlated with crop N uptake. These findings indicated that both N mineralization potential and climatic factors influenced N availability within the geographical range of the study (i.e. the state of Pennsylvania)..

In contrast with the Pennsylvania studies, Haney et al. (2001) set up a field experiment in which environmental and soil variability were minimized (only one site was included), and the size of the soil N pool was varied by applying dairy cattle manure at a range of rates from 0 to 448 kg N ha^{-1}. Not surprisingly, they found very good correlations between a laboratory index of N mineralization potential (CO_2 released during 24 hours incubation after rewetting an air dried soil), and both crop N uptake, and N mineralized during a laboratory incubation. Their research demonstrates that when variation due to soil type and environment is removed, the size of the substrate pool becomes the dominant factor determining N availability.

2.1.2 Study Objectives

The objectives of this study were:

1. To identify the laboratory nitrogen availability index that is most closely correlated with potentially mineralizable N (N_0) estimated using the Stanford and Smith (1972) long term incubation (leaching tube) method.

2. To correlate potentially mineralizable N (N_0) with a field estimate of plant-available N at five sites in Nova Scotia.

3. To study the impact of soil moisture and temperature on plant-available N at five sites in Nova Scotia.

2.2 Materials and Methods

2.2.1 Site Description and Management

Five sites were selected in the fall of 2002, all with a long history of cattle manure application. Four sites were located on commercial dairy farms, and the fifth was located at the Nappan Experimental Farm - a beef research unit. Crop rotations in all fields consisted of one or two years of corn followed by three to five years of mixed grass/legume forage. It was assumed that substrate quality and quantity at these different sites would be relatively uniform compared with the more intensive, non-livestock horticultural and cash crop farms in the province. The attempt to minimize differences in substrate quantity and quality was done so that an assessment of the environmental influence on N availability in the field could be made. Sites were located in different climatic regions of the province to maximize potential differences in temperature (Table 2-1). At each site a 10 m by 30 m plot in each field was selected and no manure or inorganic fertilizer was spread in this area in the fall of 2002 or during the spring of 2003.

This was to allow an estimate of the availability of N from the pool of native soil N present at the time of corn planting in the 2003 season.

Silage corn was planted at all sites in the spring of 2003. Black plastic horticultural mulch (IRT 76) was used to alter soil temperature and moisture conditions in a 3 m x 4 m area (mulched treatment) within each 10 x 30 m plot at each site. This material was laid down between the corn rows when the corn plants were 7.5 cm in height (3 weeks post planting). An Em5 data logger (Decagon Devices, Inc., Pullman, WA) equipped with two ECH_2O soil water sensors (Decagon Devices, Inc., Pullman, WA), and three thermistors for measuring soil and air temperature were used to monitor soil temperature and moisture on an hourly basis in both mulched and exposed areas throughout the growing season. In addition, air temperature and precipitation (using a Decagon automated rain gauge) were measured at each site. Site details and experimental management information are listed in Table 2-1 and Table 2-2 respectively.

2.2.2 Soil Sampling and Analysis

In the spring of 2003, one composite soil sample (10 cores, 0 - 15 cm) was collected from each of four randomly selected locations within the 10 by 30 m area at each site. Soil samples were taken on 30 April 2003 at all sites except Nappan, which was sampled on 1 May 2003. A composite soil sample (20 cores, 0 - 15 cm) from the 10 by 30 m area at each site was also collected on the same day that the corn was harvested (Table 2-2). All samples were stored at 5°C for no more than 24 h before air-drying at room temperature (22°C) and sieving (2 mm) prior to analysis. This was done to comply with practices that are typical for the handling and analysis of soil samples in commercial soil testing labs.

Bulk density cores (3 inch diameter, 8 per site) were collected in the fall of 2003 after corn harvest and moisture retention curves were determined using the pressure extractor technique (Sheldrick, 1984).

2.2.3 Laboratory Indices of N Availability

A series of laboratory indices of N mineralization potential were determined using the soil samples collected in the spring of 2003.

1. *KCl-extractable $NO3^-$-N and $NH4^+$-N* Soils were extracted with 2 M KCl (10g soil:100 ml extractant), shaken for one hour and filtered by gravity through Whatman No. 42 paper. Extract concentrations of NO_3^--N and NH_4^+-N were determined using a Technicon TRAACS 800 as described in Zebarth and Milburn (2003)

2. *Hot KCl-extractable $NH4^+$-N* was measured by heating 9 g soil with 60 ml 2 M KCl on a digestion block set at 100°C for 4 hours followed by filtration and analysis for NH_4^+-N as described above (Gianello and Bremner, 1986a). KCl-extractable NH_4^+-N was subtracted from the total hot KCl-extractable NH_4^+-N, to determine net hot KCl-extractable NH_4^+-N.

3. The *Illinois Soil N Test (ISNT)* was conducted using the direct diffusion method described by Khan et al. (2001). Briefly, 1 g of air dry soil was placed in a 500 ml mason jar and mixed with 10 ml of 2 M NaOH. The jar was sealed immediately using a lid with a boric acid indicator trap (4% H_3BO_3, bromocresol green, methyl red) suspended below it in a 5 ml Petri dish. The sealed jar was kept at a temperature of 48-50°C for 5 hours. The quantity of NH_4^+-N trapped in the boric acid trap was determined by titration with standardized 0.01 M H_2SO_4 and expressed as mg N kg^{-1} soil.

4. *CO_2 evolved in a 24 h incubation after rewetting air dry* soil was used as another index of soil N mineralization potential (Haney et al., 2001). A mass of 20 g air dry soil was placed in a 500 ml mason jar and wetted to 55% WFPS with distilled water. Water filled pore space was determined by estimating the bulk density of loosely packed soil in a graduated cylinder and calculating pore volume using a particle density of 2.65 g ml^{-1}. A CO_2 trap containing 5 ml of 1 M KOH was suspended from the inside of the lid of the jar. The sealed apparatus was placed in a 25°C water bath and incubated for 24 h before titrating with standardized 0.6 M HCl to determine the amount of CO_2 evolved, expressed in units of mg CO_2-C kg^{-1} soil.

5. The *chloroform fumigation extractable C (CFE flush C)* was determined as an index of soil microbial biomass (Vance et al., 1987). Duplicate sub-samples of moist, 4 mm sieved soil (20 g) were weighed into 125 ml French square bottles. The extracted organic C (EOC) in one sub-sample of each soil was immediately extracted with 50 ml of 0.5 M K_2SO_4. The second sub-sample of each soil was subjected to fumigation with chloroform under vacuum and incubated at 22°C for 24 hours before extraction of DOC as described above. The concentration of EOC in the K_2SO_4 extracts was determined using a Technicon Autoanalyzer II.

6. *Organic C and total N* were determined by dry combustion (LECO CNS-2000, LECO Corporation, St. Joseph, Michigan).

Soil pH was measured using a 1:1 soil:water suspension and particle size distribution was determined by the hydrometer method (Day, 1965). Laboratory analysis for the ISNT, 24 h CO_2 evolution and hot KCl-extractable NH_4^+-N were conducted on

each field sample in triplicate and chloroform fumigation extraction was performed in duplicate. Mean values of the laboratory replicates were used for each field sample. Prior analysis of KCl-extractable NO_3^--N and NH_4^+-N and LECO C and N had shown little variability between laboratory replicates of the same sample, so only one analysis per field sample was performed for these indices.

2.2.4 Nitrogen Mineralization Potential

Stanford and Smith (1972) originally used the leaching tube method to determine the maximum potentially mineralizable nitrogen in soils. A leaching tube apparatus accommodating the modifications described in Campbell et al. (1993) was used for the incubations. Tubes for three replicates of each of the four field samples from the five sites were set up (a total of 60 tubes). For each tube 30 g of air-dry soil was mixed with 30 g of sand, except for the Windsor soil which had more sand added due to its finer texture (20 g soil, 40 g sand). An initial leaching was conducted to remove any inorganic N. This leaching was carried out under vacuum (18 mPa) with 100 ml of 0.01 M $CaCl_2$ applied in 25 ml aliquots followed by a 25 ml aliquot of N-minus solution (Campbell et al., 1993). Soils were incubated at a temperature of 20°C and a relative humidity of 98% and further leachings were conducted on days 14, 28, 42, 57, 70, 98, 126, 154, 182, 210, 238, 266, 294, 322 and 379. All leachates were stored at -20°C until analysis for NO_3^--N using the cadmium reduction method and NH_4^+-N using the salicylic acid method on the Technicon Autoanalyzer II.

Curves were fit to the N mineralization data for each leaching tube separately. Incremental models were used in the curve-fitting process because they eliminate the additive error that results when each data point is the sum of all previously measured data points (Ellert and Bettany, 1988, Table 2-3). The N mineralized in the first 14 day

period (N_e) was not used for curve fitting because the excessively high N mineralization during this period (Figure 2-1) would result in an overestimation of the first-order rate constant. Stanford and Smith (1972) found a higher degree of precision in estimates of N_0 obtained by excluding the N mineralized from their leaching tubes during the first two weeks of the incubation. N_e represents the pool of N made available by the rewetting of previously air-dried soil (Stanford and Smith, 1972). Nitrogen mineralized in each measurement period from 28 days onwards (i.e. the incremental data) was fit to the incremental models for the single-pool first-order (SPE) and two simultaneous reactions (TSR, Ellert and Bettany, 1988) N mineralization models (Table 2-3). The TSR model is described by Ellert and Bettany (1988) and consists of a single pool of mineralizable substrate that is mineralized simultaneously at two different rates. While this model only includes a parameter estimate for one pool (N_0), the division of this pool into two fractions that mineralize at different rates, makes it essentially a two pool model.

Parameters were estimated using the FITNONLINEAR directive in GenStat 8th edition (VSN International Ltd, Rothamsted, UK). The relative rate constant (k) was constrained within the range 0.001 to 0.1 d^{-1} for the SPE model. For the TSR model the relative rate constant of the fast pool (k) was constrained within the range 0.001 to 0.1 d^{-1} while the relative rate constant of the slow pool (h) was constrained within the range 0.0001 to 0.01 d^{-1}. The extra sum of squares F test (F_{extra}) was used to assess the most effective approach to curve fitting for each site and model (Dalias et al., 2001). This statistic is calculated by:

$$F_{extra} = [(SS1-SS2)/(df1-df2)]/(SS2/df2)$$

where SS1 and SS2 refer to the residual sum of squares of the more complex (TSR) and simpler (SPE) model respectively. This statistic tests whether the reduction in the residual sum of squares achieved by adding an additional parameter to the model is statistically significant. After selecting the model which provided the best fit to the majority of samples for each site based on the significance of the F_{extra} statistic, curves were fit again using a constant value for the first-order rate constant. This was done to reduce the standard errors of the N_0 estimates (Motulsky and Christopoulos, 2004). N_0 estimates were added to N_e to generate a value for the total potentially available N (TPAN) content of the soil (Stanford and Smith, 1972).

2.2.5 Field Estimation of N Availability (PAN)

A plant bioassay method (PAN) was used to estimate N availability in the field (Haney et al., 2001; Zebarth et al., 2005). Three randomly selected 6 m lengths of corn row from the exposed treatment of the 10 by 30 m plot at each site were harvested at ground level using hand pruners. Three randomly selected 6 m lengths of corn row were also harvested in the same way from the mulched 3 x 4 m area at each site. The fresh weight of corn was determined and sub-samples were retained for determination of dry matter content. Corn dry matter yield (above-ground biomass) was calculated. Dried samples were ground to pass a 20 mesh (0.85 mm opening) sieve and analyzed for total N content by dry combustion (LECO CNS-2000, LECO Corporation, St. Joseph, Michigan). PAN for each site was calculated as:

$$PAN\ (kg\ ha^{-1}) = N_c + N_{if} - N_{is},$$

where N_c is the above-ground crop N content at harvest, N_{if} is the soil inorganic N content at crop harvest, and N_{is} is the soil inorganic N content at crop planting[1]. For sites where starter N fertilizer was applied at planting (Noel 1, Noel 2 and Musquodoboit) 75% of the total N applied was subtracted from the crop N content to account for this N source (Fox and Piekielek, 1984).

2.2.6 Accounting for Temperature Effects on N Mineralization

Hourly soil temperature readings were summed and divided by 24 to calculate STU (Honeycutt et al., 1988). Daily maximum and minimum air temperatures were used to calculate corn heat units (CHU) accumulated at each site (Bootsma, personal communication).

2.2.7 Statistical Analysis

Data was analyzed using MINITAB® Release 14 (Minitab Inc., State College, Pennsylvania) and GenStat 8th edition (VSN International Ltd, Rothamsted, UK). Site differences between parameter estimates and laboratory indices of nitrogen mineralization potential were analyzed using a one-way ANOVA with site as the main effect. Least square means were computed for each site and compared using Tukey's method which compares all possible pairs of means using a family error rate ($p=0.05$). Pearson correlation was used to examine the relationship between the estimated N mineralization parameters and laboratory indices of nitrogen mineralization potential for each field sample ($n=20$).

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[1] For Musquodoboit, Noel 2 and Windsor inorganic N content of April 30 soil samples was used for N_{is}. For Noel 1 soils used were collected on 12 June and at Nappan soil samples used were collected on 18 June.

2.3 Results

2.3.1 Measurements of Nitrogen Mineralization Potential

Laboratory indices

The site mean and coefficient of variation (CV) for each of the laboratory indices of nitrogen mineralization potential are shown in Table 2-4. There is a wide range of within field variability among the indices with relatively low variability for organic C, total N and ISNT values compared with very high variability for hot KCl-extractable NH_4^+-N. The high variability for some measures is reflected in the lack of detectable differences among the sites for those indices. There were no significant differences among the sites for hot KCl-extractable NH_4^+-N and KCl-extractable NH_4^+-N. KCl-extractable NO_3^--N, although it had a high CV (25%) still separated the sites into three groups, with the Windsor and Musquodoboit sites having the lowest value for this index and Noel 2 having the highest value. Organic C separated the sites into three groups with Windsor having the lowest value, Nappan, Musquodoit and Noel 1 having intermediate organic C contents, and Noel 2 having the highest organic C content. Site groupings for total N were similar to organic C. For most of the indices the Noel 2 site had the highest value, except for 24 h CO_2 evolution which was highest at the Nappan site.

Curve fitting

The goal of the curve fitting exercise was to obtain the best estimates of N_0 in each field sample. For four of the five sites the single-pool first-order model provided the best fit to the incremental data in the majority of tubes (10 out of 12 tubes at the Noel 1 and Noel 2 sites and 9 out of 12 tubes at the Nappan and Musquodoboit sites). For 10 out of the 12 tubes from the Windsor site the TSR model was superior to the single-pool first-order model. The fit of the models was also judged based on the standard errors (SEs) of

the parameter estimates. For the single-pool first-order model in many cases these errors were very high (Table 2-5). Field sample 2 at the Musquodoboit site had a SE of 41% of the value of the estimate for N_0. Standard errors of the relative rate constants were even higher with values greater than the estimates in some cases (e.g. Musquodoboit, field sample 1 and 2). Parameter estimates with this much variability are of little value. In order to reduce the variability of the estimates using the single-pool exponential model, the relative rate constant was constrained to a constant value. This is justifiable since an ANOVA of the response variable k indicated that there was no significant difference in this parameter estimate among the sites ($p=0.802$). The overall mean value of $k=0.004$ from the first iteration was used in the second iteration of the single-pool first-order model. Parameter estimates for N_0 with k constrained to this value had much lower SEs with a maximum for field sample 4 from the Musquodoboit site of only 17%. These estimates of N_0 were used in the calculation of TPAN. A value for N_0k, as recommended by Griffin and Laine (1983), was also calculated using the parameter estimates from the unconstrained first iteration of the single-pool first-order model.

The SEs of N_0 parameter estimates at the Windsor site using the TSR model ranged from 17 to 29% of the parameter values (Table 2-6). Standard errors of the other three parameters in the model were high with the SE of the a parameter as high as 55% of the parameter value in field sample 4. Constraining one of the parameters and refitting the curves did not improve the reliability of the parameter estimates. Problems with the curve fitting process were encountered including parameters going out of bounds and failures to converge. It was assumed that the N_0 estimates generated from the TSR curve fitting method with no parameters constrained were the best estimates of N_0 at the

Windsor site. These values were used to determine TPAN for these samples. A value for N_0k was not determined for the Windsor site.

A one-way ANOVA indicated that N_0 was highest at Noel 1, Noel 2 and Windsor while N_e was lowest at the Windsor site (Table 2-7). There was a broad range of TPAN estimates within each site, although Noel 1 had higher estimates and less variability than the other four sites (Figure 2-2). N_0k was highest at the Noel 1 site and lowest at Musquodoboit, with intermediate values for Nappan and Noel 2.

2.3.2 Correlations of Laboratory Indices with Mineralization Parameters

The mean values for N_0, N_e, N_0k and TPAN for each field sample at each site were correlated with the laboratory measures of nitrogen mineralization potential. The results of these correlations are shown in Table 2-8. None of the indices had a significant correlation with N_0. N_e was positively correlated with organic C, total N, and ISNT values, as well as being positively correlated with the sand content of the soil and negatively correlated with silt content. Total potentially available N was correlated with KCl-extractable NO_3^--N and NH_4^+-N. N_0k was positively correlated with KCl-extractable NH_4^+-N and negatively correlated with silt content. The relationship between N_0k and KCl-extractable NH_4^+-N is illustrated in Figure 2-3, which shows the wide range of values within each site for measured NH_4^+-N and the N_0k parameter estimate.

2.3.3 Predictors of PAN

Mulching increased corn yields compared with the exposed treatments at all sites except for Windsor; however, it did not result in a significant increase in PAN estimates (Table 2-9). At the Nappan and Noel 2 sites, although corn yield was higher for mulched treatments, the N content of the harvested corn was higher in the exposed treatments, which resulted in higher PAN estimates in exposed treatments. Both Noel sites had very

high soil nitrate levels at crop harvest (data not shown) which contributed to high PAN estimates.

Plots of N_0k and TPAN versus PAN are shown in Figure 2-4 and Figure 2-5. Although there are too few points to plot a meaningful regression line, these plots show that both estimates of N mineralization potential separate the sites into two distinct groups, with both Noel sites having higher in-field estimates of PAN and higher laboratory measures of N mineralization potential. For all sites except Windsor, N mineralization potential appears to be a strong predictor of PAN. The Windsor site is a separate case, with an intermediate value of N mineralization potential estimated in the laboratory, but very low in-field estimates of N availability.

Sites with more soil thermal units accumulated during corn growth, tended to have higher PAN (Figure 2-6). Within each of the Noel sites, there was a trend towards higher PAN under mulch where more soil thermal units accumulated. For the sites with PAN estimates of less than 100 kg N ha^{-1}, however, there was no relationship between PAN and soil thermal units. For these sites there was a trend towards decreasing PAN with increasing average daily soil moisture (Figure 2-7).

2.3.4 Environmental Variables

The horticultural mulch (IRT 76) increased the average daily soil temperatures only slightly, by as little as 0.3°C on average at the Musquodoboit site, to 1.3°C on average at the Noel 2 site (Figure 2-8 to Figure 2-12). The mulch effect on average daily soil temperature was significant at all sites (Table 2-10). The number of STUs accumulated during corn growth was also significantly higher in the mulched treatments. There were no significant differences among sites in mean soil temperature or STUs.

Corn heat units accumulated during corn growth were highest at the Noel 2 site and lowest at the Musquodoboit site.

Key moisture contents for each site determined by soil moisture retention analysis are shown in Table 2-11. Moisture contents between -0.01 and -0.03 MPa are considered optimal for N mineralization (Campbell et al., 1988). These values are all higher than the 30 to 60% WFPS range, which has also been proposed as non-limiting for N mineralization (Griffin et al., 2002).

The average daily soil moisture varied among the sites. Although mulch treatment did not have a statistically significant effect on mean soil moisture contents, at the Musquodoboit and Windsor sites, means were numerically higher under the mulch (Table 2-12). This is also evident from the graphs of mean daily soil moisture contents (Figure 2-13 to Figure 2-17). At Nappan and Noel 2, mulch had the opposite effect with numerically lower soil moisture contents under the mulch compared with the exposed soil. At the Noel 1 site the moisture probe in the mulched area did not function reliably and the results are not presented here.

The Windsor site had the highest average daily soil moisture values, although a malfunction of the rain gauge does not allow me to report a figure for precipitation for the season. Precipitation at a nearby Environment Canada weather station (Summerville) for the months from May to August 2003 was 303 mm. This is similar to the May – August 30 year normal for that weather station of 301 mm. The high soil moisture values at the Windsor site were also related to its location on a low-lying dykeland area, in contrast to the upland locations of the other four sites.

The average daily soil moisture value for the exposed treatment at Noel 1 was next highest after the Windsor site. Precipitation at the Noel 1 site was higher than the other four sites, even compared with Noel 2, which was less than 2 km away. The proximity of Noel 1 to the coast could have been the reason for the increased frequency of precipitation at this site. A comparison of Figure 2-15 and Figure 2-16 shows that Noel 1 experienced more rainfall events than Noel 2, and that even when rain fell on the same day, higher volumes of rain fell at the Noel 1 site than Noel 2. The soil moisture data confirms the differences in rainfall.

Precipitation data from Environment Canada weather stations near to the study sites (apart from Windsor) indicates that 2003 was a dry year. Rainfall recorded in Truro (near Noel 1 and 2), Nappan and Middle Musquodoboit, between May and August 2003 was lower than the 30-year normals in every case. For example, in Middle Musquodoboit 413 mm of precipitation were recorded between May and August in 2003, while the 30-year normal for these months is 516 mm. In all cases the precipitation recorded at the Environment Canada weather stations in 2003 was higher than the values I recorded with the data loggers. This suggests to me that the rain gauges were not very reliable. Nevertheless, 2003 was a drier than normal year at four of the five sites. Only the mulched treatment at the Windsor site experienced any days between -0.01 and -0.03 MPa; however, all sites experienced some days when soil moisture was between 30 and 60% WFPS. At all sites except for Musquodoboit, moisture conditions were between 30 and 60% WFPS on more days in the exposed than the mulched treatments. At Noel 2 and Nappan, mulched treatments were drier so they were more frequently below 30% WFPS. Conversely, at Windsor the mulched treatments were wetter, and the soil was

usually at a moisture content greater than 60% WFPS. None of the sites had moisture contents between 30 and 60% WFPS for the whole growing season. The Noel 1 exposed treatment had the most days (112 of 124 days between corn planting and harvest) at optimum soil moisture. The Windsor and Musquodoboit sites, which had the lowest PAN, also had the lowest number of days at optimum soil moisture. Moisture contents were between 30 and 60% WFPS for only 8 of the 108 days between corn planting and harvest at the Windsor site (mulched treatment); the rest of the time moisture contents were higher. At the Musquodoboit site moisture contents were between 30 and 60% WFPS for 33 of the 111 days of corn growth in the exposed treatments; on most of the other days soils were drier than 30% WFPS. These observations indicate that variations in soil moisture among sites may be a key factor driving among site differences in N availability in the field in Nova Scotia.

2.4 Discussion

2.4.1 Estimates of N Mineralization Potential

Potentially mineralizable N (N_0) estimated by the Stanford and Smith (1972) leaching tube method, is generally accepted as the most definitive means of measuring nitrogen mineralization potential (Carter and Macleod, 1987; Curtin and Wen, 1999). Constraining the relative rate constant k, to a constant value reduces the variability in N_0 (Motulsky and Christopoulos, 2004). In the current work, this approach reduced the standard error of the N_0 estimates; however, none of the laboratory indices of substrate quality or quantity were significantly correlated with these estimates of N_0. The indices selected for testing in this study have all been proposed as predictors of N availability to crops in a single growing season. The incubation in this study was conducted for 379 days at 20°C, and a total of 7580 STUs were accumulated during this period. This

represents a much longer time period for N mineralization and a higher accumulation of STUs than would occur in a typical growing season in Nova Scotia. In the field study that accompanied this laboratory study, the maximum number of STUs accumulated was 2309 (Table 2-10). It is possible that pools of N not normally accessed during a typical growing season in Nova Scotia, were mineralized during the incubation in this experiment, resulting in estimates of N_0 that were not correlated with laboratory indices that have been selected to predict mineralization of readily available pools of N in a single growing season.

The total potentially available N, a parameter that included easily mineralizable N (N_e) and N_0, was related to KCl-extractable NO_3^--N and NH_4^+-N from early spring samples. Griffin and Laine (1983) found N_0 was highly correlated to organic C and total N, but not to crop uptake of N in the field. They suggested using N_0k as a predictor of N availability in the field. This measure incorporates substrate pool size (N_0), and accessibility, which is represented by the rate constant k. The TPAN and N_0k parameter estimates in this study may be indicative of N mineralization from a pool of recently applied manure N. This would explain the high variability in this measure, which could be related to uneven spreading of manure. The factors more reflective of long term management (e.g. organic C and total N) and soil physical properties, varied less within a site.

Of the various parameters in this study, only KCl-extractable NH_4^+-N was highly correlated with N_0k. The range of KCl-extractable NH_4^+-N values was between 4 and 10 mg N kg^{-1}, but within this narrow range, there was still an increasing trend with increased N_0k values (Figure 2-3). This indicates that variability was as great within sites as

between sites for both KCl-extractable NH_4^+-N, and the N mineralization parameter estimates.

The high degree of variability within each site has practical implications for fertilizer N recommendations. It underlines the importance of taking a large number of samples from a field and mixing them to make a composite sample that will represent the average fertility of that field. This approach is suitable where fertilizers are applied at a given rate on each field. Alternatively, a precision farming approach which uses estimates of within-field variability in soil N supply to adjust fertilizer application rates, may be advisable. This finding also indicates that precision farming approaches may be particularly appropriate on farms with a history of manure application, where variability in the size of soil N pools may have developed as a result of this management.

The ISNT, organic C and total N were all correlated with N_e. In this study the amino sugar-N measured using the ISNT represented a readily-available pool of N that was the same proportion of the SOM at four of the five sites (equal to 0.01, except at Windsor, where the proportion was 0.02). The ISNT was developed as a tool to identify soils that would not be responsive to additions of N fertilizer, using a group of soils with similar amounts of total hydrolysable N (Khan et al., 2001). Since the soils in my experiment could already be separated based on total N contents, there would be no advantage to using the ISNT for this group of soils. Comparing the ISNT values for my soils to the values reported by Khan et al. (2001), the Windsor site would have fallen into the group that responds to N fertilizer, while the other four sites would have been identified as non-responsive (test values >237 mg N kg^{-1}).

The lack of correlation between most of the laboratory indices of nitrogen mineralization potential and the N mineralization parameters in this study, could be partially due to the small range in nitrogen mineralization potential represented by these five manure–amended soils. Estimates of N_0 for each site ranged between 82 and 187 mg N kg^{-1}. Curtin and Wen (1999), found a correlation between hot KCl-extractable NH_4^+-N and both N_e and N_0k, but the N_0 estimates in their study ranged from 71 to 630 mg N kg^{-1}. Carter and Macleod (1987) found strong relationships between CFE flush N and N_0, but the soils in their study had mineralization potentials ranging from 44 to 247 mg N kg^{-1}. I expected CFE flush C to be one of the better predictors of potentially mineralizable N since it is usually included as a pool of soil N with a relatively fast turnover rate in soil N models (e.g. the active SOM in the Century model, Parton et al., 1987). The microbial biomass also catalyses the decomposition process (Van Veen et al., 1984; Fang et al., 2005a), and rates of decomposition in soils may be related to the size of the biomass. Fang et al. (2005a) have argued that the size of the biomass should be a rate-modifying factor in decomposition models, since soil basal respiration is closely related to changes in microbial biomass. However, in my study, CFE flush C had a relatively small range (48 to 135 mg C kg^{-1}) and was not correlated well with N mineralization parameters. Similarly, 24 h CO_2 evolution, which should be related to the potential of the decomposer community to metabolize substrate, was not correlated to any of the N mineralization parameters. A broader range of substrate qualities and quantities might have resulted in more spread in index and parameter values, and stronger relationships between indices and parameters.

2.4.2 Relationships Between PAN, N Mineralization Potential, and Environmental Variables

Litter decomposition rates are controlled by climatic factors on a regional scale, and substrate quality and quantity locally (Adl, 2003). In this experiment I selected five sites from across Nova Scotia in order to assess the variability in soil temperature, moisture and precipitation. According to the CHU designations within Nova Scotia, in a typical year the Musquodoboit site should have accumulated 1950 CHU compared with 2450 CHU at Windsor. In the 2003 season, however, temperature differences were not this great, and CHUs ranged from 2191 CHU at Musqodoboit to 2351 CHU at Noel 2. Since the sites that accumulated the most CHU and STUs, also had the highest estimates of nitrogen mineralization potential, it was not possible to determine which factor was driving differences in PAN among sites. From the mulched and exposed data at the Noel sites, we can see a slight trend towards higher PAN with higher STUs, but this trend was not apparent at the other three sites.

At the Windsor site higher values for nitrogen mineralization potential estimates did not result in higher PAN. Reduced PAN values at this site could be due to high average daily moisture levels. Since average soil moisture contents in both the mulched and exposed treatments at the Windsor site were greater than 60% WFPS, it is likely that denitrification limited available N (Elmi et al., 2005). At the other four sites average moisture contents were between 30 and 60% WFPS, and N availability should not have been limited due to water (Griffin et al., 2002).

Temperature may not be the primary factor driving differences in N availability among sites in Nova Scotia, but it should control the seasonal dynamics of N release at a given site. This is the thesis of the work presented by Honeycutt and his colleagues

(Honeycutt et al., 1988; Honeycutt and Potaro, 1990; Griffin and Honeycutt, 2000; Griffin et al., 2002) who are developing a system based on soil temperature expressed as STUs, to predict N availability from crop residues and livestock waste during the growing season. Incubating three different soil types under controlled temperature conditions, they found that cumulative nitrification from added swine slurry could be fit to a single pool first-order model, with time and temperature being expressed as a compound variable, STUs. The parameters of the model were specific to each soil type, indicating that the soil microbial community and/or physical characteristics (e.g. texture) became important factors controlling the mineralization process when temperature and substrate were removed as variables (Griffin et al., 2002).

This study highlighted the importance of soil texture in N mineralization. As expected and previously reported in the literature (El-Shinnawi et al., 1988), higher contents of sand had a positive impact on N mineralization, when it was estimated as TPAN, and silt reduced estimates of N mineralization (both N_e and N_0k).

2.5 Conclusions

Both environmental parameters and substrate quantity and quality (N mineralization potential) were shown to vary among the five sites in the study. Because of the limited number of sites in this preliminary study, it was not possible to draw any conclusions about which factors were driving among site differences. It was noted that within site variability was high for some of the better predictors of N mineralization potential (e.g KCl-extractable NO_3^--N and NH_4^+-N). Further studies are needed to determine the relative importance of soil temperature, compared with N mineralization potential and soil moisture conditions, in determining among site differences in N

availability. These studies should include a range of sites, and could use a multiple regression approach to determine the relative importance of these factors.

Information about how soils differ in their response to changing temperatures is necessary for predicting the seasonal dynamics of N availability. The research of Griffin et al. (2002) has shown that the temperature response of N mineralization is not only affected by the size and quality of the pool of N substrate. Other factors, including the microbial community and the effect of soil type, may be affected by temperature and ultimately determine the rate of N mineralization. Unraveling the interactions between substrate quality, the microbial community, and temperature, is the focus of the remaining chapters in this thesis.

Table 2-1. Locations, soil physical characteristics, pH, classification and corn heat units for five Nova Scotia sites used in a soil N mineralization study in 2003.

Site	Location	Sand[z] ($g\ kg^{-1}$)	Silt[z] ($g\ kg^{-1}$)	Clay[z] ($g\ kg^{-1}$)	Bulk density ($Mg\ m^{-3}$)	pH	Classification (Canadian system of soil classification)	Corn Heat Unit Zone
Musquodoboit	44°59'N, 63°6'W	393	428	178	1.3	5.8	Gleyed Brunisolic Gray Luvisol	1950
Nappan	45°3'N, 64°6'W	524	338	138	1.3	6.0	Orthic Humo-Ferric Podzol	2000
Noel 1	45°19'N, 63°43'W	519	313	168	1.2	5.5	Orthic Humo-Ferric Podzol	2250
Noel 2	45°18'N, 63°42'W	456	355	188	1.2	6.6	Orthic Humo-Ferric Podzol	2250
Windsor	45°45'N, 64°15'W	204	588	208	1.4	5.7	Gleyed Regosol	2450

[z] 0-15 cm depth; hydrometer method (Day, 1965)

Table 2-2. Experimental and crop management details for five Nova Scotia sites used in a soil N mineralization study in 2003.

Location	Previous crop	Corn hybrid	Starter fertilizer rate (kg N ha^{-1})	Planting date	Mulching date	Harvest Date
Musquodoboit	corn	Dekalb 2712	44	31 May	19 June	18 Sept.
Nappan	grass/legume forage	Pioneer 39w54	0	5 June	18 June	23 Sept.
Noel 1	alfalfa	Dekalb 2715	50	19 May	4 June	19 Sept.
Noel 2	corn	Dekalb 2715	37	23 May	12 June	22 Sept.
Windsor	corn	Pickseed 2565	0	10 June	26 June	25 Sept.

Table 2-3. Incremental models fit to N mineralization data from a 379 day incubation study of soils from five Nova Scotia sites in 2003.

Single-pool first-order (SPE)model	$N_{it} = N_0 e^{-kt} (e^{ki}-1)$	Where N_{it} is quantity of N released during interval i preceding time t, in units of mg N kg^{-1} soil, k is the first-order rate constant in units of d^{-1} and N_0 is the maximum potentially mineralizable N.
Two simultaneous reactions (TSR) model	$N_{it} = N_0 a e^{-kt} (e^{ki}-1) + N_0(1-a)e^{-ht} (e^{hi}-1)$	Where N_{it} is quantity of N released during interval i preceding time t, in units of mg N kg^{-1} soil and N_0 is the maximum potentially mineralizable N. There are two pools of mineralizable N which add up to N_0, fraction one is a with a rate constant k in units of d^{-1} and fraction two is $1-a$ with a rate constant h in units of d^{-1}

Table 2-4. Site means for laboratory indices of nitrogen mineralization potential for soils from five Nova Scotia sites in 2003.

Index	CV[z] (%)	Site Means[y]				
		Windsor	Nappan	Musquodoboit	Noel 1	Noel 2
Organic C (g kg^{-1})	8	12.5 c	24.8 b	24.0 b	26.0 b	32.5 a
Total N (g kg^{-1})	8	1.14 c	1.72 b	1.83 ab	2.06 ab	2.11 a
CFE flush C (mg C kg^{-1})	16	48.4 b	87.3 ab	96.4 ab	89.7 ab	135.1 a
24 h CO_2 evolution (mg C kg^{-1})	14	25.9 d	93.2 a	72.4 b	42.1 c	87.8 a
ISNT (mg N kg^{-1})	8	195 b	271 a	299 a	323 a	312 a
KCl-extractable NO_3^-N (mg N kg^{-1})	25	2.93 c	4.88 bc	4.32 c	7.88 b	11.9 a
KCl-extractable NH_4^+-N (mg N kg^{-1})	23	5.07	6.97	5.81	6.93	6.44
Hot KCl-extractable NH_4^+-N (mg N kg^{-1})	34	1.78	3.09	1.82	1.84	4.55

[z]CV is the mean of the coefficient of variation within each site (n=5); [y]Means in the same row followed by the same letter are not different at the 5% significance level using Tukey's HSD pairwise comparison of means test.

Table 2-5. Parameter estimates for the single-pool exponential model with both parameters varying and with the relative rate constant (*k*) constrained to a constant value, for soils from four Nova Scotia sites used in an N mineralization study in 2003.

Site	Field sample	Both parameters varying					$k = 0.004$ (d^{-1})		
		Number of lab replicates with successful model fit	N_0 (mg kg^{-1})		k (d^{-1})		Number of lab replicates with successful model fit	N_0 (mg kg^{-1})	
			Mean	SE	Mean	SE		Mean	SE
Musquodoboit	1	3	175	33	0.0027	0.0077	3	140	12
	2	3	186	76	0.0019	0.0098	3	117	13
	3	2	78	12	0.0071	0.0020	3	91	13
	4	3	94	21	0.0053	0.0022	3	101	17
Nappan	1	2	128	20	0.0034	0.0009	3	116	12
	2	3	95	18	0.0042	0.0011	3	82	8
	3	3	163	46	0.0056	0.0019	3	166	27
	4	3	129	35	0.0027	0.0009	3	99	10
Noel 1	1	1	66	11	0.0184	0.0052	3	141	21
	2	3	185	34	0.0034	0.0008	3	161	14
	3	3	200	33	0.0046	0.0011	3	187	19
	4	3	273	87	0.0018	0.0007	3	166	15
Noel 2	1	2	184	56	0.0027	0.0015	3	122	18
	2	3	206	54	0.0029	0.0008	3	159	14
	3	3	196	38	0.0032	0.0009	3	158	15
	4	3	154	26	0.0047	0.0013	3	162	20

Table 2-6. Parameter estimates for the two simultaneous reactions model fit to N mineralization data for soils from the Windsor site in an N mineralization study in 2003.

Field sample	N_0 (mg kg^{-1})		k (d^{-1})		h (d^{-1})		a	
	Mean n=3	SE	Mean n=3	SE	Mean n=3	SE	Mean n=3	SE
1	125	21	0.078	0.0071	0.0018	0.0004	0.31	0.032
2	180	37	0.079	0.0020	0.0015	0.0007	0.29	0.090
3	109	26	0.075	0.011	0.0026	0.0012	0.28	0.089
4	176	51	0.065	0.032	0.0022	0.0010	0.22	0.120

Table 2-7. Mean parameter estimates of N_0, N_0k, N_e, and TPAN obtained from the SPE or TSR models fit to data from an N mineralization study of soils from five Nova Scotia sites in 2003.

Site	N_0[z] (mg kg^{-1})	N_0k (mg kg^{-1} d^{-1})	N_e (mg kg^{-1})	TPAN (mg kg^{-1})
Musquodoboit	112 a	0.440 a	50 a	162
Nappan	116 a	0.478 ab	41 a	157
Noel 1	163 b	0.682 b	53 a	216
Noel 2	150 b	0.586 ab	39 ab	190
Windsor	148 b	-	25 b	172

[z]Means followed by the same letter in the same column are not different at the 5% significance level using Tukey's HSD pairwise comparison of means test; $n=12$

Table 2-8. Significance of the correlation between various laboratory indices or textural class, and N mineralization parameters, for soils from five Nova Scotia sites in 2003.

N mineralization parameter	Laboratory indices and soil textural classes										
	C	N	CFE flush	24 h CO_2	ISNT	KCl NO_3^-	KCl NH_4^+	Hot KCl NH_4^+	Sand	Silt	Clay
N_0 (n=20)	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
N_e (n=20)	0.439*	0.616***	ns	ns	0.689***	ns	ns	ns	0.648***	-0.677***	ns
TPAN (n=20)	ns	ns	ns	ns	ns	0.410*	0.415*	ns	ns	ns	ns
N_0k (n=16)	ns	ns	ns	ns	ns	ns	0.658***	ns	ns	-0.463*	ns

*, **, *** significant at P<0.1, 0.05, 0.01 respectively

Table 2-9. Corn above-ground biomass and N content, and PAN measured in exposed and IRT plastic mulched areas at each of five Nova Scotia sites in an N mineralization study in 2003.

Site	Treatment	Corn Yield (t d ha^{-1})	Corn N content (mg kg-1)	PAN (kg N ha^{-1})
Musquodoboit	Exposed	9.2	99	87
	Mulched	12.4	107	57
Nappan	Exposed	9.2	79.	76
	Mulched	10.9	102	82
Noel 1	Exposed	11.9	150	244
	Mulched	13.5	156	174
Noel 2	Exposed	14.7	120	182
	Mulched	16.8	142	208
Windsor	Exposed	5.0	98	30
	Mulched	4.1	82	37

Table 2-10. Mean soil temperatures, soil thermal units and corn heat units (CHU) accumulated during corn growth at each of five Nova Scotia sites in an N mineralization study in 2003.

Site		Mean daily soil temperature[z] (°C)		Soil thermal units between planting and harvest		CHU
		Exposed	Mulched	Exposed	Mulched	
Musquodoboit		18.1	18.4	2008	2033	2191
Nappan		18.2	18.9	2021	2093	2294
Noel 1		16.9	18.0	2087	2309	2325
Noel 2		17.4	18.7	2140	2295	2351
Windsor		17.5	18.0	1988	2060	2284
Analysis of variance						
Source	df		p		p	
Site	4		ns		ns	
Treatment	1		0.014		0.036	

[z]Mean soil temperatures are calculated from daily means measured between corn planting and harvest; exposed soil temperatures were used for the mulched treatment up until the day the mulch was placed in the field.

Table 2-11. Key soil moisture contents (cm cm^{-3}) and matric potentials (MPa) for soils from five Nova Scotia sites in an N mineralization study in 2003.

Site	30% WFPS	60% WFPS	-0.03 MPa	-0.01 MPa	-1.5 MPa
Musquodoboit	0.15	0.30	0.36	0.39	0.12
Nappan	0.16	0.31	0.32	0.36	0.10
Noel 1	0.17	0.33	0.34	0.36	0.08
Noel 2	0.16	0.32	0.33	0.37	0.08
Windsor	0.14	0.28	0.39	0.41	0.29

Table 2-12. Mean precipitation and soil moisture contents between corn planting and harvest at five Nova Scotia sites in an N mineralization study in 2003.

Site	Growing season length (days)	Precipitation (mm)	Average daily soil moisture ($cm\ cm^{-3}$)		Days between -0.01 and -0.03 MPa		Days between 30 and 60% WFPS	
			Exposed	Mulched	Exposed	Mulched	Exposed	Mulched
Musquodoboit	110	196	0.12	0.17	0	0	33	71
Nappan	110	67	0.16	0.15	0	0	55	52
Noel 1	123	260	0.22	NA	0	NA	112	NA
Noel 2	122	112	0.19	0.18	0	0	80	69
Windsor[z]	107	-	0.29	0.34	0	7	52	8

[z]Rain gauge malfunctioning at this site; therefore no total precipitation amount is reported.

Figure 2-1. Incremental N mineralization for all tubes in a 379 day incubation of soils from five Nova Scotia sites in 2003.

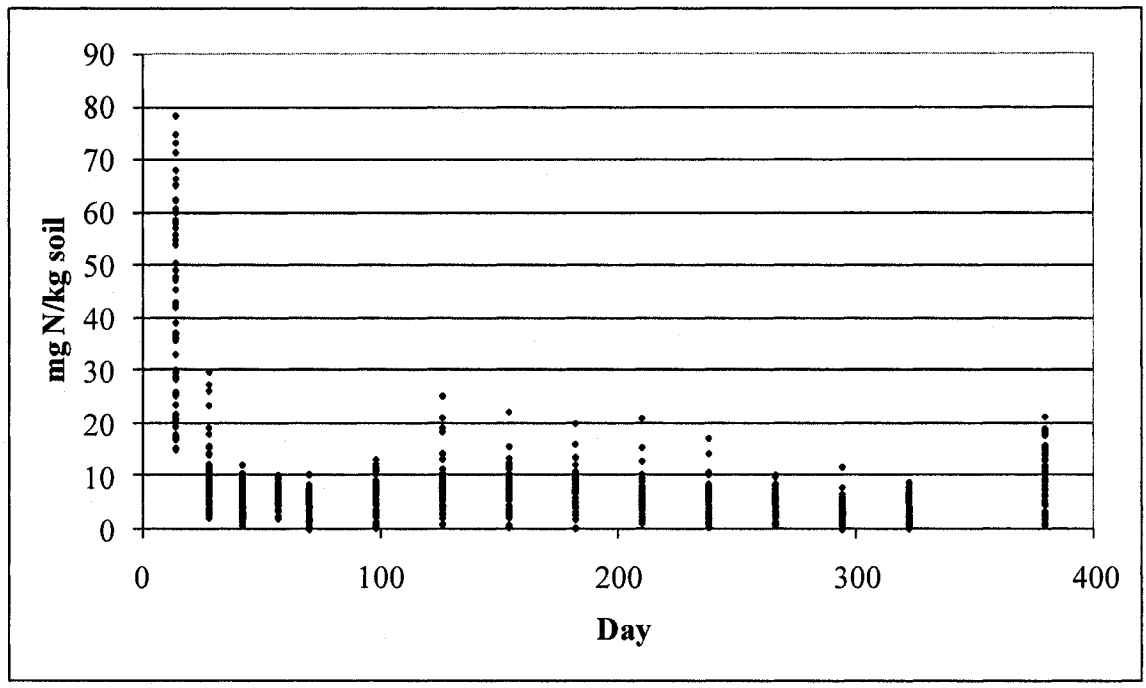
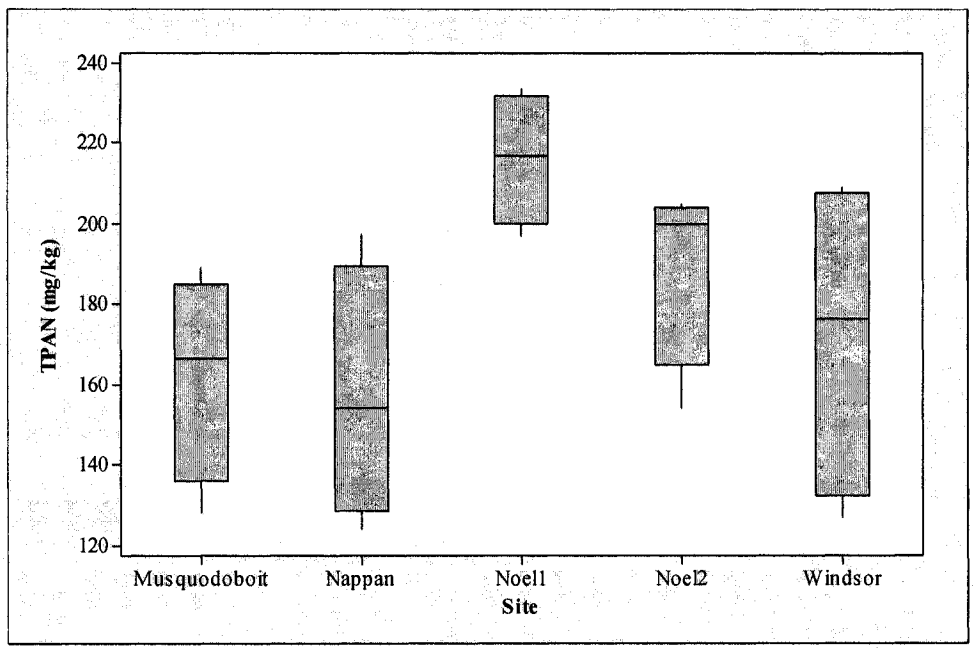


Figure 2-2. Boxplot of mean total potentially available nitrogen (TPAN) for soils from five Nova Scotia sites used in an N mineralization study in 2003.



n=4; The area within each box represents the middle 50% of the data, whiskers are the range of the data and the horizontal line in the box is the median.

Figure 2-3. Relationship between KCl-extractable NH_4^+-N and N_0k for soils from four Nova Scotia sites used in an N mineralization study in 2003.

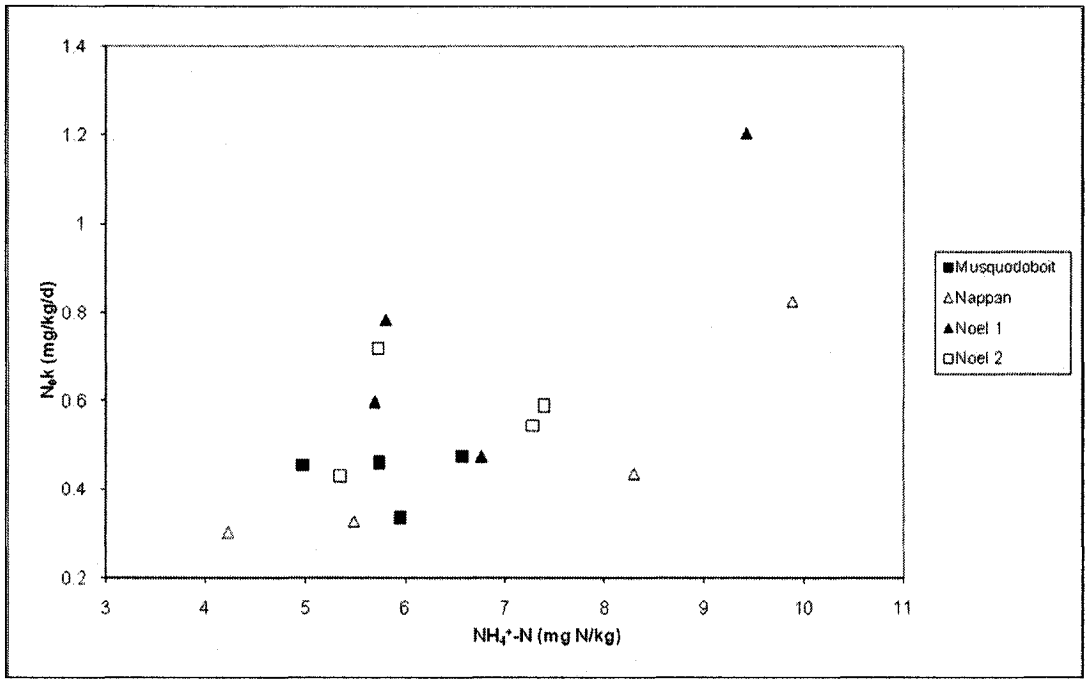
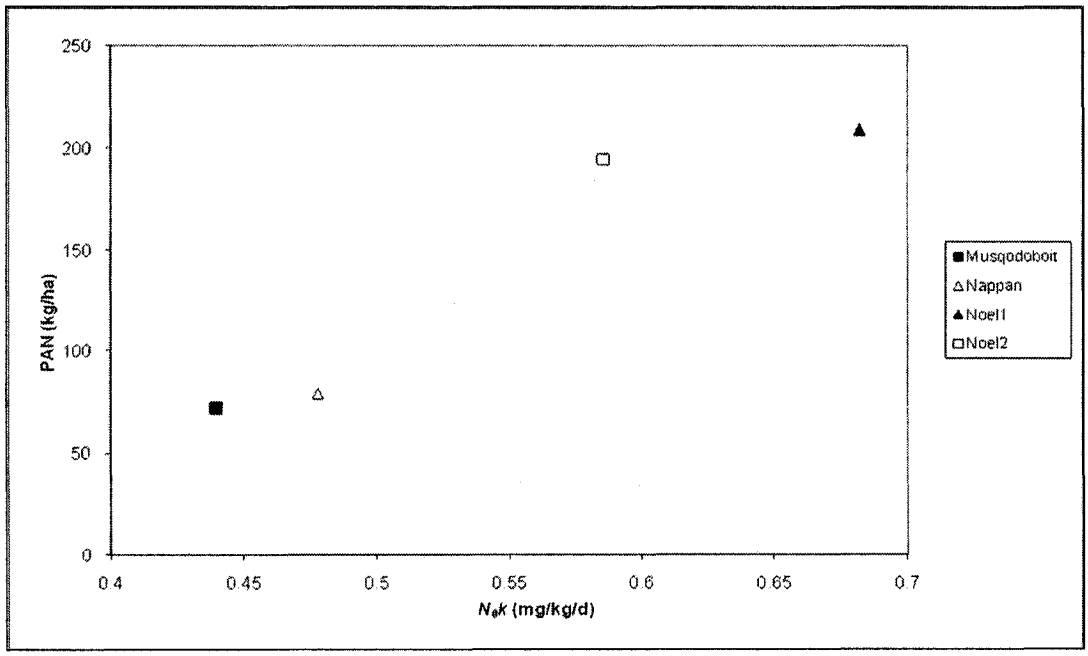
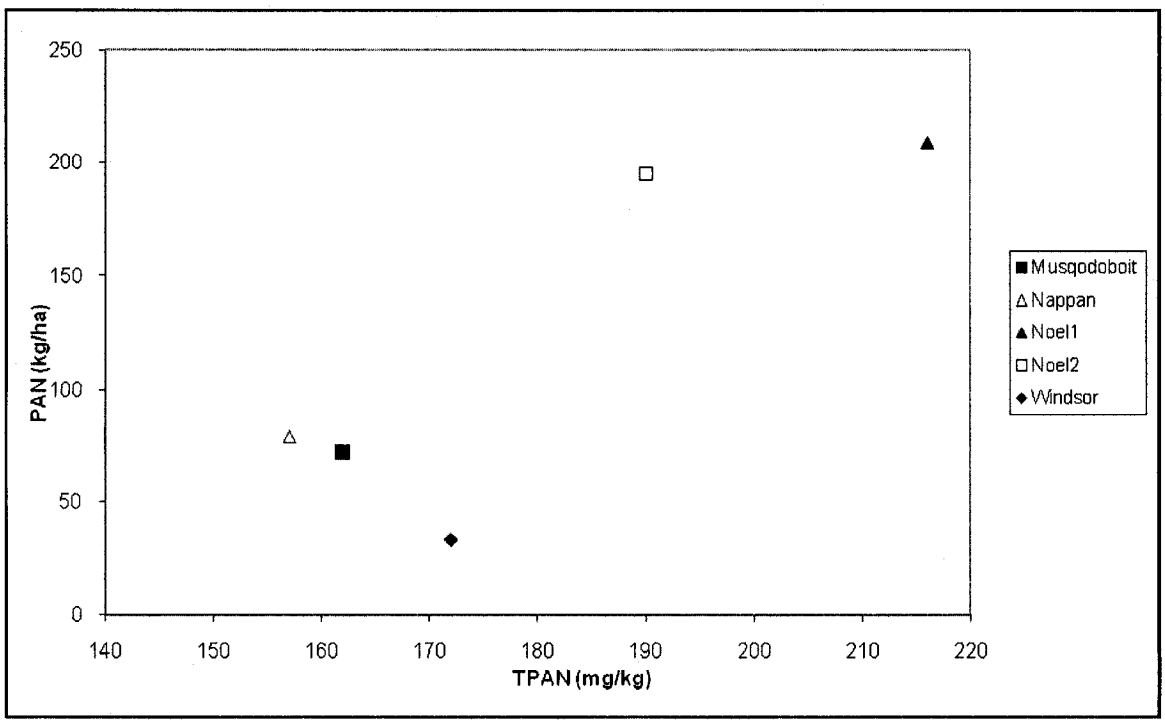


Figure 2-4. Relationship between N_0k and PAN at four Nova Scotia sites used in an N mineralization study in 2003.



.PAN estimated as the mean of mulched and exposed treatments.

Figure 2-5. Relationship between TPAN and PAN at five Nova Scotia sites used in an N mineralization study in 2003.



PAN estimated as the mean of mulched and exposed treatments.

Figure 2-6. Relationship between PAN and soil thermal units accumulated under mulched (M) and exposed (E) treatments at five Nova Scotia sites used in an N mineralization study in 2003.

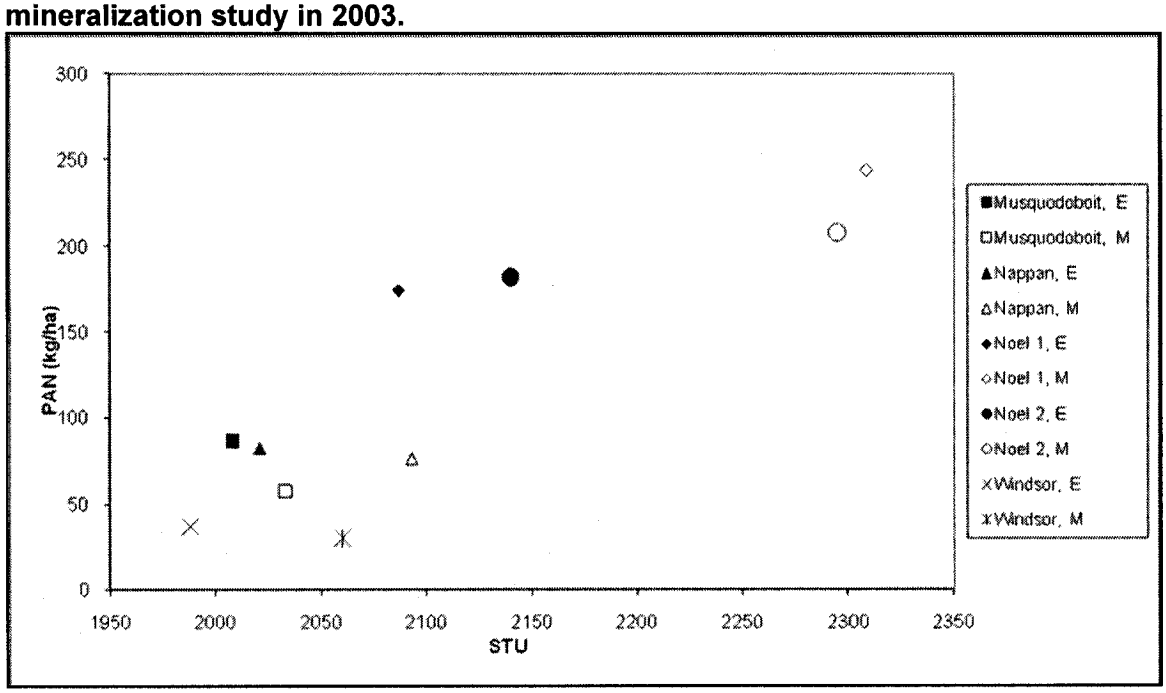


Figure 2-7. Relationship between PAN and average daily soil moisture under mulched (M) and exposed (E) treatments at five Nova Scotia sites used in an N mineralization study in 2003.

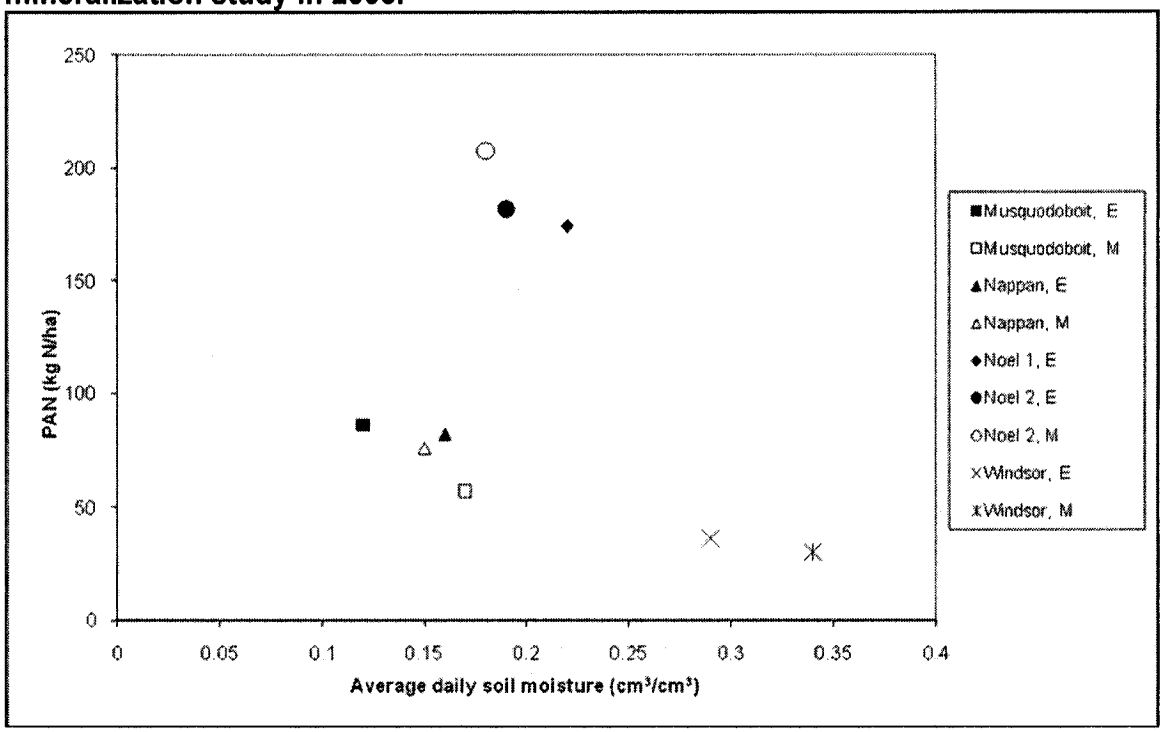


Figure 2-8. Mean daily soil temperatures between corn planting and harvest at the Musquodoboit site in an N mineralization study in Nova Scotia in 2003.

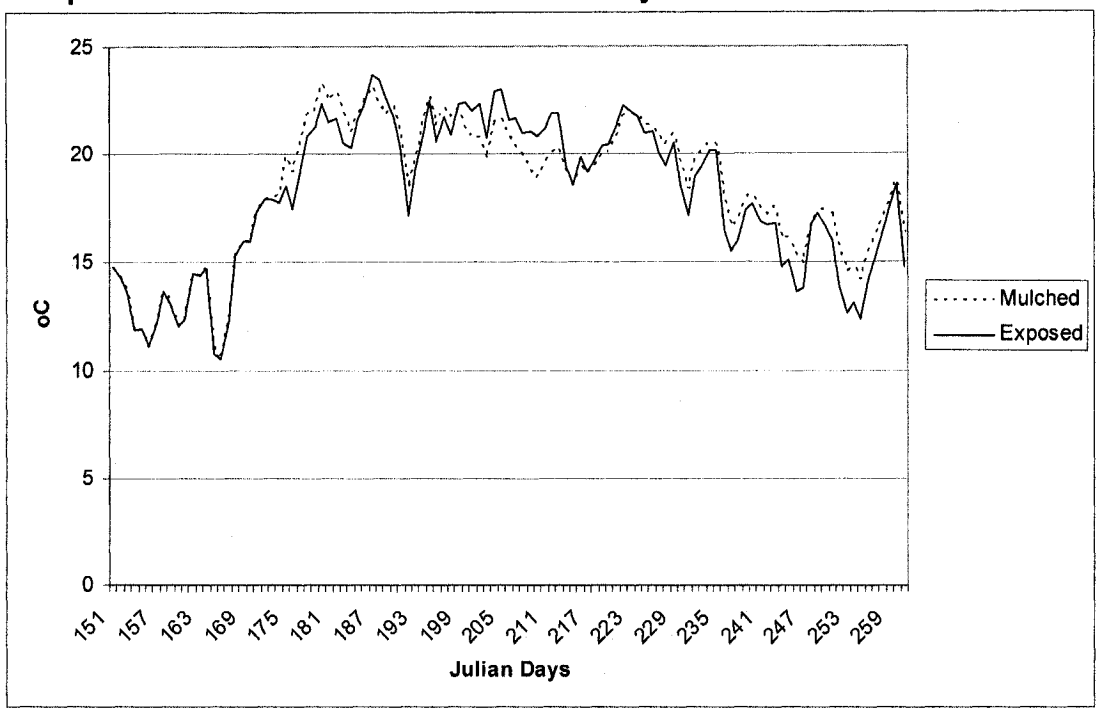


Figure 2-9. Mean daily soil temperatures between corn planting and harvest at the Nappan site in an N mineralization study in Nova Scotia in 2003.

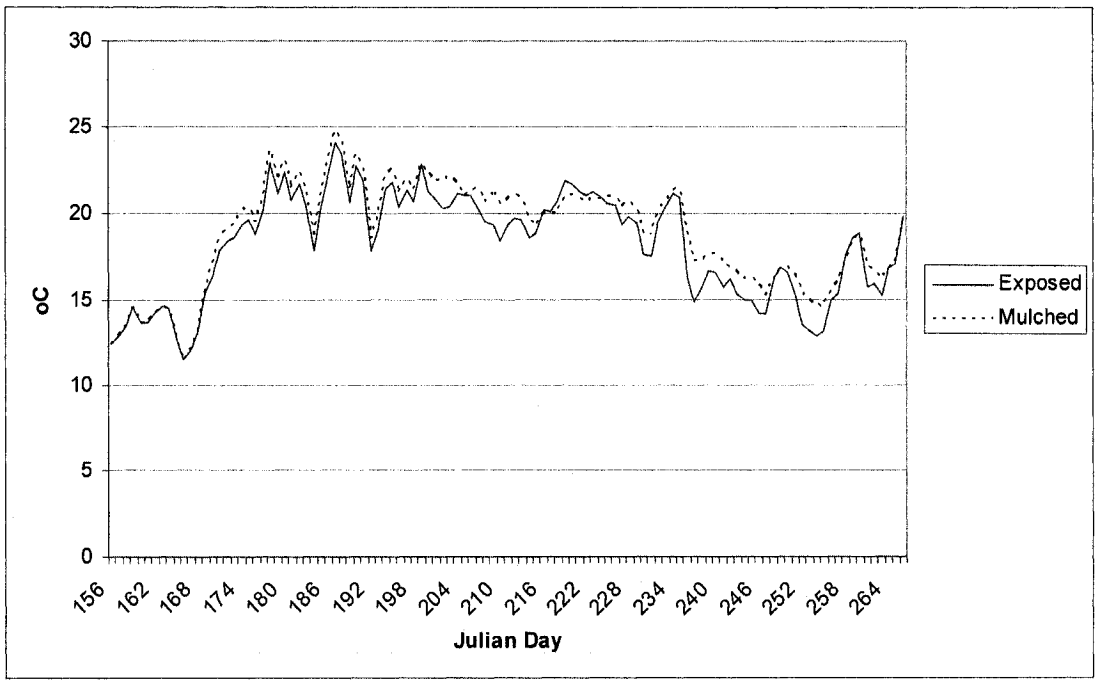


Figure 2-10. Mean daily soil temperatures between corn planting and harvest at the Noel 1 site in an N mineralization study in Nova Scotia in 2003.

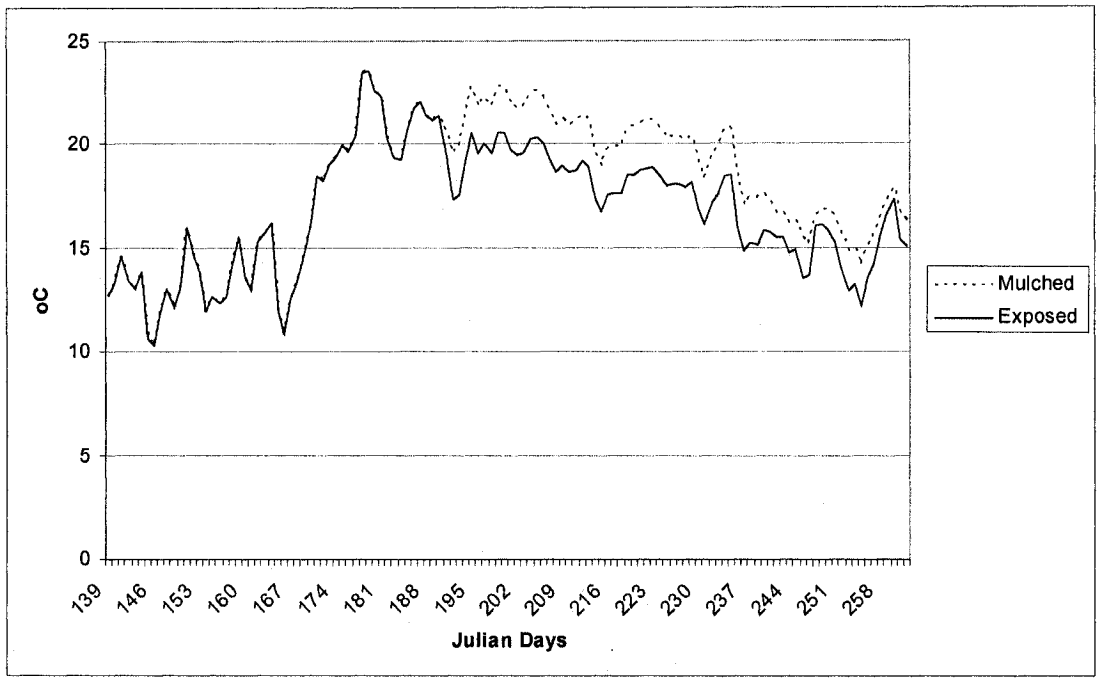


Figure 2-11. Mean daily soil temperatures between corn planting and harvest at the Noel 2 site in an N mineralization study in Nova Scotia in 2003.

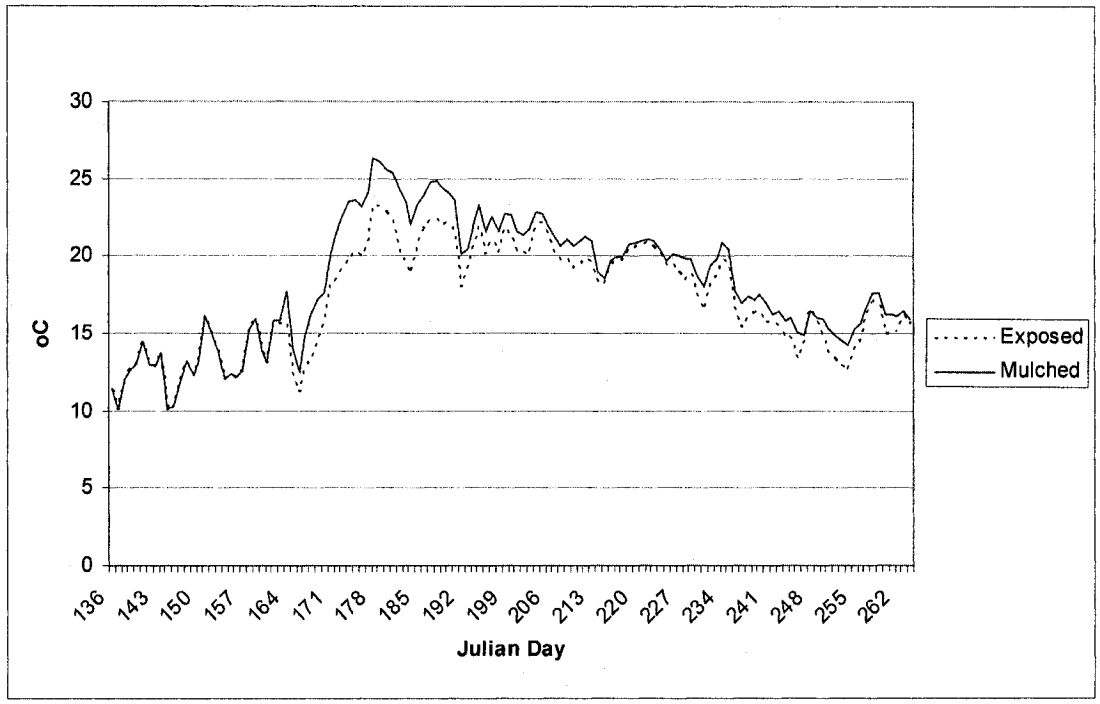


Figure 2-12. Mean daily soil temperatures between corn planting and harvest at the Windsor site in an N mineralization study in Nova Scotia in 2003.

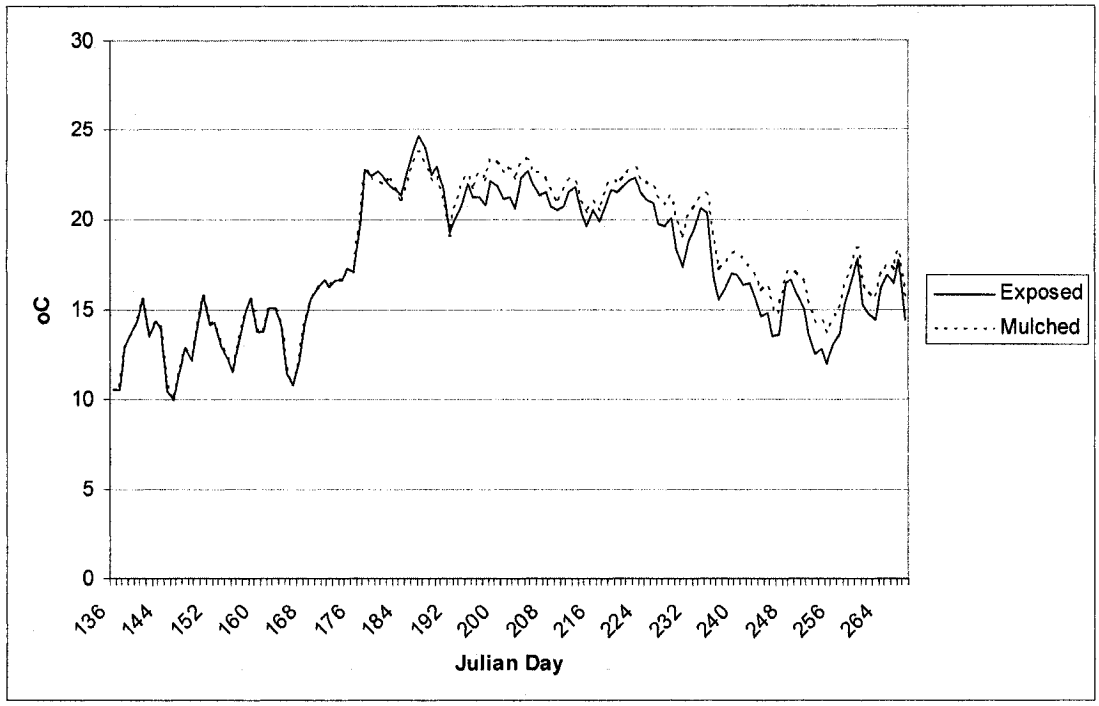


Figure 2-13. Mean daily soil moisture and precipitation at the Musquodoboit site in an N mineralization study in Nova Scotia in 2003.

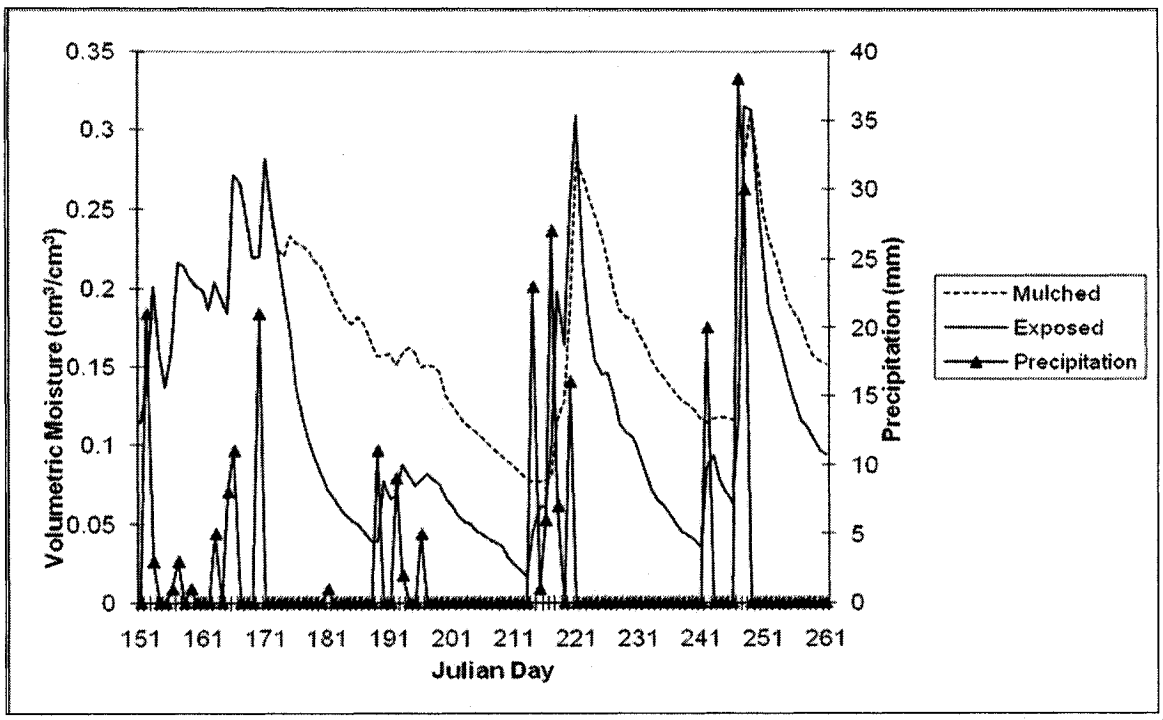


Figure 2-14. Mean daily soil moisture and precipitation at the Nappan site in an N mineralization study in Nova Scotia in 2003.

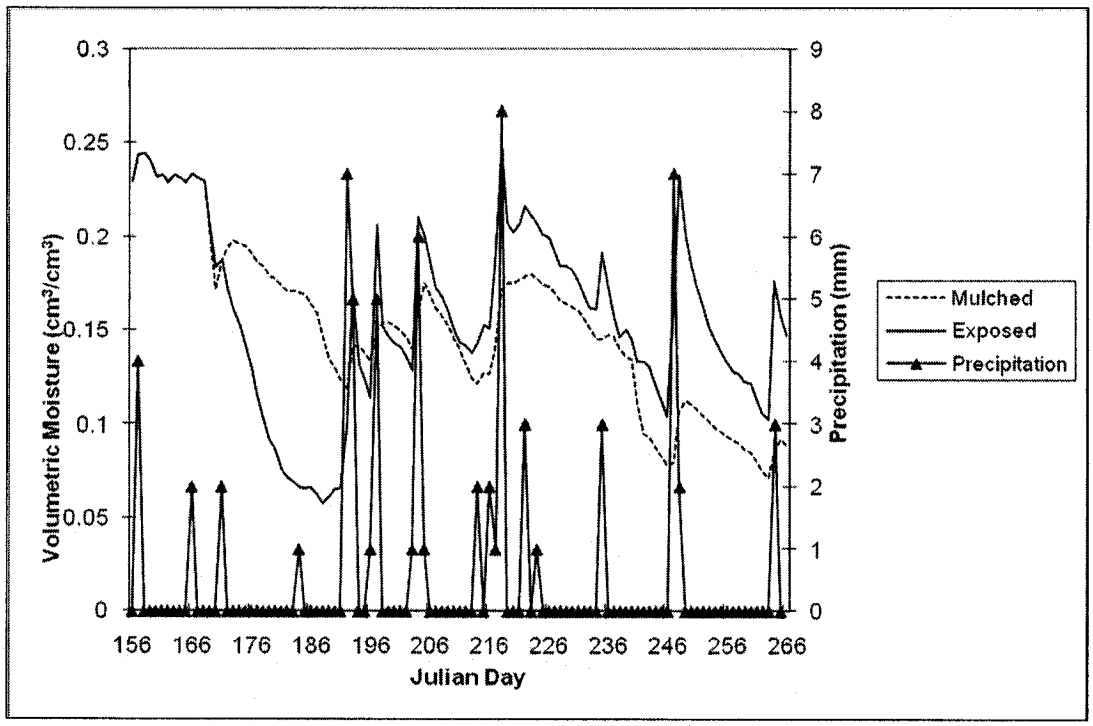
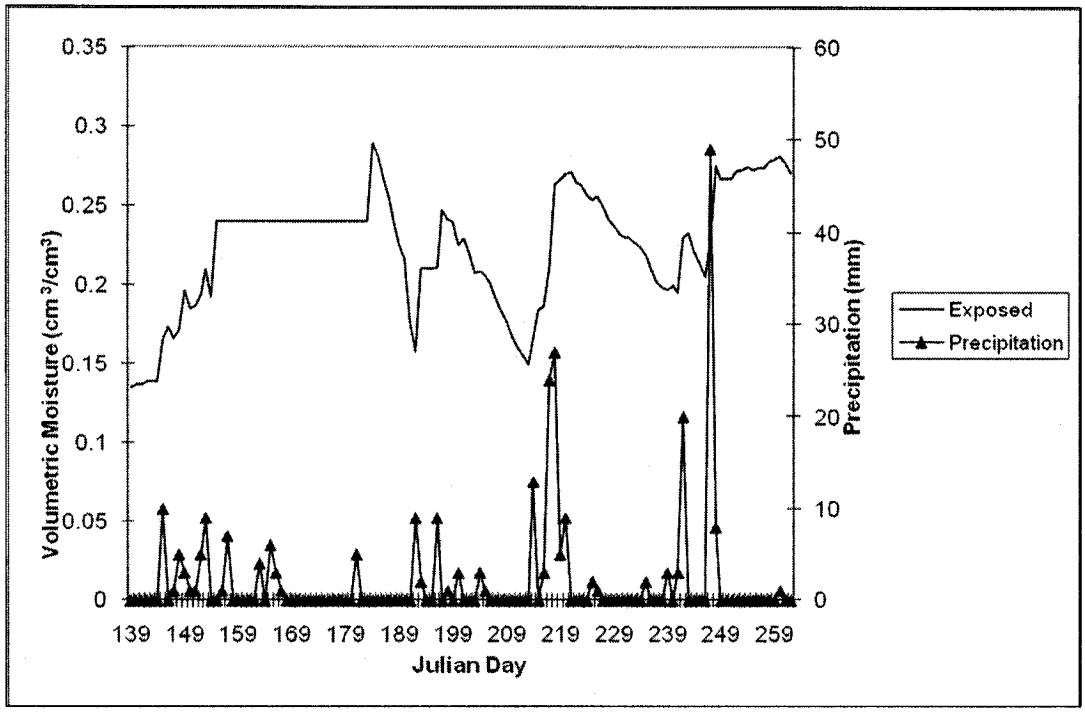


Figure 2-15. Mean daily soil moisture and precipitation at the Noel 1 site in an N mineralization study in Nova Scotia in 2003.



The mulched moisture probe was malfunctioning so only exposed data is presented here.

Figure 2-16. Mean daily soil moisture and precipitation at the Noel 2 site in an N mineralization study in Nova Scotia in 2003.

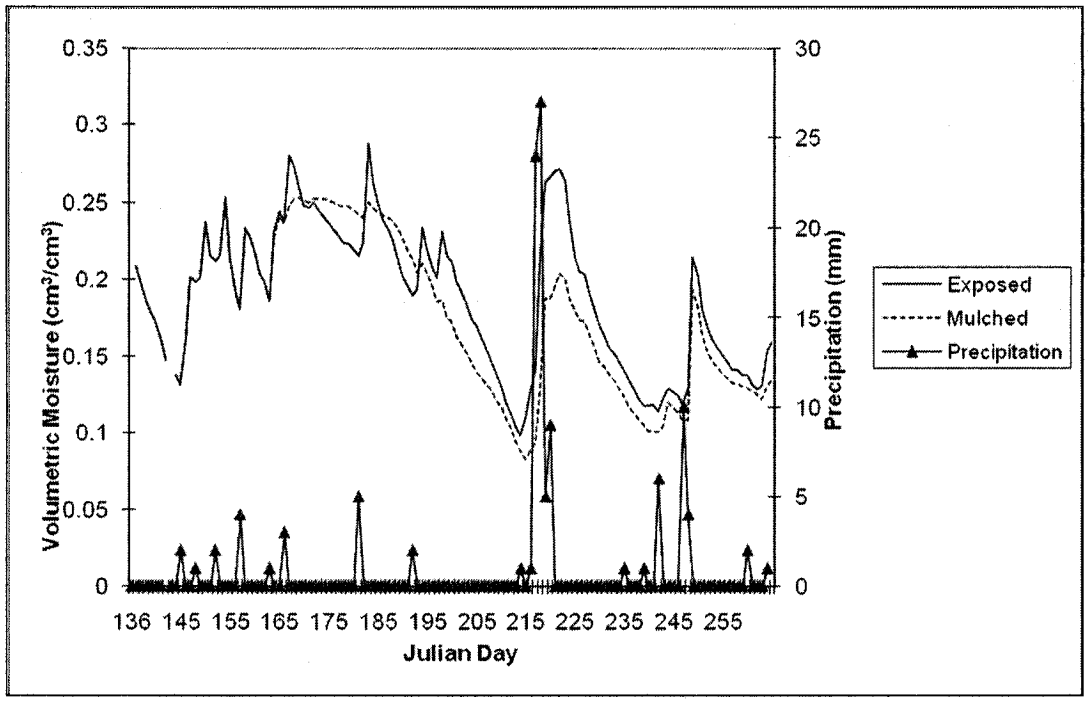
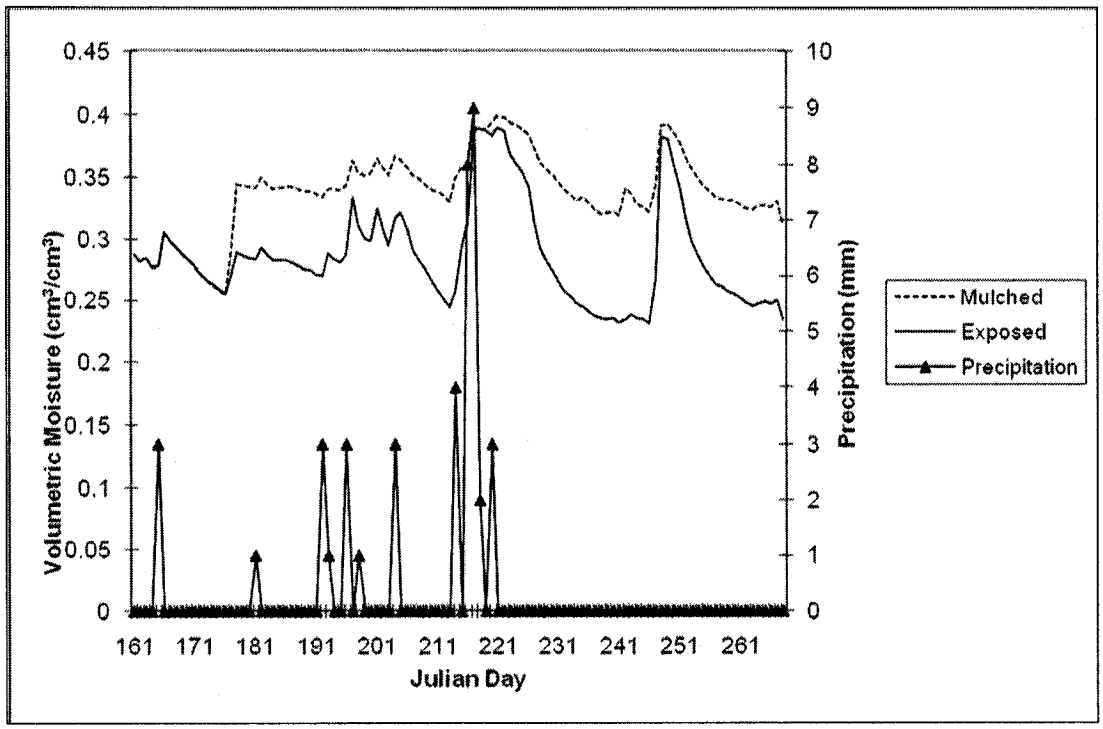


Figure 2-17. Mean daily soil moisture and precipitation at the Windsor site in an N mineralization study in Nova Scotia in 2003.



The rain gauge was not functioning after day 220.

Chapter 3: Linking C and N Mineralization from Native Pools of Soil Organic Matter at Different Temperatures with Microbial Community Characteristics

3.1 Introduction

The results of most laboratory studies into the effects of temperature on mineralization of C and N from soils have been fit to first-order models of decay (Stanford et al., 1973; MacDonald et al., 1995; Thomsen et al., 2001). Approaches to adjusting the kinetic parameters for temperature generally assume that temperature affects only the rate at which decomposition occurs, and not the size or availability of the potentially mineralizable pool of C (C_0) or N (N_0). This assumption has been referred to as the rate constant hypothesis in which the soil microbial community is assumed to be a "black box" that simply works faster as temperatures increase (Dalias et al., 2003). Rate constants in first-order models of decomposition are modified to account for the effects of temperature using one of a variety of temperature response functions; for example, the Arrhenius function as recommended by Stanford et al. (1973), or Ratkowsky's quadratic function (Ratkowsky et al., 1982). These functions have been developed to describe relatively simple processes: in the case of the Arrhenius function, the effects of temperature on rates of chemical reactions involving single substrates, and in the case of the Ratkowsky function, the effects of temperature on growth rates of bacterial communities in the laboratory. These response functions are based on the assumption that the size of the mineralizable substrate pool does not change with temperature, but only the rate at which it is mineralized. In most cases, the same temperature response functions are applied to mineralization of both C and N. Most current ecosystem models of decomposition incorporate temperature according to these assumptions (e.g. Parton et al., 1987; Jenkinson, 1990).

Dalias et al. (2003) proposed an alternative to the rate constant hypothesis: the functional shift hypothesis. This hypothesis states that the size of the pool of available substrate estimated by the first-order model may change with temperature. This may be due to a physical or chemical change in the accessibility of the substrate, or a change in the function and/or structure of the decomposer community, which makes different pools of substrate available at different temperatures. This "functional shift" has been observed by researchers who have fit the first-order model of decay to C and N mineralization data from laboratory incubations, allowing both C_0 or N_0, and the first-order rate constant, k, to vary with temperature (Honeycutt et al., 1988; Ellert and Bettany, 1992; Macdonald et al., 1995). These researchers have observed changes in the C_0 or N_0 parameter estimates at different temperatures, but the relationship between temperature and these estimates has not been predictable. In some cases C_0 or N_0 were predicted to increase with increasing temperature (e.g. Honeycutt et al., 1988), while in other cases, these estimates have decreased at higher temperatures (e.g. some of the forest soils studied by Dalias et al., 2002).

One of the possible explanations for the functional shift hypothesis is a change in the decomposer community characteristics, including its size, species composition, metabolic function, and C and N composition, at different temperatures. If we think of the decomposition of organic substrates in soil as a three step process, Figure 3-1 illustrates how each step in this process can be affected by temperature. The first step in the process is the consumption of the substrate by the decomposer community. For extracellular digesting organisms like bacteria and fungi, this step involves excretion of enzymes to directly digest the substrate. For soil fauna, the substrate must first be

ingested prior to digestion in the gut of the organism. Substrate consumption determines the gross amount of substrate potentially available to the decomposer community for utilization (either for production of biomass, or as an energy source i.e. for respiration). Temperature affects this first step in the process by influencing the structure of the decomposing community, which may differ in its substrate preferences and metabolic pathways. Temperature may also affect the substrate pool size and quality, through temperature effects on substrate solubility and through effects on the physical and chemical reactions which bind substrates and enzymes to soil colloids, and which also create aggregates within which substrates remain protected from digestion. Finally, temperature affects the rate of the digestion process, through its effects on enzyme-catalyzed reactions. These are the temperature effects on decomposition commonly included in models as Arrhenius or Q_{10} functions.

The amount of substrate actually available for utilization by the decomposer community is determined by the utilization efficiency[2] of the community. Not all of the substrate consumed by the decomposer community can be utilized. Those parts which are not readily digestible (sometimes described as recalcitrant) remain undigested by the bacteria and fungi, or are excreted as faeces by soil fauna. This unutilized material may remain in the soil for extended periods of time as "humus". The utilization efficiency of the community is partially related to the composition of the community, which may be affected by temperature. The proportion of substrate that is utilizable is also determined by the quality of the substrate, which may vary with temperature. It has been suggested that as temperature increases, recalcitrant pools of substrate are consumed more readily

[2] Hunt et al. (1987) use the term assimilation efficiency, for this parameter.

by the decomposer community (Fierer et al., 2005). The utilization efficiency of these substrates may be lower than for more readily-available substrates.

Of the carbon substrate that is utilized by the community, a portion is assimilated into the biomass, and a portion is utilized for energy (i.e. respired as CO_2). The proportion of utilized C that is retained for biomass production is referred to as the production efficiency (e_{prod}) . This, like the utilization efficiency, is a characteristic of the decomposing community, and is also likely to vary as the structure of the community varies with temperature. For example, in de Ruiter et al.'s (1993) food web model of N mineralization, both bacteria and fungi are assigned e_{prod} values of 0.30, while protozoa have e_{prod} values of 0.40, indicating that communities with high populations of protozoa, may respire less utilized substrate as CO_2.

Of the nitrogen substrate that is utilized by the community, a portion is retained and utilized for biomass production. The remainder is excreted from the organism as waste inorganic N. How much is retained in the biomass depends on the C:N ratio of that biomass. This is also a characteristic of the community which can vary, as the species composition of the community varies with temperature. Biomass C:N ratios may be particularly sensitive to the fungal to bacterial ratio of the microbial community, since bacteria can be assumed to have a C:N ratio of 4, while the C:N ratio of fungi may be as high as 10 (de Ruiter et al., 1993). All of these factors reflect those outlined in Balser et al. (2006), in which they illustrate the hypothetical relationships among environment, microbial community, and ecosystem processes.

The first objective of this research was to determine if the size and availability of the pools of native C and N in an arable Nova Scotia soil change as a function of the

temperature of incubation. Changes in substrate pool size can be estimated by fitting the first-order model to net mineralization data producing estimates of the pools N_0 and C_0. The relative rate constant of the first-order model, k, is a characteristic of the litter undergoing decomposition (Paul and Clark, 1989). These values can be used to assess the quantity and quality of the litter undergoing decomposition at each temperature.

Expressing the first-order rate constant of a cumulative mineralization curve on a soil thermal unit basis is a method of normalizing the data to remove the effects of temperature on the preliminary, depolymerization phase (Schimel and Bennett, 2004), of C and N mineralization. Temperature effects on this phase are represented in Figure 3-1 by the enzymatic rate of digestion, or Q_{10}, effect on net C and N mineralization. If the rate constant hypothesis is valid, then first-order rate constants at a range of temperatures (for the same soil and substrate) should be equivalent when expressed per soil thermal unit. Honeycutt et al. (1988) proposed using soil thermal units as a modified time variable to account for the effects of temperature on C and N mineralization from soils. They assume that for the range of temperatures over which soils are biologically active in temperate regions (5 to 35°C) the rate constant hypothesis is valid.

In cases where the data cannot be fit to the first-order model, it is assumed that the size of the substrate pool does not limit the rate of mineralization. In this case, the first-order model presented in Chapter 1 (equation 2) can be expressed as:

$$- dS/dt = kS^0 \qquad (3)$$

which can be resolved to simply:

$$- dS/dt = k \qquad (4)$$

In this expression the quantity of substrate has been raised to the power of zero, and this model is commonly referred to as a zero-order model. The zero-order rate constant is an empirically determined value, the magnitude of which is affected by substrate quantity and quality, the potential of the microbial community to metabolize the pool of substrate, and environmental conditions (temperature, moisture) that control the rate of microbial digestion of substrate. When the zero-order rate constant of decay under optimum moisture conditions, is expressed per soil thermal unit, it becomes a measure of the size and quality of the substrate pool, as well as a reflection of the soil microbial community's metabolic potential to access that pool. Comparisons of zero-order rate constants from incubations conducted at different temperatures, expressed per soil thermal unit, can therefore provide insight into temperature-related differences in substrate pool size and quality, and the microbial community's metabolic potential, at these temperatures.

The second objective of this research was to determine how the metabolic efficiency of the soil microbial community is affected by temperature. One index of the microbial community's metabolic efficiency is its metabolic quotient, qCO_2, which is the instantaneous respiration rate per unit microbial biomass. High values for qCO_2 indicate a community that is functioning inefficiently (Fliessbach et al., 2000). Metabolic quotient figures are difficult to interpret since the value is a quotient derived from two separate measurements. Changes in its value can be due to alterations in either the numerator (the rate of respiration) or the denominator (the microbial biomass), or both.

A third objective of this research was to determine how temperature affects the size of the microbial biomass. One of the assumptions of the first-order approach to modeling decomposition is that the size of the biomass does not limit the rate of

decomposition. While biomass pool is included in some organic matter decomposition models (e.g. the CENTURY model (Parton et al., 1987)), it is only treated as a pool of substrate, and its size has no impact on the rate of decomposition of other pools of substrate in the model. Fang et al. (2005a) proposed a model in which the decomposition of soil organic carbon is a function of the size of the active biomass and its specific activity. They point out that the soil basal respiration rate is closely related to changes in microbial biomass, and that there is no reason to assume that decomposition rate constants remain unchanged when soil microbial pool size varies. Since seasonal changes in the size of the biomass have been observed (Schadt et al, 2003), one of the objectives of this study was to determine if a temperature-related change in biomass size could be detected in microcosms incubated at different temperatures.

The fourth objective of the research was to compare the effects of temperature on the species composition of the bacterial and fungal communities. Changes in microbial community structure have been suggested to explain the functional shift observed by Dalias et al. (2001; 2002) and others. Zogg et al. (1997) investigated this hypothesis and detected changes in microbial community structure at different temperatures, using a phospholipid fatty acid (PLFA) technique. However, PLFAs are themselves affected by temperature, so the differences detected by Zogg et al. (1997) could have been confounded by temperature-related changes in the PLFAs. In this study, nucleic acid techniques were used to determine how temperature was affecting bacterial and fungal species composition at a range of temperatures and over a series of time-points.

In this study the effects of two management histories on decomposition in soil were contrasted: annual fertilization with a high rate of semi-solid beef manure, and the

control treatment which received no fertility amendments. These two treatments were included to address a final objective which was to determine if trends in the various parameters measured were consistent regardless of the soil's management history.

3.2 Materials and Methods

3.2.1 Site and Soils

The site chosen for the study was a long term forage fertility trial located at the Agriculture and Agri-Food Canada Research Station at Nappan, Nova Scotia, Canada. (45° 45' N, -64° 14'). The soil was developed from glacial till and is classified as a Humo-Ferric Podzol. The plots have been maintained under the same fertility management program since 1994. Initial soil properties are listed in Table 3-1.

The manure treatments received an annual early fall application of semi-solid beef manure at a rate of 300 kg total N ha^{-1} based on the manure's total N content (M300). The control treatments received no fertility amendments throughout the experiment (M0). The stand of native grass/legume forage was harvested from the plots up to three times per year based on growing conditions.

On 8 July 2004 a composite sample (40 cores per plot) was collected from the top 15 cm of each plot in three of the field replicates. A portion of each sample (1 kg wet weight) was passed through a 4 mm sieve and pre-conditioned at field moisture content (see Table 3-1) at each of the following temperatures: 5°C, 15°C, 25°C, and 35°C, for four weeks. Another portion of each soil was stored at 4°C (1 kg wet weight) and a third portion was air dried at 22°C and passed through a 2 mm sieve for later analysis (500 g wet weight).

3.2.2 Experimental Setup

The experiment was a factorial design consisting of two levels of soil management history (M0, M300) and four levels of temperature (5°C, 15°C, 25°C, and 35°C) replicated three times and analyzed as a randomized complete block design. An experimental unit consisted of a 29 x 14 x 14 cm food storage box containing 22 shell vials (44 ml; 2.9 cm OD x 4.4 cm height) of pre-weighed, field moist soil (approximately 10 g dry basis) mixed with an equal mass of acid-washed 20 mesh Ottawa sand, an additional shell vial containing acidified water (0.1 M HCl) to maintain humidity in the box, and a shell vial containing 5 ml of 1 M KOH to trap CO_2.

The total number of experimental units was 24. An additional box containing only the acidified water vial and the 1 M KOH CO_2 trap was included for each temperature in order to account for ambient CO_2 levels in mineralization calculations. Moisture contents in the soil within each vial were maintained throughout the experiment within 2% of 55% water filled pore space (WFPS), assuming a bulk density of soil in the vial of 1 g cm^{-3}. The 55% WFPS moisture ranges for the soils in the study are shown in (Table 3-1).

Sampling and analysis

Sampling was conducted on a regular basis to monitor native C and N mineralization, CFE flush C, and microbial community structure. The sampling schedule is outlined in Table 3-2. Differences in mineralization measurement schedules early in the experiment were due to slow initial rates of CO_2 release, which made it difficult to detect any mineralization by KOH trap titration for the colder incubation temperatures. Carbon dioxide contained in each trap was determined by titration with standardized 0.4 M HCl using phenolphthalein as an indicator.

Soil native N mineralization was estimated by extracting the contents of each shell vial with 30 ml of 0.5 M K_2SO_4. After shaking for 1 h, samples were filtered through Whatman No. 5 filter paper and frozen until later analysis for NH_4^+- N, using the phenoldisulphonic acid method (Technicon method #780-89T), and NO_3^- - N, using the cadmium reduction method (Technicon method #100-70W), on a Technicon Autoanalyzer II.

The chloroform fumigation extraction method (Vance et al., 1987) was used as an index of the soil microbial biomass. Duplicate vials were removed from each box. One vial was immediately extracted with 0.5 M K_2SO_4 as described above and the filtrate was stored at -20°C for later analysis. The second vial was placed in a vacuum extractor along with a beaker containing 50 ml of alcohol-free chloroform. The extractor was sealed and evacuated until the chloroform had boiled for three minutes. The vials were then left in the chloroform atmosphere overnight and extracted on the following day as described above. An aliquot of the K_2SO_4 extract was analyzed for extractable organic carbon (EOC) using the Technicon Autoanalyzer II (Technicon method #455-76W/A). CFE flush C was calculated as the difference in DOC between the fumigated and non-fumigated vial from the same box, expressed as mg C kg^{-1}soil. Although a conversion factor is frequently applied to convert CFE flush C to microbial biomass C, this factor is arbitrary and has been shown to vary depending on soil type and management history (Bailey et al., 2002); therefore, in this study, CFE flush C is reported directly with no conversion to biomass C. The metabolic quotient, qCO_2, was calculated as the rate of C respiration on the day CFE flush C was measured, divided by the CFE flush C. Shell vials for microbial species composition analysis were removed from the boxes and

immediately frozen at -20°C according to the schedule in Table 3-2. These samples were freeze dried when enough frozen samples had accumulated.

3.2.3 Microbial Community Analysis

DNA Extraction

Freeze dried samples were used for DNA extraction and analysis. Samples were extracted in sets of 96. All tubes were placed on ice between each step in the process. 1 g of freeze dried soil was placed in a 15 ml falcon tube with 2 ml of 0.12M sodium phosphate ($NaHPO_4$) in a buffered solution of 1% SDS (sodium dodecyl sulfate). The mixture was reduced to a slurry using a vortex mixer and then 1 ml of the slurry was transferred to a 2 ml tube containing glass beads in a 96 well format. The box of 96 tubes was placed on a bead beater at 30 Hz for 90 seconds three times (turning the boxes in between each cycle) and then spun at 3007 rcf for 5 minutes on a Sigma 4K15 centrifuge. Washing with phenol/chloroform/isoamyl alcohol (25:24:1) followed by chloroform/ isoamyl alcohol (24:1) was conducted as described in Pennanen et al. (2004). This was followed by transfer of the aqueous phase to a clean 2 ml tube containing 400 µl of isopropanol and 40 µl of 3M sodium acetate. The tubes were then stored in a -20°C freezer for a minimum of 2 hours before centrifugation (as above) and discarding of the supernatant. The DNA pellets were washed once with 100 µl ice cold 70% ethanol, and after centrifugation the supernatant was discarded again. The pellets were dried before re-suspending in 50 µl of molecular grade water. For the final purification phase polyvinylpolypyrrolidone (PVPP, Sigma) was packed into a 96-well Millipore multiscreen DNA cleanup plate, and stabilized three times by washing with molecular grade water (applying vacuum between water additions). Samples were applied to the

plate and eluted from the columns by vacuum. DNA extracts were stored at -80°C prior to further analysis.

DNA Amplification by PCR

Bacterial DNA was amplified by the polymerase chain reaction (PCR) using a nested approach. Reactions were carried out with the following reagents in a 15 μl mixture: template DNA, 1 μl; Expand High Fidelity (Roche) buffer, 2.5 μl; each deoxynucleoside triphosphate, 62.5 μM; bovine serum albumin, 0.6 μg; each primer, 0.2 μM; *Taq* polymerase (Expand High Fidelity enzyme mix-Roche), 1.2 U. PCR product from the first step was diluted 1:10 with molecular grade ultra-pure water and 1 μl of the diluted product was used in the second step reaction mixture. Forward and reverse primers of second round PCR products were 5'labeled with fluorescent sequencing dyes (Applied Biosystems Inc., Freemont, Calif.). The details of bacterial and fungal primer sequences and PCR conditions are outlined in Table 3-3. All PCR products were subjected to electrophoresis in ethidium bromide agarose gel (1.5%) with TBE as a buffer, and visualized under UV light. Positive and negative controls were carried through from the first round to the second round of PCR to monitor contamination.

Contamination of negative controls in the second round of the bacterial PCR was a problem. This was traced to bacterial template present in the *Taq* polymerase and was addressed by treating the first and second round master mixes for bacterial PCR with 1 U *HhaI* restriction enzyme per reaction and digesting the master mix (37°C for 40 minutes and 65°C for 10 minutes), prior to use. This method effectively removed any contaminating DNA from the master mix.

Restriction enzyme digestion of PCR products

Initial digestion with three different restriction enzymes was carried out on a few samples in order to select the best enzyme to use in the T-RFLP step. Digestion of bacterial PCR products was compared using *AluI*, *DdeI*, and *HincII* , while fungal PCR products were digested using *HinfI*, *RsaI,* and *TaqI*. Enzymes were selected that produced the highest number of restriction fragments and the best resolution between treatments. *AluI* was selected for bacterial digests and *HinfI* for fungal digests.

In the restriction enzyme digestion step, 5 μL of PCR product was digested with 1 μL of dilute (1:1) restriction enzyme (0.5 U) at 37°C for two hours followed by a 10 minute enzyme denaturation step at 65°C. The digested PCR product was then diluted 1:10 with molecular grade ultra-pure water. The dilute digestion product was mixed with 8.95 μL of formamide and 0.05 μL of an internal length standard (LIZ, Applied Biosystems Inc., Freemont, Calif.). The terminal restriction fragments marked with fluorescein were analysed by electrophoresis with an automated DNA sequencer (ABI PRISM™ 3730). Blank samples (negative PCR controls from the second PCR and water controls) were also digested and analyzed.

Data analysis

Genemapper software (version 3.7) was used to analyze the T-RFLP profiles. The samples in this analysis were part of a larger study that included DNA extracted from soils that had been amended with ^{14}C-labeled wheat (Chapter 4). For the preliminary phase of the analysis (up to and including principal components analysis) the data were processed together. Analysis of Variance of the principal component scores derived from

the T-RFLP analysis was conducted for the native C and N soils separately from the ^{14}C-labeled wheat-amended soils.

Each dye was analyzed separately. Peaks were assigned to bins manually with bin width equivalent to 1 to 1.5 base pairs, to account for slight variations in locations of peaks (Blackwood et al., 2003). Peak height thresholds for bacterial and fungal samples were determined based on thresholds observed in PCR negatives and water samples that were also digested. The minimum peak height used for the bacterial samples (both the FAM and VIC dyes) was 50 fluorescence units. A large amount of "noise" was evident in the fungal samples for both dyes. For this reason a minimum peak height of 400 fluorescence units was used when assigning peaks to bins for both the NED and PET dyes.

After initial assignment of peaks to bins, tables of peak size and area were copied to a spreadsheet (MS Excel) for further analysis. Peak areas were judged to be more representative of the relative fluorescence contributed by each fragment than peak heights, because of the spreading of peaks that occurs for larger fragments which can result in lower peak heights. While nesting of samples resulted in more even quantities of DNA for restriction enzyme digestion, in the case of bacterial samples, it also resulted in the amplification of DNA fragments in nested PCR negatives. This DNA was not visible on agarose gel, but did result in detection of fragments on Genescan gels. The bins containing these peaks were removed from the analysis (a total of 5 bins for the FAM dye and 20 for the VIC dye). No peaks were detected in PCR negatives for the fungal samples. Data for peaks that were less than one base pair apart in size were examined to determine if the two (or occasionally three) peaks ever occurred in the same

sample. If peaks for bins less than one base pair apart in size never occurred in the same sample, it was assumed that the peaks represented the same operational taxonomic unit (OTU), and these bins were merged. The data was also screened to remove bins for peaks that never represented more than 1% of the total fluorescence for any sample. Using these techniques, the total number of fragment sizes used in the statistical analysis for the FAM dye was reduced from 195 after Genemapper analysis, to 154 after screening. Similarly, the total number of fragment sizes used in the statistical analysis for the VIC dye was reduced from 241 initial fragments, to 197 after screening. The FAM and VIC dye profiles were combined for each sample before multivariate statistical analysis (a total of 351 variables in the analysis). For the PET dye, 267 fragment sizes were assigned after Genemapper analysis, but this was reduced to 197 fragments sizes after screening. After Genemapper analysis 305 fragment sizes were assigned for the NED dye, but this was reduced to 200 after screening. The NED and PET profiles were combined before multivariate analysis (a total of 397 variables in the analysis).

The band richness (S, the total number of fragments present in each sample), Shannon's diversity index (H') (Odum, 1971), and band evenness (E, calculated as Shannon's diversity index divided by the log of the number of fragments) were calculated using the relative abundance data for the bacterial and fungal T-RFLP profiles. ANOVA of these indices was used to determine treatment effects on these measures of diversity.

3.2.4 Curve-fitting of Native C and N Mineralization Data

Incremental models for zero-order, single-pool first-order (SPE) and two simultaneous reaction (TSR) mineralization were fit to the C mineralization data for each measurement period (expressed as mg C kg^{-1} soil) i.e. the incremental data (see Table 3-4) . Incremental models are superior to cumulative models because they eliminate the

additive error that results when each datapoint is the sum of all previously measured datapoints (Ellert and Bettany, 1988). They also allow the removal of datapoints that may be potential outliers, without affecting the estimates of potentially mineralizable substrate. The measured values of C respiration for day 97/98 in every case were dramatically higher than estimates on previous or subsequent days. An error in titration or acid standardization on that day was suspected, so this data was removed from the curve-fitting process. The incremental models for each type of model are presented in Table 3-4. The nonlinear models procedure in GenStat was used for the curve-fitting procedure.

The N extracted from each vial on a given date with the N present at time zero subtracted, represented the total amount of N mineralized since the beginning of the experiment i.e. the cumulative data (recognizing that mineral N lost due to denitrification or immobilized in the microbial biomass is not accounted for in this experiment). This data was fit to zero-order, SPE, and TSR models of N mineralization using the nonlinear models procedure in GenStat. For the 15°C, M0 treatments, replicate 2 showed excessively high values for accumulated inorganic N from day 82 onwards; therefore the last three datapoints for this replicate were removed from the analysis.

For the initial curve-fitting attempts, upper and lower limits were set for the parameters in the models. The relative rate constant for the SPE model ranged from 0.0001 to 0.1 d^{-1}. For the TSR model the relative rate constant of the fast pool (k) was constrained within the range 0.001 to 0.1 d^{-1} while the relative rate constant of the slow pool (h) was constrained within the range 0.0001 to 0.01 d^{-1}.

Results for curve fitting of the data from each individual experimental unit were compared with the results for fitting a curve to the pooled data from the three replicates. In all cases zero order rates constants calculated as the mean of the estimates for each experimental unit, were equal to the rate constants estimated by fitting a curve to the pooled data. First-order rate constants were also equivalent using both approaches. Estimates of potentially mineralizable C and N varied slightly depending on the approach, but averages calculated from each individual box were within the same range (confidence interval) as estimates obtained by fitting the first-order curve to the pooled data. Since one of the aims of the curve fitting exercise was to compare estimates by computing confidence intervals for each parameter, the pooled field replicate approach was used. This approach resulted in one standard error associated with each parameter estimate, and allowed confidence intervals to be easily calculated

The two types of model were compared by computing an F statistic to determine if the more complex model (TSR) i.e. the one containing more terms, improved the fit significantly over the simpler SPE model. This extra sum of squares F statistic was calculated as:

$$F_{extra} = [(SS1-SS2)/(df1-df2)]/(SS2/df2)$$

where SS1 and SS2 refer to the residual sum of squares of the simpler and more complex model respectively (Dalias et al., 2001; Motulsky and Christopoulos, 2004). The aim of the curve fitting process was to reduce the residual sum of squares to a minimum while also minimizing the number of model parameters. This statistical test provides an objective method of determining if the additional parameters included in the more complex model are balanced by a significant improvement in model fit.

A model was selected only if the p-value of the extra sum of squares test was less than 0.10 and the standard errors of the parameter estimates were less than the value of the estimates themselves. Confidence intervals for the parameter estimates were calculated as t^*SE, where t^* is a constant taken from the t distribution. Since the number of degrees of freedom for each parameter estimate was always greater than 12, a value of 2 for t^* (for 95% confidence) was used to calculate the confidence intervals (Motulsky and Christopoulos, 2004). Parameter estimates for different temperature and management history combinations were assumed to be different if the 5% confidence intervals did not overlap.

3.3 Results

3.3.1 Kinetics of C and N Mineralization

Native soil N mineralization

The measured cumulative inorganic N mineralized for each temperature by management history combination show that significantly less net N mineralization occurred at 5°C in the M300 treatment, compared with the warmer three temperatures (Table 3-5). The15°C, M300 and 25°C, M0 treatments had the highest inorganic N accumulation numerically. In fact, several other treatments (e.g. 25 and 35°C, M300) had higher levels of inorganic N earlier in the incubation, but levels had declined by the final sampling occasion. This indicates that N loss via denitrification or immobilization was becoming more prevalent during the later stages of the incubation. Although the cumulative inorganic N values were lower at 5°C, the lack of temperature effects on cumulative inorganic N per soil thermal unit suggest that temperature is only affecting the enzymatic rate of digestion, and not the pool size.

The results of the kinetic analysis, however, contradict this conclusion. The best choice of model for native N mineralization data was dependent on soil management and temperature. For soil M0, at 5 and 25°C the first-order model did not result in a significant reduction in the error SS compared with the zero-order model, therefore the zero-order model was selected, while at 15 and 35°C, the SPE model was superior. For soil M300, substrate-limitation occurred more often and the first-order model provided a superior fit to the data at every temperature except 15°C. The TSR model was difficult to parameterize and frequently resulted in parameters going out of bounds. When it could be successfully parameterized, it never resulted in a significant F_{extra} statistic, and was therefore not selected as the best model for any of the cases.

The parameter estimates for the best model at each level of temperature and management history are shown in Table 3-6. In addition, for comparison purposes the SPE parameter estimates are included where the data had also been fit to this model. For soil from the M0 treatment, there was a significant increase in the zero-order rate of N mineralization per day, as temperatures increased from 5 to 25°C. When expressed on a soil thermal unit basis, at 5°C the zero-order rate constant was still significantly lower than the 25°C zero-order rate constant, suggesting that a smaller pool of substrate was accessed at the lower temperature. The SPE N_0 and k parameter estimates are also both numerically lowest at 5°C indicating a smaller, less accessible pool of N is being utilized at this temperature. At 35°C the pool size is significantly larger as indicated by increased N_0, but the relative rate constant (k) is not, indicating a temperature-dependent change in the size of the available N pool. For soil M300, the potentially mineralizable N estimate

at 5°C was significantly lower than this estimate for the same soil at 25 and 35°C, again suggesting a smaller pool of accessible substrate at low temperatures.

The parameter estimates were used to generate graphs showing the predicted pattern of cumulative N release for each soil at each temperature (Figure 3-2 to Figure 3-9). These graphs highlight the very low rates of N release at 5°C and the significant increase, especially for the M300 soil, of predicted rates of release at 15°C and above.

Differences in mineralization parameters between the two management histories were only evident when the 35°C first-order rate constants were compared. The soil with a history of manure application had a significantly higher value for k (demonstrated in Figure 3-9 by the steep increase in net N mineralization early in the incubation), which is indicative of differences in substrate quality and the microbial community's potential to access substrate at this temperature, although the estimates of the size of this pool did not differ between the two management histories.

Native soil C mineralization

The incremental data for C mineralization was summed to obtain estimates of cumulative C mineralization (Table 3-5). There was a large degree of variability associated with these estimates and the additive error that results when a value is a sum of several separately measured values needs to be kept in mind. Nevertheless, the actual values of net C mineralization reflect the trends based on the model parameter estimates (see below): over the course of the incubation, larger amounts of C were mineralized as temperatures became progressively warmer. When rates of C accumulation per soil thermal unit were calculated, temperature had a striking effect, with the 5°C treatments respiring significantly more C per STU than the three warmer temperatures.

The zero-order model provided the best fit to the data for soil M0 at 5 and 15°C; whereas at 25 and 35°C the F_{extra} statistic was significant, and the substrate-limited model provided a better fit to the data (Table 3-7). For the M300 soil at 5°C, although the F_{extra} statistic was less than 0.10 the CV of both k and C_0 were very high; therefore, the zero-order model was considered preferable at this temperature. At 15, 25 and 35°C the first-order model significantly reduced the error SS relative to the zero-order model for soil M300. The same problems with parameterization of the TSR model were encountered for the native C mineralization data, as for the native N data.

There were few differences between the two management histories, and due to changing temperature, among the parameter estimates for native C mineralization (Table 3-7). The zero-order rate constants, and the first-order rate constants, were not significantly affected by temperature. When expressed per soil thermal unit, the 15°C zero-order rate constant for M0 was significantly lower than the 5°C estimate. There was a trend towards higher estimates of potentially mineralizable C for the M300 management history, between 15 and 25°C, but there was no further increase in C_0 above 25°C.

The parameter estimates were used to generate charts showing the predicted pattern of cumulative C release for each soil at each temperature (Figure 3-10 to Figure 3-17). These are plotted along with the cumulative C mineralization values obtained by summing up all previous measurements of CO_2 evolution for each date (keep in mind that the parameter estimates for these curves were obtained using the incremental data for CO_2 evolution). When compared with the net N mineralization curves, these graphs highlight the difference in the predicted response of net C and net N mineralization to

increasing temperature. Net C mineralization at 15°C is not significantly greater than at 5°C; whereas a large increase in net N mineralization was observed between 5 and 15°C. The C:N ratios of net C mineralization to net N mineralization at each temperature also demonstrate the differences in the temperature responsiveness of these processes (see Table 3-7). The C:N ratio of predicted net C and N mineralization is relatively high at 5°C, indicating very low N mineralization relative to C mineralization at this temperature. The ratio drops as temperatures increase and then increases slightly at 35°C. This variability in the ratios of net C:N mineralized at each temperature indicates that the temperature responsiveness of net C and N mineralization are not equal. Net N mineralization shows a large response to the temperature increase from 5 to 15°C, thereby reducing the net C:N mineralized ratio.

3.3.2 Microbial Community Size and Metabolic Efficiency

CFE flush C has been used as an index of the soil's microbial biomass (Vance et al., 1987). CFE flush C was affected by both incubation temperature and management history and there was a significant interaction between these two terms (Table 3-8). For the first three levels of temperature (5, 15 and 25°C) the soils with a history of manure application had significantly higher CFE flush than the M0 soils, although this was not the case at 35°C. For both management histories the trend was for CFE flush C values to decrease with increasing temperature. The metabolic quotient (qCO_2) is an indication of the efficiency with which the biomass is functioning; higher values generally indicate a more inefficient, stressed community (Mamilov and Dilly, 2002). There were no significant differences between the two management histories with respect to metabolic quotient. There was a significant trend towards higher qCO_2 values at warmer temperatures, especially for soils incubated at 35°C. Figure 3-18 to Figure 3-21 illustrate

the pattern in CFE flush C over time for each incubation temperature. The confidence intervals around the means are large, especially at 35°C, so there are no statistically significant differences in CFE flush C due to sampling day; however, there is a trend towards declining values with time at warmer temperatures (Figure 3-20 and Figure 3-21).

Since there were no significant effects due to management history on qCO_2, the means from both management histories at each temperature on each sampling occasion are shown in Figure 3-22. The figure shows that the metabolic quotients measured on each occasion at 5°C and 15°C remained relatively stable over time, averaging 0.06 mg CO_2 evolved mg^{-1} CFE flush C over the 120 incubation. Values at 35°C are significantly higher than the other three temperature treatments and sharply decline after peaking at 0.45 mg CO_2 evolved mg^{-1} CFE flush on day 55.

3.3.3 Bacterial and Fungal Community Structure

Relative abundances of terminal restriction fragments for both the FAM and VIC T-RFLP profiles were combined for the bacterial community structure analysis. A principal components analysis was conducted to reduce the number of variables from the initial 351. The PC scores for the first six principal components were analyzed by ANOVA (Table 3-9). Sampling day was the main factor affecting PC1, and also had a significant effect on PC4. PC3 and PC4 were both affected by incubation temperature. Management history contributed to the variability in PC1 and PC4 ($p<0.10$), suggesting that there was some difference in bacterial community structure due to management history. Figure 3-23 shows the effect of sampling day on the mean score for PC1. The value on day 123 was significantly different from the earlier four sampling occasions indicating a change in bacterial community structure over time. When the mean scores at

each temperature for PC3 were plotted against PC4 (Figure 3-24), the 35°C community was clearly separated from the 5, 15 and 25°C communities.

Management had a significant effect on the evenness of bacterial species composition with the M0 soils having a significantly higher value for E compared with the M300 soils (1.01 compared with 0.94, p=0.027). Species richness was significantly higher on day 123 for both management histories over the range of temperatures, compared with day 7 (data not shown).

Relative abundances of terminal restriction fragments for both the NED and PET profiles were combined for the fungal community structure analysis. A principal components analysis reduced the number of variables from the initial 414. It is notable that the proportion of total variability accounted for by each fungal principal component was very small, with PC1 accounting for only 2.1% of the total variability in the dataset, and subsequent PCs accounting for progressively less variability. Day and temperature were the two factors that had a significant effect on the fungal principal component scores (Table 3-10). PC1 was primarily affected by sample day. The mean scores for this principal component are shown in

Figure 3-25, which indicates that there was a significant difference in the fungal T-RFLP profile on day 123 compared with the other four sampling occasions. Variability in PC2, 3 and 4 was largely due to temperature. When mean scores at each temperature for PC2 were plotted against PC4, a separate 35°C community was evident (Figure 3-26). The 15 and 25°C communities were similar to each other, and the 5°C community was separated from the other three temperatures (by PC4). Fungal species richness was significantly reduced on day 123 with only 16 OTUs identified, compared

with values of 40, 52, 46 and 40 on days 7, 14, 27 and 55, respectively. The reduction in band richness contributed to a significant reduction in Shannon's diversity index on day 123 compared with the other days (1.36 on day 123 compared with 1.90, 1.98, 1.98 and 1.64 on days 7, 14, 27 and 55, respectively). Evenness was not affected by sampling day, but was significantly lower at 25°C compared with 5°C (1.07 compared with 1.27).

3.4 Discussion

3.4.1 Temperature Effects on C and N Mineralization

Interpretation of the net C:N mineralization ratio

The results of the kinetic analysis were difficult to interpret when the models that provided the best fit to each set of data were used. For this reason the first-order parameters, when available, were included in Table 3-6 and Table 3-7. These results indicated a temperature-driven change in the size of the mineralizable pool of N for both soils. The net C mineralization first-order rate constant was affected by temperature for the M0 soil while the value of C_0 increased with temperature for the M300 soil. These findings indicated that in some cases both C and N mineralization did not follow the principles of first-order kinetics.

Another interesting finding was that the net C:N mineralization ratio declined as temperatures increased from 5 to 15°C, indicating a large increase in net N mineralization relative to net C mineralization, over this temperature range. This can be interpreted as a difference in the temperature responsiveness of net C mineralization and net N mineralization.

Most soil organic matter models incorporate a temperature response function for even the most stable pools of SOM (Lomander et al., 1998; Fierer et al., 2005). However, recent publications (Liski et al., 1999; Giardina and Ryan, 2000) have

challenged the assumption of temperature sensitivity of low-quality pools of organic matter. Giardina and Ryan (2000) used data from long-term incubations and land conversion studies from temperate to tropical zones to show that temperature did not affect the turnover time of soil carbon. They concluded that when substrate quality is poor (i.e. in mineralization of C from native pools of organic matter), substrate supply, not temperature, is the major limiting factor in decomposition. This paper has sparked controversy and has been criticized for the use of a simple, single pool model to explain the complex process of soil organic carbon turnover (Davidson et al., 2000). Nevertheless, there does appear to be agreement that current soil organic matter models may be overestimating temperature effects on soil carbon dynamics, and that more *in situ* studies are needed to settle the issue. The results presented here suggest a relatively small response of native C pools to temperature warming between 5 and 15°C, when compared with native N mineralization. This observation challenges the notion that the same temperature response functions can be used to model both C and N dynamics.

At 25 and 35°C the increase in net C:N mineralization ratio relative to 15°C may reflect a shift in the balance between gross N mineralization and N loss pathways (immobilization and denitrification) later in the incubation. This is evident from the reductions in mineral N extracted from the microcosms at later dates (see Figure 3-4, R1, R3; Figure 3-5, R1, R2; Figure 3-8, R1, R2; and Figure 3-9, R2, R3). These reductions in inorganic N resulted in lower estimates of N_0 than if curve fitting had been conducted using only the earlier data. Therefore, values of net C:N mineralization ratios at 25 and 35°C may be overestimated.

There are two possible explanations for this shift in mineral N gain-loss pathways.

At 25°C it is likely that immobilization became more prevalent later in the incubation. For the M0 soil there was no further net C mineralization after day 82 and for M300, net C mineralization reached a plateau at day 112. At this point the efficiency of C use (as indicated by a low qCO_2 later in the incubation) was very high. It is possible that the biomass continued to metabolize very slowly and efficiently and gradually immobilized inorganic N from the soil solution.

For the M300 soil, inorganic N in the microcosm declined from day 58 on, which indicates enhanced N losses during this period. The CFE flush C data showed a trend towards net loss of biomass in the 35°C treatments from day 55; therefore, enhanced immobilization during this phase of the incubation seems unlikely. Denitrification therefore, may have played a role. The temperature sensitivity of denitrification is usually assumed to be similar to decomposition, and modeled using Arrhenius or Q_{10} approaches with a Q_{10} of 2 to 3 (Heinen, 2006). If this assumption is correct, then enhanced denitrification at 35°C is a result of other contributing factors, in particular a source of readily-available substrate: the biomass C released during die-off, and an easily accessible electron acceptor: the nitrate accumulated earlier in the incubation.

Another factor contributing to declining levels of inorganic N later in the incubation could be the utilization of lower quality substrates. While SOM models assume a constant substrate quality, in microcosm studies where substrates are not replenished, substrate qualities decline over time. Decomposing organisms initially metabolize the higher quality substrates, and utilize lower quality substrates after the readily-available substrates are depleted. Especially at 35°C, where initial rates of CO_2 respiration were high, it is likely that the readily-available substrates became depleted by

late in the incubation. Lower quality substrates have higher C:N ratios, and therefore resulted in the release of less N per unit C metabolized.

These findings indicate that the parameter estimates for N mineralization at the warmer temperatures should be accepted with caution: substrate depletion, shifts in the balance between gross N mineralization and N loss pathways, changes in the quality of the substrate undergoing metabolism, and changes in the efficiency of metabolism, were all occurring over time. These factors could have resulted in the underestimation of the size of the pool of mineralizable N, especially at 35°C.

Biomass characteristics impacting net C:N mineralization ratio

Biomass characteristics that might lead to changes in the ratio between net C and net N mineralization at varying temperatures can be investigated using the conceptual model in Figure 3-1. This figure shows that biomass characteristics, including assimilation and production efficiencies, and C:N ratio, are key factors in determining the ratio between net C and N mineralization. Changes in these characteristics with temperature, can lead to differences in the ratio of net C to net N mineralization, as observed in this experiment.

The assimilation and production efficiencies of the biomass were not directly measured in this experiment. The biomass specific respiration, or metabolic quotient, has been proposed as an indicator of C utilization efficiency (Fliessbach et al., 2000), with high qCO_2 values indicating low C utilization efficiency. The metabolic quotient has also been proposed as an indicator of environmental stress and degree of succession (Wardle and Ghani, 1995). In this experiment the qCO_2 increased with increasing temperature, indicating a decline in the efficiency of C use.

In addition to indicating a degree of stress in the microbial biomass, the high metabolic quotient may also be a result of the characteristics of the substrate being metabolized. Efficiency of substrate use is related to the quality of the substrate (Jensen and Magid, 2002). Readily available substrates like glucose can have a substrate use efficiency of as high as 70%, while more recalcitrant substrates may have an efficiency of use of only 15 to 20%. The high metabolic quotients measured at warmer temperatures in this experiment may be indicative of metabolism of more recalcitrant substrates. The use of lower quality substrates at higher temperatures has also been reported by Andrews et al. (2000). The reduced efficiency of use could also reflect aeration stress, and is supported by the evidence for enhanced denitrification at warmer temperatures later in the incubation as discussed above.

Considering that the biomass was least efficient in its use of C at 35°C, an increase in net C mineralization compared with the lower temperatures, is expected. However, in this study, the size of the potentially mineralizable pool of C was not significantly higher at 35°C. The trend towards declining biomass (as indicated by CFE flush C) may have compensated for the reduced efficiency of C use at warmer temperatures. While the rate of respiration per unit biomass was much higher at warm temperatures, the size of the respiring biomass steadily declined with increasing temperatures. Zogg et al. (1997) also observed a reduction in the size of the biomass at increasing temperatures in an incubation experiment similar to this one. The reductions in biomass size and efficiency of respiration provide further evidence to support the hypothesis that denitrification is a significant N loss pathway later in the incubation at

35°C. Nitrate is a less efficient electron acceptor than oxygen, therefore less biomass is produced per unit of CO_2 respired i.e. anaerobic respiration is less efficient.

In contrast, Cookson et al. (2002) observed increases in biomass N and immobilization in the field at low temperatures, which they concluded indicated a growth in the size of the biomass. Growth in the biomass at low temperatures could be due to a reduction in microfaunal grazing, resulting in an increase in the size of the bacterial and fungal populations. Soil microfauna play a key role in turning over microbial biomass (Griffiths, 1994). Mamilov and Dilly (2002) found a significant reduction in microbial biomass in microcosms that included nematodes, and attributed this to nematodes grazing on fungal hyphae. The activity of microfauna is reduced at low temperatures (Anderson and Coleman, 1982). It is likely that in the experiment reported here the activity of microfauna was inhibited in the 5°C treatment so that only low temperature-tolerant bacteria and fungi were functioning, acting as primary decomposers of the native soil organic matter. This community may have been able to grow in size because its usual predators, nematodes and protozoa, were not active. This lack of predation could have resulted in an accumulation of C and N in bacterial and fungal biomass. As temperatures warmed, soil microfauna may have become more active and consumed the bacterial and fungal biomass, resulting in the steadily declining CFE flush C measured at increasing temperatures. This decline in biomass due to grazing, would also be accompanied by a release of surplus N, as reported in various foodweb studies (Clarholm 1985; Ingham et al., 1985), and as observed in this experiment over the 5 to 15°C temperature range.

3.4.2 Temperature effects on biomass characteristics

The previous discussion has already highlighted some of the temperature effects on biomass specific respiration (metabolic quotient) and biomass size, as measured by CFE flush C. One of the explanations that was proposed for the functional shift hypothesis is that temperature induces a change in microbial community structure and function (Dalias et al., 2003). Zogg et al. (1997) reported shifts in community structure and function (indicated by changes in the C_0 and k parameter estimates) at different temperatures. They also noted reductions in biomass size with increasing temperature. Both of these phenomena were observed in this experiment as well. Recently Larkin et al. (2006) reported differences in microbial community function, estimated using the community level substrate utilization (CSLU) method (BIOLOG Inc., Hayward, CA, USA), and microbial community structure indicated by changes in fatty acid methyl ester profiles, for soils incubated at 18°C compared with 25°C. However, it is not clear whether the differences in CLSU profiles would be reflected in differences in soil processes, in particular C and N mineralization.

The changes in the quality of the pool of N being accessed (indicated by the zero-order rate expressed as mg N kg^{-1} STU^{-1}) between 5 and 25°C (M0) parallels differences in fungal community structure observed over this temperature range. Although the proportion of fungal to bacterial biomass was not assessed in this study, it can be hypothesized that fungal organisms were more dominant at lower temperatures. Various studies have shown that a higher proportion of the soil microbial community is composed of fungal organisms at low temperatures (e.g. <10°C) (Zogg et al., 1997; Lipson et al., 2002; Pietikäinen et al., 2005). Schadt et al. (2003) found that soil microbial biomass reached its peak under snow and that the fungal to bacterial biomass ratio dropped from

14.9 under snow to 6.6 during the summer months. They suggested that microbial growth in winter was fuelled by decomposition of organic polymers and phenolic compounds, hence the dominance of fungi which usually play a role in attacking the less decomposable compounds in the soil.

A distinct microbial community was present at 35°C, which also had a significantly higher biomass specific respiration rate than the communities at the three lower temperatures. It is possible that bacteria were more dominant at higher temperatures. Pietikäinen et al. (2005) found that while optimum growth rates for fungi and bacteria were in the 25-30°C range, growth rates for fungi decreased more rapidly than for bacteria at higher temperatures. This distinct community could also reflect an increase in the proportion of denitrifying bacteria present, as has already been suggested by the declines in inorganic N evident at 35°C.

The diversity indices were largely unaffected by temperature. The predominant factor appeared to be sample day, with day 123 frequently having different diversity measures than the previous sampling days. Unfortunately, it was not possible to separate the effects of DNA extraction, PCR conditions, enzyme digestion, and sequencer run, from sampling day. Sampling days 7 to 55 were included in one 96 well plate that was carried through to the sequencer stage, and day 123 samples were on a separate plate. Therefore, any plate effects (due to machine or operator variability) would be included as a sampling day effect. This may explain the effects of sampling day on diversity measurements. Nevertheless, it is possible that band diversity did change over time, as specialized microbial communities adapted to conditions in the microcosm developed.

3.4.3 The Role of the Biomass in C and N Mineralization Models

The role of specific biomass characteristics and their effects on C and N mineralization has been overlooked in most decomposition models (Schimel, 2001; Fang et al. 2005a). Biomass C and N are usually only included as pools of substrate, with the size of the biomass pool not impacting on the rate of mineralization from other pools. It is generally assumed that the size of the microbial biomass does not impact on rates of decomposition as long as substrate is a limiting factor (Swift et al., 1979). It is acknowledged, however, that the soil biomass, including soil microfauna and microflora, represent the main driving force for decomposition (Kjøller et al., 2000). The size of this community may therefore impact the magnitude of net C and N mineralization, both as a pool of available substrate, and as facilitators of the decomposition process.

The inclusion of biomass size in models of decomposition has been proposed by Fang et al. (2005a). Schimel (2001) has also urged the inclusion of microbial community effects, including impacts from differences in species composition and biomass size, in biogeochemical models. Currently biomass characteristics are included in first-order models of decomposition implicitly, in the first-order rate constant. Environmental effects on the biomass characteristics that are "buried" in the first-order rate constant are incorporated into the rate modifying functions used to adjust for environmental factors (e.g. temperature and moisture). If a specific environmental factor always influences biomass characteristics in the same way (e.g. declining biomass size with increasing temperature or increasing fungal to bacterial biomass ratio with declining temperature), then there will be no improvement in model efficiency by including more biomass parameters. If, however, biomass characteristics and environmental factors interact to

produce different responses to ecosystem disturbance it may be advantageous to incorporate more biological parameters into the model.

In this study I used soils with two different management histories in order to test the effects of temperature, and the interaction of temperature and management history, on biomass characteristics. The soil with a long history of manure application (M300) had a significantly larger biomass (estimated using CFE flush C). There were also some differences in bacterial T-RFLP profiles between the two management histories, but no significant effect on fungal T-RFLP profiles. The metabolic efficiency of both soils was not significantly different. In spite of the measurable differences in some biomass characteristics, management history did not have a significant effect on the first-order rate constants at a given level of temperature (except for net N mineralization at 35°C). This result indicates that management history had not resulted in the development of differences in biomass characteristics significant enough to affect the kinetics of decomposition. Differences in estimates of C_0 between the two soils reflected management history effects on the pool of mineralizable substrate. Organic C was higher in the soil with a history of manure application (Table 3-1) and biomass C itself was likely a major source of mineralizable C. Estimates of potentially mineralizable N were not significantly higher for the M300 soil, in spite of the higher total N content of this soil. Problems with N loss in the microcosms as discussed in section 3.4.1, may have resulted in underestimation of this parameter.

Temperature had a significant effect on all of the biomass characteristics measured, but there was only a significant management history by temperature interaction for CFE flush C. For this characteristic, at 35°C values were not significantly

different for each soil. This result could explain reductions in potentially mineralizable C_0 estimates reported by others at high temperatures (Honeycutt et al., 1988; Dalias et al., 2001) i.e. regardless of the size of the biomass at lower temperatures, biomass size decreases to an equally low value at high temperatures.

This study did not provide any evidence to suggest that making net C and N mineralization models more mechanistic, by including more biomass characteristics, will improve model predictions. Management history had not impacted on the soil microbial biomass enough in this case, to create two communities with significant differences in their potential to mineralize organic substrates (i.e. k). It was therefore not possible to determine if soils with different relative rates of decomposition react differently to changing temperature.

The results of this experiment provided more insights into the biological mechanisms that can explain some of the previously observed temperature effects on decomposition. Declining biomass size with increasing temperature may partially explain reductions in the temperature response i.e. declining Q_{10}s at increasing temperatures. Differences in the C:N ratio of net mineralization at different temperatures has highlighted the importance of developing separate temperature response functions for each process.

The results from this experiment indicated differences in biomass specific respiration due to changes in temperature. These differences are difficult to interpret since the metabolic quotient does not differentiate between actively respiring biomass, and resting biomass. Small values for qCO_2 could be due to a large biomass with only a small component respiring at a high rate, or a small biomass, that is respiring relatively

efficiently. Biomass specific respiration rates do not indicate the actual production or assimilation efficiencies of the biomass. These values are best estimated using radio- or stable isotope studies. In future chapters of this thesis results from studies using ^{13}C- and ^{14}C-labeled substrates will be reported.

3.5 Conclusions

This study is unique in that it links the results of a typical kinetic analysis of C and N mineralization at a range of temperatures, with a variety of measures of microbial community characteristics. This research has confirmed previously reported temperature-driven changes in C and N substrate pool size and availability in laboratory incubations. It has revealed differences in the temperature response of net C and N mineralization, with large increases in net N mineralization between 5 and 15°C. This resulted in a decline in the ratio of net C mineralization to net N mineralization over this temperature range. It is hypothesized that C and N accumulate in the biomass at 5°C, due to a lack of predation by microfauna at this temperature. The large increase in net N mineralization between 5 and 15°C could be due to faunal grazing on the biomass and the release of biomass N.

The biomass specific respiration (qCO_2) has been suggested as an indication of the efficiency of C use by the biomass, with higher values indicative of low metabolic efficiency. In this experiment, average qCO_2 values were highest at the warmest incubation temperature (35°C) but these values declined over time, likely due to an exhaustion of available C. Higher qCO_2 values are expected at warmer temperatures, due to the Arrhenius effect of temperature on the enzymatic rate of digestion, yet these temperature-enhanced respiration rates do not always lead to increases in net C mineralization from soils. This phenomenon could be due to concomitant reductions in

biomass size at higher temperatures which could explain the reductions in potentially mineralizable C sometimes reported at high temperatures in soil incubation studies.

Some effects of temperature on bacterial and fungal species composition were observed, with distinctive communities forming at both the high (35°C) and low (5°C) temperatures. Since these differences in species composition were observed along with changes in biomass size and specific respiration, it is not possible to conclude which microbial community characteristics were causing the differences in zero-order and first-order mineralization rate parameters observed. There were no temperature by management interactions for any of the biomass characteristics except for CFE flush C at 35°C (a temperature outside the normal range for the soils in this study). This research therefore does not support the inclusion of more biomass characteristics in C and N mineralization models. Temperature effects on all biomass characteristics showed the same trends regardless of management history. These trends would have been incorporated within the temperature response function of the first-order rate constant. There were not enough soil types and replications in this experiment to determine the most appropriate TRF, but the evidence suggests that the TRF of both soils in this study is the same. While the experiment has not provided support for the inclusion of biomass characteristics explicitly in decomposition models, it has provided more insight into mechanisms behind temperature effects on decomposition. In particular, the effects of temperature on biomass characteristics (microbial biomass, community composition) that may help to explain temperature-driven functional shifts, have been explored.

Table 3-1. Initial pH, C and N, and moisture contents of a Nova Scotia soil from a long term fertility trial with (M300) and without (M0) a history of manure application.

Fertility management	pH	Organic C[z] mg g^{-1}	Total N mg g^{-1}	C:N	Field moisture range g H_2O g^{-1}	55% WFPS g H_2O g^{-1}
Control (M0)	6.1	24±2	1.5±0.2	16	0.24-0.27	0.27-0.30
Manure (M300)	6.3	30±4	2.0±0.3	15	0.26-0.31	0.32-0.33

[z] Determined by dry combustion, means ±SD reported; n=4

Table 3-2. Chemical and biological parameter sampling schedule for microcosms of soil from a Nova Scotia long term fertility trial incubated at four temperatures for up to 123 days.

Procedure	Temperature	Days from Initiation
Native C mineralization measurement	5	1, 2, 4, 7, 9, 11, 14, 17, 20, 27, 34, 41, 48, 55, 62, 69, 76, 82, 97, 111, 123
	15	2, 4, 5, 6, 7, 9, 11, 14, 17, 20, 27, 34, 41, 48, 55, 62, 69, 75, 82, 97, 111, 121
	25	1.5, 3, 4, 5, 6, 7, 9, 11, 14, 17, 20, 27, 34, 42, 49, 56, 61, 70, 77, 84, 98, 112, 121
	35	1.5, 3, 4, 5, 6, 7, 9, 11, 14, 17, 20, 27, 34, 41, 48, 55, 62, 69, 76, 83, 97, 111, 119
Inorganic N extraction	5	7, 14, 20, 27, 41, 55, 82, 111, 123
	15	7, 14, 20, 27, 41, 55, 82, 111, 121
	25	7, 14, 20, 27, 42, 56, 84, 112, 121
	35	7, 14, 20, 27, 41, 55, 83, 111, 119
Freeze drying for later microbial community analysis, CFE biomass measure	5	7, 14, 27, 55, 123
	15	7, 14, 27, 55, 121
	25	7, 14, 27, 56, 121
	35	7, 14, 27, 55, 119

Table 3-3. Primer pairs and sequences and PCR conditions for first and second round amplifications targeting the SSU rDNA region for bacteria and ITS region for fungi used in T-RFLP analysis of DNA extracted from microcosms of soil from a Nova Scotia long term fertility trial incubated at four temperatures for up to 123 days.

Domain	PCR round	Primer pairs and sequences
Bacteria	1	16f27 (5'-AGA GTT TGA TCC TGG CTC AG)
		1494r (5'-TAC GG(CT) TAC CTT GTT ACG AC)
	2	63f (5'- FAM-CAG GCC TAA CAC ATG CAA GTC)
		1405r (5'- VIC-CGG GCG GTG TGT ACA AG)
Fungi	1	EF3RCNL (5'- CAA ACT TGG TCA TTT AGA GGA)
		ITS4 (5'- TCC TCC GCT TAT TGA TAT GC)
	2	ITS1f (5'- NED-CTT GGT CAT TTA GAG GAA GTA A)
		ITS4 (5'-PET- TCC TCC GCT TAT TGA TAT GC
PCR amplification conditions		4 min at 94°C 30 s at 94°C, 30 s at 51°C, 1 min 30 s at 68°C for 35 cycles[z] 10 min at 72°C

[z]25 cycles for the second round bacterial PCR

Table 3-4. Incremental models used for curve fitting native C mineralization data measured from microcosms of soil from a Nova Scotia long term fertility trial incubated at four temperatures for up to 123 days.

Zero-order model	$Cit = ki$	Where C_{it} is the amount of C released during interval i preceding time t, in units of mg C kg^{-1} soil and k is the zero-order rate constant in units of mg C $kg^{-1}d^{-1}$
Single-pool exponential model	$Cit = C_0e^{-kt}\ (eki\text{-}1)$	Where C_{it} is the amount of C released during interval i preceding time t, in units of mg C kg^{-1} soil and k is the first-order rate constant in units of d^{-1} and C_0 is the maximum potentially mineralizable C in units of mg C kg^{-1} soil.
Two simultaneous reaction (TSR) model	$Cit = C_0ae^{-kt}\ (e^{ki\text{-}1}) + C_0(1\text{-}a)e^{-ht}\ (e^{hi\text{-}1})$	Where C_{it} is amount of C released during interval i preceding time t in units of mg C kg^{-1} soil and C_0 is the maximum potentially mineralizable C in units of mg C kg^{-1} soil. There are two pools of mineralizable C which add up to C_0, fraction one is a with a rate constant k in units of d^{-1} and fraction two is (1-a) with a rate constant h in units of d^{-1}

Table 3-5. Measured cumulative C and N mineralization from microcosms of a Nova Scotia soil from a long term fertility trial with (M300) and without (M0) a history of manure application, incubated at four temperatures for up to 123 days.

Temperature	Management history	Cumulative C mineralization		Cumulative N mineralization	
		Total (mg C kg^{-1} soil)	Rate (mg C kg^{-1} soil STU^{-1})	Total (mg N kg^{-1} soil)	Rate (mg N kg^{-1} soil STU^{-1})
5	M0	287.3 d	0.47 a	9.3 a	0.015 a
15	M0	450.3 d	0.24 b	31.4 a	0.025 a
25	M0	615.0 bcd	0.20 b	142.1 c	0.046 a
35	M0	874.8 abc	0.20 b	122.9 bc	0.029 a
5	M300	404.9 d	0.66 a	20.1 a	0.033 a
15	M300	524.5 cd	0.28 b	134.0 c	0.073 b
25	M300	941.8 ab	0.31 b	125.3 bc	0.041 a
35	M300	1086.3 a	0.31 b	117.0 bc	0.027 a
			ANOVA		
Temperature		$p<0.001$	$p=0.002$	$p<0.001$	$p=0.054$
Management		$p<0.001$	ns	ns	$p=0.049$
Temperature x Management		ns	ns	$p=0.028$	$p=0.057$

Means followed by the same letter in the same column are not different at the 5% significance level using Tukey's HSD pairwise comparison of means test.

Table 3-6. Parameter estimates (5%CI) for native N mineralization data from microcosms of a Nova Scotia soil from a long term fertility trial with (M300) and without (M0) a history of manure application, incubated at four temperatures for up to 123 days and fit to the zero-order and SPE models.

Temperature	Management history	Zero-order rate constant, z (mg N kg^{-1} d^{-1})	Zero-order rate constant, z (mg N kg^{-1} STU^{-1})	SPE relative rate constant, k (d^{-1})	N_0, (mg kg^{-1})
5	M0	0.088 (0.028) a	0.018 (0.006) a	*0.010 (0.026)* ab	*13 (22)* a
15	M0			0.028 (0.024) ab	27 (5) a
25	M0	1.362 (0.153) d	0.055 (0.006) d	NA	NA
35	M0			0.011 (0.007) a	177 (74) b
5	M300			0.066 (0.071) ab	18 (5) a
15	M300	0.988 (0.200) c	0.066 (0.013) d	NA	NA
25	M300			0.011 (0.011) a	177 (104) b
35	M300			0.068 (0.037) b	108 (16) b

Means in the same column followed by the same letter are not significantly different at the 5% probability level
Italicized SPE parameter estimates are included for comparison, although zero-order models provided a better fit.
NA indicates that the model could not be parameterized.

Table 3-7. Parameter estimates (5%CI) for incremental native C mineralization data from microcosms of a Nova Scotia soil from a long term fertility trial with (M300) and without (M0) a history of manure application, incubated at four temperatures for up to 123 days and fit to the zero-order and SPE models.

Temp-erature	Management history	Zero-order rate constant, z (mg C kg^{-1} d^{-1})	Zero-order rate constant, z (mg C kg^{-1} STU^{-1})	SPE relative rate constant, k (d^{-1})	C_0, (mg kg^{-1})	C:N ratio of net C and N mineralization (estimated to day 123)
5	M0	2.743 (0.854) a	0.549 (0.171) a	NA	NA	31.2
15	M0	3.750 (0.846) ab	0.250 (0.056) b	*0.002 (0.007)* b	*1642 (3258)* ab	17.6
25	M0			0.015 (0.006) a	889 (185) a	4.5
35	M0			0.011 (0.006) a	1398 (464) ab	7.9
5	M300	3.438 (1.394) ab	0.688 (0.279) a	*0.008 (0.012)* a	*744 (662)*	23.5
15	M300			0.011 (0.007) a	757 (260) a	4.6
25	M300			0.011 (0.004) a	1522 (364) b	9.8
35	M300			0.013 (0.004) a	1528 (280) b	10.5

Means in the same column followed by the same letter are not significantly different at the 5% probability level
Italicized SPE parameter estimates are included for comparison, although zero-order models provided a better fit.
NA indicates that the model could not be parameterized.

Table 3-8. Chloroform fumigation extraction flush and metabolic quotient for microcosms of a Nova Scotia soil from a long term fertility trial with (M300) and without (M0) a history of manure application, incubated at four temperatures for up to 123 days.

Temperature	Management history	CFE flush (mg C kg^{-1})	qCO_2 (mg CO_2-C mg^{-1}CFE flush d^{-1})
5	M0	103.7 bc	0.0090 a
5	M300	162.8 a	0.0130 a
15	M0	91.2 cd	0.0664 ab
15	M300	144.8 a	0.0413 ab
25	M0	78.0 d	0.1127 b
25	M300	115.7 b	0.0728 ab
35	M0	39.3 e	0.2666 c
35	M300	44.0 e	0.2751 c
		ANOVA	
Temperature		$p < 0.001$	$p < 0.001$
Management		$p < 0.001$	ns
Temperature x Management		$p < 0.001$	ns

Values presented are the means of 5 sampling occasions. Means followed by the same letter in the same column are not different at the 5% significance level using Tukey's HSD pairwise comparison of means test.

Table 3-9. Probability values from analysis of variance of first four principal component scores for bacterial T-RFLP profiles from a Nova Scotia soil with (M300) and without (M0) a history of manure application, incubated at four temperatures for up to 123 days, measured on five occasions.

Source[z]	df	PC1 (9.8%)[y]	PC2 (4.0%)	PC3 (2.8%)	PC4 (2.5%)
Temp	3	0.491	0.664	0.009	<0.001
Mgt	1	0.059	0.474	0.877	0.080
Day	4	0.006	0.649	0.198	0.020
Rep	2	0.865	0.211	0.122	0.834
Temp*Mgt	3	0.827	0.477	0.729	0.806
Temp*Day	12	0.320	0.726	0.061	0.526
Mgt*Day	4	0.759	0.598	0.9	0.497
Error	68				
Total	97				

[z]Mgt=Management history; Temp=Temperature; Day=Sampling occasion
[y] Values in parentheses are the percentage of the variability accounted for by each PC.

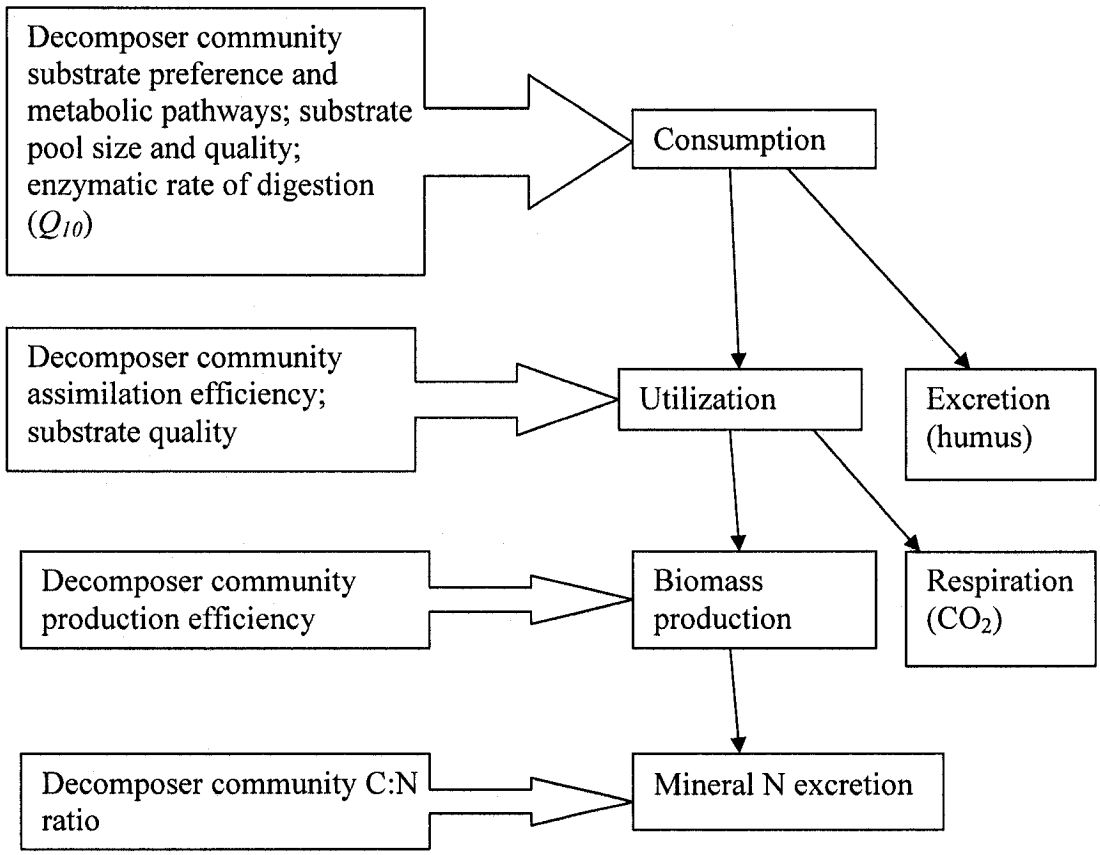
Table 3-10. Probability values from analysis of variance of first four principal component scores for fungal T-RFLP profiles from a Nova Scotia soil with (M300) and without (M0) a history of manure application, incubated at four temperatures for up to 123 days, measured on five occasions.

Source[z]	df	PC1 (2.8%)[y]	PC2 (2.1%)	PC3 (2.0%)	PC4 (1.9%)
Temp	3	0.082	0.025	<0.001	<0.001
Mgt	1	0.438	0.491	0.107	0.471
Day	4	<0.001	0.224	0.047	0.015
Rep	2	0.972	0.941	0.114	0.22
Temp*Mgt	3	0.985	0.715	0.128	0.716
Temp*Day	12	0.826	0.906	0.118	0.309
Mgt*Day	4	0.278	0.119	0.635	0.828
Error	55				
Total	84				

[z]Mgt=Management history; Temp=Temperature; Day=Sampling occasion

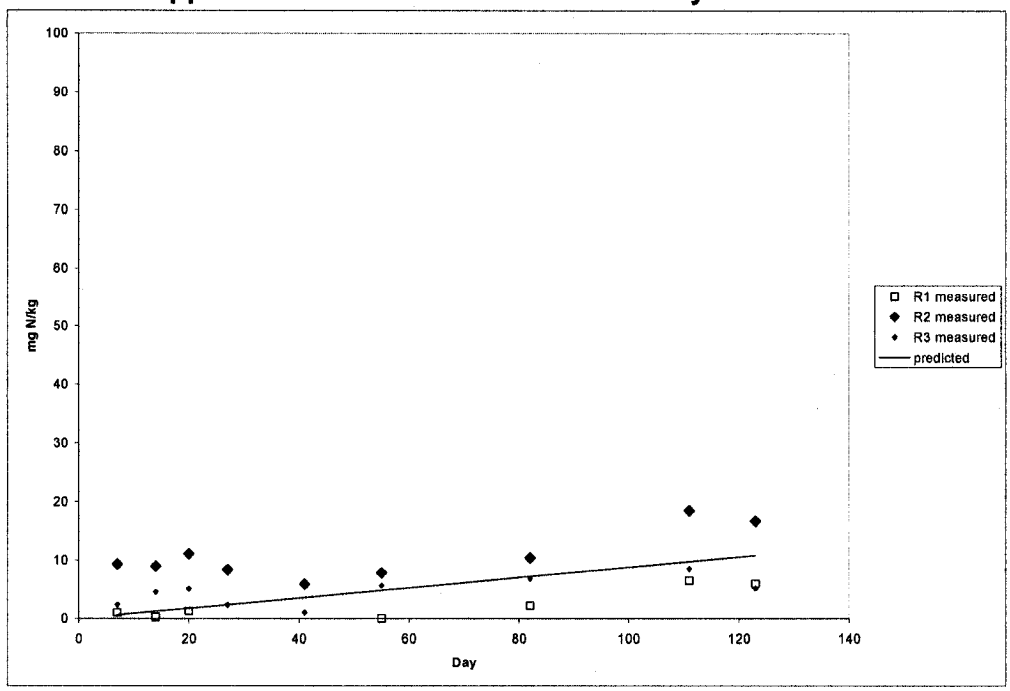
[y] Values in parentheses are the percentage of the variability accounted for by each PC.

Figure 3-1. Steps in the decomposition of organic substrates in soil, and biological and chemical factors that may be affected by temperature and influence these steps.



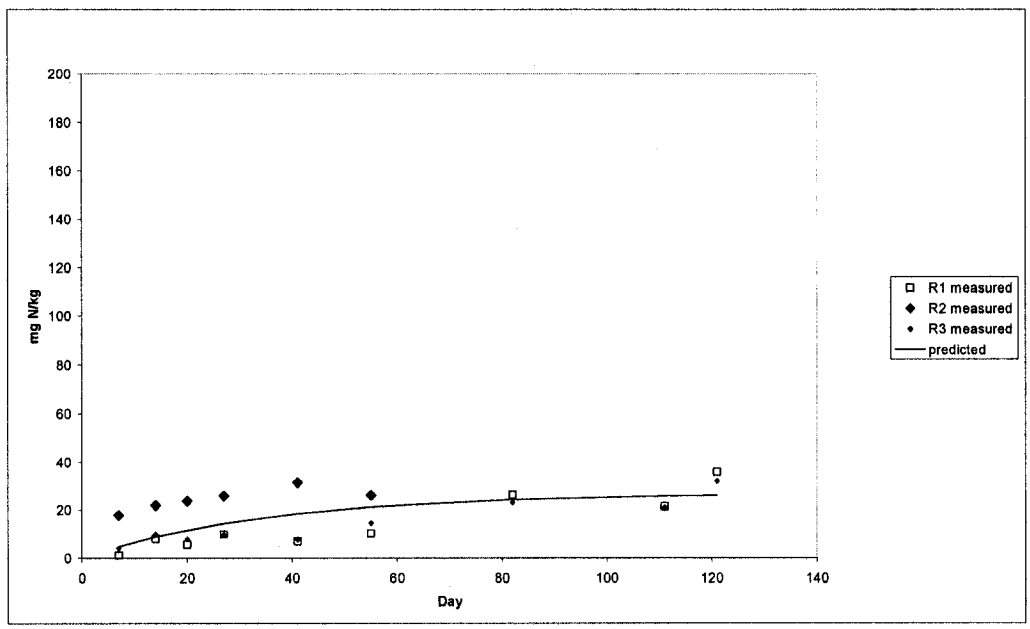
Steps represented by rectangles; biological and chemical factors represented by rectangular arrows; linear arrows represent the flow of C and N substrates. Figure modified from Hunt et al. (1987).

Figure 3-2. Measured and predicted cumulative net N mineralization from microcosms of a Nova Scotia soil from a long term fertility trial without a history of manure application incubated at 5°C for 123 days.



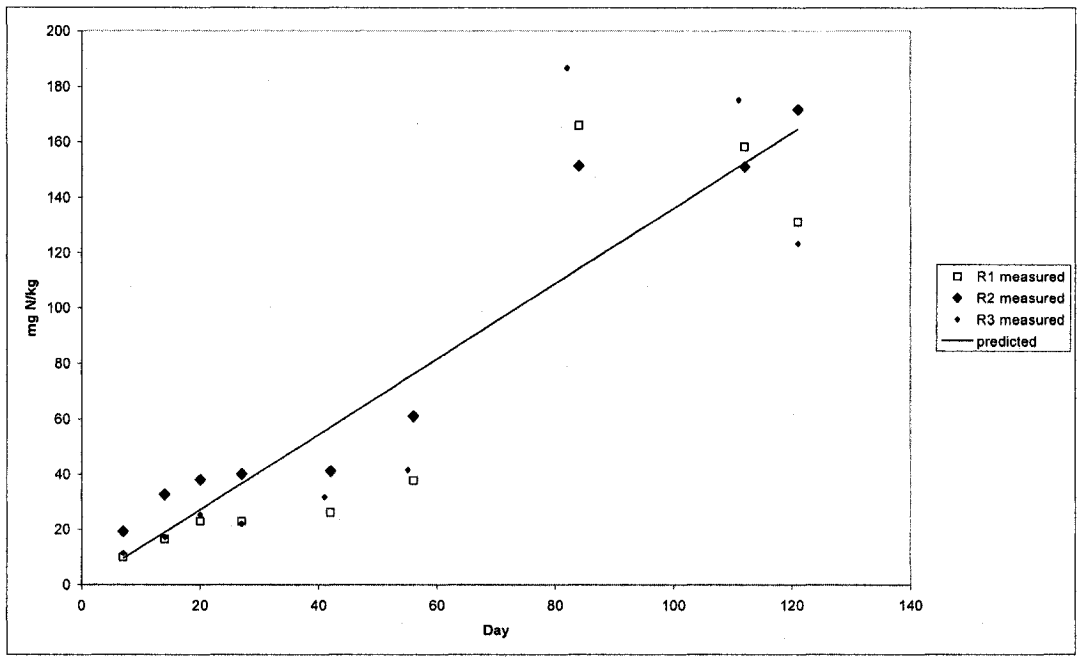
R refers to replicate.

Figure 3-3. Measured and predicted cumulative net N mineralization from microcosms of a Nova Scotia soil from a long term fertility trial without a history of manure application incubated at 15°C for 121 days.



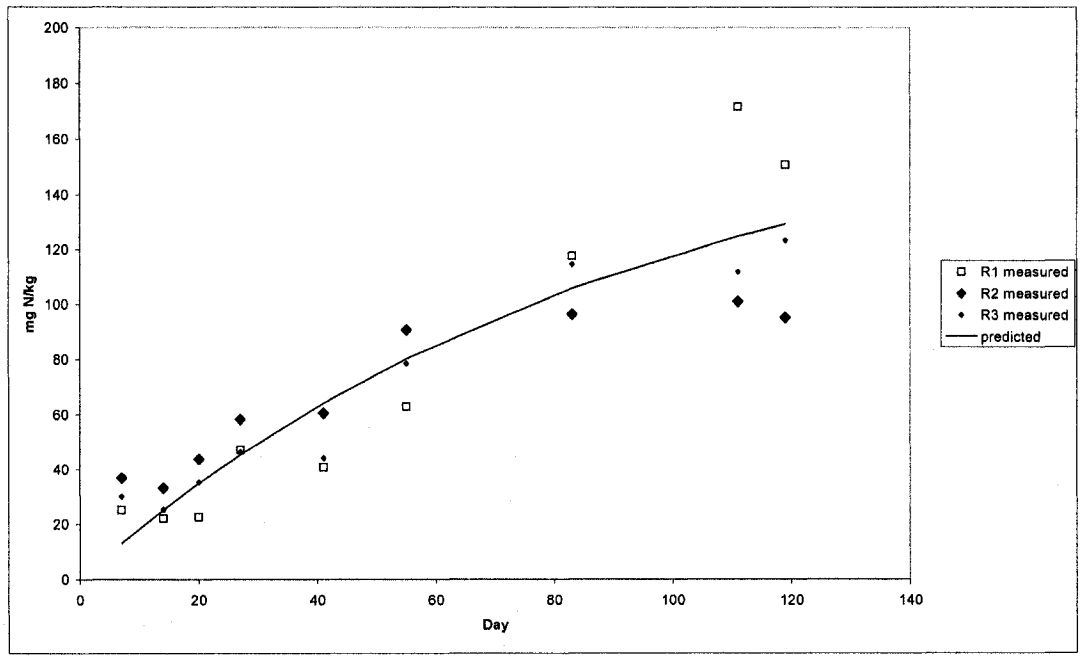
R refers to replicate.

Figure 3-4. Measured and predicted cumulative net N mineralization from microcosms of a Nova Scotia soil from a long term fertility trial without a history of manure application incubated at 25°C for 119 days.



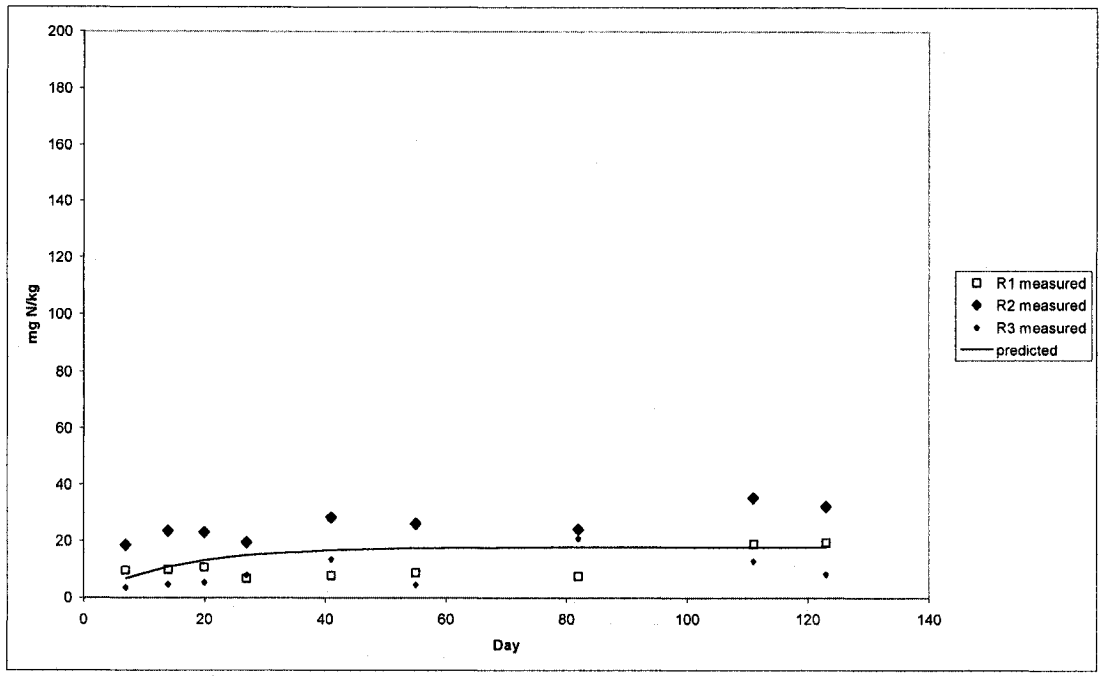
R refers to replicate.

Figure 3-5. Measured and predicted cumulative net N mineralization from microcosms of a Nova Scotia soil from a long term fertility trial without a history of manure application incubated at 35°C for 117 days.



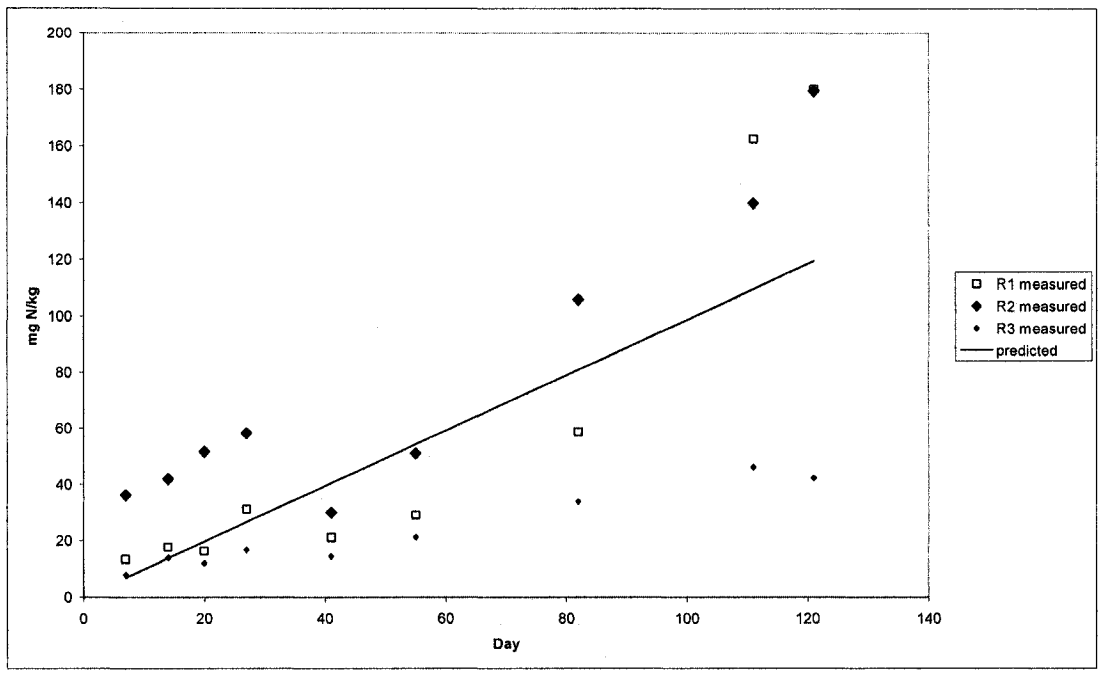
R refers to replicate.

Figure 3-6. Measured and predicted cumulative net N mineralization from microcosms of a Nova Scotia soil from a long term fertility trial with a history of manure application incubated at 5°C for 123 days.



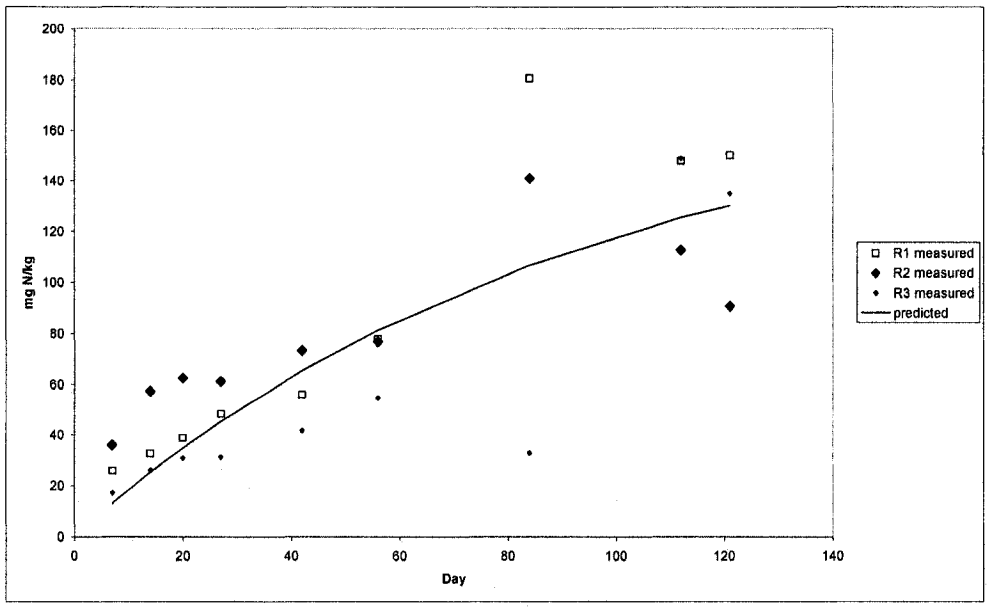
R refers to replicate.

Figure 3-7. Measured and predicted cumulative net N mineralization from microcosms of a Nova Scotia soil from a long term fertility trial with a history of manure application incubated at 15°C for 121 days.



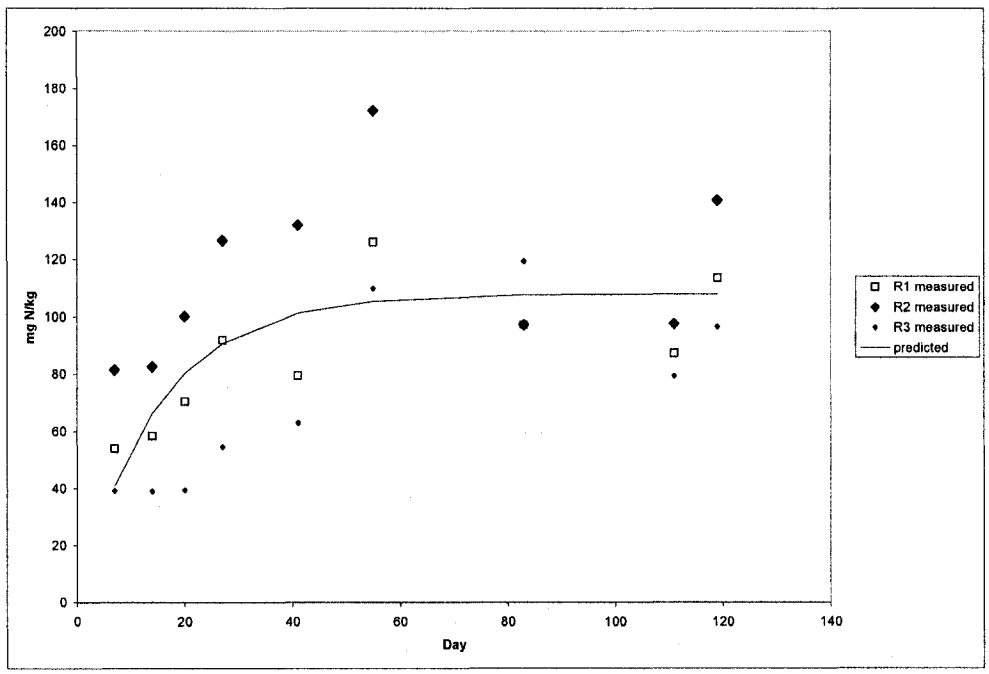
R refers to replicate.

Figure 3-8. Measured and predicted cumulative net N mineralization from microcosms of a Nova Scotia soil from a long term fertility trial with a history of manure application incubated at 25°C for 119 days.



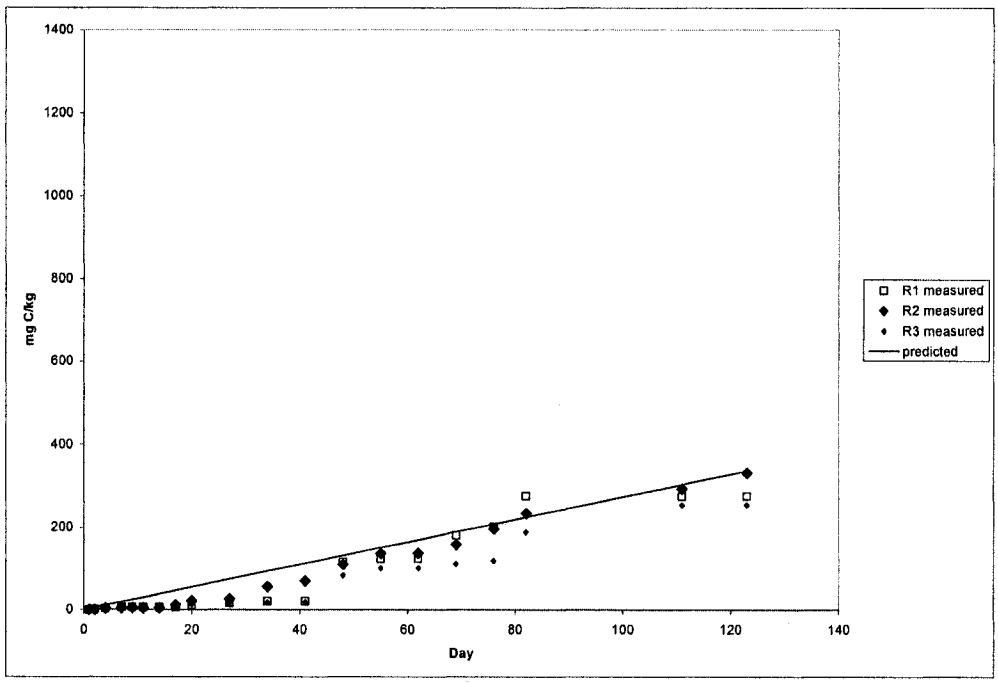
R refers to replicate.

Figure 3-9. Measured and predicted cumulative net N mineralization from microcosms of a Nova Scotia soil from a long term fertility trial without a history of manure application incubated at 35°C for 117 days.



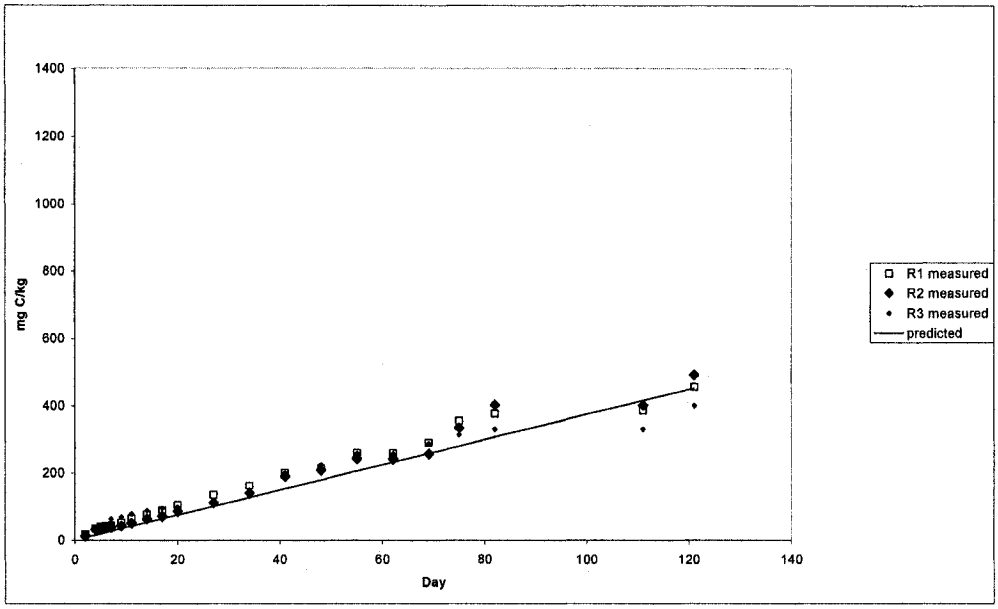
R refers to replicate.

Figure 3-10. Measured and predicted cumulative C mineralization from microcosms of a Nova Scotia soil from a long term fertility trial without a history of manure application incubated at 5°C for 123 days.



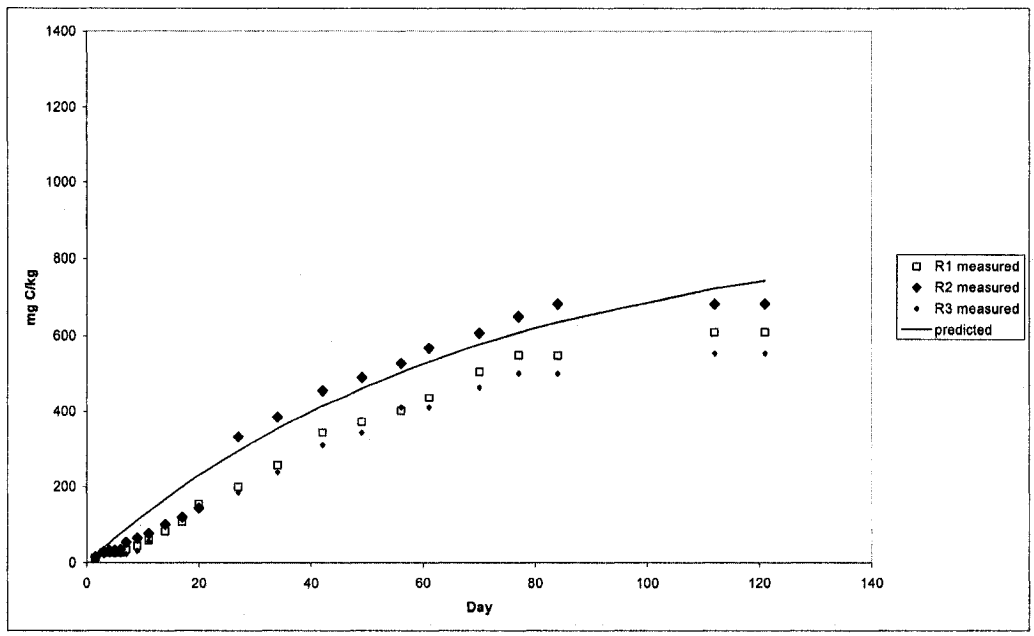
R refers to replicate.

Figure 3-11. Measured and predicted cumulative C mineralization from microcosms of a Nova Scotia soil from a long term fertility trial without a history of manure application incubated at 15°C for 121 days.



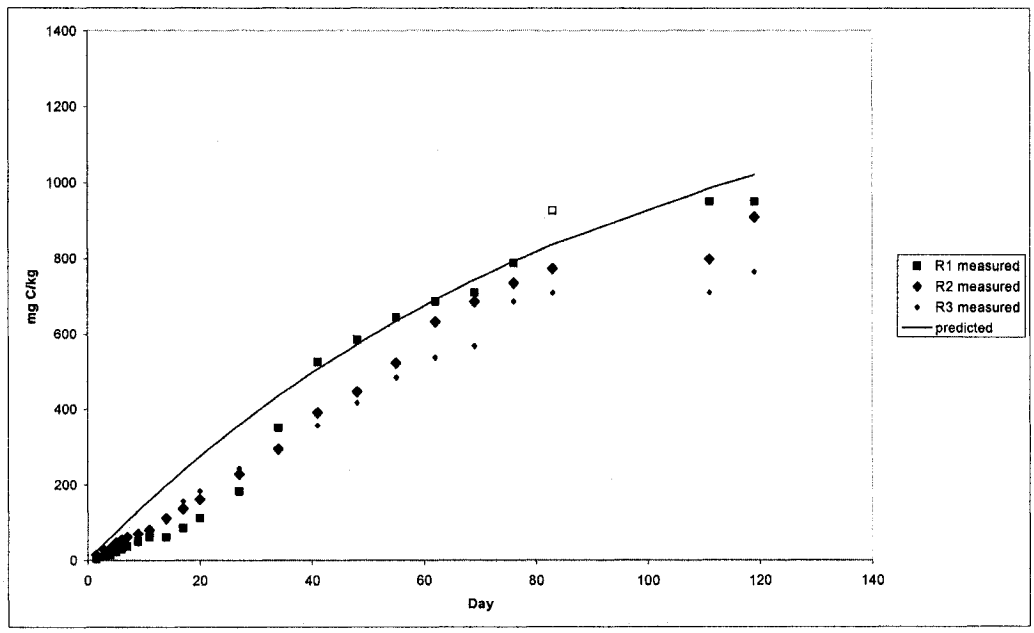
R refers to replicate.

Figure 3-12. Measured and predicted cumulative C mineralization from microcosms of a Nova Scotia soil from a long term fertility trial without a history of manure application incubated at 25°C for 119 days.



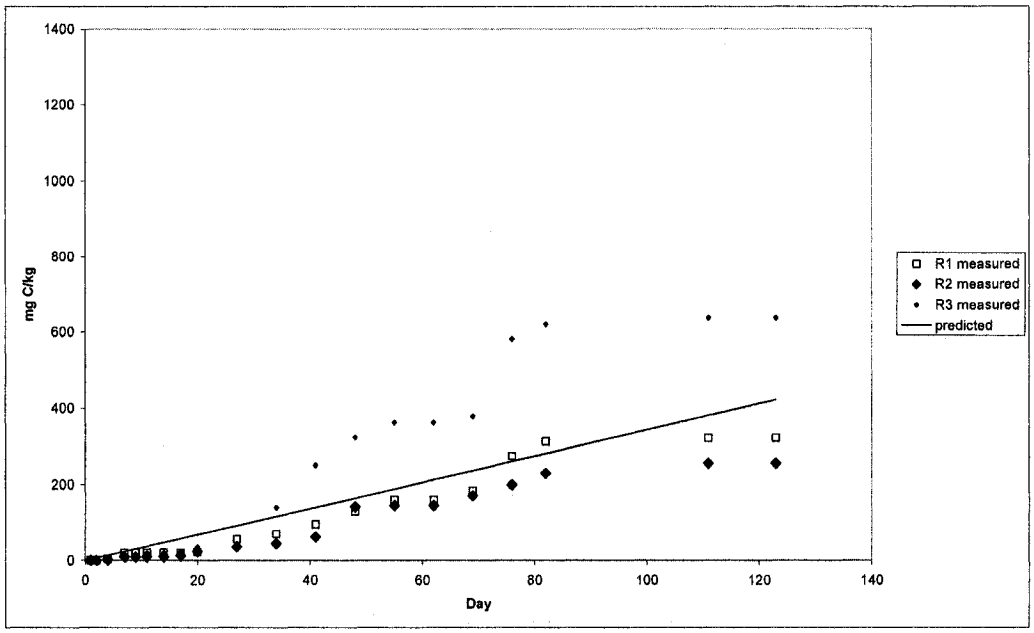
R refers to replicate.

Figure 3-13. Measured and predicted cumulative C mineralization from microcosms of a Nova Scotia soil from a long term fertility trial without a history of manure application incubated at 35°C for 117 days.



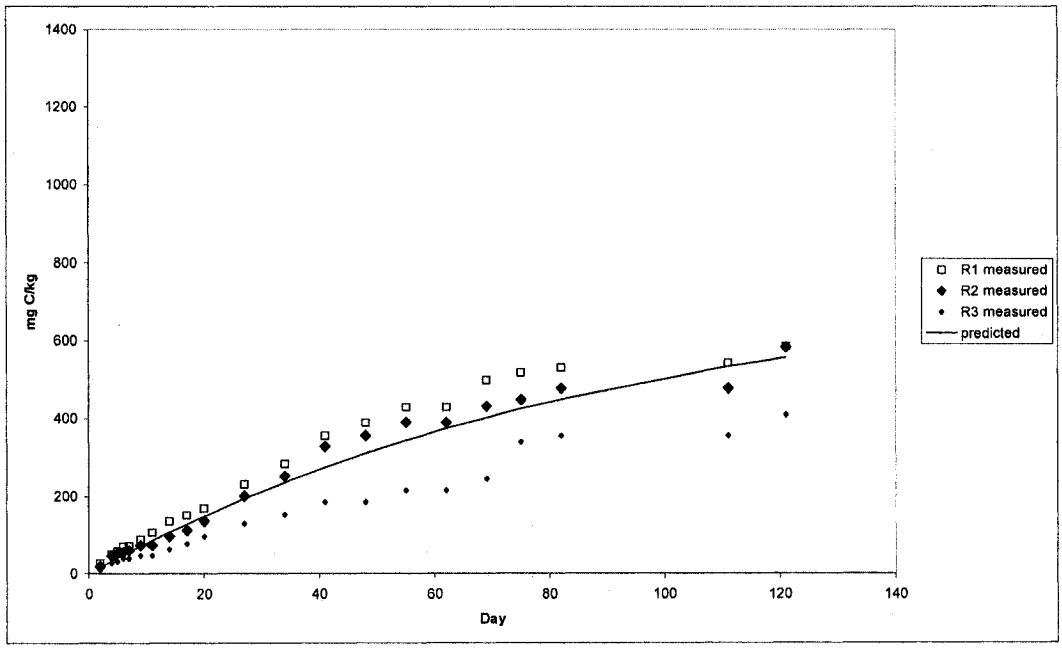
R refers to replicate.

Figure 3-14. Measured and predicted cumulative C mineralization from microcosms of a Nova Scotia soil from a long term fertility trial with a history of manure application incubated at 5°C for 123 days.



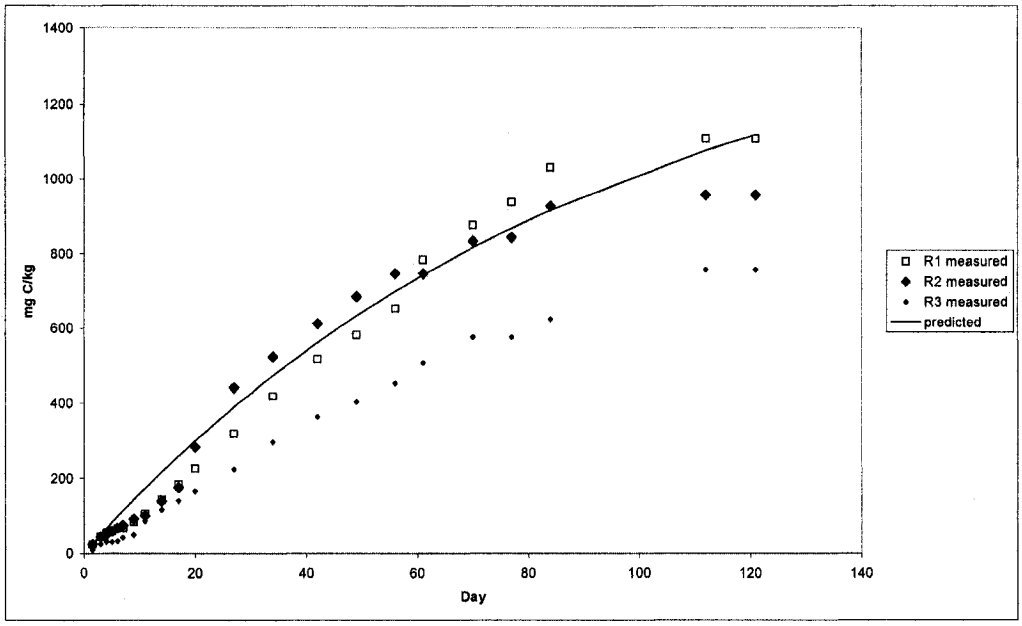
R refers to replicate.

Figure 3-15. Measured and predicted cumulative C mineralization from microcosms of a Nova Scotia soil from a long term fertility trial with a history of manure application incubated at 15°C for 121 days.



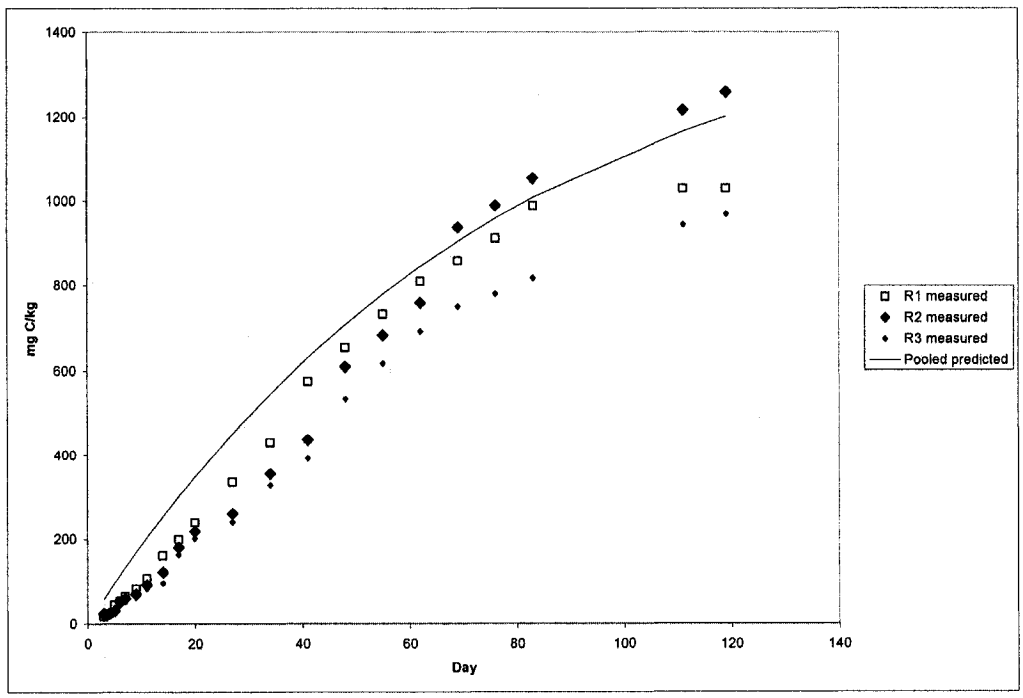
R refers to replicate.

Figure 3-16. Measured and predicted cumulative C mineralization from microcosms of a Nova Scotia soil from a long term fertility trial with a history of manure application incubated at 25°C for 119 days.



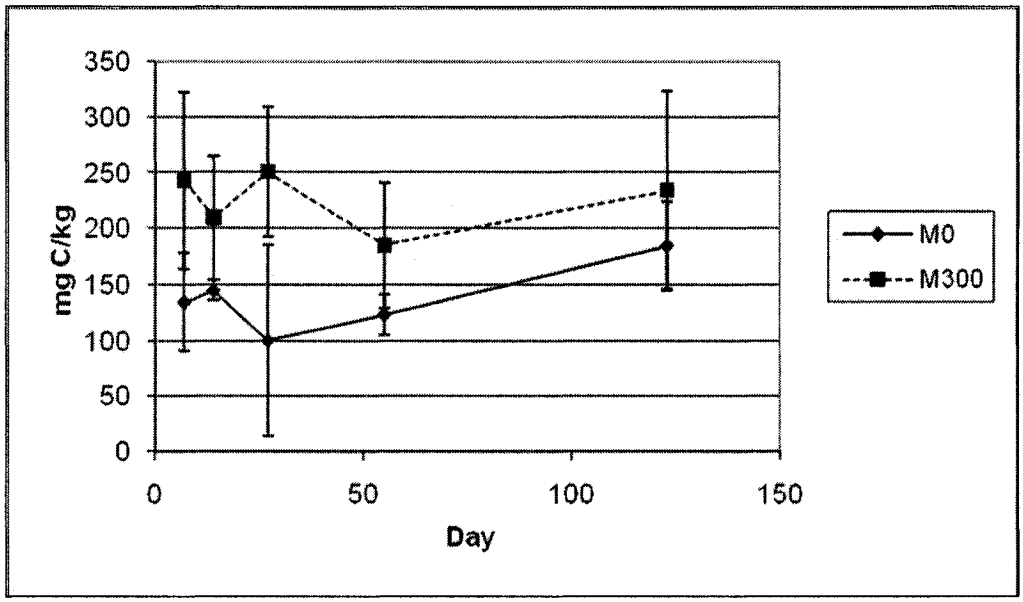
R refers to replicate.

Figure 3-17. Measured and predicted cumulative C mineralization from microcosms of a Nova Scotia soil from a long term fertility trial with a history of manure application incubated at 35°C for 117 days.



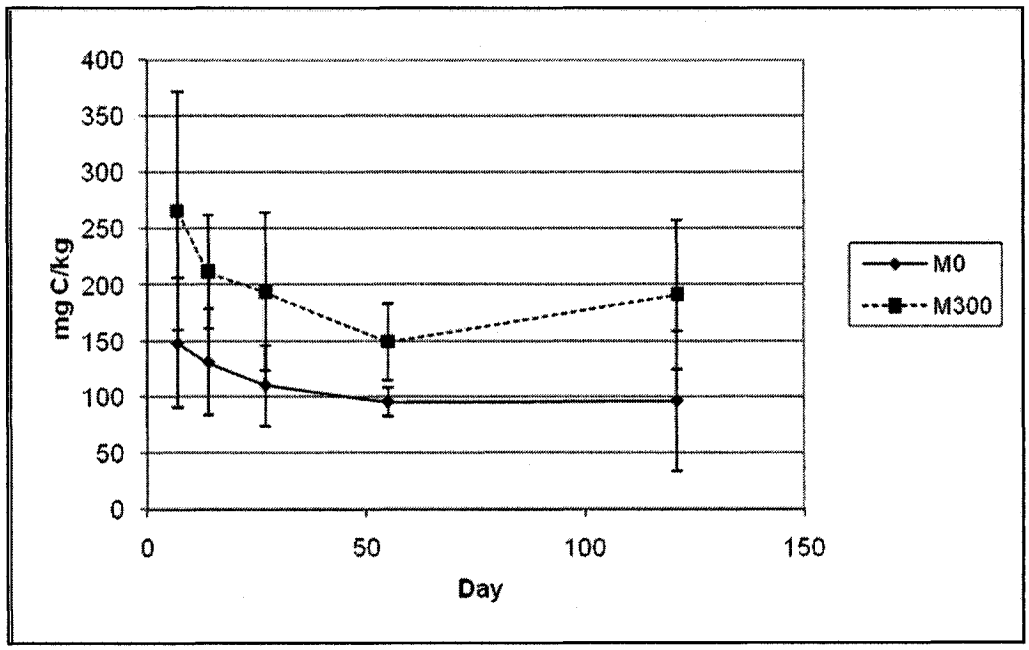
R refers to replicate.

Figure 3-18. CFE flush C measured on five occasions in microcosms of a Nova Scotia soil from a long term fertility trial with (M300) and without (M0) a history of manure application incubated at 5°C for 123 days



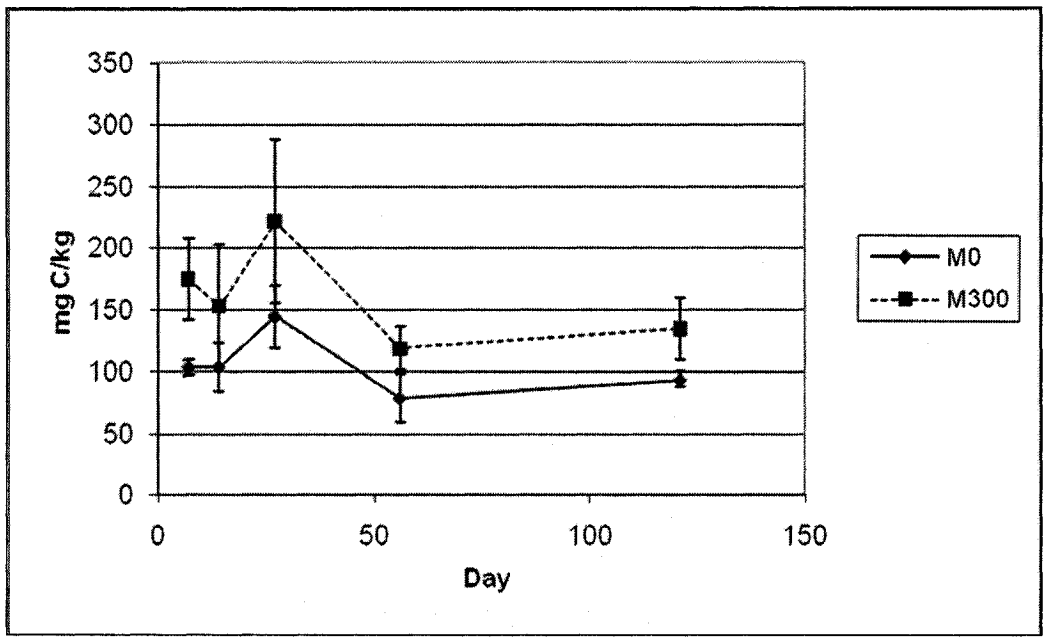
Error bars are 95% CI of the mean.

Figure 3-19. CFE flush C measured on five occasions in microcosms of a Nova Scotia soil from a long term fertility trial with (M300) and without (M0) a history of manure application incubated at 15°C for 121 days (error bars 95% CI).



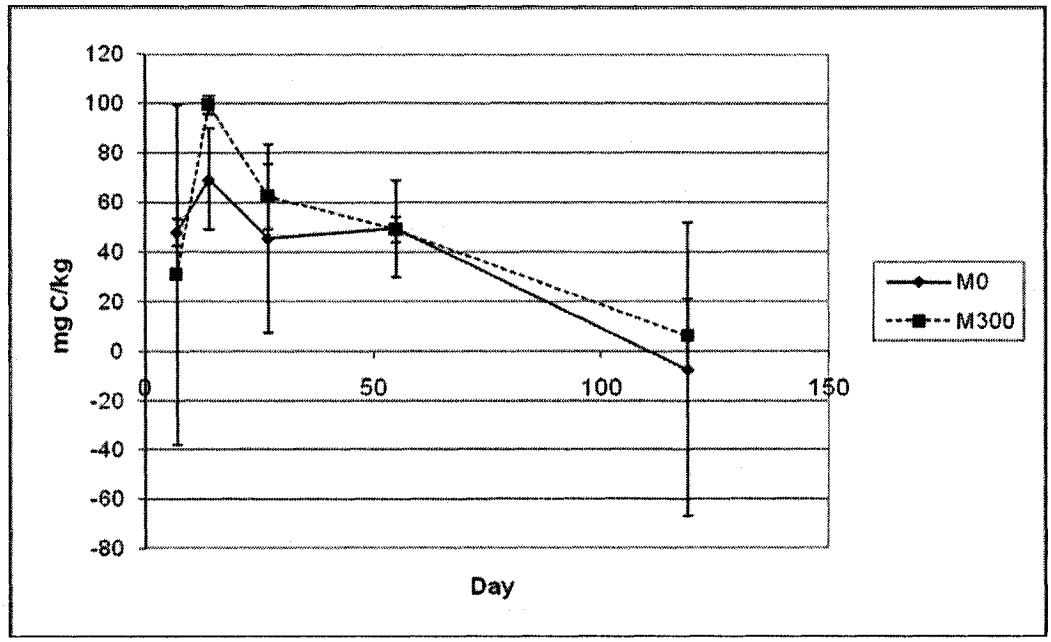
Error bars are 95% CI of the mean.

Figure 3-20. CFE flush C measured on five occasions in microcosms of a Nova Scotia soil from a long term fertility trial with (M300) and without (M0) a history of manure application incubated at 25°C for 119 days.



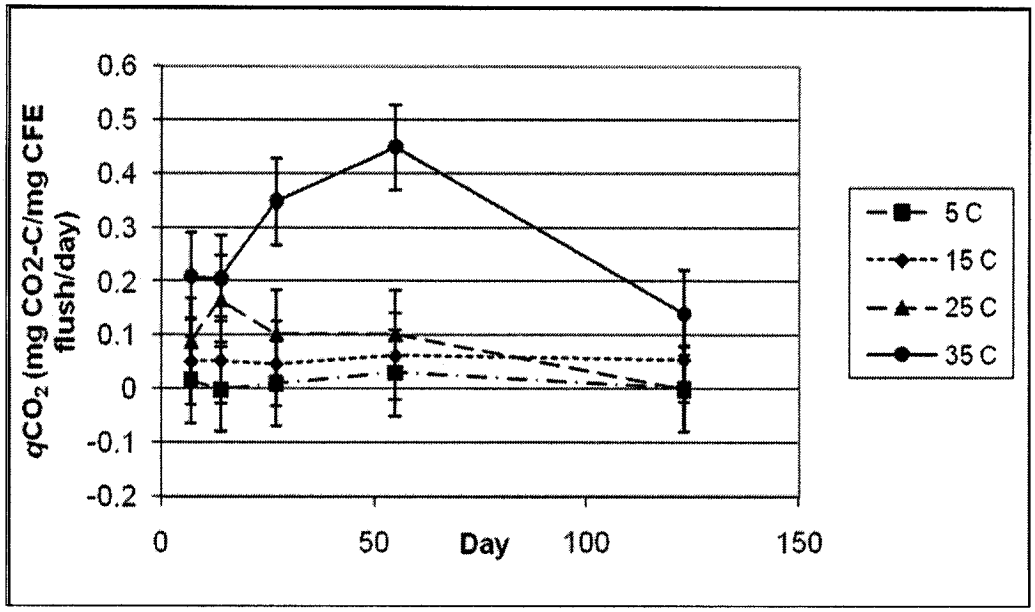
Error bars are 95% CI of the mean.

Figure 3-21. CFE flush C measured on five occasions in microcosms of a Nova Scotia soil from a long term fertility trial with (M300) and without (M0) a history of manure application incubated at 35°C for 117 days.



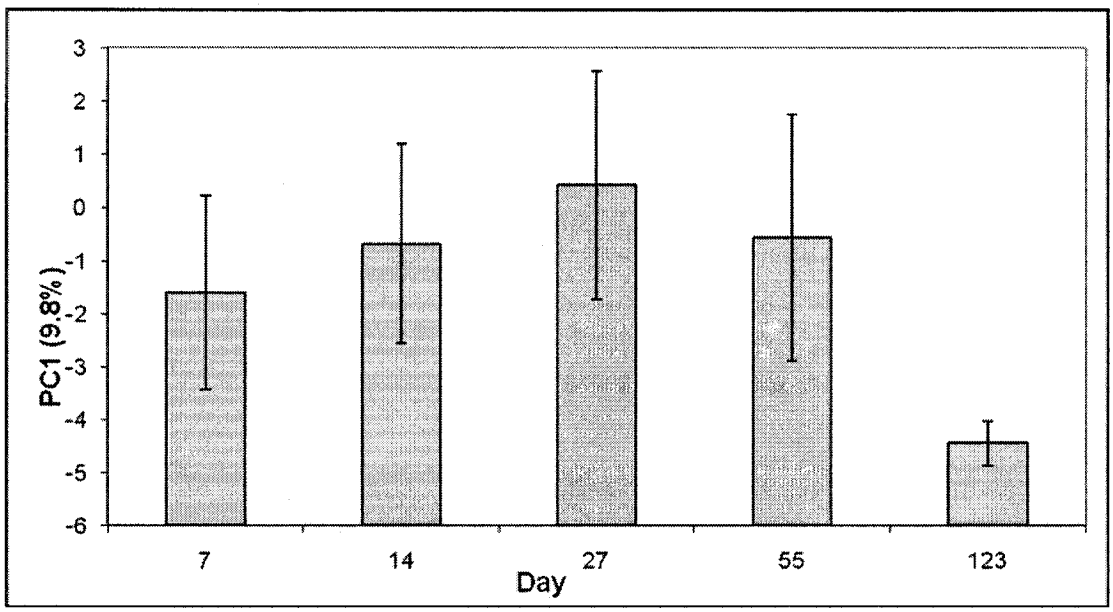
Error bars are 95% CI of the mean.

Figure 3-22. Effect of sample day on the metabolic quotient (qCO_2) of a Nova Scotia soil from a long term fertility trial with and without a history of manure application incubated at four temperatures for up to 123 days.



Both soils combined at each level of temperature; error bars 95% CI.

Figure 3-23. Bacterial T-RFLP scores on each sampling day for a Nova Scotia soil from a long term fertility trial with and without a history of manure application incubated at four temperatures for up to 123 days.



Error bars indicate the 95% confidence intervals of each mean

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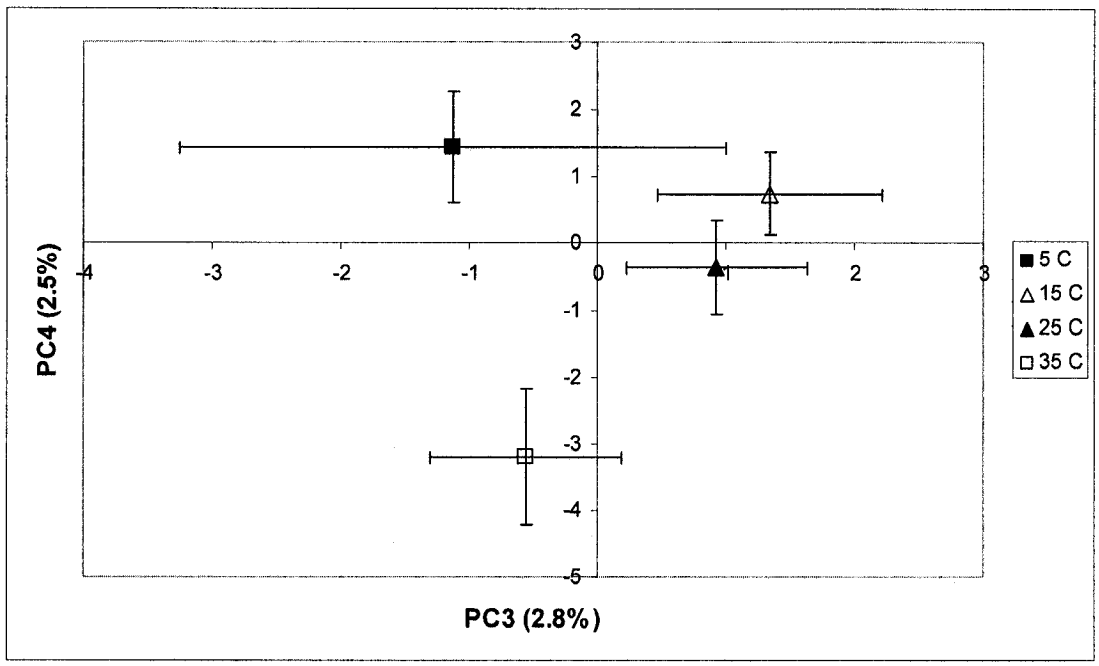
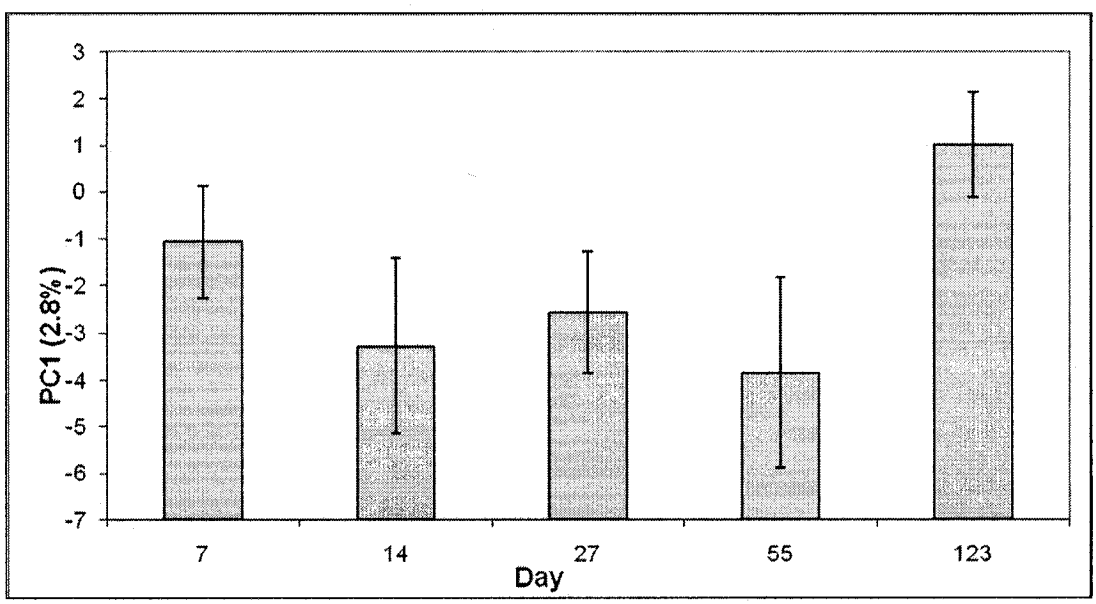
Figure 3-24. Mean scores for principal components of bacterial T-RFLP profiles for a Nova Scotia soil from a long term fertility trial with and without a history of manure application incubated at four temperatures.



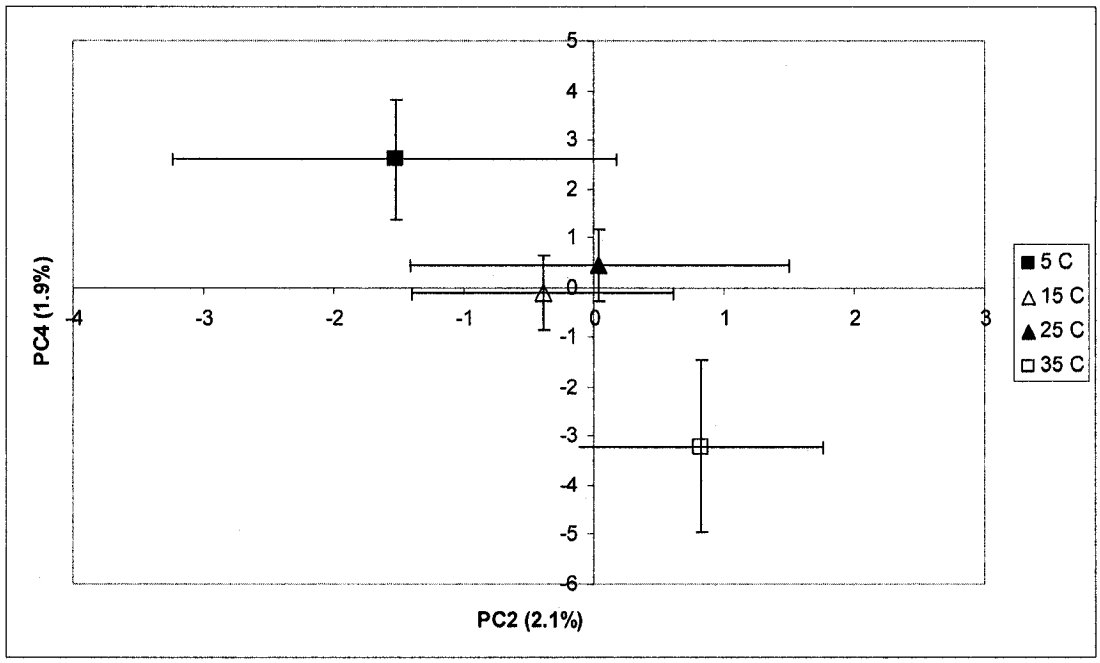
Error bars indicate the 95% confidence intervals of each mean.

Figure 3-25. Fungal T-RFLP scores on each sampling day for a Nova Scotia soil from a long term fertility trial with and without a history of manure application incubated at four temperatures for up to 123 days.



Error bars indicate the 95% confidence intervals of each mean.

Figure 3-26. Mean scores for principal components of fungal T-RFLP profiles for a Nova Scotia soil from a long term fertility trial with and without a history of manure application incubated at four temperatures for up to 123 days.



Error bars indicate the 95% confidence intervals of each mean.

Chapter 4: Temperature Effects on Mineralization of ^{14}C-labeled Wheat and Microbial Community Characteristics

4.1 Introduction

First-order kinetics are commonly used when modeling soil organic matter dynamics (Neill and Gignoux, 2006). This approach, sometimes called the rate constant approach, is based on the assumption that the rate of reaction is a function of the size of the substrate pool (C_o). When examining how these relations are altered to reflect the effects of temperature, two key assumptions are commonly made: first, that the size of the pool of metabolizable substrate (usually referred to with the symbol C_0 in carbon mineralization studies) does not change with temperature, and, second, that the effect of temperature on the rate of mineralization can be predicted using a temperature response function. Temperature response functions used in first-order models vary, but all are empirical functions based on observed effects of temperature on soil respiration rates or net C mineralization rates.

There is mounting evidence that these assumptions are incorrect. Several researchers have observed in laboratory incubations that the size of the mineralizable pool of C and N estimated using the first-order model varies with changing temperature (Ellert and Bettany, 1992; Macdonald et al., 1995; Zogg et al., 1997; Zak et al., 1999; Dalias et al., 2002). In all of these studies both the relative rate constant, k, and the potentially mineralizable substrate pool (C_0 or N_0) were allowed to fluctuate in the curve-fitting process. Because these parameters are often correlated, it is therefore difficult to determine which of the parameters is displaying temperature sensitivity (Davidson and Janssens, 2006). Nevertheless, the findings indicate some temperature-driven changes in mineralization kinetics that violate the assumptions of first-order kinetic approaches to

soil organic matter modeling.

Biological mechanisms have been proposed to explain deviations of some experimental results from first-order kinetic assumptions (Dalias et al., 2001; Dalias et al., 2002; Honeycutt et al., 1988). Dalias et al. (2003) suggested that organisms with different functions capable of accessing different pools of substrate predominate at different temperatures: they term this the functional shift hypothesis. If this functional shift occurs consistently over the same temperature range and is predictable in direction and magnitude, then its effects could be incorporated into an improved empirical temperature response function; however, this has not been the case. Honeycutt et al. (1988) found that a shift towards a larger pool of substrate C (from added papermill sludge) occurred at 30°C. Dalias et al. (2001; 2002) studied C and N mineralization of an added substrate in 7 different European forest soils and reported functional shifts occurring at a variety of different temperatures with no clear trend towards larger or smaller substrate pools at specific temperatures. This suggests that the temperature response of net C and N mineralization in soils is affected by the soil microbial community, or the native pools of C and N in the soil, or the mineralogy of the soil. All of these characteristics could have varied among the soils in the Dalias et al. (2002) study, because they came from a variety of European forest locations.

There is a lack of consensus on how well soil C models based on first-order kinetics can be used to predict the effects of climate change on soil carbon (Davidson and Jansenns, 2006). Giardina and Ryan (2000) used a single-pool approach to demonstrate that soil C is relatively insensitive to increases in temperature; however, this has been disputed by Davidson et al. (2000) who argued that the single-pool approach masks the

differences in temperature sensitivity of the various pools of soil C. Some authors have advocated for the incorporation of microbial parameters representing biomass size and function into soil process models in order to improve their accuracy (Balser et al, 2006; Neill and Gignoux, 2006; Fang et al., 2005a; Grenon et al., 2004; Schimel, 2001). The incorporation of additional biological parameters into soil decomposition models will be useful if the links between temperature, biological parameters, and the final soil process rate can be made. The study reported in this chapter was designed to investigate the effects of temperature on soil biological characteristics and the capacity of the microbial community to metabolize an added substrate.

The following questions were addressed:

1. Is the first-order model suitable for describing C respiration from plant litter added to soil across a range of temperatures?
2. How does incubation temperature affect the size of the pool of metabolizable C, and how is this related to estimates of C_0 obtained using the first-order model?
3. How does incubation temperature affect the bacterial and fungal community structure and microbial use of added substrate C?
4. Is respiration of added plant litter affected by the past management of the soil?

To answer these questions, an experimental system was developed to examine the mineralization of a standard ^{14}C-labeled substrate (wheat) added to two soils with differing management histories, but with similar mineralogical characteristics. The release of ^{14}C-CO_2, assimilation of ^{14}C into microbial biomass, efficiency of the active

biomass, and bacterial and fungal community structure, were monitored over a 123 day incubation period at four temperatures (5, 15, 25 and 35°C).

4.2 Materials and Methods

4.2.1 Site and Soils

Soils were sampled from a long term field trial at the Agriculture and Agri-Food Canada Research Station at Nappan, Nova Scotia, Canada (45° 45' N, -64° 14'), in July 2004. Soils were taken from forage (native grass/clover mixture) plots that had either received semi-solid beef manure (300 kg total N ha^{-1} rate) annually for the previous ten years, or no fertilizer amendment. Initial soil properties and moisture characteristics are listed in Table 3-1. Full details of the site chosen, sampling protocol and experimental setup, are included in Chapter 3, Section 3.2.

4.2.2 Production of ^{14}C-labeled Wheat

A pulse labelling method was used to label 4 flats (25 x 50 x 5cm) of wheat. Wheat was grown in a well-ventilated greenhouse at ambient temperatures for 8 weeks. Labelling was conducted on 8 occasions during the growing period using the following protocol. Two flats of plants were placed inside a plexiglass chamber (60 x 60 x 60 cm). The lid of the plexiglass chamber was fitted with two subaseal stoppers below each of which was suspended the lid of a 12 ml Exetainer (Labco, UK) with the rubber stopper removed. Pulse labelling was conducted by attaching a vial containing $Na_2{}^{14}CO_3$ (0.375 Mbq for occasion 1 and 2, 1 MBq for occasions 3, 4 and 5, and 1.25 MBq for occasions 6, 7 and 8) and 0.05 g $Na_2{}^{12}CO_3 \cdot H_2O$ to one of the suspended lids. Another vial containing 0.596 g $Na_2{}^{12}CO_3 \cdot H_2O$ was attached to the second lid. The plexiglass chamber was closed and 0.5 ml of 2 M HCl was injected into the collection vial containing the $Na_2{}^{14}CO_3$. The carbon dioxide content of the chamber was monitored with an infrared

gas analyzer (Quibit Systems, Kingston, ON). When CO_2 levels reached a minimum level (approximately 20 ul l^{-1}), 0.5 ml of 2 M HCl was added to the vial containing the $Na_2{}^{12}CO_3 \cdot H_2O$ in order to elevate chamber CO_2 levels again and effectively "chase" any remaining ^{14}C-CO_2 into the plant.

All above-ground plant material (at Zadok's growth stage 25) was harvested, dried at 60°C and ground to pass through a 40 mesh (0.42 mm) sieve. The activity of the wheat was determined by liquid scintillation counting after digestion of 50 to 100 mg ground tissue with 1 mL Scintigest for 24 h at room temperature. Average activity of the wheat was 0.036 kBq mg^{-1} (n=4).

4.2.3 Experimental Setup

The experiment was a factorial design consisting of 2 levels of soil management history and 4 incubation temperatures (5°C, 15°C, 25°C, and 35°C). It was replicated three times using the field replicates, and analyzed as a randomized complete block design. The total number of experimental units was 24.

An experimental unit consisted of a 29 x 14 x 14 cm food storage box containing 22 shell vials (44 ml – 2.9 cm OD x 4.4 cm height) of pre-weighed, field moist soil (approximately 10 g dry basis) mixed with an equal mass of acid-washed 20 mesh Ottawa sand, a shell vial containing acidified water (0.1 M HCl) and a shell vial containing 5 mL of 1 M KOH.

For each treatment combination, one experimental unit was set up with the soil/sand mixture only and another box was set up with soil/sand plus ^{14}C-labeled wheat added at a carbon addition rate corresponding to 3% of native soil organic C (Bottner et al., 1998). Only the results for the ^{14}C-labeled wheat amended soils are reported in this chapter. An additional box containing only the acidified water vial and the 1 M KOH

CO_2 trap was included for each temperature in order to account for ambient CO_2 levels in mineralization calculations. Moisture contents in the soil within the vial were maintained throughout the experiment within 2% of 55% water filled pore space, assuming a bulk density of soil in the vial of 1 g cm^{-3}. The 55% WFPS moisture ranges for the soils in the study are shown in Table 3-1.

4.2.4 Experimental Sampling and Analysis

Sampling was conducted on a regular basis to monitor ^{14}C respiration, ^{14}C-CFE flush C, and microbial community structure. The sampling schedule followed is outlined in Table 4-1.

Differences in mineralization measurement schedules among treatments early in the experiment were due to slow initial rates of CO_2 release, which made it difficult to detect any mineralization by KOH trap titration for the colder incubation temperatures. On each sampling occasion, a 0.5 ml aliquot of KOH was taken from the trap, mixed with 4 ml of Ecolume and stored for 2 days prior to analysis by liquid scintillation counting (Beckman LS 3801). The remaining 4.5 ml of KOH was titrated with standardized 0.4 M HCl using phenolphthalein as an indicator.

The chloroform fumigation extraction method (Vance et al., 1987) was used as an index of the soil microbial biomass. Duplicate vials were removed from each box. One vial was immediately extracted with 0.5 M K_2SO_4 as described above and frozen for later analysis. The second vial was placed in a vacuum extractor along with a beaker containing 50 ml of alcohol-free chloroform. The extractor was sealed and a vacuum applied to allow the chloroform to boil for three minutes. The vials were then left in the chloroform atmosphere overnight and extracted on the following day as described above. An aliquot of the K_2SO_4 extract was analyzed for dissolved organic carbon using the

Technicon Autoanalyzer II. Another aliquot (0.5 ml) of the K_2SO_4 was mixed with 4 ml of Ecolume, left to sit for 48 hours, and the activity was then counted using the Beckman LS 3801. ^{14}C-CFE flush C was calculated as the difference in the percentage of the initial ^{14}C ($\%^{14}C_i$) in the K_2SO_4 extract from the fumigated and non-fumigated vials. The metabolic quotient of ^{14}C, qCO_2-^{14}C, was calculated as the rate of respiration of $\%^{14}C_i$ on a given day divided by the ^{14}C-CFE flush C on the same day. Substrate use efficiency was calculated by dividing the ^{14}C-CFE flush C by the sum of the ^{14}C-CFE flush C and the total $\%^{14}C_i$ respired in the period since the previous CFE measurement or the beginning of the experiment for the first occasion. Shell vials for microbial community analysis were removed from the boxes and immediately frozen at -20°C according to the schedule in Table 4-1. Samples were freeze dried when enough frozen samples had accumulated.

4.2.5 Microbial Community Analysis

The microbial community analysis protocol is outlined in detail in section 3.2.3. Briefly, DNA was extracted from the soil and amplified using the polymerase chain reaction (PCR). Separate, nested, polymerase chain reactions of bacterial and fungal DNA were conducted using flourescently labeled forward and reverse primers in the second step of the nested procedure. The fluorescently labeled PCR products were then digested with a restriction enzyme (*AluI* for bacteria and *HinfI* for fungi) and the terminal restriction fragment polymorphism (T-RFLP) profiles were analyzed using Genemapper software (version 3.7). Treatment effects on microbial community composition were assessed by summarizing the variability in the dataset using principal components analysis, followed by an Analysis of Variance and comparison of means of the scores for the main principal components. Diversity was assessed by computing Shannon's

Diversity Index (H') using the peaks in each profile as operational taxonomic units (OTUs).

4.2.6 Curve Fitting of ^{14}C Mineralization Data

Potentially mineralizable ^{14}C was estimated by fitting the incremental models for zero-order, single-pool first-order (SPE) and two simultaneous reactions (TSR) data from the pooled field replicates for each measurement period (i.e. the incremental data). The incremental models used are listed in Table 4-2. The details on the procedure followed are outlined in section 3.2.4. The total ^{14}C mineralized during the incubation was calculated as the sum of the incremental values of ^{14}C mineralization.

4.3 Results

4.3.1 ^{14}C Mineralization

Mineralization of added ^{14}C as a percentage of the initial added substrate ($\%^{14}C_i$) was fit to the zero-order, single-pool first-order (SPE) and two simultaneous reaction (TSR) models. In every case the SPE was a significant improvement over the zero-order model ($p=0.10$). The SPE model also resulted in relatively small confidence intervals for both of the parameter estimates (Table 4-3) and relatively high coefficients of determination (R^2). The TSR model was difficult to parameterize and frequently resulted in parameters going out of bounds. When it could be successfully parameterized, it never resulted in a significant F_{extra} statistic, and was therefore not selected as the best model for any of the cases. The results of the curve fitting process using the TSR model are not presented in this chapter.

Both the first-order rate constants and the size of the potentially mineralizable pool were affected by temperature. As temperature increased, the first-order rate constant increased, whereas the value for C_0 declined. The product of the rate constant and the

potentially mineralizable pool estimate (C_0k) increased steadily with temperature (Table 4-3), indicating a strong temperature effect on the initial, absolute rate of the reaction.

Approximately 50% of the added ^{14}C was potentially mineralizable (C_0) at 5°C, which was significantly higher than C_0 at any of the other temperatures (Table 4-3). Soils from both management histories incubated at 15°C and the M0 treatment at 25°C had predicted C_0 values from 34 to 38% of the added ^{14}C. The remaining temperature and management history combinations (25°C, M300 and both management histories at 35°C) had predicted C_0 values from 25 to 30% of the added ^{14}C. The differences in model parameter values reflect a changing pattern of C release due to temperature, since there was no difference in the total percentage of added ^{14}C respired by the end of the incubation at each temperature (refer to the last column in Table 4-3). Figure 4-1 to Figure 4-4 illustrate the daily rates of ^{14}C release. Initial rates of C release at 35°C were very high, but they declined rapidly, resulting in a lower predicted value for C_0 than at 5°C, where lower rates of ^{14}C respiration persisted for longer. This effect is due to the nature of the first-order model which is sensitive to rates of respiration early in the incubation, which determine the steepness of the cumulative C respiration curve. The shape of the first-order curve is also affected by the change (i.e. reduction) in respiration rates over time, which affects the point of inflection of the curve, and ultimately the estimate of C_0. In the 5°C incubation there was a 27 day lag phase before maximum daily rates of added ^{14}C were measured. This was not the case for the warmer temperatures where maximal ^{14}C respiration rates were reached on the first day (at 25 or 35°C), or by day 3 (15°C).

The specific activity of the respired CO_2 was calculated as the disintegrations per minute (dpm) of the respired CO_2 divided by the total mg CO_2 respired (Bailey et al., 2006). This value indicates the proportion of the respired CO_2 on each measurement date which originated from the added ^{14}C-labeled substrate. In order to clarify the presentation of this data, only the means for both management histories, without confidence intervals of the means, are presented in Figure 4-5. This data indicates a difference in C utilization by microorganisms at 5°C compared with the other three warmer temperatures. Until day 9, specific activities of respired CO_2 were similar for all temperatures. From day 9 until day 69 the 5°C treatments respired significantly more ^{14}C-CO_2 as a proportion of the total CO_2 respired, compared with the other three temperatures. At 5°C organisms appeared to need a period of adaptation to the new substrate, before they could maximize their use of it. They then preferentially utilized this substrate for a much longer period than the organisms functioning at warmer temperatures. Presumably the ^{14}C is relatively more accessible than the background, native C, at 5°C. At warmer temperatures, pools of native C are available for metabolism, so the proportion of respired C originating from the ^{14}C is relatively small.

4.3.2 Substrate Use

The turnover rate of the biomass was determined by monitoring the decline in levels of ^{14}C-labeled biomass on four measurement occasions. The data for ^{14}C-CFE flush at 25°C and 35°C were fit to an exponential decay model to explain changes in ^{14}C-labeled biomass with time. The 25 and 35°C curves and decay models are presented in Figure 4-6 to Figure 4-9. Biomass turnover times were calculated from the exponential decay constant (k), as the inverse of $365k$ (Wessels Perelo and Munch, 2005). The turnover time for the M0 soil at 25°C was 0.238 years while for the M300 soil it was

longer, 0.321 years. At 35°C the turnover time for the biomass in the M0 soil was slower (0.128 year) compared with the M300 soil (0.033 year). However, there were relatively large standard errors (as high as 75% of the parameter estimate in the case of the M300 soil at 35°C) associated with the estimates of *k*, so these turnover times can only be taken as approximations. The data for day 27 at 5 and 15°C were missing. In addition, there was considerable scatter in the data with no clear trend over time, therefore it was not possible to estimate biomass turnover at the lower two temperatures.

The microbial communities active at 5°C were more efficient in their use of ^{14}C than the other three communities. The specific substrate use efficiency (SSUE) was calculated on day 7 as the ratio of the ^{14}C in the biomass (^{14}C-CFE flush) to the sum of the ^{14}C in the biomass and the ^{14}C respired as CO_2 (Table 4-4). This value was highest for the 5°C community, and became progressively lower as temperature increased.

The amount of ^{14}C in the biomass on day 55 was used as an indicator of partitioning of the added wheat C at each temperature (Table 4-4). The amount of ^{14}C found in the biomass was significantly lower at 35°C than the other three temperatures; although the amount of ^{14}C respired up to day 55 did not differ among the temperature treatments. The amount of ^{14}C in the biomass was added to the amount of ^{14}C respired by day 55 to obtain an estimate of the total amount of added wheat C utilized by the soil decomposer community by day 55. This was highest at 25°C and lowest at 15°C with intermediate values for 5 and 35°C.

Total C respired was also calculated up to day 55 in order to compare these values with the ^{14}C respired by day 55 (Table 4-4). Total CO_2 respired increased steadily with increased temperature, in contrast to the ^{14}C respiration which did not increase as

temperatures became warmer. Soil management history did not have any effect on the efficiency of wheat C use or partitioning of wheat C between the biomass and respiration.

4.3.3 Microbial Community Structure

Bacteria

As explained in Chapter 3, relative abundances of terminal restriction fragments for both the 63f and 1405r T-RFLP profiles were combined for the bacterial community structure analysis. A principal components analysis of the relative abundance data for the bacterial T-RFLP profiles was done to reduce the initial number of variables from 351. An analysis of variance for the first four principal components indicated that Day was frequently a significant factor (for PC1, PC3 and PC4) (Table 4-5) and that it frequently interacted with temperature (PC1 and PC4) and management (PC1). For this reason, the PC scores were divided up for each day and a separate ANOVA conducted to determine how management and temperature affected microbial community structure throughout the experiment.

On day 7, management was a significant factor for PC3 ($p=0.043$). Management was not significant on the other sampling days except for day 123 when it was significant for PC3, PC4 and PC6 ($p=0.016$, 0.023 and 0.040 respectively). Temperature had an effect on PC4 on day 27 (Figure 4-10) when the 35°C community was significantly different from the communities formed at the other three incubation temperatures. Similarly, on day 123, the 35°C communities for soils from both management histories were separated from communities at the other three temperatures (Figure 4-11). Management had a significant effect on both Shannon's diversity index and the species richness (number of OTUs identified), at all levels of temperature and on each sampling day. The soil with no history of manure application had a higher H' overall compared

with the M300 soil (1.94 versus 1.77), as well as more OTUs (109 compared with 91). In most cases temperature had no effect on the bacterial measures of diversity. The only exception was for the M0 treatment which had slightly lower evenness (0.89) at 35°C compared with 15°C (1.02). This effect was not significant for the M300 treatment.

Fungi

As explained in Chapter 3, relative abundances of terminal restriction fragments for both the ITS1f and ITS4 profiles were combined for the fungal community structure analysis. A principal components analysis reduced the number of variables from the initial 414, with the proportion of total variability accounted for by each principal component very small. An ANOVA of the scores for the first four principal components indicated that only temperature was a significant factor, and only for PC2 and PC4 (Table 4-6). When mean scores for PC2 and PC4 at each level of temperature were plotted, fungal communities at 5, 15 and 35°C were clearly separated, while the 25°C community overlapped with the 5 and 15°C communities. All measures of fungal diversity were significantly lower at 35°C compared with the other three temperatures, and there were no differences in diversity measures among the 5, 15 and 25°C communities (Table 4-7).

4.4 Discussion

4.4.1 Suitability of the First-order Model

Although the use of a single pool first-order model to describe mineralization of organic C has been debated extensively in the literature, it consistently provided the best fit to the data in this study. This finding concurs with Van Schöll et al. (1997) who found that the first-order model provided a good fit to N mineralization data from an incorporated catch crop in the 1 to 15°C temperature range. The Q_{10} they reported for the 5 to 15°C range was 2.18, which is just slightly higher than the 1.59 to 1.87 range we

reported for ^{14}C mineralization in this study. The first-order model is designed to describe mineralization under conditions where the quantity of the substrate limits the instantaneous rate of the process. It is therefore suitable for models of plant litter dynamics, where the pool of substrate is finite and relatively homogenous in quality, compared with the larger pool of soil organic C. Cases in which two pool models have proven superior usually involve studies of mineralization of C and N from native pools of organic matter, as reported in Benbi and Richter (2002), although the experiments of Dalias et al. (2001) were similar to ours, and they selected a two pool model.

While the first-order model always provided the best fit to the data in this experiment, this was only the case when C_0 and k were both allowed to vary. As reported by Dalias et al. (2001), under these conditions, C_0 becomes temperature dependent. It is not certain, however, that the declines in the C_0 estimates with increasing temperature observed in this experiment reflect real differences in C availability. Both C_0 and k are inversely correlated, as clearly shown in this study, where decreases in C_0 were accompanied by significant increases in k. Differences in the estimates for k were related to the pattern of C release. As shown in Figure 4-1 to Figure 4-4, maximum rates of C mineralization were achieved on different days according to the incubation temperature. The soils incubated at 5°C had a low value for k because there was a 27 day lag phase before they reached maximum ^{14}C respiration rates. This period is similar to the 20 day lag phase reported by Cookson et al. (2002) before they observed maximum rates of gross N mineralization from soils amended with clover green manure at 5°C. The lag phase in my experiment should not have been associated with acclimation of the communities to temperature, since the soils were all preconditioned at their respective incubation

temperatures for three weeks before experimental setup. It may have been related to the "conditioning of the substrate" phase described by Swift et al. (1979), which is the period during which the structural material of plant tissue is disrupted, prior to enzymatic digestion. This period may be temperature dependent. It may also correspond to the development of a decomposer community adapted to metabolism of the added substrate.

Addition of the ^{14}C-labeled wheat had an impact on both fungal and bacterial community structure. In both cases, the first principal component generated from the T-RFLP data clearly separated all wheat-amended communities from non-amended (native C metabolizing) communities (data not shown). The lag phase in respiration at 5°C could therefore have been due to the development of fungal and bacterial communities adapted to metabolism of the added wheat. Development of these communities was slower than at the warmer incubation temperatures, due to temperature-related reductions in rates of cell division.

Low estimates of C_0 at warmer temperatures in this study may have been due to differences in the pattern of C release during the incubation. Nevertheless, when the sum of all ^{14}C respired throughout the incubation was calculated, no significant differences in the percentage of the added ^{14}C respired due to temperature were detected. This implies that the quantity of respirable C was equal at all temperatures, leaving the biological meaning of C_0 unclear. Estimates of C_0 may not have been accurate, and since they varied with temperature, the constant pool size assumption of the first-order model was violated. These findings indicate that application of first-order approaches to modeling soil organic matter dynamics under fluctuating temperatures is problematic due to uncertainty and variability in C_0 estimates.

4.4.2 Temperature Effects on the Pool of Accessible C

While the C_0 estimated using the first-order approach indicated a decline in the accessibility of the added C with increasing temperature, other measures of substrate use did not show the same trend. The sum of biomass ^{14}C and ^{14}C respired by day 55 (^{14}C utilized) is a more accurate estimate of the accessibility of the added C. It provides an indication of the gross mineralization of the added C and shows that the maximum amount of wheat C was metabolised at 25°C. The sum of biomass ^{14}C and ^{14}C respired by day 55 was significantly lower at 15°C compared with 25°C. This could have been due to a temperature-related reduction in the rates of the enzymatic reactions involved in the breakdown of wheat C, or a change in the types of enzymes excreted by the primary saprotrophs, or a change in the direct solubility of the substrate. Since there were never any differences between bacterial or fungal community structures at 15°C compared with 25°C, it is not likely that there was a temperature-related change in the types of enzymes excreted over this temperature range. The reduced accessibility of wheat C at 15°C was more likely due to simple enzyme kinetics, with slower reaction rates and reduced solubilities of substrates contributing to reduced rates of wheat utilization.

Differences in substrate accessibility at changing temperatures are clear when the trends in ^{14}C utilization at each temperature are compared with total CO_2 respiration. Temperature increased the total amount of CO_2 respired significantly with maximum respiration at 35°C; however there was little change in ^{14}C utilization due to temperature, with no difference between 5 and 35°C. The results support the hypothesis that at warmer temperatures certain inaccessible substrates become more accessible. These substrates could have diluted the ^{14}C pool and increased the total CO_2 respired. Temperature-driven changes in substrate accessibility could be due to increased substrate

solubility and/or a shift in microbial community function. This shift in function could accompany the differences in bacterial and fungal species composition that I observed in this study. Andrews et al. (2000) observed differences in soil microbial community composition at 4°C compared with 22 and 40°C. They also reported a less negative isotopic ratio of CO_2 at 4°C and hypothesized that this was due to a smaller fraction of lignin or lignin-like compounds being used as substrates at that temperature.

The relatively high level of wheat C utilization at 5°C compared with the three warmer temperatures (Table 4-4), also suggests that there are biological mechanisms that compensate for reduced enzymatic reaction rates and substrate solubility at low temperatures. Increasingly, researchers are reporting relatively high levels of soil decomposer community activity at low temperatures. Uchida et al. (2005) studied CO_2 emissions at low temperatures in the lab and the field, and reported significant respiration, even at temperatures as low as -2°C. Monson et al. (2006) reported that wintertime respiration from soil under snow contributed to from 7 to 10% of the total annual ecosystem respiration. Most dramatically, Panikov et al. (2006) have reported respiration in Arctic and Antarctic soils at temperatures as low as -39°C.

4.4.3 Temperature Effects on Microbial Biomass Characteristics

In my study microbial biomass characteristics at 5°C were distinct from those measured at the warmer temperatures. Substrate use efficiency of the 5°C community was significantly higher than the other temperature treatments. Wheat C was also used preferentially by the 5°C community compared with the other temperature treatments. The T-RFLP analysis indicated a trend towards the formation of a distinct fungal community at 5°C, while there were no differences in bacterial community structure between 5, 15 and 25°C. Balser and Firestone (2005) also reported temperature-driven

changes in fungal community structure in a field study on environmental effects on soil processes and microbial community dynamics, but no change in PLFA biomarkers for bacteria. It is likely that a cold temperature-adapted fungal community was functioning in the 5°C treatment. Uchida et al. (2005) also reported increases in fungal biomass during the snow-covered season.

Significantly less wheat C was accessed by the 35°C community compared with the 25°C community. This suggests that at 25°C conditions were optimal for metabolism of the added wheat. At 35°C the microbial community characteristics indicated changes in species composition and metabolism. The warmest temperature bacterial communities were separated from the other three incubation temperatures, and a distinct 35°C fungal community was present, which had significantly lower diversity than the fungal communities at the other three temperatures. The efficiency of substrate use for the 35°C community was also relatively low, and can be interpreted in a similar way to high values of the metabolic quotient (qCO_2): as indicative of a community that is under stress or functioning under suboptimal conditions (Fliessbach et al., 2000; Balser and Firestone, 2005).

The microbial community characteristics at 35°C suggest a response to temperatures outside of the normal range. Summer soil temperatures in Nova Scotia would rarely exceed 35°C. Balser and Firestone (2005) also reported high values for the metabolic quotient (qCO_2) when soil from a conifer ecosystem was incubated at a site with soil temperatures outside of its 'normal' range. The standard protocol for estimating the size of potentially mineralizable C and N pools in soils is to conduct incubations at 35°C in order to shorten the time required (Stanford and Smith, 1972; Wang et al., 2003).

This practise needs to be re-examined considering that the biomass in most arable soils in temperate regions will be under stress at this temperature. Rather than simply accelerating the rate of decomposition, the organisms involved and their metabolism (efficiency in particular) are likely to be altered by high incubation temperatures.

4.4.4 Implications for the Use of Temperature Response Functions

In first-order approaches to modeling C respiration from added substrates, the first-order rate constant is adjusted to account for temperature effects on the rate of respiration, using a temperature response function. These empirical functions bury the biology of the process in an algorithm that has been shown, through experimentation, to accurately predict temperature effects on respiration rates. If a given temperature response function can accurately predict respiration rates at high temperatures (e.g. 35°C), then it could be argued that changes in microbial community composition and metabolism at these temperatures are irrelevant; however, the temperature response of soil communities has been shown to vary with soil type. Dalias et al. (2001) reported that for some forest soils the C_0 of added plant litter increased with increasing temperature, while for other soils, the opposite was true. This finding implies that there are soil-related differences in the metabolism of added plant litter at different temperatures. Whether these differences are related to differences in native pools of substrate, microbial community composition, or mineralogy, is not clear. Stanford et al. (1973) advised using one temperature response function to predict net N mineralization from native pools of organic matter, for all of the arable soils in their study. In my study, although the two soils had differing management histories, and differed in their bacterial diversity, there were few differences in their metabolism of the added wheat at varying temperatures. Are there soil-related differences in the temperature response of decomposition of added

plant litter in arable soils? Future experiments should include agricultural soils from different crop rotations e.g. forage-based versus cash crop and of different soil types, in order to address this question.

4.5 Conclusions

In this study some significant questions about the use of the first-order model to predict C mineralization from soils at a range of temperatures have been investigated. Most decomposition models currently use the rate constant approach in which only the rate constant, k, is modified by the temperature response function (TRF) (Scenario A, Figure 4-13). This approach is based on the assumption that the size of the potentially mineralizable substrate pool in the soil does not change with temperature, however, this has been disproved by various researchers, as well as in my experiment. Best fits of the first-order model to net C mineralization data are obtained when both the k and C_0 parameters are allowed to fluctuate. Temperature response functions that incorporate a changing substrate pool size along with changing rates of mineralization would need to be developed to address this phenomenon (Scenario B, Figure 4-13). An alternative approach is to consider the impacts of temperature on the soil decomposer community. Temperature response functions could then be developed for the biological characteristics that have been shown to change with temperature (Scenario C, Figure 4-13). These characteristics could include C use efficiency, biomass size, and biomass C:N ratios, all of which could impact on net C and N mineralization at different temperatures. Currently these biological characteristics are implicit within the TRF. There will be no benefit to adopting Scenario C, if the biological characteristics that impact on decomposition are affected in the same way by temperature. If, however, microbial communities that differ (e.g. in species composition, metabolic efficiency, size) respond differently to changing

temperature, model predictions may be improved by including the relevant measures and temperature response functions explicitly.

In this study, I observed changes in microbial community structure, with a distinct fungal community developing at 5°C. At this temperature the metabolism of the community was also affected, with higher efficiency of substrate use than at warmer temperatures. At the warmest incubation temperature (35°C) the soil microbial community was under stress and used the added wheat carbon relatively inefficiently. There was also a trend towards the development of a distinct bacterial community at 35°C. All of these characteristics could impact on net C mineralization, but there were no differences between the two soils in the temperature response of added wheat mineralization. This suggests that there would be no advantage in this case to including these biological parameters in a model of wheat C metabolism at different temperatures. An empirically derived TRF would have adequately described any of the indirect effects of these biological characteristics on net C mineralization.

Recent advances in techniques to study soil biological processes provide an exciting opportunity for scientists to delve into the mechanisms underlying the mineralization of C from soil organic matter. This information will improve our understanding of the biology behind the complex process of decomposition in soils and the impacts of ecosystem disturbance on this process. The current study provided no justification for the inclusion of more biology in current decomposition models, but did support the notion of temperature-dependent substrate pools. This information may allow the improvement of existing empirical models, and contribute to the development of a new generation of soil process models that combine the advantages of empirical models,

with the improved accuracy that is promised when biological principles are more explicitly represented in these models.

Table 4-1. Chemical and biological parameter sampling schedule for microcosms of soil from a Nova Scotia long term fertility trial amended with ^{14}C-labelled wheat and incubated at four temperatures for up to 123 days.

Procedure	Temperature (°C)	Days from setup
^{14}C respiration	5	1, 2, 4, 7, 9, 11, 14, 17, 20, 27, 34, 41, 48, 55, 62, 69, 76, 82, 97, 111, 123
	15	2, 4, 5, 6, 7, 9, 11, 14, 17, 20, 27, 34, 41, 48, 55, 62, 69, 75, 82, 97, 111, 121
	25	1.5, 3, 4, 5, 6, 7, 9, 11, 14, 17, 20, 27, 34, 42, 49, 56, 61, 70, 77, 84, 98, 112, 121
	35	1.5, 3, 4, 5, 6, 7, 9, 11, 14, 17, 20, 27, 34, 41, 48, 55, 62, 69, 76, 83, 97, 111, 119
^{14}C-CFE flush measurement	5	7, 14, 27, 55
	15	7, 14, 27, 55
	25	7, 14, 27, 56
	35	7, 14, 27, 55
Freeze drying for later microbial community analysis	5	7, 14, 27, 55, 123
	15	7, 14, 27, 55, 121
	25	7, 14, 27, 56, 121
	35	7, 14, 27, 55, 119

Table 4-2. Incremental models used for curve fitting ^{14}C mineralization data from microcosms of soil from a Nova Scotia long term fertility trial amended with ^{14}C-labelled wheat and incubated at four temperatures for up to 123 days.

Zero-order model	$C_{it} = ki$	Where C_{it} is amount of ^{14}C released during interval i preceding time t, in units of $\%^{14}C_i$ and k is the zero-order rate constant in units of $\%^{14}C_i\ d^{-1}$
Single-pool exponential model	$C_{it} = C_0 e^{-kt}(e^{ki}-1)$	Where C_{it} is amount of ^{14}C released during interval i preceding time t in units of $\%^{14}C_i$, k is the first-order rate constant in units of d^{-1} and C_0 is the maximum potentially mineralizable $\%^{14}C_i$.
Two simultaneous reaction (TSR) model	$C_{it} = C_0 a e^{-kt}(e^{ki}-1) + C_0(1-a)e^{-ht}(e^{hi}-1)$	Where C_{it} is amount of $\%^{14}C_i$ released during interval i preceding time t, C_0 is the maximum potentially mineralizable $\%^{14}C_i$. There are two pools of mineralizable $\%^{14}C_i$ which add up to C_0, fraction one is a with a rate constant k in units of d^{-1} and fraction two is $(1-a)$ with a rate constant h in units of d^{-1}

Table 4-3. Parameter estimates (±95%CI) for the SPE model fit to incremental ^{14}C mineralization data from a Nova Scotia soil from a long term fertility trial with (M300) and without (M0) a history of manure application, amended with ^{14}C-labelled wheat and incubated at four temperatures for up to 123 days.

Temperature (°C)	Management history	SPE relative rate constant, k (d^{-1})	Q_{10} k	C_0, $\%^{14}C_i$ respired	C_0k	R^2	Total $\%^{14}C_i$ respired[z]
5	M0	0.014±0.003 a		50.0±6.3 a	0.72	0.87	38.1 a
15	M0	0.027±0.004 b	1.87	37.7±4.2 bc	1.02	0.85	35.9 a
25	M0	0.052±0.006 c	1.93	37.2±3.2 b	1.94	0.89	40.4 a
35	M0	0.134±0.028 d	2.57	25.5±4.2 d	3.41	0.74	33.0 a
5	M300	0.014±0.004 a		50.2±6.7 a	0.75	0.86	39.0 a
15	M300	0.024±0.004 b	1.59	34.1±3.4 bc	0.81	0.89	31.4 a
25	M300	0.057±0.009 c	2.40	30.2±3.5 cd	1.71	0.83	34.8 a
35	M300	0.168±0.038 d	2.97	25.0±4.3 d	4.21	0.75	34.3 a

Table 4-4. Measures of ^{14}C use and partitioning by the biomass in microcosms of a Nova Scotia soil from a long term fertility trial with and without a history of manure application amended with ^{14}C-labelled wheat and incubated at four temperatures for up to 123 days.

Temperature	Substrate use efficiency on day 7	^{14}C-CFE flush day 55 (%$^{14}C_i$)	^{14}C respired day 55 (%$^{14}C_i$)	^{14}C-CFE flush + total ^{14}C respired day 55 (%$^{14}C_i$)	Total CO_2 respired to day 55 (mg C kg^{-1})
5	0.82 a	6.45 a	25.18 a	31.55 ab	230.7 a
15	0.50 b	4.66 a	25.52 a	30.27 a	606.6 b
25	0.37 bc	5.26 a	32.19 a	37.45 b	721.0 bc
35	0.28 c	2.86 b	27.49 a	32.62 ab	883.0 c

Means followed by the same letter in the same column are not different at the 5% significance level using Tukey's HSD pairwise comparison of means test.

Table 4-5. Probability values from analysis of variance of the first four principal component scores for bacterial T-RFLP profiles from a Nova Scotia soil with and without a history of manure application, amended with ^{14}C-labelled wheat and incubated at four temperatures for up to 123 days, sampled on five occasions.

Source[z]	df	PC1 (9.8%)[y]	PC2 (4.0%)	PC3 (2.8%)	PC4 (2.5%)
Temp	3	0.366	0.840	0.368	0.000
Mgt	1	0.924	0.440	0.167	0.013
Day	4	0.000	0.400	0.014	0.075
Rep	2	0.386	0.024	0.824	0.875
Temp* Mgt	3	0.582	0.645	0.663	0.638
Temp* Day	12	0.057	0.528	0.201	0.004
Mgt*Day	4	0.039	0.771	0.386	0.370
Error	77				
Total	106				

[z]Mgt=Management history; Temp=Temperature; Day=Sampling occasion

[y] Values in parentheses are the percentage of the variability accounted for by each PC.

Table 4-6. Probability values from analysis of variance of the first four principal component scores for fungal T-RFLP profiles from a Nova Scotia soil from a long term fertility trial with and without a history of manure application, amended with ^{14}C-labelled wheat and incubated at four temperatures for up to 123 days, sampled on five occasions.

Source[z]	df	PC1 (2.8%)[y]	PC2 (2.1%)	PC3 (2.0%)	PC4 (1.9%)
Temp	3	0.151	0.000	0.130	0.000
Mgt	1	0.819	0.190	0.112	0.288
Day	4	0.720	0.308	0.275	0.396
Rep	2	0.404	0.649	0.295	0.369
Temp* Mgt	3	0.259	0.461	0.419	0.147
Temp* Day	12	0.106	0.890	0.894	0.580
Mgt*Day	4	0.791	0.694	0.355	0.305
Error	81				
Total	110				

[z]Mgt=Management history; Temp=Temperature; Day=Sampling occasion

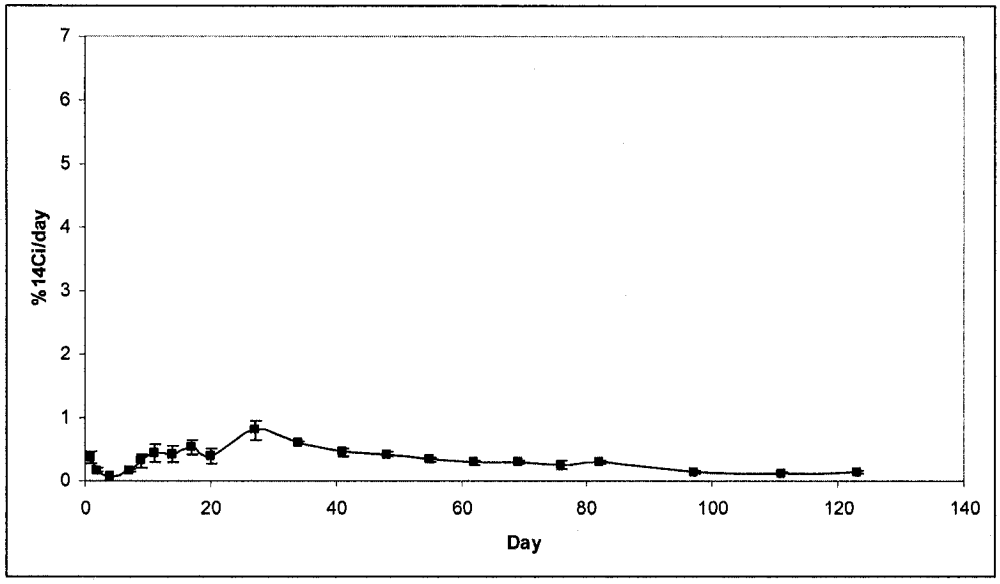
[y] Values in parentheses are the percentage of the variability accounted for by each PC.

Table 4-7. Summary of Shannon's diversity index (H'), species richness (S) and evenness (E) calculated using fungal T-RFLP data from a Nova Scotia soil from a long term fertility trial with and without a history of manure application, amended with ^{14}C-labelled wheat and incubated at four temperatures for up to 123 days.

Temperature	H'	S	E
5	1.76 a	29 a	1.23 a
15	1.62 a	27 a	1.15 a
25	1.62 a	27 a	1.17 a
35	0.96 b	17 a	0.80 b

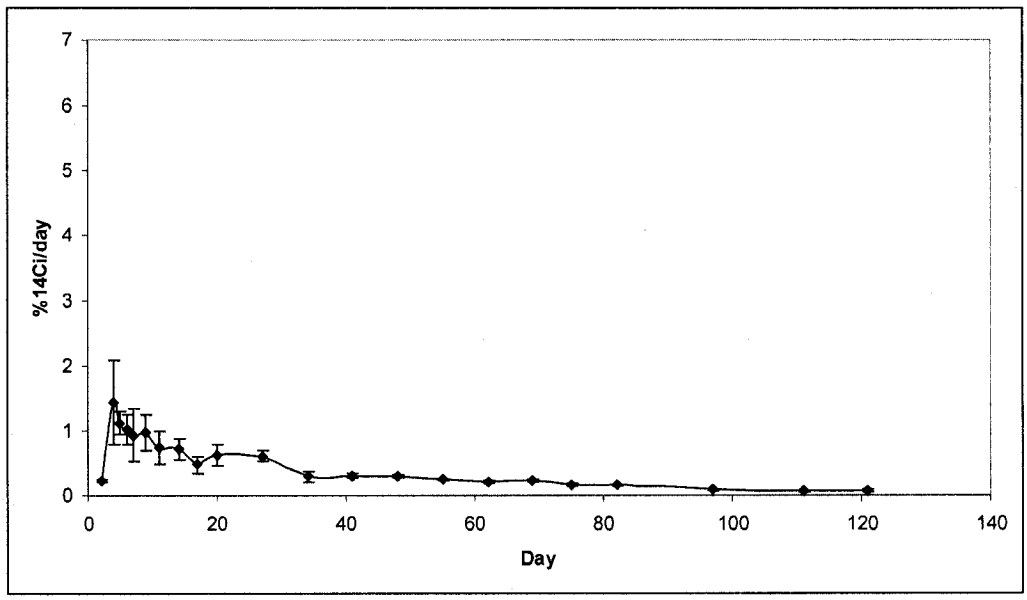
Means followed by the same letter in the same column are not significantly different at the 5% significance level, Tukey's HSD test. Values are the average of five sampling occasions and both management histories.

Figure 4-1. Mean daily rates of ^{14}C respiration as a percentage of the initial ^{14}C measured in microcosms of a Nova Scotia soil from a long term fertility trial amended with ^{14}C-labelled wheat and incubated at 5°C for 123 days.



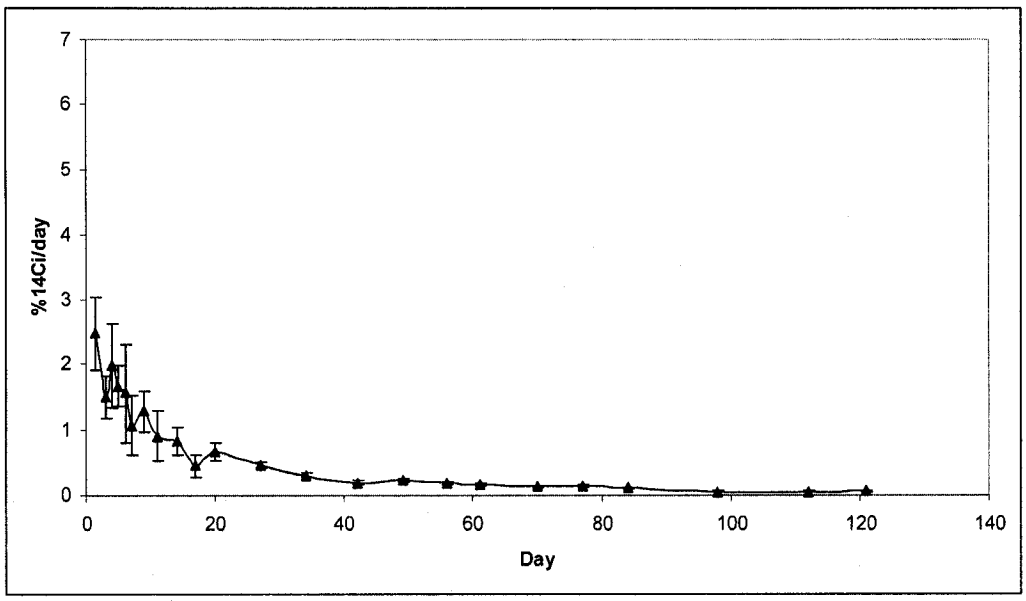
Values are the average of both management histories; error bars represent 95% CI of the daily means.

Figure 4-2. Mean daily rates of ^{14}C respiration as a percentage of the initial ^{14}C measured in microcosms of a Nova Scotia soil from a long term fertility trial amended with ^{14}C-labelled wheat and incubated at 15°C for 121 days.



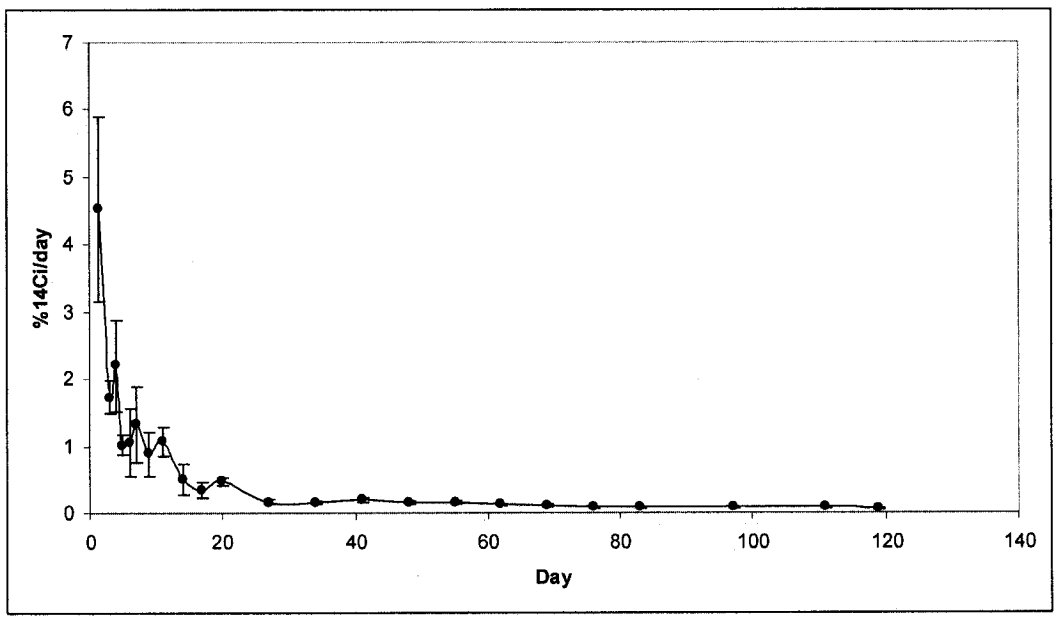
Values are the average of both management histories; error bars represent 95% CI of the daily means.

Figure 4-3. Mean daily rates of ^{14}C respiration as a percentage of the initial ^{14}C measured in microcosms of a Nova Scotia soil from a long term fertility trial amended with ^{14}C-labelled wheat and incubated at 25°C for 119 days.



Values are the average of both management histories; error bars represent 95% CI of the daily means.

Figure 4-4. Mean daily rates of ^{14}C respiration as a percentage of the initial ^{14}C measured in microcosms of a Nova Scotia soil from a long term fertility trial amended with ^{14}C-labelled wheat and incubated at 35°C for 117 days.



Values are the average of both management histories; error bars represent 95% CI of the daily means.

Figure 4-5. Mean specific activity of respired CO_2 averaged across management history for a Nova Scotia soil from a long term fertility trial amended with ^{14}C-labelled wheat and incubated at four temperatures for up to 123 days.

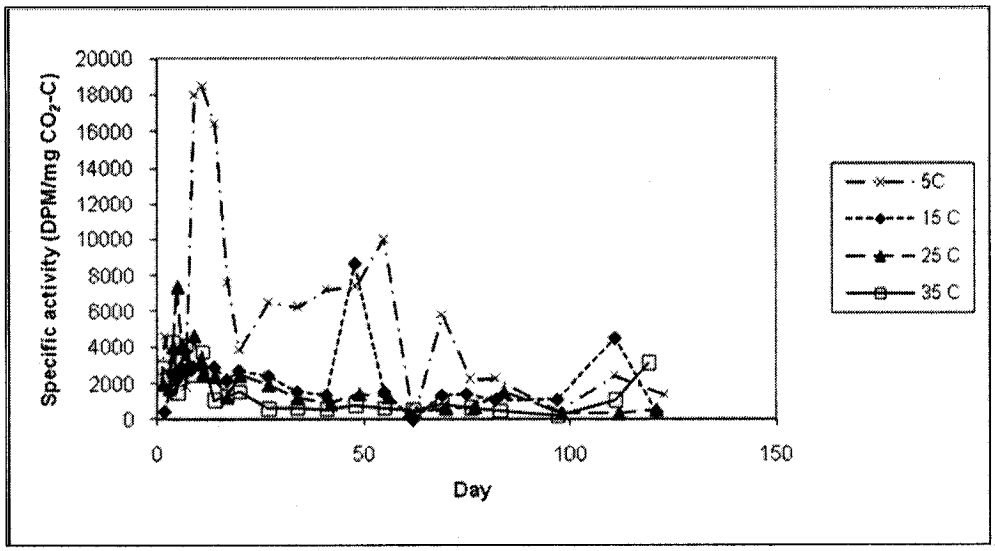


Figure 4-6. ^{14}C-CFE flush data for a Nova Scotia soil from a long term fertility trial without a history of manure application, amended with ^{14}C-labelled wheat and incubated at 25°C for 119 days, fit to an exponential decay model.

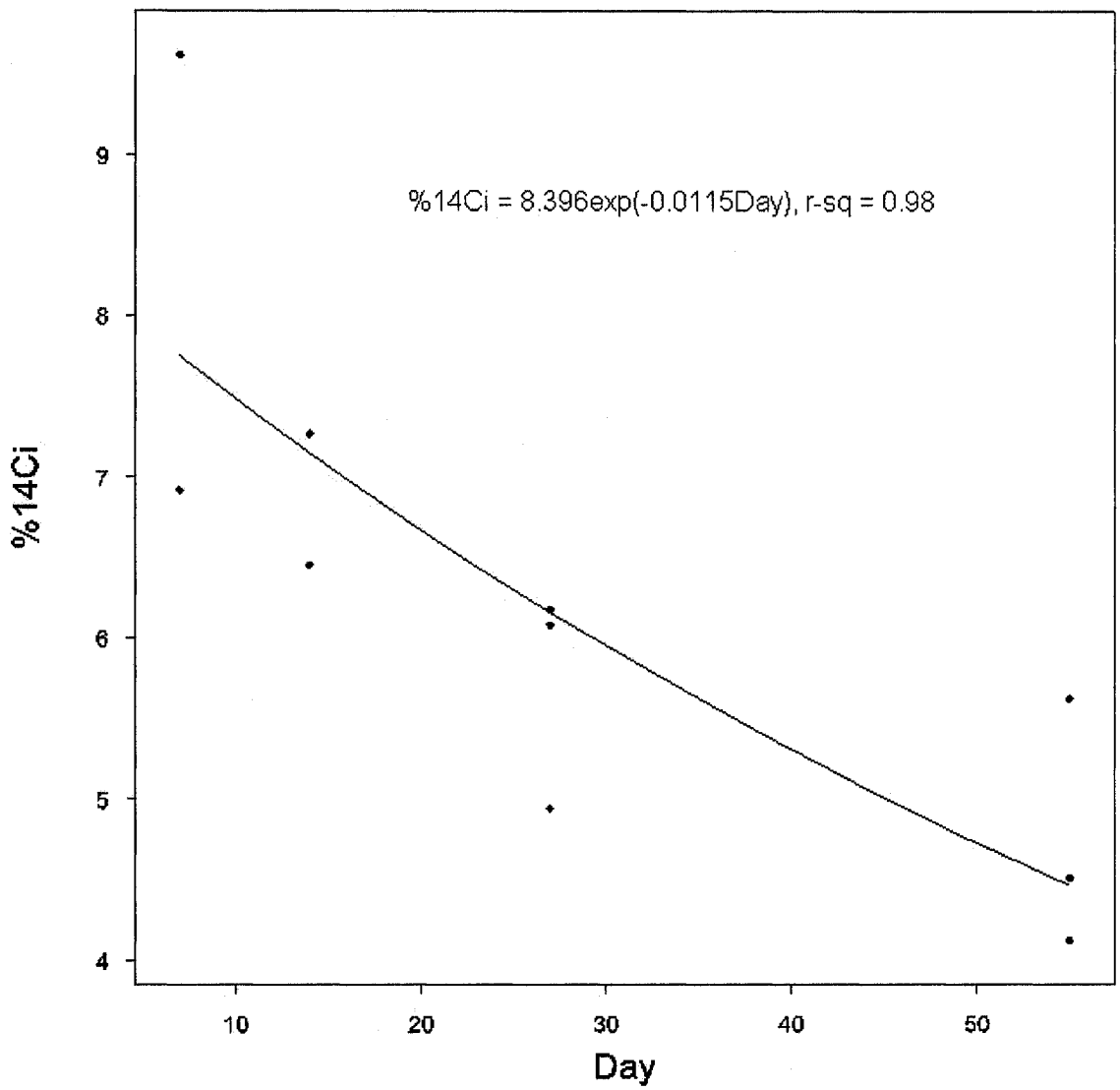


Figure 4-7. ^{14}C-CFE flush data for a Nova Scotia soil from a long term fertility trial with a history of manure application, amended with ^{14}C-labelled wheat and incubated at 25°C for 119 days, fit to an exponential decay model.

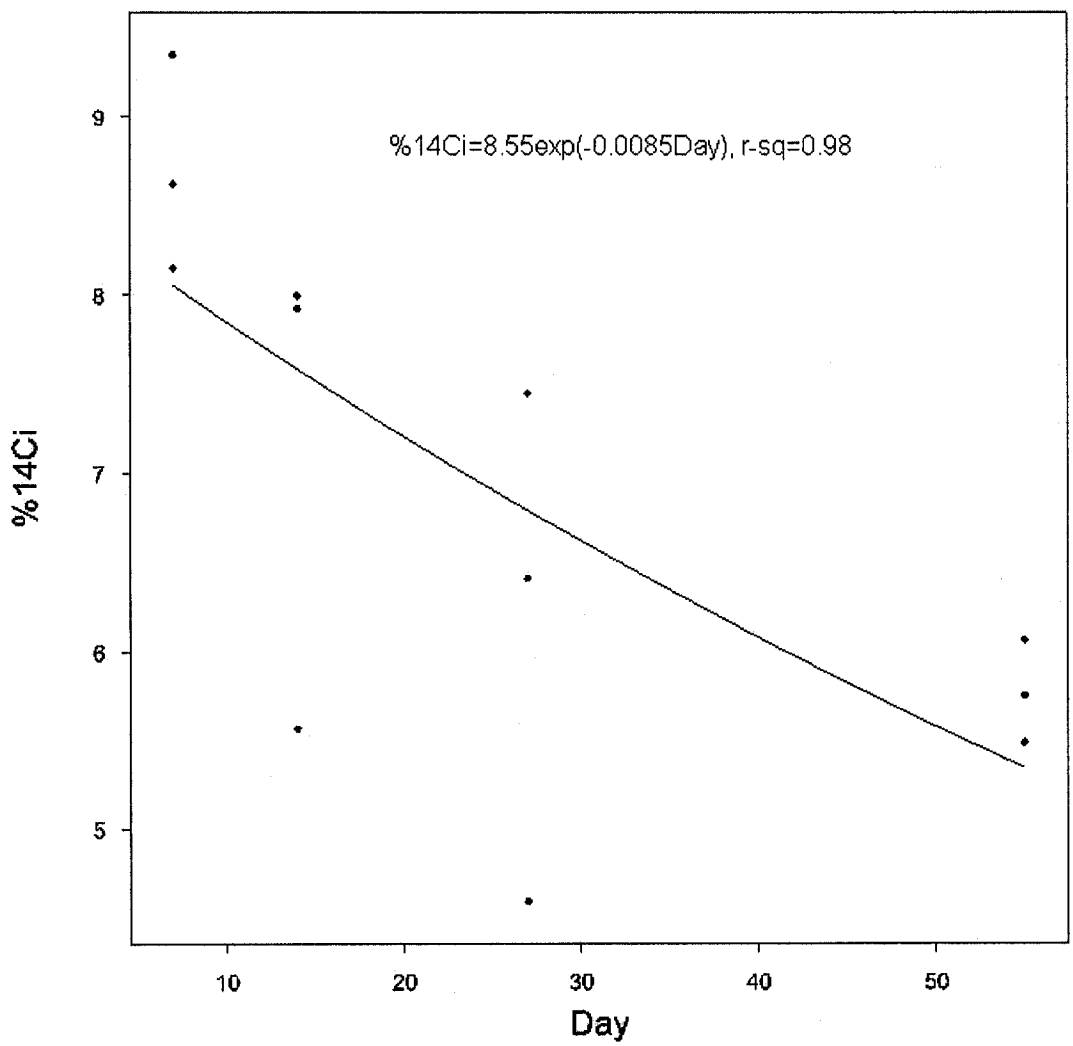


Figure 4-8. ^{14}C-CFE flush data for a Nova Scotia soil from a long term fertility trial without a history of manure application, amended with ^{14}C-labelled wheat and incubated at 35°C for 117 days, fit to an exponential decay model.

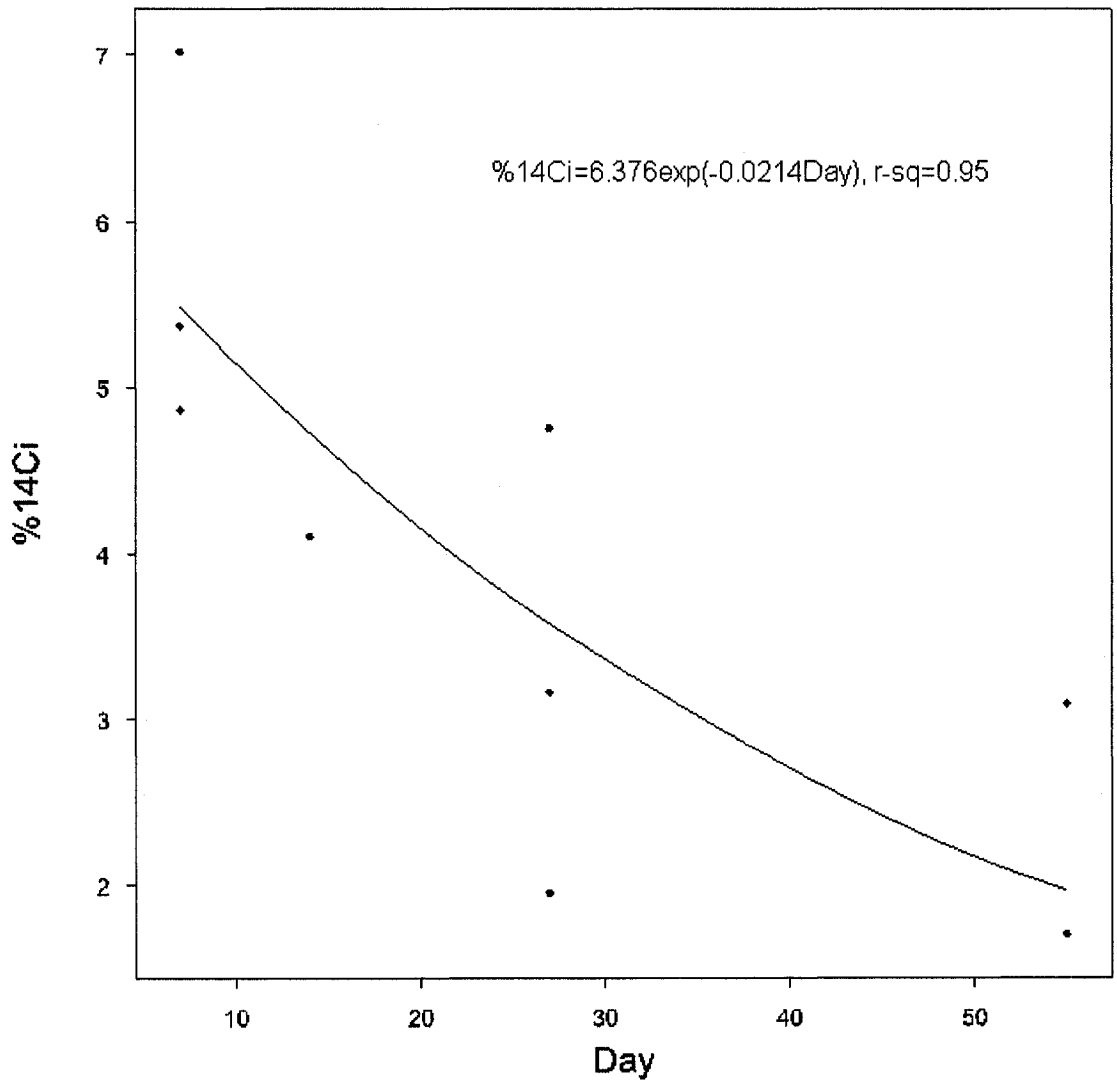


Figure 4-9. ^{14}C-CFE flush data for a Nova Scotia soil from a long term fertility trial with a history of manure application, amended with ^{14}C-labelled wheat and incubated at 35°C for 117 days, fit to an exponential decay model.

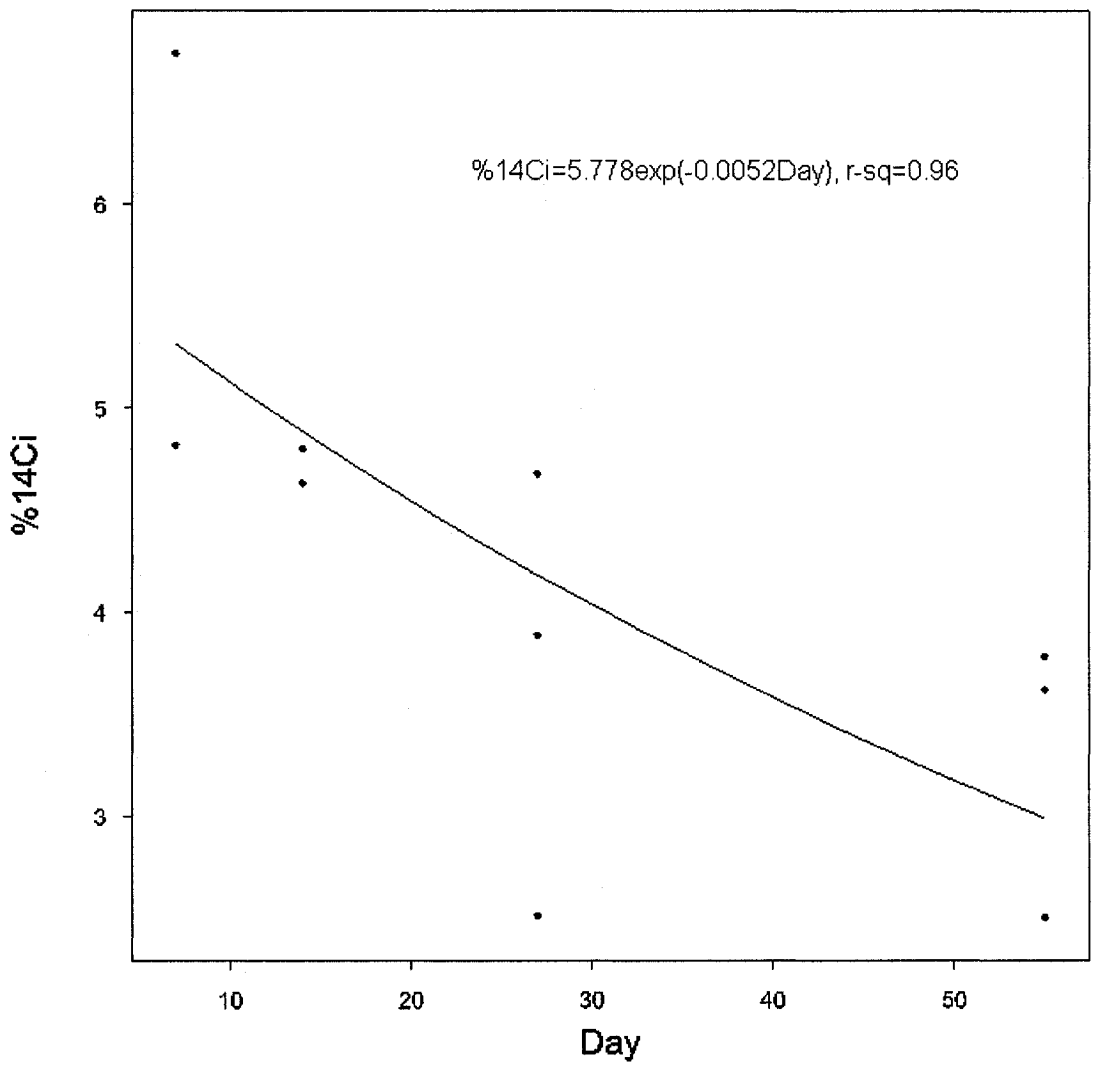
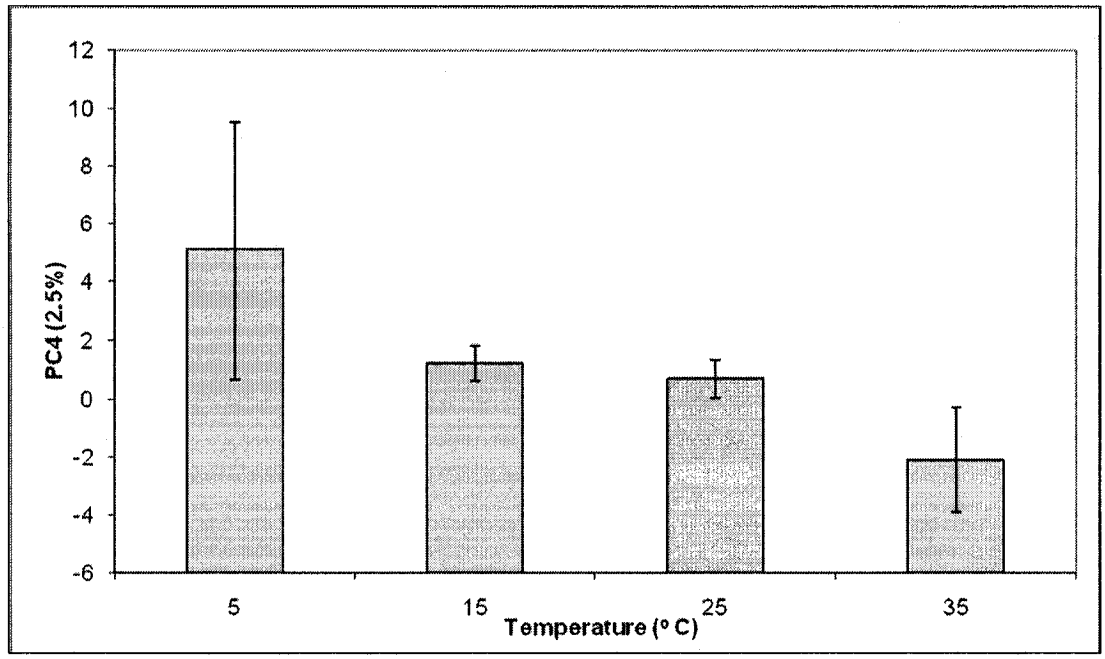


Figure 4-10. Bacterial T-RFLP scores on day 27 for a Nova Scotia soil from a long term fertility trial with and without a history of manure application amended with ^{14}C-labelled wheat and incubated at four temperatures for up to 123 days.



Error bars indicate the 95% confidence intervals of each mean.

Figure 4-11. Bacterial T-RFLP scores on day 123 for a Nova Scotia soil from a long term fertility trial with (M300) and without (M0) a history of manure application amended with ^{14}C-labelled wheat and incubated at four temperatures for up to 123 days.

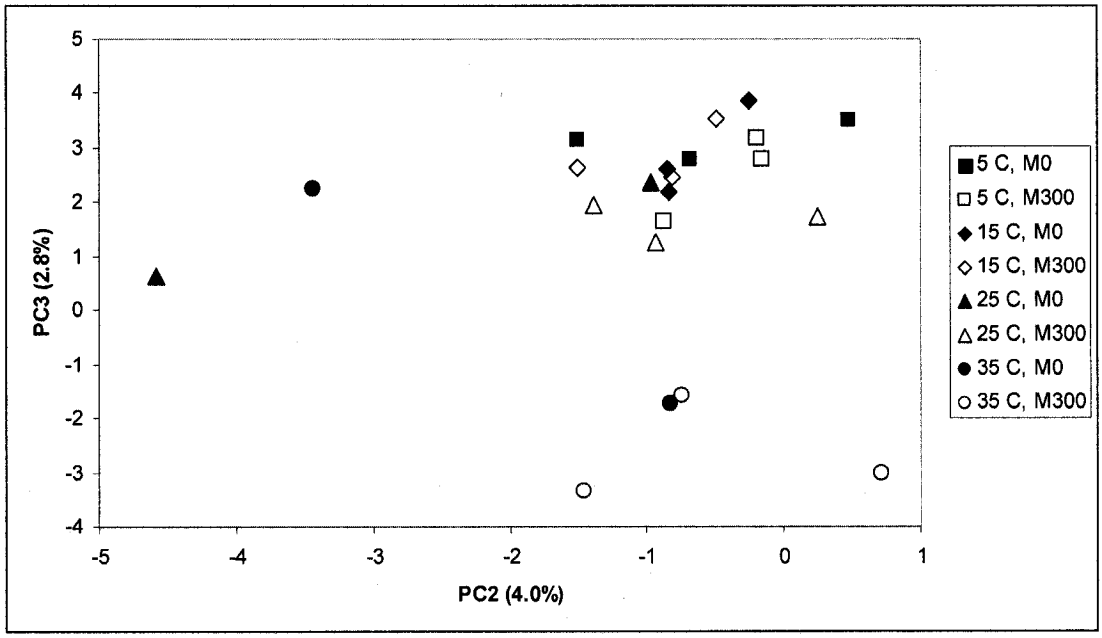
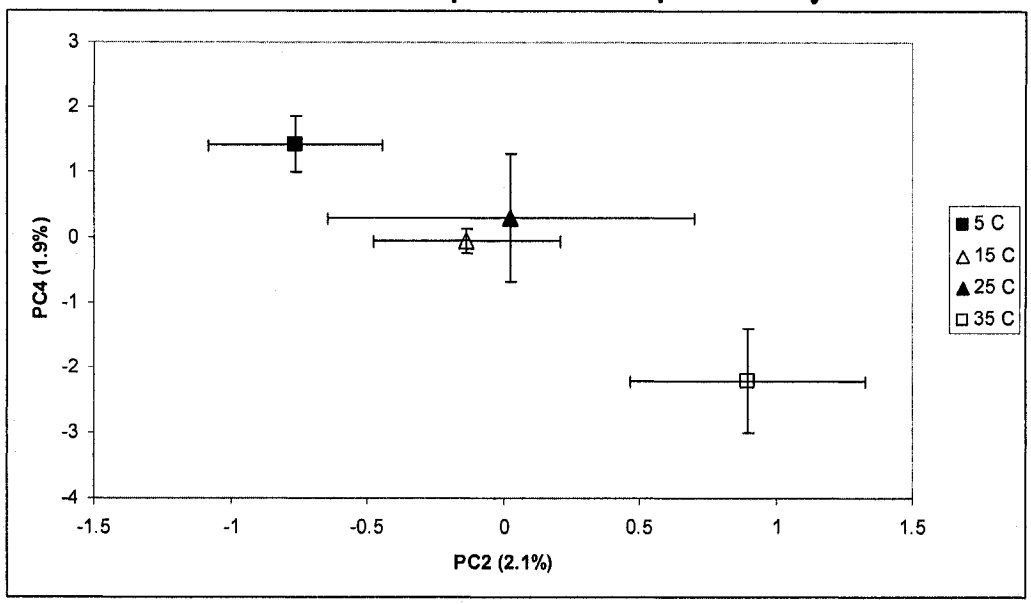
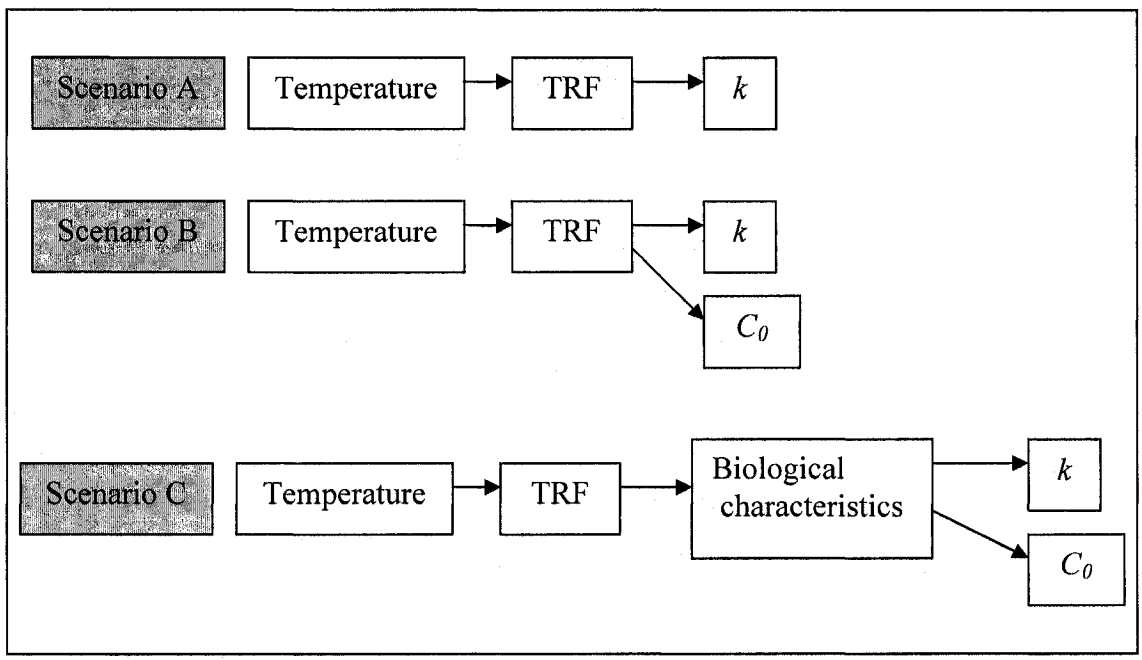


Figure 4-12. Fungal T-RFLP scores for a Nova Scotia soil from a long term fertility trial with and without a history of manure application amended with ^{14}C-labelled wheat and incubated at four temperatures for up to 123 days.



Mean scores for both management histories; error bars indicate the 95% confidence intervals of each mean.

Figure 4-13. Three scenarios for incorporating temperature into decomposition models: the rate constant approach (Scenario A), the functional shift approach (Scenario B), and the biological approach (Scenario C).



TRF=temperature response function

Chapter 5: Temperature Effects on Decomposition of a Complex ^{13}C-labeled Substrate: Substrate Partitioning and Microbial Community Dynamics

5.1 Introduction

The effect of temperature on soil microbial community composition and dynamics is a subject of considerable debate in the literature (Kirschbaum, 2006). Global climate models use relatively simple approaches to estimate the effect of changing temperature on the release of C and N from soils. These approaches include the use of Q_{10} or Arrhenius functions to modify first-order rate constants. Arrhenius, or rate-constant, approaches are based on the assumptions that neither size nor availability of soil substrate pools are affected by temperature. In laboratory incubation studies these assumptions have not been supported. The size of the potentially mineralizable pool of substrate estimated using first-order models has been shown to vary with temperature (Honeycutt et al., 1988; MacDonald et al., 1995; Dalias et al., 2002), as has the structure of the microbial community (Zogg et al., 1997). This change in community structure could lead to a change in the metabolic capacity to utilize substrate pools as organisms possessing different metabolic pathways may dominate at different temperatures.

Current techniques in molecular biology, such as denaturing gradient gel electrophoresis (DGGE) and terminal restriction fragment length polymorphism (T-RFLP), can be used to study the species composition of soil microbial communities as affected by environmental conditions such as temperature (Saleh-Lakha et al., 2005). Terminal restriction fragment length polymorphism relies on the variation in the position of restriction sites among sequences and determination of the length of fluorescently labeled fragments by high resolution gel electrophoresis (Dunbar et al., 2001). When complex environmental patterns are studied using T-RFLP, each band is said to represent

an operational taxonomic unit (OTU), and T-RFLP profiles can be used as an indicator of microbial community structure (Hartmann and Widmer, 2006). Recently, the development of DNA- and RNA-stable isotope probing (SIP) techniques has enabled the identification of microbial communities involved in the metabolism of a specific, ^{13}C-labeled substrate (Radajewski et al., 2000). In this method a ^{13}C-labeled substrate is supplied to microorganisms that incorporate the ^{13}C into their nucleic acids during cell growth and division. After sufficient time has passed for the ^{13}C metabolism to occur, the microbial DNA is extracted and separated by density on a density gradient (usually caesium chloride or caesium trifluoroacetate) produced by centrifuging at high relative centrifugal forces. The densest nucleic acids are assumed to be those containing the ^{13}C-labeled DNA or RNA. These nucleic acids can then be studied using standard molecular techniques (e.g. PCR, sequencing, T-RFLP). This technique has been used to determine the phylogenetic relationships between organisms responsible for metabolism of ^{13}C-glucose, ^{13}C-phenol, ^{13}C-caffeine, and ^{13}C-naphthalene in soils (Padmanabhan et al., 2003). It has also been used to study plant-microbial interactions by tracing the fate of root exudates from $^{13}CO_2$ pulse-labeled plants (Prosser et al., 2006). While McMahon et al. (2005) used ^{13}C-labeled ryegrass to distinguish communities involved with its decomposition using PLFA techniques, the use of DNA-SIP to study decomposition processes using a complex substrate such as a plant material has not been reported.

Understanding the effects of temperature on the composition, size and function of the microbial biomass may explain some of the "unexplained error and surprises" in current biogeochemical studies (Schimel, 2001). Fang et al. (2005a) suggested that the size of the microbial biomass should be included in biogeochemical models of

decomposition, since not only is it the biomass that catalyzes the decomposition process, it is also one of the most dynamic substrate pools in the soil. Microbial community characteristics such as efficiency of substrate use may also be affected by temperature. If microbial community composition, including fungal to bacterial biomass ratios, are affected by temperature as demonstrated by Zogg et al. (1997), then temperature-driven changes in substrate use efficiency may result. Sakamoto and Oba (1994) demonstrated that the metabolic quotient of microbial communities decreased with an increase in the fungal to bacterial ratio, and suggested that this was due to differences in efficiency of C use by fungi compared with bacteria. More recently, however, Thiet et al. (2006) were not able to demonstrate any differences in C use efficiency among soil microbial communities with differing fungal:bacterial ratios. While the effects of microbial community composition on substrate use efficiency remains unclear, a temperature effect on substrate use efficiency was demonstrated by Devêvre and Horwáth (2000). In their study, rice paddy soils were incubated under non-flooded conditions at 5, 15 and 25°C, and a clear decrease in the efficiency of C use with increasing temperature was observed (from 61% of added straw C at 5°C to 35% at 25°C). This difference was attributed to differences in substrate source at different temperatures, with the more labile constituents only being degraded at the lowest temperature, and both labile and recalcitrant substrates being degraded at the warmest temperature. If C use efficiency declines at higher temperatures, then a greater proportion of substrate will be respired as CO_2 than at cooler temperatures. This effect is not considered in Arrhenius-based descriptions of the effect of temperature on soil metabolism and as a result these approaches could underestimate rates of C respiration at warmer temperatures.

The first objective of this study was to measure the partitioning of ^{13}C-labelled plant material into respired CO_2, biomass C, and unmetabolized C, in soils with two management histories incubated at three temperatures (4, 22 and 35°C). Since ^{13}C-labelled plant material was used in this experiment, a second objective was to determine if DNA-SIP could be used to differentiate wheat C metabolizing communities from the native C metabolizing communities in one of the two soils used in the experiment. The third objective of the study was to use DNA-SIP to compare the community structure of wheat C and native C metabolizing communities at three different temperatures.

5.2 Materials and Methods

5.2.1 Production of ^{13}C-labeled Wheat

Wheat seeds (*cv. Consort*) were planted into pure perlite in a 28 x 28 x 5 cm tray and germinated in a glasshouse. The seedlings were grown to 5 cm in height before being transferred to a gas-tight chamber (30.5 x 30.5 x 45.7 cm, Nalgene™ Dessicator Cabinet) on 29 March 2005. The chamber was connected to an infrared gas analyzer (IRGA) so that levels of CO_2 could be monitored periodically. Carbon dioxide labeled with ^{13}C was introduced into the chamber by adding a volume of 1 M $NaH^{13}CO_3$ through a syringe into a beaker that was suspended within the chamber and converting it to CO_2 gas by adding a volume of 6 M H_2SO_4 (1.5 times the volume of $NaH^{13}CO_3$). Volumes of $NaH^{13}CO_3$ added on each occasion were 2, 4 or 6 ml, depending on the amount of plant material within the chamber. Carbon dioxide was generated in the chamber twice a day throughout the wheat's growing period. The plants were harvested twice during the growing period: at 42 and 91 days after planting. A total of 12 g dry leaf material and 12.5 g dry root/perlite mixture was produced. The C, N and at.%^{13}C values of the wheat were analyzed by isotope ratio mass spectrometry (IRMS). While material

harvested at the end of April was still diluted with ^{12}C and had a maximum level of enrichment of 66 at.% ^{13}C, by the June harvest, material was more highly enriched with all parts of the leaf enriched to 84 at.% ^{13}C. The leaf material harvested in June was used in the incubation experiment described in section 5.2.2.

5.2.2 Experimental Setup

Soils from a long-term fertility experiment at the Nappan Experimental Farm (45° 45' N, -64° 14') in Nova Scotia, Canada were collected on 17 Aug 2005. Two treatments were selected: one had received no fertility amendment for the previous eleven years (M0), while the other had received 300 kg N ha^{-1} annually as semi-solid beef manure (M300). A total of 20 cores from the 0 to 15 cm horizon from each plot were pooled. For each of the two treatments in the study, 3 field replicates were sampled. Soils were stored at 4°C until shipping by air to the Scottish Crop Research Institute in Dundee, Scotland. Upon arrival, soils were sieved through a 4 mm sieve, split into three equal portions, and immediately stored at one of three temperatures: 4, 22 or 35°C. Throughout the pre-conditioning and incubation period, soils were maintained at 55% water filled pore space (WFPS), assuming a bulk density of sieved soil of 1 g cm^{-3}. The experiment was set up on 8 and 9 December 2005. Approximately 12 g fresh soil (10 g dry weight) was mixed with ^{13}C-wheat (84 at.% ^{13}C) at a rate equivalent to 3% of the soil C as wheat C, and added to a 25 mm x 75 mm glass vial. Each experimental unit consisted of twelve vials that could be destructively sampled as required. Vials were sealed with Nescofilm™ and incubated at the same temperature at which they had been preconditioned.

5.2.3 Sampling Methods

Sampling for respiration, biomass C and N , and microbial community analysis, was conducted according to the schedule in Table 5-1. Gas samples were obtained by sealing one vial from each experimental unit with a subaseal stopper for four hours, and then taking a 5 ml gas sample with a syringe. Gas samples were analyzed for total C content and at.%^{13}C on the IRMS. Respiration was expressed as mg native C kg^{-1}soil and mg wheat C kg^{-1}soil, as well as a percentage of the initial added wheat C (%C_w).

Pairs of vials from each experimental unit were selected for biomass C and N determination by chloroform fumigation extraction (Vance et al., 1987) and DNA extraction. The first vial had a sub-sample (approximately 4 g) of moist soil removed and frozen immediately in liquid nitrogen, before storing in the freezer (-20°C) until later DNA extraction. Biomass C and N were determined using the remaining soil in each vial. One vial had 15 ml of 0.5 M K_2SO_4 added immediately, followed by mixing on a roller bed for one hour, centrifugation (10 minutes at 1300 g), and filtration of the supernatant through a Whatman GF/F disc, before freezing (-20°C). The second vial was placed in a vacuum extractor containing chloroform, fumigated, and left for 24 hours before extracting in the same manner as the first vial (Vance et al., 1987). All K_2SO_4 extracts were frozen and then freeze dried. The freeze dried K_2SO_4 extract was analyzed for total C and N and at.%^{13}C on the IRMS.

One of each duplicate frozen sample from day 22 and day 57 was used to track the losses of wheat C from the microcosms. This was done to provide an alternative method to the monitoring of ^{13}C in respired CO_2, after it became apparent that estimates of at.%^{13}C in the gas samples from early sampling occasions were not reliable. Frozen soil samples were dried at 105°C and ball milled before analysis for total C and at.%^{13}C

using the IRMS. The quantities of ^{13}C in these soil samples were compared with the initial quantities of wheat C based on the recorded values for masses of wheat added at experimental setup.

5.2.4 ^{13}C Calculations

Proportions of wheat C in the various fractions measured were determined using a binary mixing model (Scrimgeour and Robinson, 2003). The IRMS provided results for ^{13}C enrichment as at.% ^{13}C. This is calculated as:

^{13}C at.% = $[R_{sample}/(R_{sample} + 1)] \times 100$; where R_{sample} is the $^{13}C/^{12}C$ ratio determined by IRMS. The proportion of C originating from the added labeled material was calculated using the ^{13}C at.% value by: x = (L at.% - C at.%)/(X at.% - C at.%) where x is the proportion of C originating from component X (in this case, ^{13}C-labeled wheat), L at.% is the ^{13}C enrichment measured in the mixed sample, C at.% is the ^{13}C enrichment of wheat at the same growth stage growing in an unenriched atmosphere, and X at.% is the ^{13}C enrichment of our labeled wheat. Labeled wheat used in this incubation was 84 at.% ^{13}C, while wheat grown under natural conditions (harvested from outdoor plots at the Scottish Crop Research Institute) was 1.1 at.% ^{13}C. These figures were used to calculate the proportion of respired C and CFE flush C originating from the added wheat. Respiration of added wheat C was expressed as a percentage of the initial mass of wheat C added to each soil.

5.2.5 DNA Extraction

Soil for DNA extraction was placed directly into 15 ml falcon tubes (4 g wet weight), immediately frozen in liquid nitrogen, and later stored in a -20°C freezer. These samples were mixed with 3 ml of 0.12*M* $NaHPO_4$ in a buffered solution of 1% sodium dodecyl sulfate containing 5 x 10^6 copies ml^{-1} of a mutated clone of Muyzer et al. (1993)

universal bacterial primers (342f, 534r). The mixture was reduced to slurry using a vortex mixer. The rest of the extraction procedure was conducted as outlined in Chapter 3, Section 3.2.4.

5.2.6 Density Gradient Centrifugation

Optimization of centrifugation conditions

Density gradient solutions of caesium trifluoroacetate (CsTFA) (Amersham Biosciences) were used to separate DNA into fractions containing different proportions of ^{13}C. The first step in this process was to establish a relationship between CsTFA buoyant density and DNA ^{13}C content. DNA (1 μg) extracted from the ^{13}C-labeled wheat (previously determined to be 84 at.%^{13}C) was loaded into a centrifuge tube (Beckman, 13 x 51 mm, 5.0 ml thinwall polyallomer) containing 5 ml of CsTFA (average buoyant density 1.6 g ml^{-1}) and centrifuged (Beckman Optima Max Ultracentrifuge) in a swing out rotor (MLS-50) at 30,000 rpm (72,300 *g*) and 20°C for 72 h. The DNA extracted from wheat grown with atmospheric CO_2 was centrifuged in a separate tube under the same conditions. Salt solutions were fractionated within 20 minutes of the end of the run, by piercing a hole in the bottom of the tube with a syringe (0.6 x 25 mm) and collecting fractions (average volume 150 μl) in a microtitre plate. The buoyant density of each fraction was determined gravimetrically by weighing 100 μl on an analytical balance. DNA, in 10 μl aliquots from each fraction, was quantified using the Quant-iT™ PicoGreen dsDNA kit (Molecular Probes). Because of the quenching effect of salts on the fluorescence measured using the PicoGreen assay, a standard curve was developed relating the fluorescence of a known quantity of DNA at a range of salt contents, to fluorescence without salts present. This curve was used to convert fluorescence in the CsTFA solutions to unquenched fluorescence. I assumed that relative amounts of DNA

in each fraction were directly related to the fluorescence units; therefore fluorescence units were not converted to actual quantities of DNA. Fluorescence was graphed versus buoyant density for each DNA extract. As shown in Figure 5-1, the ^{13}C and ^{12}C DNA could be separated by an approximately 0.05 g ml^{-1} difference in buoyant density, with maximum ^{13}C DNA in the 1.6 g ml^{-1} fraction and maximum ^{12}C DNA in the 1.55 g ml^{-1} fraction.

Centrifugation conditions were optimized to allow maximum separation of 1.55 g ml^{-1} density from 1.6 g ml^{-1} within the centrifuge tube. The remainder of the fractionation runs used in the study were conducted at 25,000 rpm (50,200 *g*) and 20°C for 72 h.

The density of each fraction was measured using the technique described above. For each sample, a regression equation was fit to the density estimates using fraction number as a predictor. This removed the slight variability associated with density measurements obtained using the gravimetric approach. The regression equation for each sample was used to calculate corrected densities for each fraction in a given sample. The corrected densities were used in the statistical analyses conducted on each fraction.

Density gradient centrifugation, fractionation, and precipitation of soil extracts

The quantity of DNA in each soil extract was determined spectrometrically using a NanoDrop instrument (NanoDrop Technologies, Wilmington, DE) and 1 μg of DNA was added to 5 ml of CsTFA (average density 1.6 g ml^{-1}) for centrifuging. Solutions were centrifuged at 25,000 rpm (50,200 *g*) for 72 h at 20°C and fractionated as outlined above. Fractionated DNA was stored at -20°C until precipitation.

DNA was precipitated by adding 1 µl of glycogen (20 mg ml^{-1}) and 10 ul of 3 *M* sodium acetate to a 25 µl aliquot of each fraction. Ice cold 70% ethanol (63 µl) was also added and the mixture was stored at -20°C for at least 2 hours. The mixture was centrifuged at 4100 rpm (3007 g, SIGMA 4K15 centrifuge) for 20 minutes at 4°C before pouring off the ethanol and washing again with 100 µl ice cold 70% ethanol. After centrifuging again, the ethanol was poured off and the DNA pellet was dried, then re-suspended in 20 µl of ultra-pure molecular grade water. Samples were stored in a -20°C freezer for later analysis. A precipitation blank was included with each plate to allow detection of any contamination that may have occurred during the precipitation process.

5.2.7 T-RFLP Analysis

PCR and restriction enzyme digestion

The DNA-SIP portion of the experiment was only conducted on soils from the M0 treatment. A previous study had shown that management had minimal effect on T-RFLP profiles. Six samples were chosen for analysis of the bacterial and fungal community structure and diversity. These were day 3 samples from replicates 1 and 2 of the M0 treatment, at each of the three incubation temperatures. All samples had been subjected to density gradient centrifugation and fractionation into approximately 24 fractions, except for the day 3, 20°C samples, which were fractionated into 48 fractions. These samples were used for a more in depth preliminary analysis of fungal and bacterial community structure and diversity, before analysis of the remaining 4 samples.

T-RFLP was conducted as outlined in Chapter 3, section 3.2.4, except that the *Taq* polymerase used was Invitrogen Platinum Hi Fidelity, which requires an extension temperature of 68°C. Briefly, separate nested, polymerase chain reactions of bacterial and fungal DNA were conducted using flourescently labeled forward and reverse primers

in the second step of the nested procedure. Details concerning primer sequences and PCR conditions are as listed in Chapter 3, Table 3-3. The fluorescently labeled PCR products were digested with a restriction enzyme (*AluI* for bacteria and *HinfI* for fungi) and the terminal restriction fragment polymorphism (T-RFLP) profiles were analyzed using Genemapper software (version 3.7). Treatment effects on microbial community composition were assessed by summarizing the variability in the dataset using principal components analysis, followed by an Analysis of Variance and comparison of means of the scores for the main principal components. Diversity was assessed by computing Shannon's Diversity Index (H') using the peaks in each profile as operational taxonomic units (OTUs).

Data analysis

Genemapper software (version 3.7) was used to analyze the T-RFLP profiles. Each dye was analyzed separately. Peaks were assigned to bins using the autobin feature of the software, with bin width set at 3 bp to account for variations in sizing among capillaries. Peak height thresholds for bacterial and fungal samples were determined based on thresholds observed in PCR negatives and the peak-free areas of samples. The minimum peak height used for the bacterial samples (both the FAM and VIC dye) was 100 fluorescence units. The minimum peak height used for fungal samples was 200 fluorescence units for the PET dye and 400 fluorescence units for the NED dye.

Tables of peak size and area were copied to Excel for further analysis. Peak areas were judged to be more representative of the relative fluorescence contributed by each fragment than peak heights, because of the spreading of peaks that occurs for larger fragments which can result in lower peak heights. For each sample, peak areas were

converted to relative abundance values by dividing the fluorescence contributed by each peak, by the total fluorescence of the sample. Peaks appearing in precipitation blanks and PCR negatives were examined and deleted from the analysis if they appeared in the majority of samples. All peaks that never represented more than 1% of the total fluorescence of a sample were also removed from the analysis. Using this screening approach, the total number of fragment sizes (bins) for the PET dye was reduced from 196 to 166, and for the NED dye from 170 to 149. Samples with no peaks present after the screening process were removed from the analysis. In the case of the fungal analysis, the initial 235 samples in the analysis were reduced to 206 after removal of failed samples. Using the same screening approach the number of fragment sizes for the FAM dye were reduced from an initial 122 identified with the autobinning feature, to 76 post-screening. Similarly, 143 bins for the VIC dye were identified by autobinning, but this number was reduced to 84 after screening.

T-RF patterns for each dye were combined, resulting in a total of 315 variables for the fungal analysis and 160 variables for the bacterial analysis. The number of variables in the analysis was reduced using principal components analysis (based on the covariance matrix). The relationship between community structure and density was investigated by correlating the principal component scores with density.

The species richness (the total number of fragments present in each sample), Shannon's diversity index (H') (Odum, 1971), and species evenness (calculated as Shannon's diversity index divided by the log of the number of fragments) were calculated using the relative abundance data for the bacterial and fungal T-RFLP profiles. Analysis

of Variance of these indices was used to determine treatment effects on these measures of diversity.

5.2.8 Quantitative PCR

The efficacy of the density gradient centrifugation procedure for separating ^{12}C from ^{13}C-DNA was tested by conducting real-time PCR on one of the in depth samples. The mutated clone of the universal bacterial primers 342f/534r was composed only of ^{12}C DNA, and would therefore serve as a marker to indicate the location of the ^{12}C DNA in the density gradient. Quantities of mutated clone in selected fractions were determined using the Roche LightCycler™.

5.3 Results

5.3.1 Respiration of C from Native and Wheat C Pools

Rates of respiration of both the native C and the wheat C increased with increasing temperature (Figure 5-2 to Figure 5-7). It is interesting to note that the peak native C respiration rates were equal for both soils at 4°C (approximately 40 mg C kg^{-1} d^{-1}), but rates of wheat C respiration were higher by approximately 20 mg C kg^{-1} d^{-1} for the soils with a history of manure application (M300). At 22°C, both native and wheat C peak respiration rates were higher for the M300 treatment than the M0 treatment. At 35°C, there was no difference in the peak rate of wheat C respiration, but the native C respiration was almost twice as high in the M300 treatment compared with the M0 treatment.

When the areas under the respiration rate curves for wheat C were calculated, the amount of wheat respired was greater than 100% within the first 15 days, at 22 and 35°C. Maximal rates of ^{13}C-wheat respiration at 22°C were 20% per day compared with 3% per day at 25°C in the ^{14}C experiment, and 38% of added ^{13}C-wheat per day at 35°C

compared with 7% per day in the ^{14}C experiment. During the first 14 days of the ^{14}C-wheat experiment (see Chapter 4), at 5°C total rates of C respiration ranged from 10 to 30 mg C kg^{-1} d^{-1}. As illustrated in Figure 5-2 and Figure 5-3, at 4°C total rates of C respiration (estimated by summing native C and wheat C respiration rates) in the stable isotope experiment were as high as 125 to 150 mg C kg^{-1} d^{-1}. Rates were also higher for the 22 and 35°C treatments in the stable isotope experiment compared with the ^{14}C experiment (see Chapter 4). These results suggest that CO_2 measurements using the IRMS in this experiment, were not accurate. As an alternative method of estimating wheat C respiration during the incubation, ^{13}C-enrichment of soil samples taken from the experiment on day 22 and day 57, was measured and the amount of wheat remaining was calculated as a percentage of the original material applied (Table 5-3). This analysis showed that by day 22, at the most, 57.6% of added wheat had been respired at 35°C for the M300 soil. Based on these investigations, it was concluded that the values for wheat C respiration rates in the this experiment were overestimated by the IRMS.

The shape of the curves in Figure 5-2 to Figure 5-7 suggests a priming of native pools of C during the first 15 days of the incubation, since respiration rates of this pool were enhanced during this period. This effect, however, is likely an artefact of the problems with enrichment estimates already discussed. Some priming of the native pools of organic C may have occurred in the 22 and 35°C treatments. Priming was observed in the ^{14}C-wheat experiment reported in Chapter 4 at 25 and 35°C, but the effect was only apparent in the first 5 days of the experiment, and never resulted in native C respiration rates in amended soils exceeding basal respiration in non-amended soils by more than 26 mg C kg^{-1} d^{-1} (in the M300 soil at 25°C).

When the proportion of total respired C originating from the added wheat during the latter (after day 14) phase of the incubation was plotted against time, a clear difference in C utilization based on temperature was apparent (Figure 5-9). At 4°C, proportionally more of the respired C was originating from the added wheat than at 22 or 35°C. The lowest proportion of respired C originated from the wheat at 35°C. These effects were apparent until the end of the incubation on day 57. However, when the same proportions were plotted versus soil thermal units (Figure 5-10), at 4°C too few soil thermal units were accumulated during the incubation to make a direct comparison with the two warmer temperatures. At 22°C, a larger proportion of the respired C originated from the added wheat C than at 35°C when the equivalent number of soil thermal units had accumulated.

5.3.2 Biomass Characteristics and N Mineralization

There were significant interactions between temperature and management and temperature and day for CFE flush C; therefore the results for this parameter were analyzed separately for each soil. Figure 5-11 shows the results for the soil with no history of manure application, where few differences in CFE flush C were detected. For the M300 treatment (Figure 5-12), CFE flush C at 35°C was lower than the other two temperatures on all days except for day 57 at 22°C. On day 1, CFE flush C at 4 and 22°C was higher than the other three days.

A summary of the dynamics of wheat C respiration for two periods, day 0 to day 22, and day 0 to day 57, is shown in Table 5-3. Temperature had a significant effect on the percentage of added wheat C respired for both periods, and the respiration rate per STU and percentage of wheat C in the biomass. For both soils the percentage of wheat C respired was significantly lower at 4°C compared with 35°C. When the rate of wheat C

respiration over the first 22 days was expressed per soil thermal unit, for only the M300 soil, the rate was significantly higher at 4°C. For both soils, there was significantly more wheat C in the biomass at 4°C compared with 35°C. This indicates a significance difference in the rate of biomass turnover at these two temperatures.

Net mineral N production during the incubation at each temperature was calculated by subtracting the inorganic N measured on day 1 from the subsequent measurements (days 7, 38 and 57). Results for each soil are shown in Figure 5-13 and Figure 5-14. For both soils, there was significantly more net mineral N production at 35°C than for the other two temperatures by the final sampling day. The negative estimates of net mineral N production for both soils incubated at 22°C indicates that either immobilization or denitrification of N were dominant processes in the first 38 days under these conditions. While immobilization and denitrification were also likely occurring at 4 and 35°C, they may have been balanced by relatively higher amounts of N mineralization, which resulted in increasing estimates of net mineral N production at 35°C over time and constant estimates of net mineral N production at 4°C during the incubation.

5.3.3 Efficacy of the Density Gradient Centrifugation

Real-time PCR was conducted to confirm that ^{12}C DNA was being separated from ^{13}C DNA by the centrifugation process,. The real-time PCR was conducted to amplify the mutated clone which had been added to the soil during the DNA extraction process. Since this clone consisted of ^{12}C-DNA only, it served as a tracer to monitor the location of the ^{12}C DNA in the fractionated samples. As shown in Figure 5-15, most of the clone was amplifiable in the lighter fractions, especially the fractions that were approximately 1.54 g ml^{-1} in density. Some of the clone was amplified even from the most dense

fraction which indicates that separation of ^{12}C DNA from ^{13}C DNA was not complete using this approach.

5.3.4 Fungal and Bacterial Community Structure at a Range of Densities

Fungal communities

The fungal T-RFLP profiles for the 5' and 3' ends of the PCR product were combined and used in a principal components analysis. Although there were a large number of OTUs identified for both the 5' and 3' ends of the PCR product (149 for the 5' end and 166 for the 3' end), an examination of the loadings for the first four principal components (accounting for a total of 38.9% of the variance) indicated that relatively few of these contributed significantly (with absolute values of loadings >0.1) to the total variation of the dataset (Table 5-4). A 5'-labeled fragment approximately 183 bp in size was dominant for all four PCs. Five fragments (5'-labeled) ranging from 302 to 309 bp were also important. The small size range for these 5 OTUs suggests that variability in size estimates may have resulted in the creation of more bins than were necessary. A separate 3'-labeled fragment 84 bp in size was also significant. Fragments (3'-labeled) of 280 and 285 bp were significant, as were two OTUs of 374 and 377 bp. Both of these pairs of fragments are similar in size, again indicating the potential for incorrect identification of bins. This analysis shows that at the most, twelve OTUs were predominant in the analysis, although it seems likely that the true number of predominant fungal species is less than this.

The relationship between fungal T-RFLP profiles (band size and fluorescence) and sample density was examined to determine if there were differences in the communities separated by density gradient centrifugation. The PC scores for each density fraction from the in depth samples (22°C) were related to sample density using

linear regression. This relationship was highly significant for PC2, PC3 and PC4 ($p<0.01$) and showed a significant trend ($p<0.1$) for PC1; however, in all cases the r^2 values were very low (from 3.9 to 27.8%) indicating that this relationship explained only a small percentage of the total variation.

The Shannon's diversity values declined as density increased (Figure 5-16). The data indicated at least four distinct regions of diversity, based on the pattern of peaks. The first region was in the 1.53 to 1.56 g ml^{-1} density range, where ^{12}C-DNA was expected to accumulate. A second peak in diversity occurred between 1.56 and 1.61 g ml^{-1}, which was the range where the ^{13}C-labeled DNA had been predicted to predominate. A further two peaks were located between 1.61 and 1.66 g ml^{-1} and between 1.66 and 1.7 g ml^{-1}.

Bacterial communities

The bacterial T-RFLP profiles for the 5' and 3' ends of the PCR product were combined and used in a principal components analysis. The loadings with absolute values of greater than 0.1 for the first three principal components (accounting for a total of 42.7% of the variance) are shown in Table 5-5. Scores for principal component 1 were largely determined by the relative abundance of reverse primer (VIC-labeled) fragments, especially a fragment approximately 336 bp in size. This fragment was an important determinant of the score for PC2 and 3 as well. A forward primer (FAM-labeled) fragment approximately 180 bp in size and VIC-labeled fragments 69, 117, 133, and 333 bp in size, all contributed significantly to the loadings of 3 or more of the first 6 principal components.

Compared with the fungal samples, there were fewer significant relationships between the PC scores for bacterial T-RFLP and sample density (day 3, 22°C only). Only PC5 and PC6 were significantly correlated with density ($p=0.011$ and $p<0.0001$ respectively). This indicates that most of the variability in the dataset was due to factors other than density. An Analysis of Variance of the PC scores with temperature as a fixed effect and replicate, sample and fraction number as nested random effects, indicated that temperature did not have a significant effect on any of the first six PC scores.

There was a trend towards declining diversity at increasing densities (Figure 5-17) although for replicate 1, there was a peak in diversity at approximately 1.6 g ml^{-1}, while this effect was not apparent for replicate 2. The two bacterial samples showed differences in overall diversity, with much lower H' values for replicate 2 compared with replicate 1. For 7 of the fractions in replicate 2, no OTUs at all were detected in the T-RFLP analysis, and these were deleted from the analysis.

5.3.5 Temperature Effects on ^{12}C- and ^{13}C-metabolizing Communities

Fungal communities

In order to investigate the relationship between temperature and the metabolism of native C and wheat C, the principal component scores derived for each sample were divided up according to density ranges. The density ranges used for the fungal analysis were based on the distinct groupings resulting from the Shannon's diversity analysis of the 22°C samples. From Figure 5-16, density ranges of less than 1.57 g ml^{-1} (representing the native, ^{12}C-metabolizing community), 1.57 to 1.61 g ml^{-1} (representing an "intermediate" community), and from 1.61 to 1.66 g ml^{-1} (representing the wheat C metabolizing community), were selected. A fourth community with densities greater than

1.66 g ml^{-1} was not included in the investigation because it consisted of only 13 samples, 12 of which were from the 20°C treatment.

The plots of mean fungal PC scores for each temperature and density range are shown in Figure 5-18. The chart shows a clear separation of fungal communities based on temperature, but no differences due to density within a given temperature.

The Shannon's diversity results (Figure 5-19) indicated significantly lower diversity at 35°C compared with 4°C, but no effect of density on diversity, except at 22°C where the diversity for the densest fraction was lower than for the least dense fraction. The species richness (based on numbers of OTUs, or peaks in a sample) ranged from a low of 38 at 22°C for the 1.61 to 1.66 g ml^{-1} density range, to a high of 73 at 4°C for the 1.57 to 1.61 g ml^{-1} density range.

Bacterial communities

The scores for the principal components analysis were divided into three different density ranges to represent three different bacterial communities: density ranges of less than 1.55 g ml^{-1}, representing the ^{12}C-metabolizing community; the 1.55 to 1.65 g ml^{-1} density range, representing intermediate communities; and the greater than 1.65 g ml^{-1} range, representing the primarily ^{13}C metabolizing communities. When the mean scores for each temperature over each density range for the first five principal components were plotted, few differences between temperatures or densities were observed (data not shown). However, Figure 5-20 illustrates the means for each temperature and density range for PC6. In this case, some differences between temperatures are apparent with a 35°C community in the mid-density range that is separate from the 22 and 4°C communities. It is also very interesting to note that PC6 scores for the 22°C communities

are clearly separated over the three density ranges, suggesting that specialized communities have developed at this temperature depending on the substrate being metabolized. There were no differences in Shannon's diversity index due to temperature or density (Figure 5-21), except for the 22°C treatment where diversity was slightly higher at the lowest density, compared with the highest density.

5.4 Discussion

5.4.1 Partitioning of Wheat C at Different Temperatures for Different Soil Management Histories

The first objective of this study was to trace the partitioning of ^{13}C-labelled plant material into respired CO_2, biomass C, and unmetabolized C, in soils with two management histories incubated at three temperatures (4, 22 and 35°C).

The proportion of respired CO_2 originating from the added wheat was affected by temperature. This was very clear when proportions were plotted versus time in days after day 14 (Figure 5-9), but also true when soil thermal units were used as a modified time variable (Figure 5-10). In fact at 22°C, proportionally more wheat C per STU was being respired than at 35°C. This supports the hypothesis that different pools of C are accessed at different temperature ranges (Dalias et al., 2002). The addition of wheat C may have stimulated the microbial biomass to access previously inaccessible pools of C, i.e. the priming effect (Fontaine et al., 2003). This priming of native pools of C appears to be greater at warmer temperatures as shown by the higher rates of native C respiration, especially for the M300 treatment at 35°C (Figure 5-7).

Temperature effects on the size of the pool of accessible substrate could be due to the development of unique microbial communities at each temperature. In this study, fungal communities were more affected by temperature than the bacterial communities, as indicated by the greater separation of fungal community T-RFLP profiles due to

temperature than for the bacterial communities. Especially at the coldest temperature, a unique fungal community capable of accessing the more recalcitrant pools of C in the added wheat could have been active, since fungal organisms secrete extracellular enzymes that allow them to access these substrates (Sylvia et al., 1999). Various researchers have reported an increase in fungal biomass relative to bacterial biomass at low temperatures (Zogg et al. 1997; Lipson et al. 2002; Pietikäinen et al. 2005). This specialized fungal community could have been assimilating much of the N that was contained within the added wheat, since microbial biomass was highest at the coldest temperature. This could explain the low levels of net mineral N production at 4°C (**Figure 5-13** and Figure 5-14).

In contrast, at the warmest temperature, more of the respired C originated from native C sources. This source may have been the microbial biomass pool itself, since it was much smaller at 35°C. The microbial biomass was smallest for the M300 soil at 35°C which was also the treatment with the highest mineral N in the soil at the end of the incubation. Turnover of the microbial biomass could therefore have been a major source of both C and N in the microcosms incubated at 35°C.

The biomass was larger in the soil with a history of manure application (M300)l and it respired more wheat C than the soil that had not received annual applications of manure (M0) ,during the first 22 days of the incubation (Table 5-3). There was also evidence for a management-related difference in the efficiency of wheat C use, since higher amounts of wheat C were assimilated into the soil with a history of no manure amendment. None of these differences were evident later in the incubation, but by this time the initial flush of activity due to the added wheat C had subsided, and soil

respiration rates had returned to basal levels. This finding suggests that the size of the microbial biomass may be an important factor driving the rate of mineralization of C and N from added organic materials, especially during the period immediately after addition to the soil.

These findings suggest the development of specialized microbial communities with specialized functions at different temperatures. However, it is the differences in temperature effects on the soils with different management histories that are most relevant to the question: does more microbiology need to be incorporated into soil C models? This study provided some evidence to suggest differences in wheat C utilization between the two management histories at 4°C. The M300 soil metabolized approximately 13.7% more of the wheat C than the M0 soil at this temperature. The effect was less pronounced as temperatures increased, with 5.3% more wheat C metabolized by the M300 soil at 22°C and just 2.7% more at 35°C. These findings suggest that management-related differences in microbial community composition and function have the greatest effect on decomposition processes at cold temperatures.

5.4.2 Use of DNA-SIP to Differentiate Wheat C and native C Metabolizing Communities

By using DNA-SIP and dividing the density gradient into 48 fractions, we were able to separate wheat C metabolizing fungal communities from native C metabolizing fungal communities using T-RFLP. This allowed a very high resolution of communities and showed that communities are divided by density-gradient centrifugation along a continuum. At 22°C for both bacteria (Figure 5-21) and fungi (Figure 5-19), the lightest fractions were the most diverse, supporting the hypothesis that these fractions represent the whole soil microbial community and its full diversity: a community utilizing the

broad range of substrates available within the SOM pool. As fractions became progressively denser, the diversity of the community decreased. At the highest density the community was the least diverse and represented those organisms involved in the primary metabolism of the added wheat C. Since the wheat was labeled with 13C continuously, this community would still be reasonably complex consisting of organisms with the ability to metabolize a range of substrates, from simple to complex sugars, and cellulose. Nevertheless, this community was still considerably less diverse than the whole community.

The fungal diversity graph (Figure 5-19) seems to indicate the presence of two, intermediate communities at densities between 1.56 and 1.64 g ml^{-1}. These communities could be wheat C metabolizing communities that have only replicated once and therefore contain only 1 strand of DNA; however, if this were the case, we would expect the diversity to be similar to the more dense wheat C metabolizing communities. Using similar logic, if these communities represent a mixing of ^{12}C and ^{13}C DNA in the centre of the gradient, we would expect the diversity to be similar to the communities identified at the lightest end of the gradient. The intermediate diversity of these samples supports the hypothesis that these communities are distinct from the specialized wheat C metabolizing community and the whole soil community. These communities could consist of organisms capable of metabolizing specific substrates found in the wheat, that are also present in the wider soil organic pool, hence the mixture of ^{12}C and ^{13}C-labeled DNA found in this community.

The less clear resolution of bacterial communities by density gradient centrifugation (Figure 5-17) may be due to a low overall usage of the wheat C at the early

stage of the incubation. Since fungi have the ability to secrete xylanases and cutinases (Sylvia et al., 1999), they may have been utilizing relatively more of the wheat C on day 3 than the bacteria. This could have resulted in relatively low labeling of the bacterial DNA with ^{13}C and poor separation on the density gradient.

In previous studies using simple substrates, the DNA from only one narrow density band within the gradient has been precipitated and used to represent the community responsible for metabolizing the substrate (Radajewski et al., 2000; Padmanabhan et al., 2003). Rangel-Castro et al. (2005b) created more complex ^{13}C-labeled substrates by pulse-labelling plants with $^{13}CO_2$ and extracting RNA from the rhizosphere. They used fractions 4 to 6 from their density gradient to represent the ^{12}C community and fractions 8 to 10 to represent the ^{13}C community. By dividing density gradients into as many as 48 fractions, we have been able to detect subtle shifts in community composition that may not be detectable when larger density ranges are grouped together. In fact, we could not detect a relationship between the community composition (i.e. PC scores from T-RFLP profiles) and density, for the 4 and 35°C samples, and this may have been due to the reduced resolution we obtained by only dividing these gradients into 24 fractions.

5.4.3 Differences Between the Wheat C and Native C Metabolizing Communities at Different Temperatures

Distinct fungal communities developed at each of the three incubation temperatures, similar to the results reported in Chapter 4 where the 5°C fungal community in the ^{14}C-wheat amended soils was clearly separated from the 35°C community. At each temperature, the communities from each of the three density ranges were grouped closely together. This suggests that the added substrate did not have as

significant an effect on fungal community structure as the temperature. While the density gradient separation technique was able to detect a trend towards a change in community structure with changing density, this was not apparent when the data were grouped into three density ranges. It is likely that there is considerable overlap in communities from adjacent fractions. More clear separation of communities from each density range may be possible if a few "buffer" fractions between each density range are excluded from the analysis. The diversity analysis nonetheless indicated trends towards decreased fungal diversity with increased fraction density, as well as decreases in diversity with increasing temperature.

The bacterial communities appeared to be less affected by temperature than the fungal communities in this study. There was a trend towards less diversity of bacterial communities at 4°C which complements the increased fungal diversity at this temperature. At this stage of the incubation it is possible that the bacteria had not assimilated enough labelled C to allow good separation by density in the CsTFA solution. It is, however, surprising that there was no separation of the bacterial T-RFLP profiles due to temperature. This effect was clear in the experiment reported in Chapter 3 and 4, however, in those experiments community structure was measured after a much longer period of incubation (up to 123 days) so that temperature had exerted its selection pressure on the microbial communities for longer and the effect was more clear.

In this study, a few samples were selected for study of all fractions using DNA-SIP. The effects of temperature on native C and wheat C metabolizing communities could be further studied by conducting T-RFLP on only selected fractions from a larger number

of samples. In addition, the use of RNA-SIP would allow more precise identification of those organisms involved in metabolism of both the native C and wheat C.

5.5 Conclusions

As reported in Chapters 3 and 4, biomass characteristics were affected by temperature and to a lesser extent, management history. Biomass (as indicated by CFE flush C) declined at higher temperatures and was also lower overall in the soil with no history of manure application. A difference in the respiration process was apparent at 4°C, where the rate of wheat C respired per STU, was significantly higher than at the two warmer temperatures. This difference in process could be a result of the temperature-related differences in microbial community structure already reported in this chapter and elsewhere. There was also a difference between the two management histories at this temperature, with the soil with a history of manure amendment, metabolizing a higher proportion of the added wheat C than the soil with no history of manure amendment.

DNA-SIP was used to differentiate wheat C metabolizing communities from native C metabolizing communities. By dividing density gradients into a large number of fractions (as many as 48), I was able to detect a relationship between fungal T-RFLP profiles (summarized using principal components analysis) and fraction density. There was also a clear relationship between fraction density and fungal diversity. These relationships were not so apparent in those samples that were only fractionated into 24 fractions. The relationships were also not so apparent for the bacterial T-RFLP results.

DNA-SIP was also used to compare wheat C and native C metabolizing communities at three different temperatures. For fungal communities, there was a clear difference between fractions taken from samples at different temperatures. This reflected temperature-driven differences in fungal community structure already reported in

Chapters 3 and 4. These temperature-driven differences, however, were not so apparent for the bacterial communities. There were no differences between the wheat C and native C metabolizing fungal communities at a given temperature, when the communities were divided into three density ranges. This may have been due to the overlap of communities at the boundaries of these ranges. Fractions that are located at the boundaries of a particular density range may be equally similar to the adjacent fraction in the next density range, as they are to the adjacent fraction within their density range. Clearly, there is a continuum of species across the range of densities and it was not possible to select specific densities that could be used to separate ^{12}C from ^{13}C communities.

The use of stable isotopes to study soil C dynamics is a well-established technique. In this study, while there were problems with the estimates of wheat C respiration rates, it was still possible to use this technique to trace the partitioning of added wheat C into respired, biomass and un-metabolized C. DNA-SIP is still a relatively new technique. In this study it showed some potential for differentiating soil microbial communities based on substrate source. Further refinement of the technique is required before it can be used reliably as a tool to address questions about microbial species composition and utilization of a complex substrate.

Table 5-1. Chemical and microbiological sampling schedule for microcosms of a Nova Scotia soil from a long term fertility trial with and without a history of manure application amended with ^{13}C-labelled wheat and incubated at 3 temperatures for up to 57.5 days.

Procedure	Temperature	Days from setup[z]
Gas sampling	4	1.5, 3.5, 5.5, 7.5, 15.5, 22.5, 29.5, 36.5, 43.5, 50.5, 57.5
	22	1, 3, 5, 7, 15, 22, 29, 36, 43, 50, 57
	35	0.5, 2.5, 4.5, 6.5, 14.5, 21.5, 28.5, 35.5, 42.5, 49.5, 56.5
CFE-flush determination; inorganic N	4	2.5, 7.5, 38.5, 57.5
	22	1, 7, 38, 57
	35	0.5, 6.5, 37.5, 56.5
Microbial community samples frozen	4	2.5, 3.5, 5.5, 7.5, 22.5, 38.5, 57.5
	22	1, 3, 5, 7, 22, 38, 57
	35	0.5, 2.5, 4.5, 6.5, 21.5, 37.5, 56.5

[z] In order to simplify references to sample day, in the text the 22°C days from setup figures are used for both 4 and 35°C treatments. Readers can refer to this table for the exact days from setup figures.

Table 5-2. Primer pairs and sequences and PCR conditions for first and second round amplifications targeting SSU rDNA region for bacteria and ITS region for fungi used in T-RFLP analysis of DNA extracted from microcosms of soil from a Nova Scotia long term fertility trial incubated at three temperatures for up to 57.5 days.

Domain	PCR round	Primer pairs and sequences
Bacteria	1	16f27 (5' AGA GTT TGA TCC TGG CTC AG 3') 1494r (5' TAC GG(CT) TAC CTT GTT ACG AC 3')
	2	63fFAM (5' CAG GCC TAA CAC ATG CAA GTC 3') 1405rVIC (5' CGG GCG GTG TGT ACA AG 3')
Fungi	1	EF3RCNL (5' CAA ACT TGG TCA TTT AGA GGA 3') ITS4 (5' TCC TCC GCT TAT TGA TAT GC 3')
	2	ITS1fNED (5' CTT GGT CAT TTA GAG GAA GTA A 3') ITS4PET
PCR amplification conditions		4 min at 94°C 30 s at 94°C, 30 s at 51°C, 1 min 30 s at 68°C for 35 cycles[z] 10 min at 72°C

[z]25 cycles for the second round bacterial PCR

Table 5-3. Dynamics of wheat C respiration up to day 22 and day 57 in microcosms of a Nova Scotia soil from a long term fertility trial with (M300) and without (M0) a history of manure application incubated at three temperatures.

Management history	Temperature °C	Day 0 - 22		Day 0 - 57		
		Wheat C respired (%C_w)	Respiration rate (%wheat C STU^{-1})	Wheat C respired (%C_w)	Wheat C in Microbial Biomass (%C_w)	Unmetabolized /Excreted
M0	4	15.7 a	0.14 b	18.1 a	6.3 bc	75.6
M0	22	39.4 bc	0.08 b	43.7 b	4.5 bc	51.8
M0	35	50.1 cd	0.06 b	55.7 c	0.5 a	43.8
M300	4	31.0 ab	0.28 a	29.7 ab	8.4 c	61.9
M300	22	45.8 bcd	0.09 b	50.9 bc	2.6 b	46.5
M300	35	57.6 d	0.08 b	57.2 c	1.7 ab	41.1
				ANOVA		
Temp[z]		p<0.0001	p=0.001	p<0.0001	p=0.001	
Mgt[y]		p= 0.007	p=0.027	ns	ns	
Temp*Mgt		ns	ns	ns	ns	

[z]Temp=Temperature; [y]Mgt=Management; [x]Calculated as 100 subtract the amount respired by day 57 and the amount still in the biomass on day 57. Values expressed as a percentage of the wheat C initially added. Means followed by the same letter in the same column are not different at the 5% significance level using Tukey's simultaneous pairwise comparison test

Table 5-4. Principal component coefficients (loadings) and sizes of predominant OTUs in the first four principal components of the fungal T-RFLP analysis of a Nova Scotia soil amended with ^{13}C-labelled wheat and incubated at three temperatures for up to 57.5 days.

Dye[z]	Size (bp)	PC1 (12.7)[y]	PC2 (11.2)	PC3 (7.9)	PC4 (7.1)
NED	182.84	0.115	-0.269	-0.433	0.339
	301.94	-0.817	0.197	-0.354	-0.135
	303.12	0.194	0.216	0.185	0.210
	304.85	-0.301		0.345	0.406
	307.56				-0.118
	309.20	0.313	0.574	-0.274	
	314.93			0.117	-0.220
PET	83.60	0.126	0.220	-0.124	
	280.05	0.113	-0.318	-0.477	0.382
	284.54	0.121	-0.233	0.120	-0.417
	373.96		0.453	-0.152	
	377.31		0.151	0.207	0.310

[z] NED dye was used to label the forward primer and PET dye was used to label the reverse primer;
[y]Percentage of variation accounted for by each principal component in parentheses.

Table 5-5. Principal component coefficients (loadings) and sizes of predominant OTUs in the first three principal components of the bacterial T-RFLP analysis of a Nova Scotia soil from a long term fertility trial amended with ^{13}C-labelled wheat and incubated at three temperatures for up to 57.5 days.

Dye[z]	Size (bp)	PC1 (24.7)[y]	PC2 (11.1)	PC3 (7.9)
FAM	72.06			
	102.96			
	116.01			
	154.66			
	170.47			
	173.15			
	179.72		0.311	0.788
	184.79			-0.136
	202.66			
	210.39			-0.104
VIC	51.75			
	69.09	0.409	-0.444	-0.134
	112.41			
	113.67			
	117.10	-0.265	0.191	-0.160
	129.84			
	130.29			
	132.96		0.133	0.332
	182.19			
	330.25			
	333.19	0.300	-0.615	0.356
	336.19	0.811	0.502	-0.179

[z] FAM dye was used to label the forward primer and VIC dye was used to label the reverse primer. [y] Percentage of variation accounted for by each principal component in parentheses.

Figure 5-1. Fluorescence of fractionated DNA from ^{13}C wheat and ^{12}C wheat versus solution density.

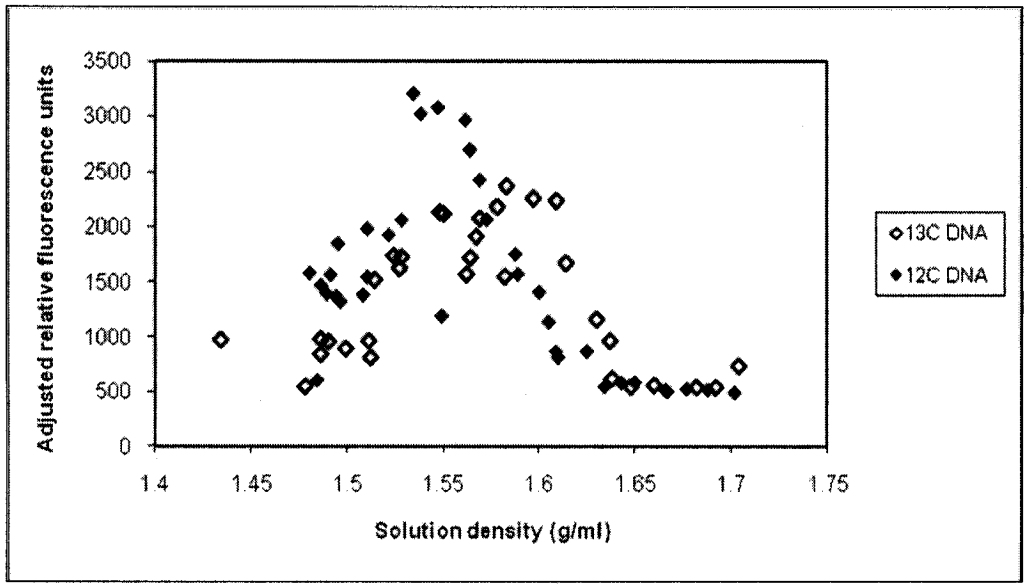
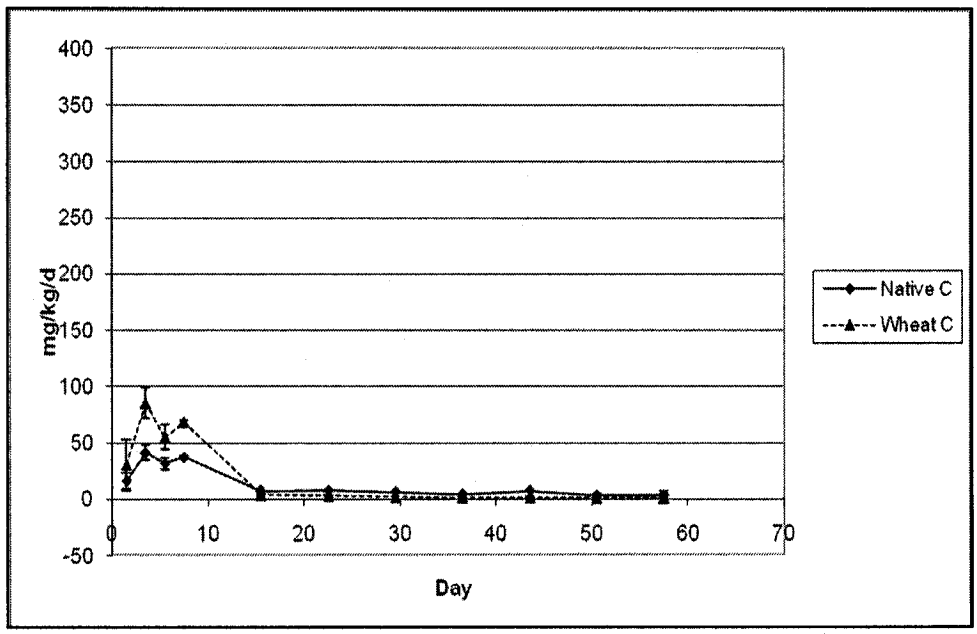
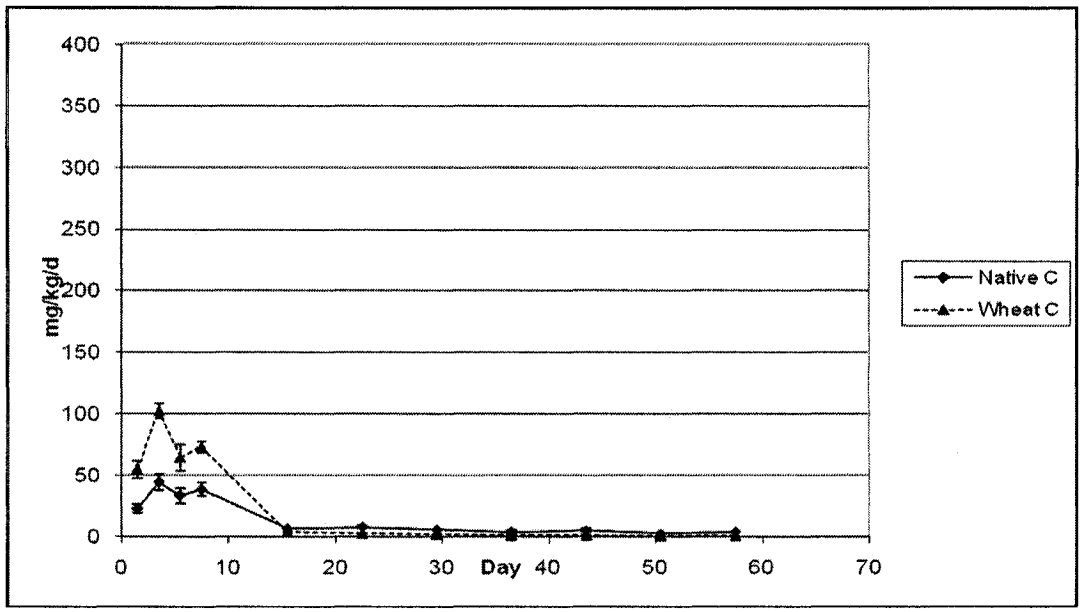


Figure 5-2. Respiration rates of native C and wheat C measured in microcosms of a Nova Scotia soil from a long term fertility trial without a history of manure application incubated at 4°C.



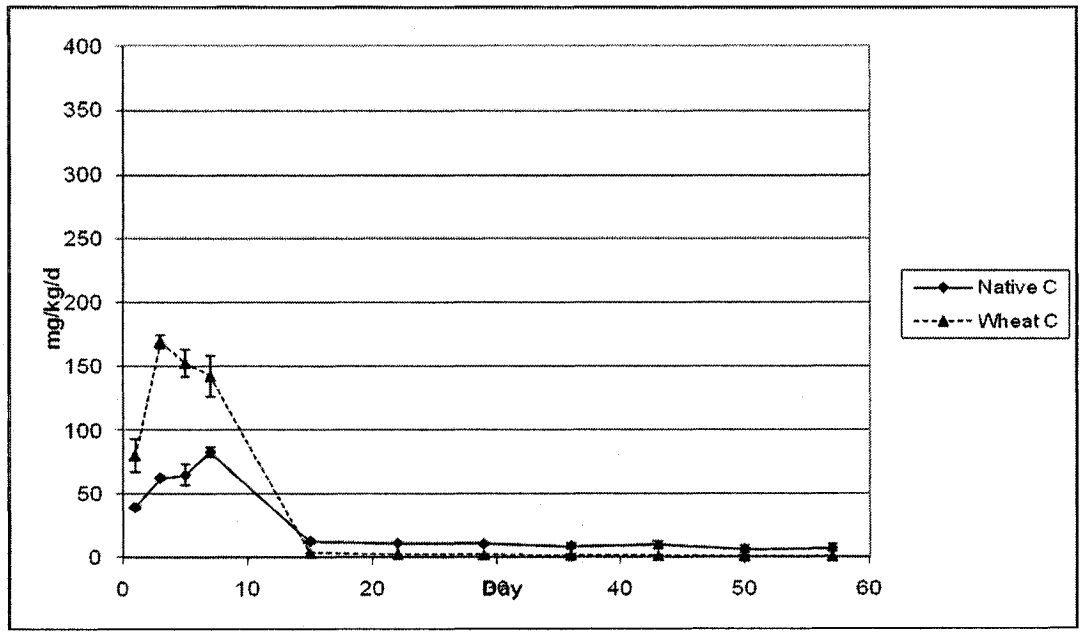
Error bars represent 95% CI of the daily means.

Figure 5-3. Respiration rates of native C and wheat C measured in microcosms of a Nova Scotia soil from a long term fertility trial with a history of manure application incubated at 4°C.



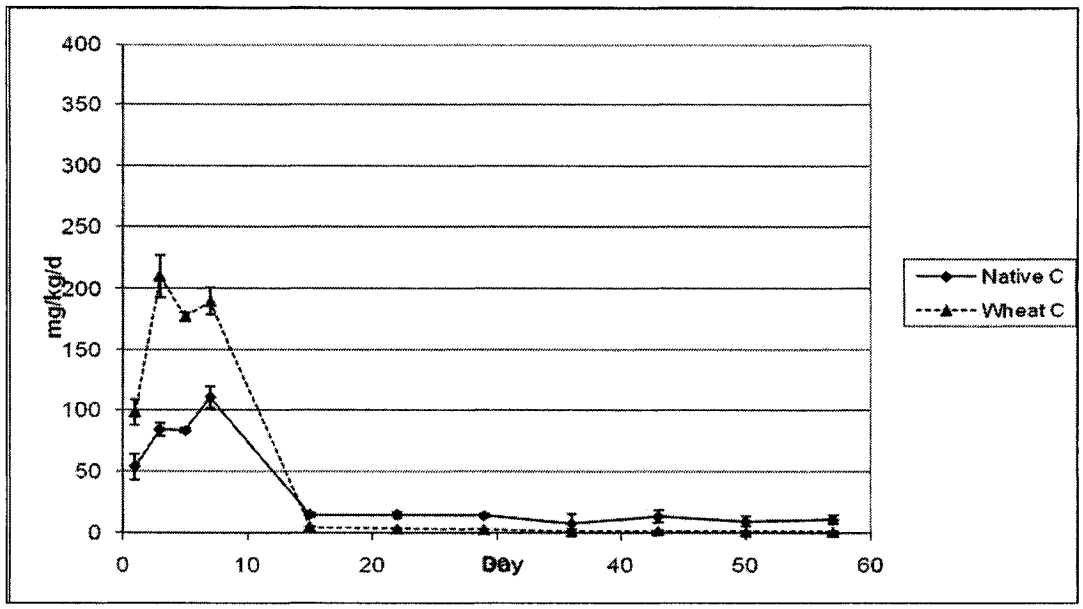
Error bars represent 95% CI of the daily means.

Figure 5-4. Respiration rates of native C and wheat C measured in microcosms of a Nova Scotia soil from a long term fertility trial without a history of manure application incubated at 22°C.



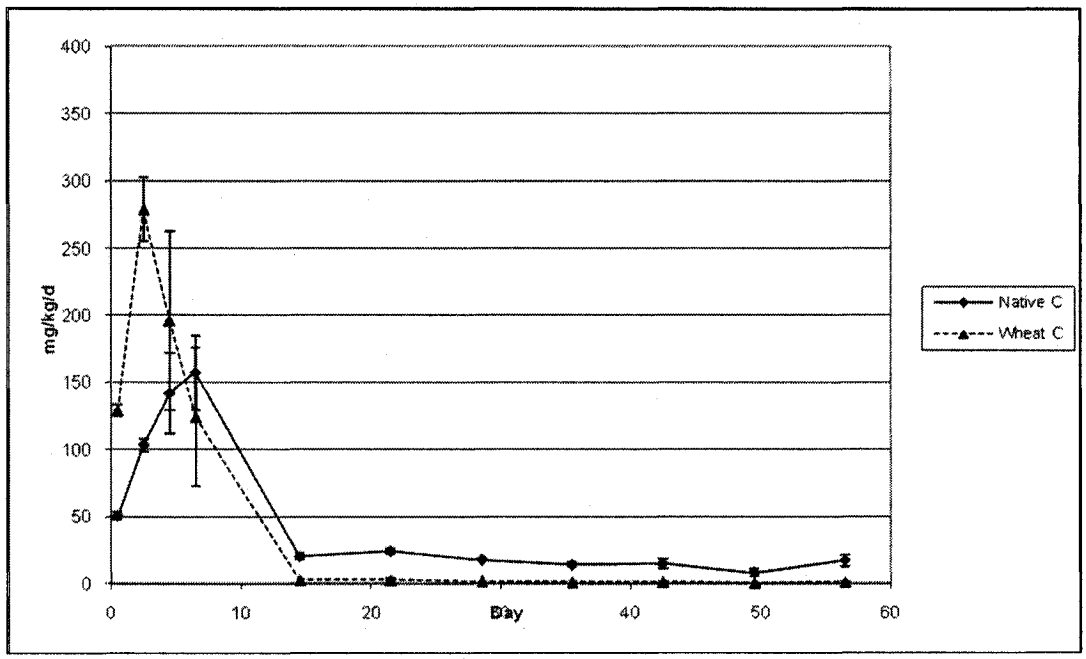
Error bars represent 95% CI of the daily means.

Figure 5-5. Respiration rates of native C and wheat C measured in microcosms of a Nova Scotia soil from a long term fertility trial with a history of manure application incubated at 22°C.



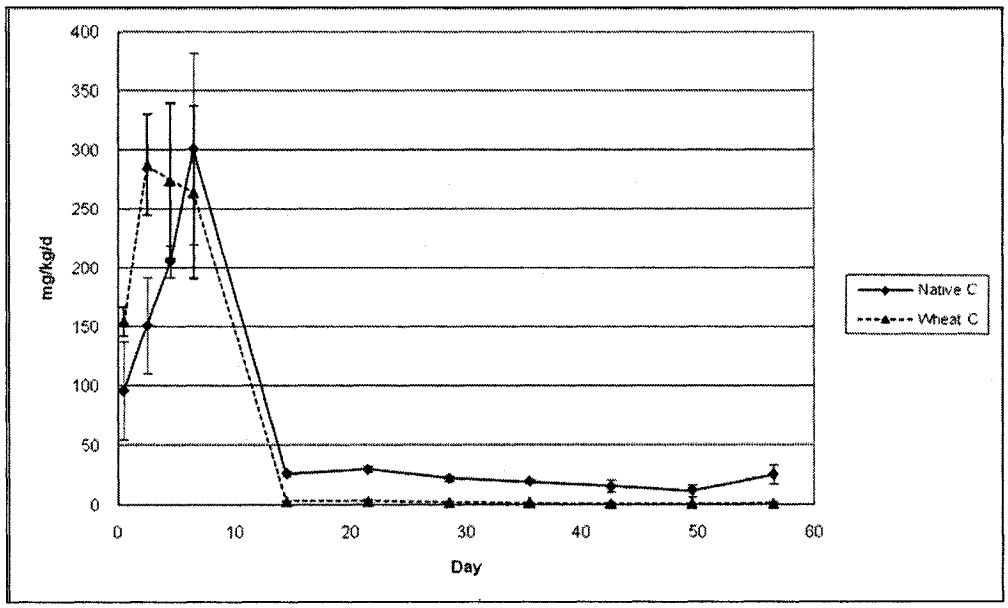
Error bars represent 95% CI of the daily means.

Figure 5-6. Respiration rates of native C and wheat C measured in microcosms of a Nova Scotia soil from a long term fertility trial without a history of manure application incubated at 35°C.



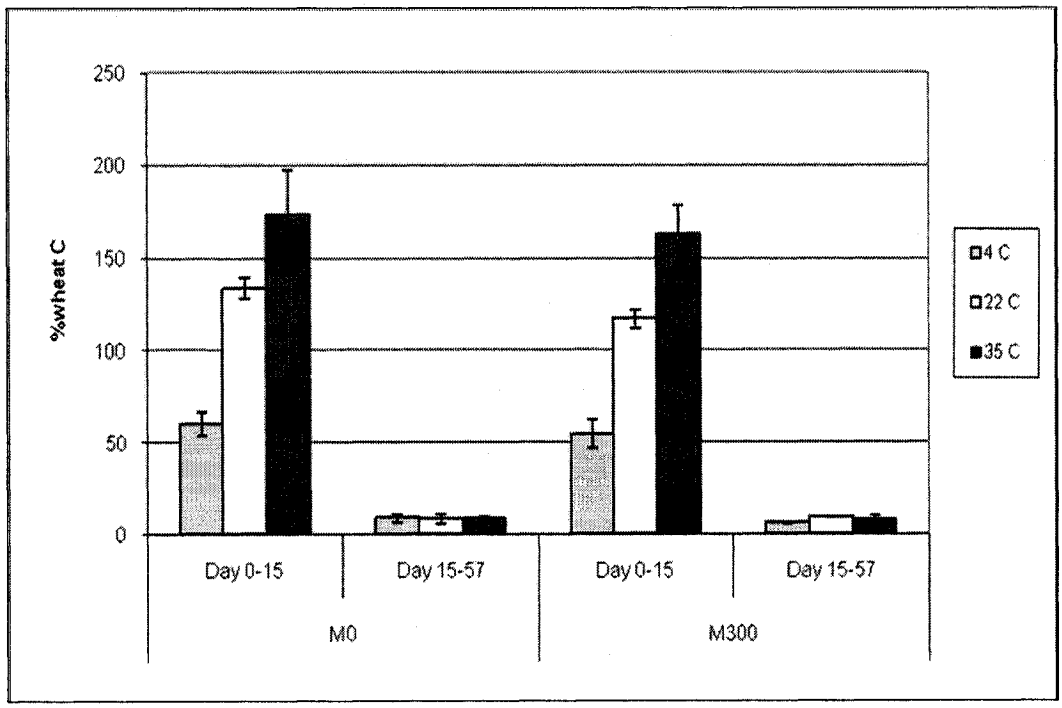
Error bars represent 95% CI of the daily means.

Figure 5-7. Respiration rates of native C and wheat C measured in microcosms of a Nova Scotia soil from a long term fertility trial with a history of manure application incubated at 35°C.



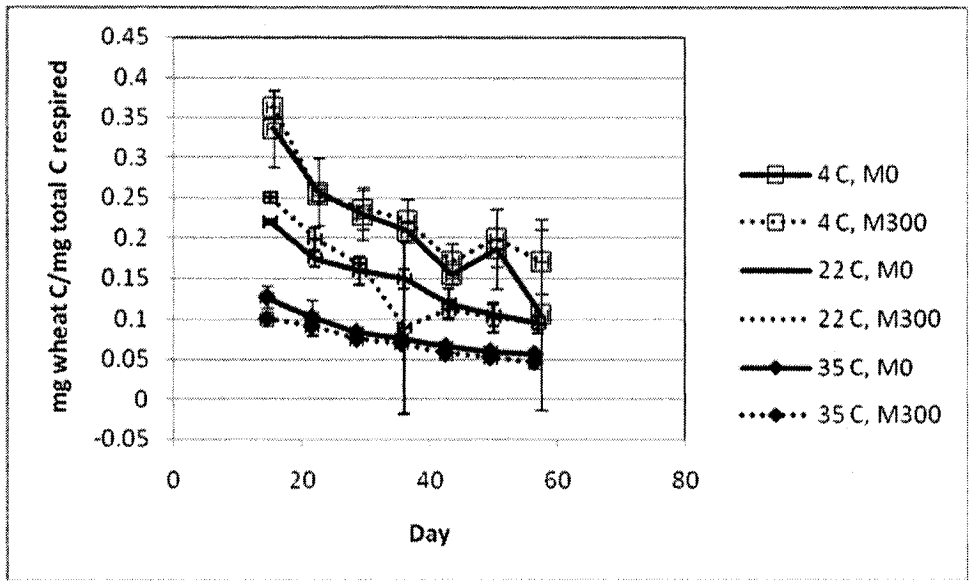
Error bars represent 95% CI of the daily means.

Figure 5-8. Estimates of the percentage of added wheat C respired during two phases of an incubation of a Nova Scotia soil from a long term fertility trial with (M300) and without (M0) a history of manure application, at three temperatures.



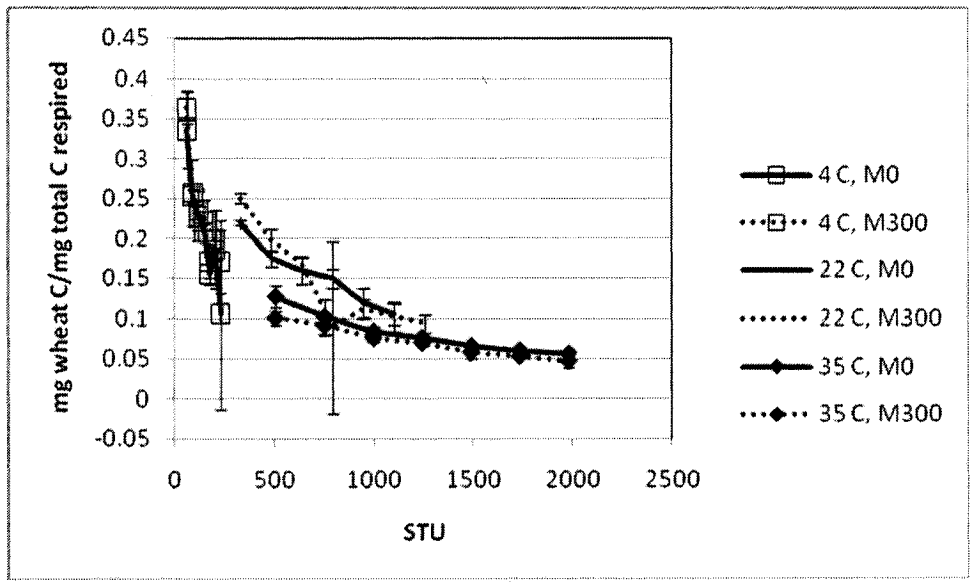
Error bars represent 95% CI of the means.

Figure 5-9. Proportion of total respired C originating from added wheat, versus time in days measured from day 14 to day 57.5 in microcosms of a Nova Scotia soil from a long term fertility trial with (M300) and without (M0) a history of manure application, incubated at three temperatures.



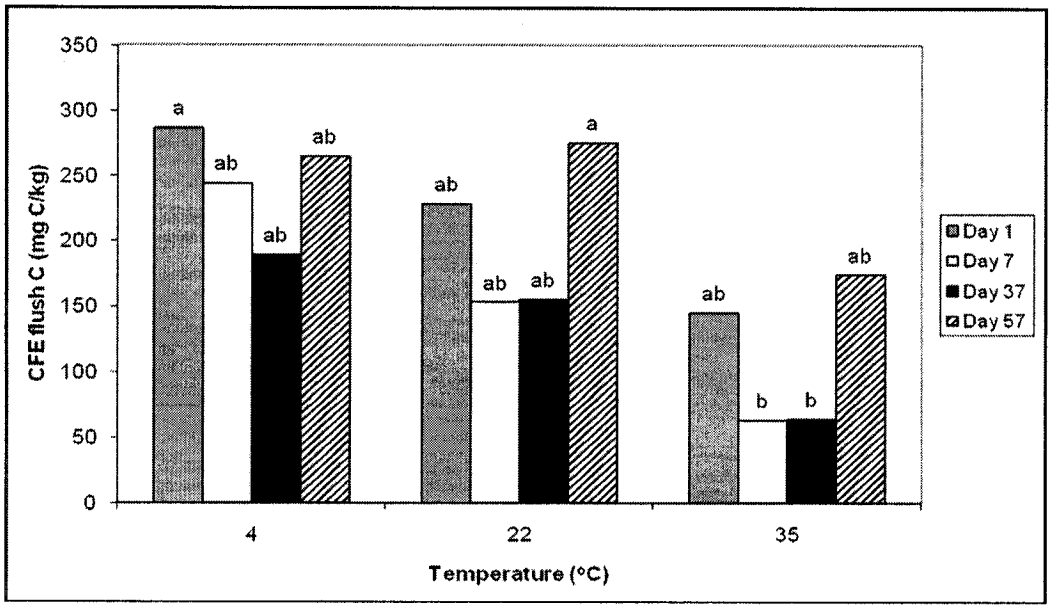
Error bars represent 95% CI of the daily means.

Figure 5-10. Proportion of total respired C originating from added wheat, versus time in STU measured from day 14 to day 57.5 in microcosms of a Nova Scotia soil from a long term fertility trial with (M300) and without (M0) a history of manure application, incubated at three temperatures.



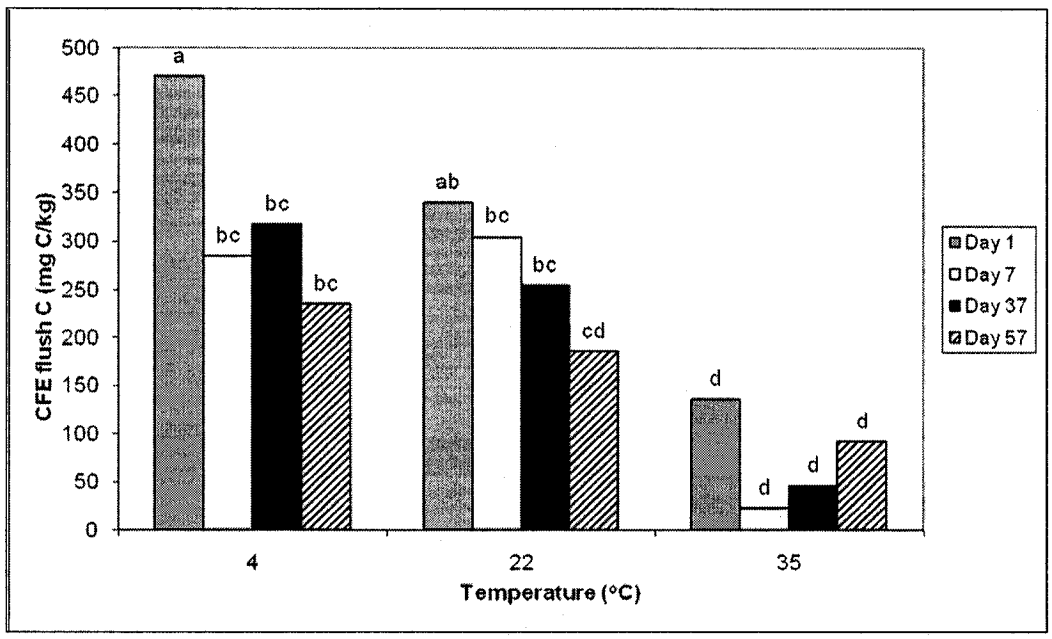
Error bars represent 95% CI of the daily means.

Figure 5-11. Mean CFE flush C measured in microcosms of a Nova Scotia soil from a long term fertility trial without a history of manure application, incubated at three temperatures for 57.5 days.



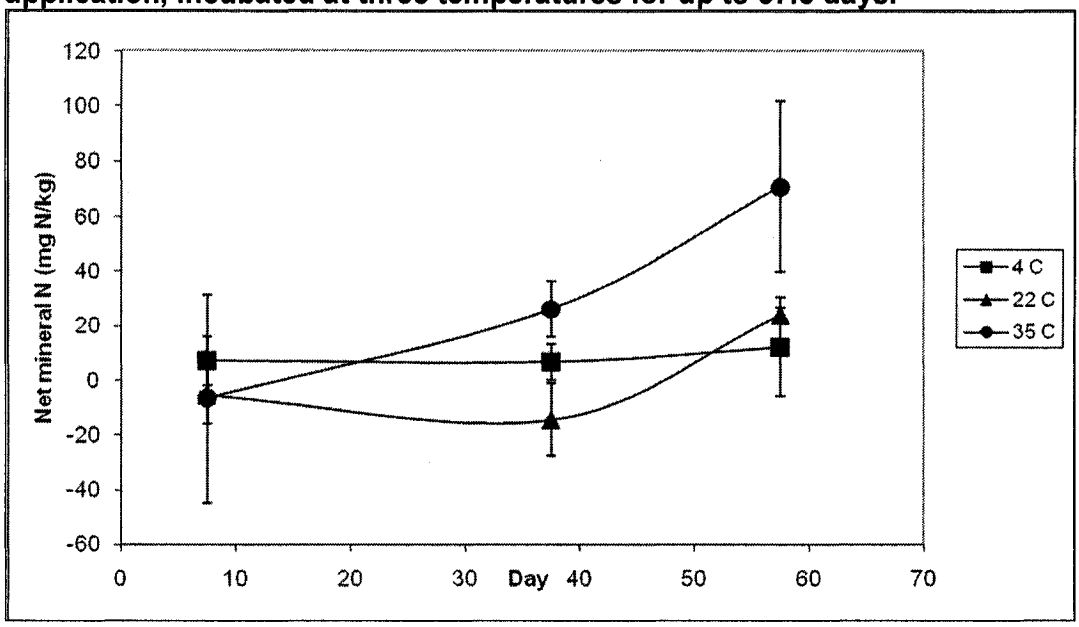
Means followed by the same letter are not different at the 5% significance level using Tukey's simultaneous pairwise comparison test .

Figure 5-12. Mean CFE flush C measured in microcosms of a Nova Scotia soil from a long term fertility trial with a history of manure application, incubated at three temperatures for 57.5 days.



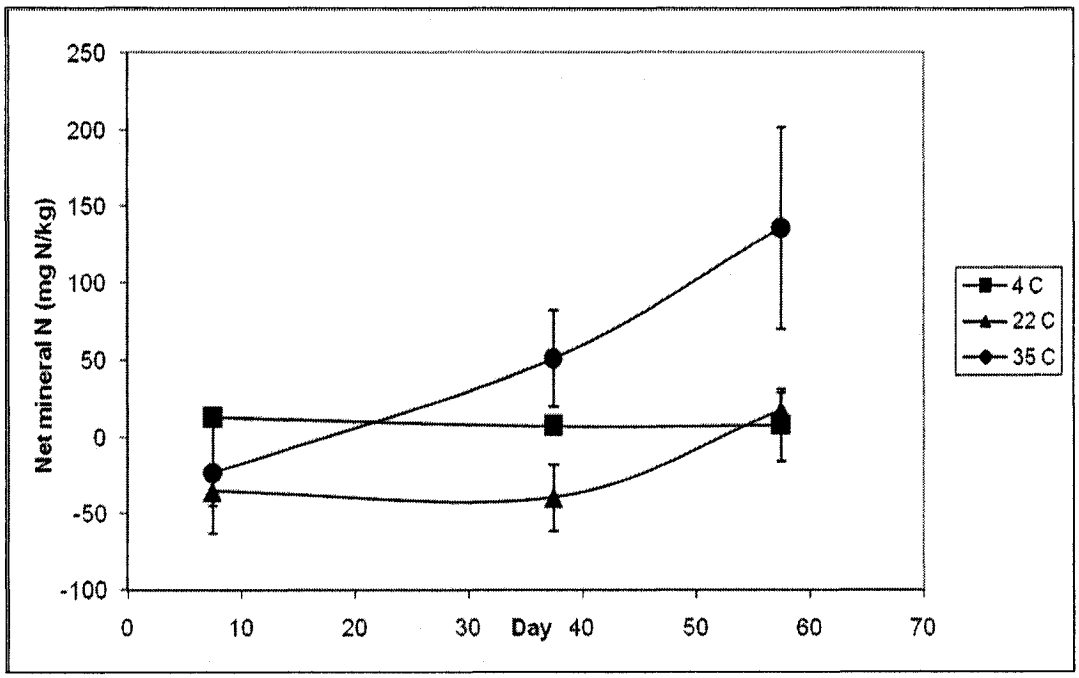
Means followed by the same letter are not different at the 5% significance level using Tukey's simultaneous pairwise comparison test

Figure 5-13. Trends in net mineral N production measured in microcosms of a Nova Scotia soil from a long term fertility trial without a history of manure application, incubated at three temperatures for up to 57.5 days.



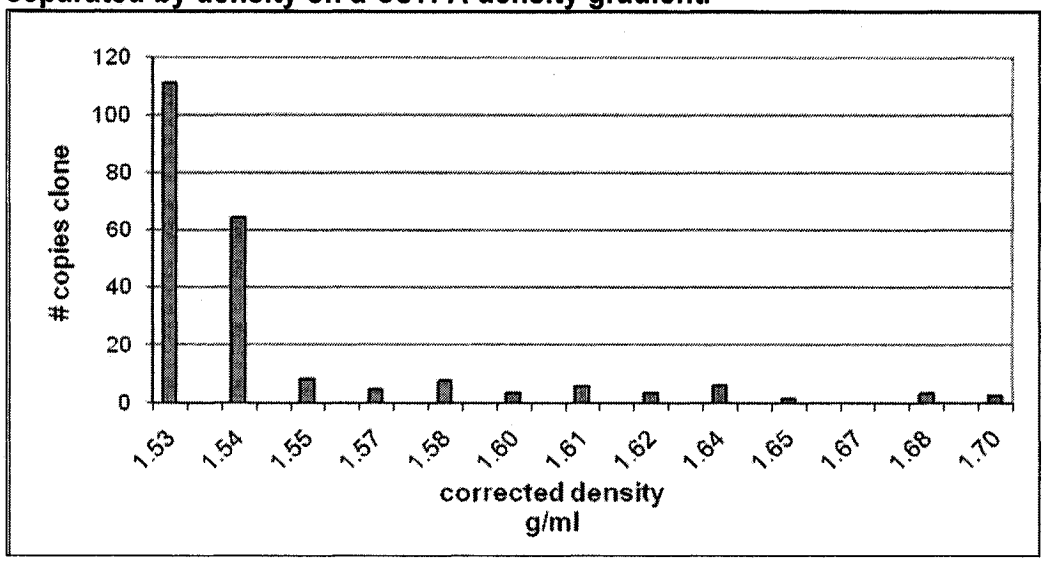
Error bars represent 95% CI of the daily means.

Figure 5-14. Trends in net mineral N production measured in microcosms of a Nova Scotia soil from a long term fertility trial with a history of manure application, incubated at three temperatures for up to 57.5 days.



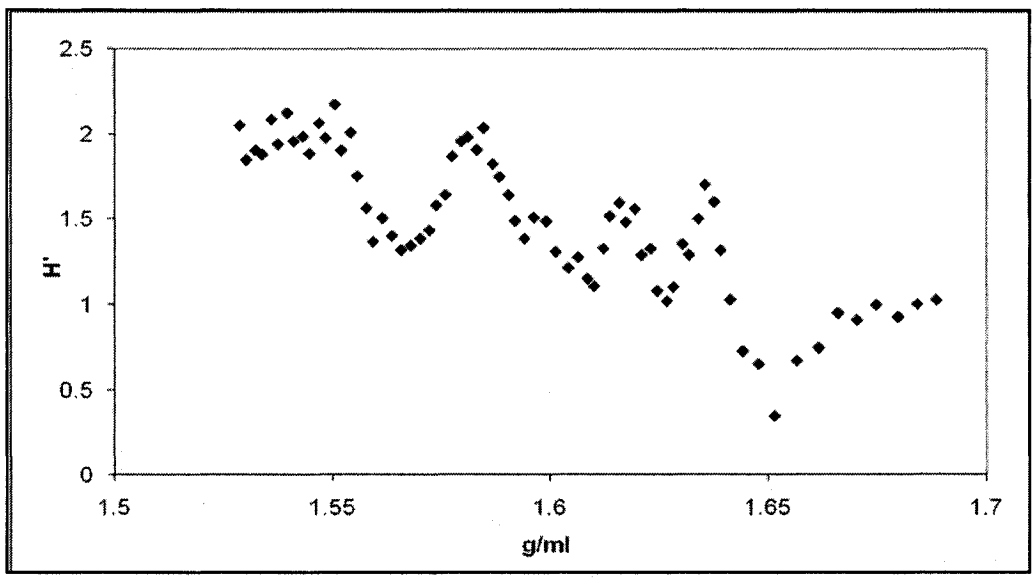
Error bars represent 95% CI of the daily means.

Figure 5-15. Number of copies of a mutated ^{12}C DNA clone amplified from DNA[z] separated by density on a CsTFA density gradient.



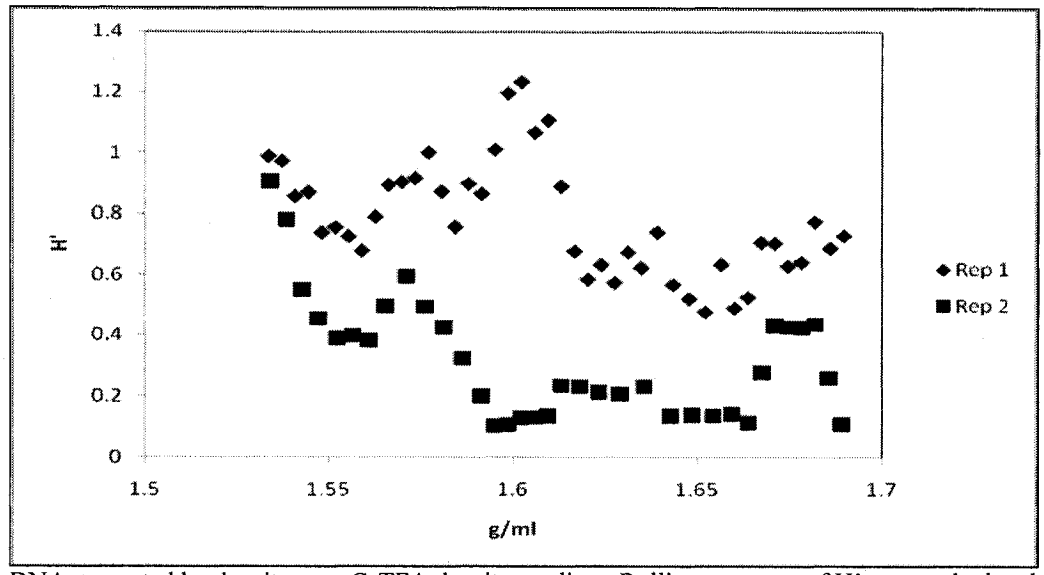
[z]DNA extracted from a soil amended with ^{13}C-labelled wheat and incubated at 22°C for 3 days.

Figure 5-16. Shannon's diversity of fungal T-RFLP samples from DNA extracted from a soil amended with ^{13}C-labelled wheat and incubated at 22°C for 3 days, plotted versus solution density.



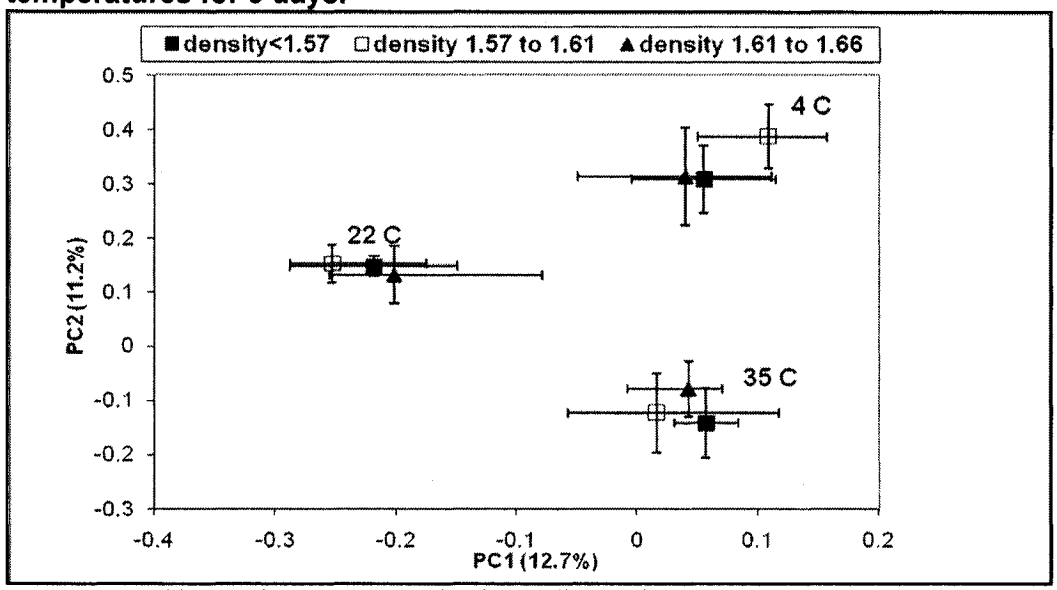
DNA separated by density on a CsTFA density gradient. Rolling averages of H' were calculated as the mean of the previous 5 values and plotted versus the mean of the previous 5 values of corrected densities. This was done to improve visualization of the trend in H' with changing density.

Figure 5-17. Shannon's diversity (H') of bacterial T-RFLP samples from DNA extracted from a soil amended with ^{13}C-labelled wheat and incubated at 22°C for 3 days.



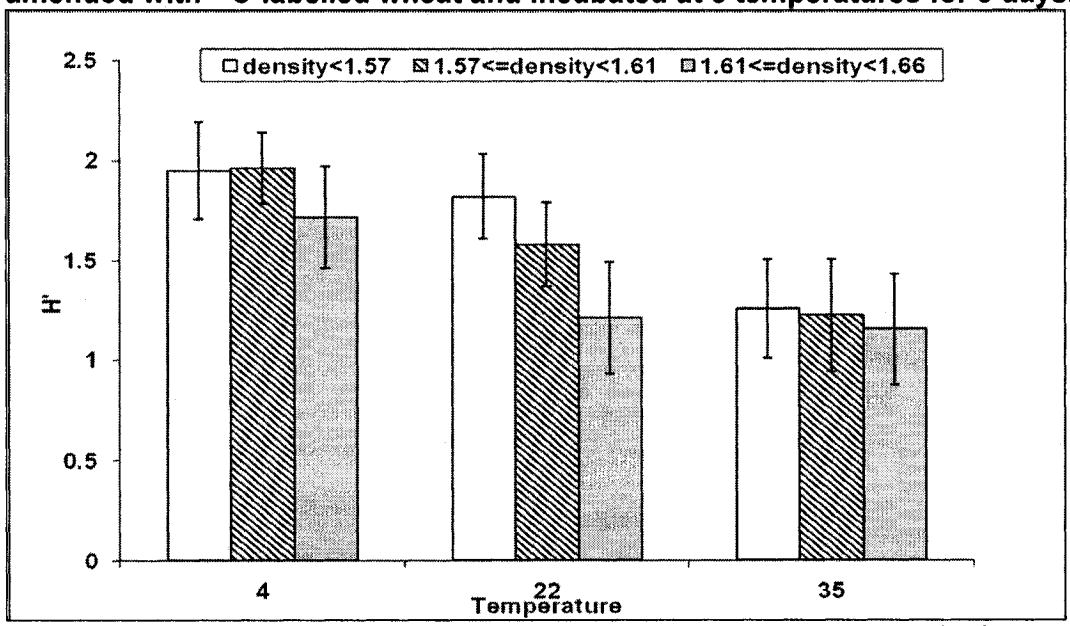
DNA separated by density on a CsTFA density gradient. Rolling averages of H' were calculated as the mean of the previous 5 values and plotted versus the mean of the previous 5 values of corrected densities. This was done to improve visualization of the trend in H' with changing density.

Figure 5-18. Mean scores for principal components of fungal T-RFLP profiles of DNA extracted from a soil amended with ^{13}C-labelled wheat and incubated at 3 temperatures for 3 days.



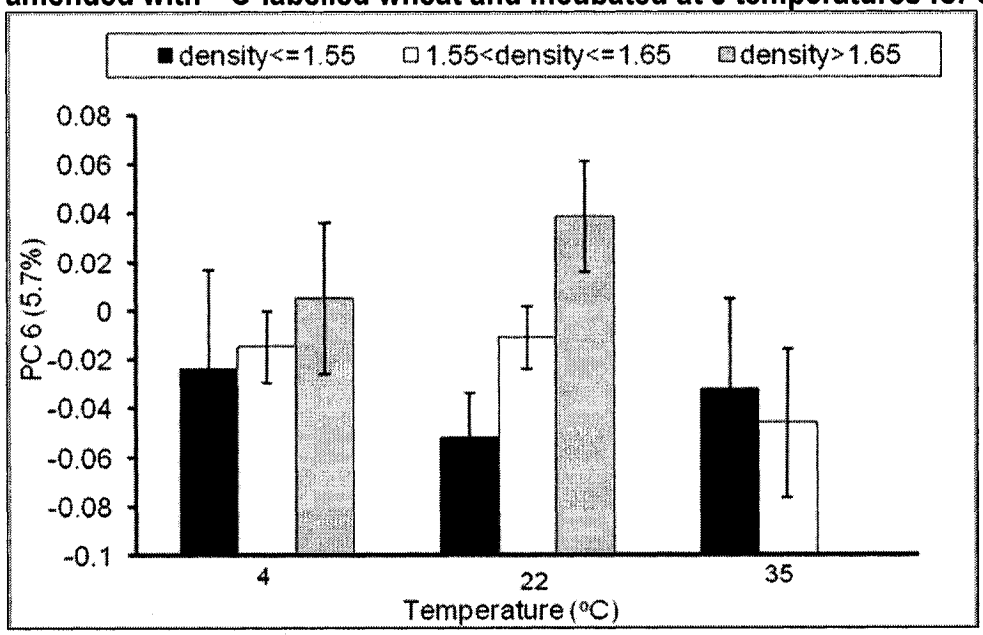
DNA separated by density on a CsTFA density gradient and scores averaged over 3 density ranges; error bars indicate the 95% confidence intervals of each mean.

Figure 5-19. Shannon's diversity index (H') of fungal T-RFLP profiles of a soil amended with ^{13}C-labelled wheat and incubated at 3 temperatures for 3 days.



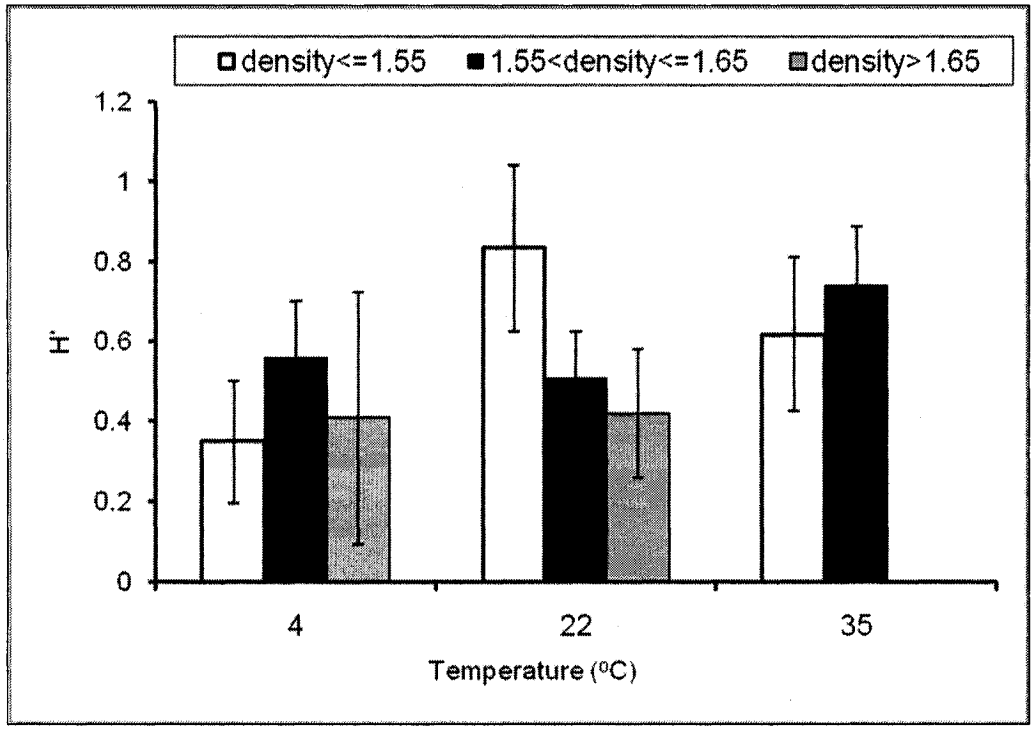
DNA separated by density on a CsTFA density gradient and diversity averaged over 3 density ranges; error bars indicate the 95% confidence intervals of each mean.

Figure 5-20. Principal component scores for bacterial T-RFLP profiles of a soil amended with ^{13}C-labelled wheat and incubated at 3 temperatures for 3 days.



DNA separated by density on a CsTFA density gradient and scores averaged over 3 density ranges; error bars indicate the 95% confidence intervals of each mean.

Figure 5-21. Shannon's diversity index (H') of bacterial T-RFLP profiles of a soil amended with ^{13}C-labelled wheat and incubated at 3 temperatures for 3 days.



DNA separated by density on a CsTFA density gradient and diversity averaged over 3 density ranges; error bars indicate the 95% confidence intervals of each mean.

Chapter 6: Temperature Effects on the Biology of Decomposition: Considerations for the First-order Model

6.1 Inconsistencies with First-order Model Assumptions

This thesis reports the results from a variety of experiments about the effects of temperature on decomposition in soils. Net C and N mineralization were used as indicators of the decomposition process. Although these processes are usually described with first-order kinetics across a range of temperatures, I found that at lower temperatures, the mineralization of C and N from native pools of soil organic matter was a zero-order process, not limited by substrate availability. At higher temperatures, C and N mineralization was substrate-limited and the process could be described by the first-order model, with k, C_0 and N_0 varying with temperature. In the study of plant litter decomposition kinetics at different temperatures (Chapter 4), the estimated size of the mineralizable pool of substrate declined with increasing temperatures. According to first-order kinetic theory, the size of the mineralizable pool of substrate is not affected by temperature; however, changes in pool size have been reported by a number of researchers. This phenomenon has been termed a "functional shift" (Dalias et al., 2003).

Another common assumption when using first-order kinetics to model C and N mineralization is that the temperature response of both elements is the same. In my experiment into temperature effects on mineralization of native pools of substrate, when the ratios of C to N mineralization at each temperature were compared, the values were not constant. This ratio declined with increasing temperature, especially between 5 and 15°C, indicating that the temperature responses of C and N mineralization are not equivalent. Since most decomposition models use the same temperature response functions to modify the first-order rate constants for both C and N mineralization, this is

an intriguing finding that deserves further investigation. A number of biological mechanisms could explain this phenomenon, including an increase in the grazing activity of soil fauna at temperatures above 10°C.

Several biological mechanisms have been discussed in this thesis to explain potential inconsistencies in first-order theories of decomposition kinetics. These mechanisms include:

1. *A change in the size of the microbial biomass with temperature.* Larger biomass should have the potential to metabolize more substrate than smaller biomass. While the potential of the soil microbial community to metabolize substrate has always been assumed to be a non-limiting factor in the decomposition subsystem, recently this notion has been challenged (Fang et al., 2005a; Schimel, 2001). The size of the biomass may affect the instantaneous rate of decomposition in soils. If its size is affected by temperature, some of the anomalies in temperature effects on decomposition that have been reported, such as reductions in estimates of substrate pool size (C_0 and N_0) could be explained by changing biomass size.
2. *A change in substrate use efficiency with temperature.* These experiments demonstrated that the metabolic quotient of the biomass increased with temperature while substrate use efficiency declined. Less efficient use of C at higher temperatures could lead to higher than expected rates of C respiration as temperatures increase, and higher estimates for the C_0 parameter estimate.
3. *Changes in the C:N ratio of the microbial biomass with changing temperature.* In these experiments changes in soil microbial community structure with temperature were reported. Specifically, distinct fungal communities developed at the coldest

temperatures. These communities would likely have higher C:N ratios in their biomass than the biomass developed at warmer communities which would be dominated by bacteria (de Ruiter et al., 1993). Changes in biomass C:N ratios only impact the net release of N from a given substrate; these changes should not impact on net release of C, which is controlled by substrate use efficiency, substrate availability, biomass size, and temperature effects on enzymatic digestion of substrates. Organisms with high C:N ratios do not need to assimilate as much N from the substrate they are metabolizing as organisms with a lower C:N ratio (i.e. a higher biomass N content). If biomass C:N ratio is affected by temperature, then it is feasible that this could cause a change in the ratio of C:N mineralized at different temperatures.

Since the microbial community analysis portion of this research clearly showed that temperature influences species composition of the fungal and bacterial communities in the soil decomposition subsystem, it is possible that microbial community characteristics, like efficiency of substrate use, and C:N ratio, also vary with temperature. In these experiments, I also detected a clear relationship between temperature and biomass size, with decreasing CFE flush C at warmer temperatures.

6.2 Model simulations

Although it was not the intention of this thesis to develop a complete model of decomposition, a simple simulation model was created using MS Excel to demonstrate the potential impacts of changes in biomass characteristics on the first-order kinetics of C and N mineralization at different temperatures. The model was designed to simulate first-order decomposition, with microbial characteristics impacting on the first-order rate constant. Four simulations are considered.

In the initial simulation, the pool of mineralizable C was set at 100 units. Efficiency of substrate use at all temperatures was assumed to be 0.5. A base value for the first order rate constant of 0.001 d^{-1} was used. This was calculated based on the average first-order rate constant recommended by Stanford and Smith (1972) for the arable soils in their study (0.054 wk^{-1} at 35°C) and adjusted to 5°C using a Q_{10} of 2. The first-order rate constants at 15 and 25°C were also modified for temperature using a Q_{10} of 2. The rate constant was also modified to account for temperature-related changes in the size of the microbial biomass. The effect of temperature on the biomass was estimated using the values for CFE flush C that were estimated in the native C mineralization study (see Chapter 3) at each of the four temperatures, on day 7. These values were standardized so that the biomass was equivalent to 1 at 5°C, and declined with increasing temperature. The temperature-adjusted rate constant was multiplied by the standardized biomass, to give a biomass and temperature-adjusted first-order rate constant estimate.

The results of the first simulation at each of four temperatures are shown in Figure 6-1. This simulation illustrates how a decline in the size of the microbial biomass can result in reductions in the net mineralization of C at high temperatures. In this example, the net mineralization of C at 35°C is very similar to the 25°C curve. At 15°C, mineralization of C is relatively close to 25°C. This approach demonstrates how the observations for native C mineralization reported in Chapter 3 (Figure 3-10 to Figure 3-17) could have been due in part to temperature-related changes in microbial biomass size that counteracted the increased enzymatic rates of digestion at higher temperatures.

In the second simulation, the first-order rate constant was modified to account for the effects of temperature on the efficiency of substrate use. The metabolic quotient can be used as an indicator of substrate use efficiency (Fliessbach et al. 2000), with higher values for qCO_2 indicative of a reduction in substrate use efficiency. The metabolic quotient at 5°C was made equivalent to 1, and the values at the other 3 warmer temperatures were adjusted from this standard value. The values used were 3.18, 5.50, and 12.86 for 15, 25 and 35°C respectively, and were based on the measured metabolic quotients on day 7 in the native C mineralization experiment. The base value of the first-order rate constant (0.001 d^{-1}) was multiplied by the standardized metabolic quotient value and the standardized biomass value as in simulation 1, in order to produce a rate constant that was adjusted for temperature effects on the biomass size and substrate use efficiency. Increases in respiration rates due to temperature effects on the enzymatic rates of digestion (i.e. Q_{10} or Arrhenius effects) were implicit within the standardized qCO_2 values, so it was not necessary to adjust the first-order rate constant using a Q_{10} of 2, as in the first simulation. The results of this simulation (Figure 6-2) are very similar to the previous simulation, in which a Q_{10} temperature response function was used to adjust the first-order rate constant. This finding suggests that the biological mechanisms behind observed changes in first-order rate constants with temperature may be due to changes in both biomass size and metabolic efficiency.

A third simulation was run to investigate the hypothesis that changes in the biomass C:N ratio with temperature could affect net N mineralization. In this simulation, changes in biomass size with temperature and adjustments to the rate constant using a Q_{10} of 2 were included as described in scenario 1. In addition, a decline in the microflora

biomass C:N ratio was included with this value equal to 10 at 5°C and dropping to 8, 6 and 4 as temperatures increased to 15, 25 and 35°C. Gross N mineralization was assumed to be a first-order process paralleling net C mineralization. Net C assimilation was assumed to be equal to net C mineralization (based on an assumed C use efficiency of 50%). N immobilization was calculated as net C assimilation divided by the C:N ratio of the biomass, and net N mineralization was calculated as gross N mineralization with N immobilization subtracted. The calculations were done in Excel using a daily time step.

The results of this simulation illustrate how increases in microbial biomass C:N ratios result in increased immobilization of N from substrates with the same starting C:N ratio (Figure 6-3). At 35°C, net N mineralization was negative (net immobilization), indicating that the hypothetical substrate did not contain enough N to balance the N needs of the metabolizing biomass. At 5°C net N mineralization was equal to N immobilization.

The biomass C:N ratios selected in this simulation were chosen to reflect C:N ratios used by de Ruiter et al. (1993), who used a C:N ratio of 10 for fungi and 4 for bacteria in their food web model of N mineralization. Assuming that fungal organisms are active at low temperatures, as has been reported by a number of authors (Zogg et al. 1997; Lipson et al. 2002; Pietikäinen et al. 2005), it is reasonable to predict changes in the C:N ratio of the active biomass as I have done in this simulation. The results of the simulation, however, do not reflect my findings in the native N mineralization portion of this study (Chapter 3). I observed a dramatic increase in net N mineralization when temperatures were increased from 5 to 15°C, and further increases in net N mineralization at warmer temperatures. In this simulation there was a slight increase in net N

mineralization when the 15°C simulation was compared with 5°C; however, at 25 and 35°C net N mineralization was reduced. This effect reflects the lower C:N ratios used in the model at warmer temperatures, and increased immobilization of N by the microflora.

The negative net N mineralization at warmer temperatures is only evident when one trophic level (the microflora in this case) is included in the model. In the fourth simulation (Figure 6-4), soil fauna was included as a second trophic level that consumes a portion (50%) of the microflora biomass. The soil fauna were assigned a C:N ratio of 10 (Ferris et al., 1996), and the microflora C:N ratio was kept at a constant value of 6. This resulted in a significant increase in net N mineralization between 5 and 15°C, with little difference in net N mineralization among the warmer three temperatures. This pattern was similar to the predicted pattern of net N mineralization using the parameters estimated from the native N mineralization data in Chapter 3 (Figure 3-2 to Figure 3-9). The role of soil fauna in enhancing the turnover of microbial biomass and the release of immobilized nutrients has been recognized (Griffiths, 1994; Ferris et al., 2004). As suggested in Chapter 3, inhibition of faunal grazing at temperatures below 10°C may be resulting in an accumulation of N in the microflora biomass. This is consistent with the larger size of the biomass estimated by CFE flush C, at the lower temperatures in the experiments I have conducted.

I have included these simulations to illustrate how some of the biological characteristics I observed in these experiments could impact net C and N mineralization. Reductions in biomass at higher temperatures could have resulted in the lack of a significant increase in the parameter estimates for C_0 or k from native pools of organic matter, between 25 and 35°C (Table 3-7). However, for plant litter C (Table 4-3), while

the C_0 parameter estimate declined with increasing temperature, the first-order rate constant was significantly higher at warmer temperatures. The biological meaning of these parameters is not certain, since they are empirically derived. It has been suggested that k is a parameter that encompasses substrate quality and accessibility, and also the potential of the microbial biomass to metabolize substrate. However, in reality, attributing biological meaning to these parameters is purely speculative.

6.3 Limitations of Microcosm Studies

It is important to keep in mind that the reductions in biomass at increasing temperatures that I observed in my experiments may be an artefact of the microcosm method. This method only reflects the decomposition process in the bulk soil, so inputs of C from the rhizosphere are excluded. The ongoing plant litter inputs to the soil C pool that would be characteristic of the decomposition subsystem *in situ*, are also not present. By the end of the incubation the biomass in the microcosm was likely becoming severely C-limited, especially at the warmer incubation temperatures. This was the mechanism proposed by Zogg et al. (1997) who also observed declines in microbial biomass with increasing temperature and changes in substrate utilization.

It remains to be seen whether these changes would also be observed in the field where pools of C are not likely to become depleted under optimal conditions for decomposition (i.e. warm, moist environment) since these conditions are also optimal for plant growth. In field studies a clear link between temperature and microbial biomass size has not been shown (Wardle, 1998). Lipson et al. (2000) found increases in the size of the microbial biomass during winter months and linked this to substrate supply and rates of metabolism, with winter microbial communities respiring slowly and able to access sufficient carbon. When temperatures increased respiration rate increased, C

became limiting, and microbial biomass decreased. While Raubuch and Joergensen (2002) also reported seasonal fluctuations in microbial biomass, they could not relate these to net C and N mineralization rates. In the field study portion of my research, initial values for microbial biomass from samples taken in the spring were not correlated with the potentially mineralizable N content of the soil (Table 2-8) or plant uptake of N (PAN). Therefore, a direct relationship between the soil microbial biomass and rates of C and N mineralization has not yet been proven, although the biological argument for this relationship has been made (Fang et al., 2005a; Schimel, 2001).

The changes in microbial community structure observed later in the experiments may also have been related to substrate-depletion. Communities adapted to metabolize more recalcitrant substrates in the soil may have become more dominant later in the incubation, as the more readily-available substrates in the soil were consumed. Since these substrates were not being replenished as they would be in a natural ecosystem, changes in microbial community structure later in the incubation may not reflect changes that would occur in the bulk soil under field conditions.

All of these biomass characteristics: declines in size, efficiency and changes in structure, are likely to become more exaggerated over time in a microcosm experiment. For this reason, it is more realistic to use estimates of these parameters from early in the incubation. This is why the day 7 estimates for microbial biomass size and metabolic quotient were used in the simulations described above.

6.4 Directions for Future Research

This thesis reports on temperature effects on net C and N mineralization from native and added substrates, both in the field and in laboratory microcosms. The biological mechanisms for temperature effects on these processes have been investigated.

Changes in substrate pool size and accessibility with temperature were observed at different temperatures. This is reflected in first-order models of decomposition as variations in the C_0 or N_0 values of each pool. The accuracy of predictions from decomposition models based on first-order principles could therefore be improved by making substrate pool size and accessibility a function of temperature. This would require observation of the effects of temperature on these pools and the development of empirical temperature response functions to modify pool size and accessibility.

In this study, the soils with two different management histories did not differ significantly in their kinetic characteristics; therefore, it is likely that the same temperature response function could be used to modify pool size and accessibility for both soils. A priority for researchers will be to determine if there is a temperature by soil type/management history interaction for the biological characteristics that affect net C and N mineralization. If this interaction does not occur, then an empirical approach to describing the kinetics of decomposition at different temperatures is still applicable. Temperature effects on the biological characteristics described in this thesis can remain implicit within the temperature response functions used to modify decomposition rates and substrate pool size and accessibility.

Alternatively, a food web model approach could be used where the biological characteristics identified in this thesis as being temperature dependent (biomass size, substrate use efficiency), could be included as a function of temperature. In food web

models the decomposition process is driven by the size and metabolic function of the soil microbial biomass. While these models can be complex and include a number of functional groups (de Ruiter et al., 1994a), a simple model with only one or two "functional groups" e.g. microflora and microfauna, may be sufficient if the key characteristics of each group (size, efficiency, C:N ratio), are included. Such a model should still include a temperature-dependent pool of litter for consumption by the microflora (i.e. the primary saprotrophs) as described above. The Arrhenius function could still be used to modify feeding rates (enzymatic digestion of substrates), since these are controlled by temperature once temperature effects on substrate pool size and biomass physiology are accounted for.

This thesis has provided further insight into the biological factors affected by temperature that may be impacting on net C and N mineralization kinetics. Further research using a range of soils with differing mineralogies and management histories is required to determine if the inclusion of more biology in current decomposition models will improve their accuracy.

Figure 6-1. Simulation of C mineralization using a first-order model with the rate constant adjusted for temperature effects on the enzymatic rate of digestion and the size of the metabolizing biomass.

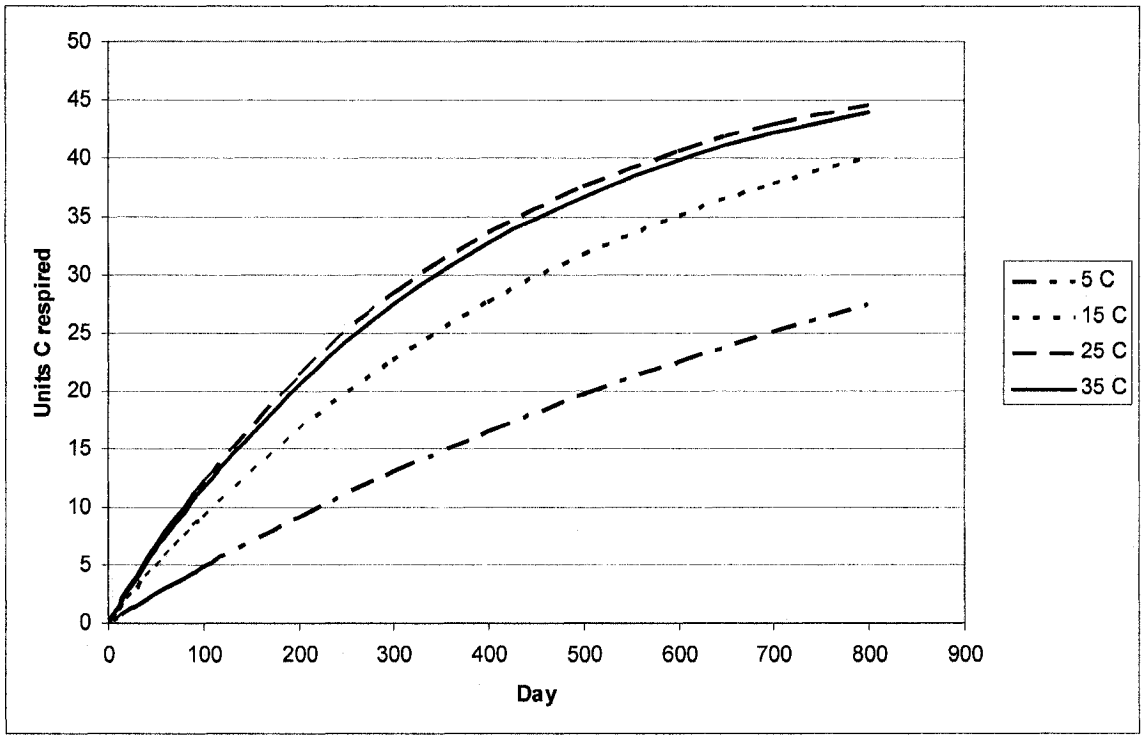


Figure 6-2. Simulation of C mineralization using a first-order model with the rate constant adjusted for temperature effects on the efficiency of substrate use and the size of the metabolizing biomass.

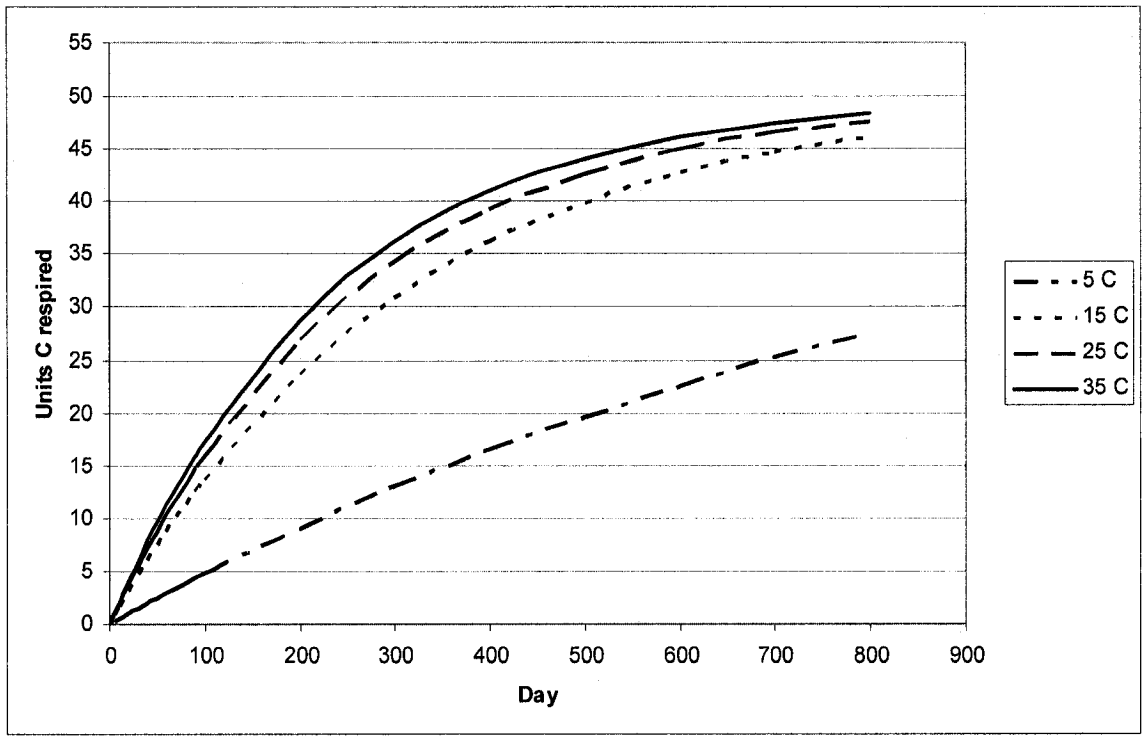
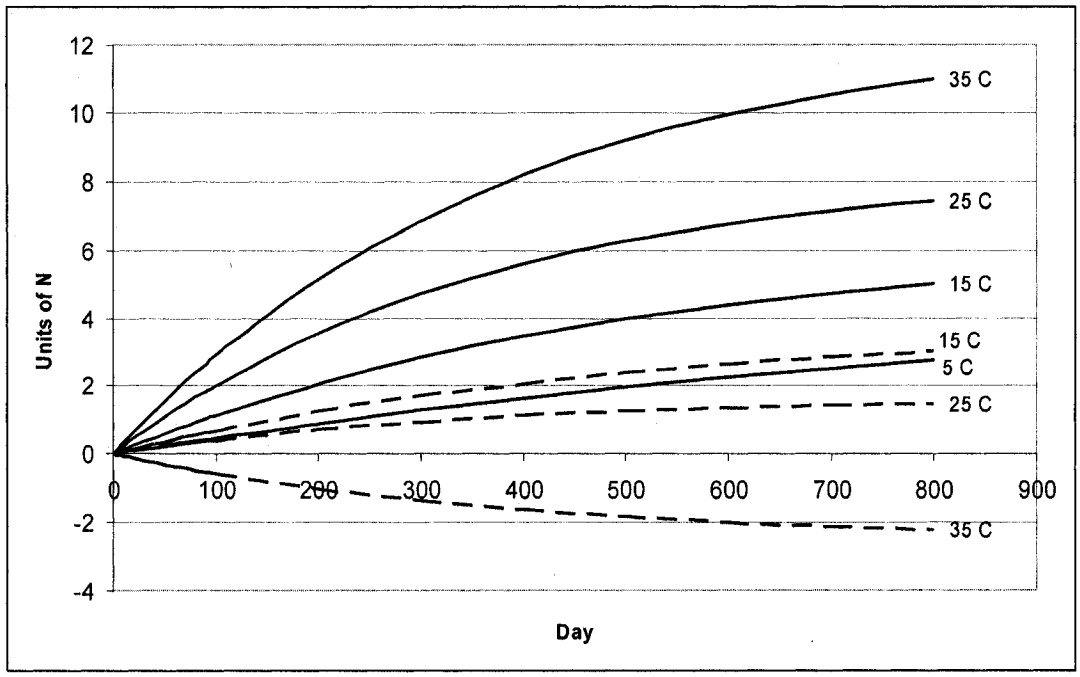


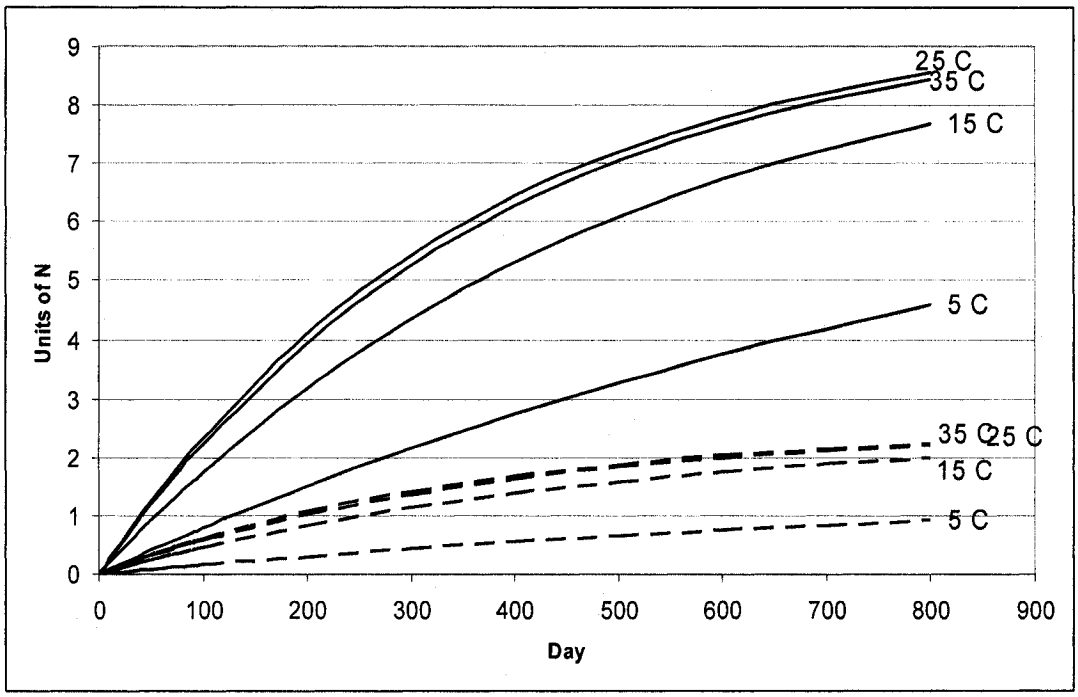
Figure 6-3. Simulation of net N mineralization and gross immobilization with the rate constant adjusted for temperature effects on the enzymatic rate of digestion and the size of the metabolizing biomass, and microflora C:N ratio a function of temperature.



net N mineralization = - - - - ; gross immobilization = ——

NB: at 5°C net N mineralization was equal to net N immobilization.

Figure 6-4. Simulation of net N mineralization and gross immobilization with the rate constant adjusted for temperature effects on the enzymatic rate of digestion and the size of the metabolizing biomass, including grazing by soil fauna.



net N mineralization = - - - - ; gross immobilization = ——

Chapter 7: Conclusions

First-order kinetic models have been commonly used to describe the mineralization of C and N from soils. The effects of temperature on this process are usually incorporated into model coefficients using a temperature response function that modifies the first-order rate constant, while the estimate of potentially mineralizable C and N is assumed not to be temperature dependent (the rate constant hypothesis). One method of testing the validity of this approach is to use soil thermal units as a modified time variable that incorporates temperature effects on the rate of the process into the expression for time. When reaction rates per STU are equivalent, it can be assumed that temperature only affects the rate of the process and not the size of the pool of substrate. This is consistent with current first-order approaches to modeling temperature effects on decomposition. In this thesis the applicability of first-order kinetics for describing soil C and N mineralization was investigated at a range of temperatures in agricultural soils of Nova Scotia. The effects of temperature on the kinetics of C and N mineralization, and on a range of biological characteristics, were studied in order to determine if the inclusion of more biological parameters in C and N mineralization models is justified.

Laboratory indices of N availability are not always related to the total N mineralization potential (N_0) of the soil. In this study, during a constant temperature incubation used to determine N_0, more STUs were accumulated than during a typical growing season in Nova Scotia. This could explain the lack of correlation between laboratory indices selected to predict N availability during a growing season, and N_0 which is determined over a much longer period of thermal time. It is important to conduct soil incubations at a temperature that is within the "normal" range for the soil being studied. If the estimate of N_0 is to be related to N supply to a crop in a field season,

then the length of the incubation in thermal time should be similar to the length of a typical growing season.

An estimate of substrate quality that included the N mineralized during the first two weeks of the incubation (TPAN) proved to be a better predictor of N availability in the field. This value was correlated with KCl-extractable inorganic N, indicating that a simple mineral N extraction in the spring may still be the best predictor of the N supplying potential of agricultural soils in Nova Scotia. Temperature was also correlated with N availability in the field, making it difficult to determine whether substrate quality or temperature were the predominant factors driving N supply in the field. This result lead to a focus on temperature effects on C and N mineralization in the remainder of the thesis work.

When an agricultural soil with and without a history of manure application was incubated at four temperatures (5, 15, 25 and 35°C), rates of C mineralized per soil thermal unit were significantly higher at 5°C than at the warmer three temperatures. This contradicts first-order principles of mineralization, which are based on the assumption that only the rate of the process is affected by temperature. In fact, there may be significant changes in the biology of the decomposition subsystem at 5°C, which results in the use of different pools of substrate and different pathways of metabolism. In this study, a variety of biological parameters were found to differ at 5°C compared to the three warmer incubation temperatures. Both bacterial and fungal species composition were significantly different, as well as microbial biomass, which was larger, and metabolic quotient (qCO_2), which was lower indicating more efficient respiration at this temperature. The soils in this study should have been well adapted to metabolizing at

5°C since the mean annual temperature in Nova Scotia is 5 to 7°C, so this could explain the higher than expected C respiration at this temperature. The application of temperature response functions derived for soils from one climatic region of the world, to soils from another climatic region, may result in erroneous predictions of temperature effects on soil C mineralization.

Both C and N mineralization are usually modeled using the same temperature response functions in current biogeochemical models. In this study, predictions of the ratio of net C to net N mineralization were not constant across the range of four temperatures. Again, the 5°C case appeared to be unique with a much higher ratio of predicted net C:N mineralization compared to the three warmer temperatures. It is possible that soil fauna are a key factor affecting the temperature response of net N mineralization in the lower temperature range. The role of soil fauna in enhancing the cycling of mineral N has already been demonstrated at a single incubation temperature (Clarholm, 1985; Ingham, 1985). The results observed in the current study could have been caused by an inactive soil faunal population at the coldest temperature, allowing N to accumulate in the microbial biomass. At the three warmer temperatures microbial biomass was smaller, indicative of faunal grazing on the biomass, which would be accompanied by the release of mineral N. This process could explain the relatively large increase in N mineralization between 5 and 15°C.

When ^{14}C-labeled plant litter was added to the same pair of Nova Scotia soils and incubated at four different temperatures (see above), the release of $^{14}C\text{-}CO_2$ was best described by a single pool, first-order model. Best fits of the model were obtained when the potentially mineralizable C pool was allowed to vary with temperature, with

estimated pool size declining at warmer temperatures. This was in contrast to the actual, measured amounts of ^{14}C-respired, which did not vary due to temperature. For this relatively labile substrate, mineralization did not appear to be temperature sensitive. By the 55th day of the incubation, equal amounts of ^{14}C had been respired at each temperature. The total CO_2 respired by this day, however, was much greater at the warmest incubation temperature. This finding suggests that the temperature sensitivity of different pools of substrate in the soil is not equivalent. As already proposed by Fierer et al. (2005), it is likely that more readily available substrates, like the ^{14}C-labeled wheat material in this experiment, are relatively insensitive to increasing temperature. In contrast, native C pools seem to be sensitive to temperature with significantly more native CO_2 respired by day 55 as temperatures increased.

Utilization of C from added wheat followed a similar pattern in a later experiment that used ^{13}C-labeled wheat as a substrate at three incubation temperatures (4, 22, and 35°C). Early in the incubation (day 0 -22) temperature had a significant effect on the respiration rate of added wheat C when expressed per STU, with the highest rate at 4°C in the soil with a history of manure application. In this later experiment, a few effects of soil management history on soil biological parameters were observed, with the soil with a history of manure application utilizing more of the added wheat C at 4°C than the soil that had not received annual applications of manure. These differences were most pronounced at the coldest temperature.

The most dramatic differences in soil biological parameters were noted at 5°C, where differences in microbial biomass size, substrate use efficiency, metabolic efficiency, substrate use per STU, and microbial community structure, were all

significantly different than the other three temperatures. These changes in biological parameters can be used to explain the temperature response of net C and N mineralization when incorporated into a more mechanistic, food-web simulation model, as demonstrated in Chapter 6 of this thesis.

In first-order approaches to modeling decomposition, the first-order rate constant is assumed to represent the overall capacity of the soil to metabolize organic substrates. Any effects of temperature on parameters like efficiency and biomass size are implicitly included in the temperature response function that is used to modify *k*. This empirical approach can be relied upon as long as we are confident that the soil biological parameters that ultimately control decomposition and the release of C and N as waste products, respond in the same way to changing temperatures regardless of soil management history or soil type. In the experiments reported in this thesis in most cases, the management history of the soil did not interact with temperature to produce management history by temperature interactions. While measurement of biological parameters provided useful insights into the mechanisms behind variations in estimates of substrate pool size at different temperatures, evidence was not provided for the inclusion of biological parameters explicitly within soil decomposition models. A wider range of soil types and management histories need to be studied in order to confirm that the specific inclusion of biological parameters in soil decomposition models will not significantly improve the accuracy of predictions from these models.

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