

EXPLORING THE ENVIRONMENTAL SENSITIVITY OF
NATURAL SOIL CO₂ EMISSIONS: DO ROOTS AND SOIL
MICROBES RESPOND TO DIFFERENT CLIMATIC
CUES?

By
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Abstract

Carbon dioxide is respired by soil microbes during organic matter decomposition, and by plant roots. Together these natural soil processes constitute the largest land-atmosphere CO₂ flux in the global carbon cycle, exceeding human emissions by ten times. Soil respiration is a poorly understood component of landscape carbon balance and clarification is needed in many areas, in particular the individual climatic response of microbes and roots. Methodological challenges currently limit our ability to separate soil respiration into its component parts. This thesis seeks to tackle methodological issues, and to address a central question: Do roots and soil microbes respond to different environmental cues? Isotopic signatures of vegetation and soil were characterized as a foundation for isotopic partitioning efforts, but despite improved sampling techniques, isotopic partitioning suffered from large uncertainties. Errors were associated with temporal variability in root respiration signatures, and high spatial variability in soil gas transport that was likely a stronger determinant of forest soil CO₂ signatures than isotopically distinct source contributions. Other techniques were, however, successful and elucidated new information about the relative climatic sensitivity of root and microbial processes. Physical approaches were used to monitor microbial decomposition in several distinct soil organic matter pools, and root respiration in different soil layers. Both microbes and roots were sensitive to temperature but displayed unique behavior; microbial decomposition rates increased in an exponential manner, and in contrast, roots respiration rates followed near logarithmic rates of increase, reaching a plateau above 15° C. This suggests that soil microbial respiration could become proportionately more important in a warmer

world. Root activity was universally related to temperature across study sites, while microbial responses were site-specific. This unique behavior highlights the importance and utility of partitioning methodologies in soil carbon research.

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Antigonish, Nova Scotia

Dave Risk

April 28th, 2006

Chapter 0

Introduction

0.1 Background

Soil CO₂ emissions comprise an important component of the global carbon cycle, and represent the largest terrestrial source of CO₂ to the atmosphere. Globally, soils emit 75 Pg C/yr to the atmosphere, compared with 5 Pg C/yr from all CO₂ emissions attributable to mankind (76). Soil CO₂ respiration has been identified as the primary and least well understood variable in the landscape carbon balance, or the ability of terrestrial ecosystems to store photosynthetically-derived carbon in excess of loss as respired CO₂ (157). Soil respiration is projected to increase as a result of increasing global temperatures and shifting moisture regimes(85), potentially tipping the carbon balance of terrestrial ecosystems from a net sink to a net source, further contributing to climate warming. Whether the balance will lie in a net gain or loss of soil carbon is a topic of current debate (87; 60; 154; 85; 52).

In addition to the massive global source of CO₂ to the atmosphere, soils contain significant reservoirs of carbon (76) and as a result, small but widespread increases in respiration rates have the capacity to profoundly affect atmospheric CO₂ concentrations over both short and long time intervals. If soil carbon losses of (0.06 %/yr) were expected as a consequence of global climate change and were unmatched by an increase in gross primary productivity, they would approach 1PgC/yr, roughly two

thirds the annual anthropogenic C emissions of the United States. Losses of this magnitude could likely be sustained for some time.

Soil respiration rates are sensitive to many factors. Temperature is a primary control on CO₂ production in most soils, according to site-specific studies (35; 127; 122) and in recent reviews of soil respiration data (85). Other secondary determinants of soil respiration rates are summarized by Davidson and Janssens (35), and may include factors such as soil moisture, texture, aggregation and gas diffusivity rates. Additional geophysical parameters such as soil thermal conductivity, heat capacity and heat content (127) may also play important roles. Many of these secondary determinants are climate-sensitive and their relative importance is not well understood. It is conceivable, however, that at many sites they could cumulatively outweigh temperature as the primary control on soil respiration in a changing climate. High latitude soils are an excellent example, where significant soil carbon reservoirs are protected from decomposition by high moisture contents (35).

Carbon dioxide is produced naturally in soils through two pathways; microbial decomposition by soil microbes (primarily bacteria, fungi), and respiration by plant roots. Both contribute in roughly equal quantities based on vegetation type (67). The temperature sensitivity of soil microbes, who exploit the various different pools of soil organic matter as their feedstock, are relatively well understood from the many laboratory incubation experiments to have tackled this issue (35; 56; 124; 99). Much less is known about the relative climatic sensitivities of root and soil microbial processes. Root respiration is poorly characterized and much of our understanding is of an indirect nature. Information points to the fact that roots and microbes respond to different microclimatic and biological factors, from studies involving seasonal fine

root turnover and dynamics (18; 75), isotopic information about the coupling of root respiration to photosynthesis and aboveground processes (74), and partitioning studies elucidating the individual seasonal and temperature-specific dynamics of these processes (159; 92; 22). These and other studies provide some evidence as to climatic determinants on these processes, but the potential responses of root respiration rates to climate change are still poorly characterized. Biochemical controls on root respiration are complex (5) and research in this area is needed. Hanson (67) cites resolution as the major problem in determining, and eventually predicting, the individual dynamics of microbial and root respiration. Traditionally, soil respiration approaches have examined total soil CO₂ efflux (121) and this information forms the basis for our current understanding (136), but it is methodologically difficult to separate root and microbial soil respiration. Techniques that can reliably measure “partitioned” soil respiration (split into its root and microbial components) will help to clarify the individual climatic responses of root and soil microbial sources, and improve our abilities to better predict the fate of vast quantities of carbon stored in soils globally.

0.2 Isotopic Tools to Partition Microbial and Root Respiration

Recently, stable carbon and oxygen isotope analyses have been used to identify the relative contributions of root and microbial respiration. The second most abundant carbon isotope behind ¹²C, ¹³C, is a stable isotope measured relative to the Pee Dee Belemnite (32) and expressed as permil deviation from this standard ($\delta^{13}\text{C}$). The most distinct difference in $\delta^{13}\text{C}$ distribution in terrestrial ecosystems is due to the photosynthetic pathways of the plants present, whether they belong to the C3 (Calvin

cycle), C4 (Hatch-Slack cycle) or CAM (Crassulacean acid metabolism) types. These photosynthetic pathways are described in detail elsewhere (e.g. (113)), but the main point here is that the differences in diffusion, fractionation, and carboxylation mechanisms among these vegetation types lead to distinctive carbon isotope signatures. CAM plants tend to have a $\delta^{13}\text{C}$ signature around -11 permil, C4 plants of -14 permil and C3 vegetation normally has a far more depleted signature, at approximately -26 permil (113). At high latitudes, C3 vegetation dominates; the vast tracts of boreal and temperate forests in eastern Canada are home exclusively to C3 plants. Grasslands, however, are often composed of C4 species while CAM plants are typically found only in very arid environments.

Even where plant metabolism type is constant, there are variations across latitudinal transects. Bird (16) found a 1-2 permil enrichment in forest soils between 49 and 68 degrees, from approximately -26.8 +/- 0.3 (south) to 26.2 +/- 0.1 (north) in exclusively C3 ecosystems.

The distinguishing features of vegetation type determine the specific isotopic signature of carbon moving through the terrestrial biosphere at any particular location, and also that of relic organic matter incorporated through pedogenesis. Plant tissues and soil organic matter share similar isotopic ratios, because little or no fractionation is associated with the process of microbial decomposition (164; 46). As a result, the $\delta^{13}\text{C}$ signature of CO_2 produced by microbes accurately reflects the microbial feedstock. There is a tendency towards $\delta^{13}\text{C}$ enrichment in soil organic matter with depth that is a function of increasing incorporation of isotopically-enriched dead microbial biomass (46) and is roughly related to soil organic matter age. This may control $\delta^{13}\text{C}$ signatures of CO_2 produced through depth by soil microbes.

Root-derived $\delta^{13}\text{CO}_2$ also reflects the feedstock for respiration in the root zone. In this case, new sugars produced aboveground are allocated to the roots for metabolism. The $\delta^{13}\text{C}$ of these sugars are determined aboveground by complex biochemical reactions. Physical tree tissues that most closely reflect the $\delta^{13}\text{C}$ of current photosynthate (and that of root respiration) are the newest tree tissues, either shoots or buds (97).

Based on natural abundance differences in $\delta^{13}\text{C}$ between root and microbial CO_2 , researchers have been attempted to distinguish isotopically between these sources of soil CO_2 . A recent study by Ekblad and Hogberg (48) sheds light on the relative seasonality of root and microbial respiration, and the controlling variable of moisture on root processes. They employed a predicted value (113) for the plant photosynthates that are catabolized in the root zone during respiration, and predicted a fraction of root respiration for the system using detailed $\delta^{13}\text{C}$ data. The authors noted that soil respiration was overall controlled by soil temperature, but aboveground moisture (in particular relative humidity) strongly controlled the $\delta^{13}\text{C}$ of soil CO_2 respiration, likely reflecting the changing contribution of root respiration to total soil respiration resulting from variations in plant stomatal closure (48).

To complement carbon isotope data, oxygen isotopes have been applied to studies of soil respiration. The stable isotope of oxygen, ^{18}O , is also measured in permil relative to a standard, in this case Standard Mean Ocean Water (SMOW). The primary control on CO_2 - $\delta^{18}\text{O}$ ratios is the water with which it has been in contact (164). The isotopic signature of precipitation is the single largest determinant on soil CO_2 isotopic signatures, but evaporation at the soil surface causes a significant enrichment in $\delta^{18}\text{O}$ at shallow depths (164).

Three-component, dual-isotope partitioning models use $\delta^{18}\text{O}$ measurements in

conjunction with $\delta^{13}\text{C}$ data. Lin et al. (97) first used the oxygen isotope ratio of respired soil CO_2 to partition the respiration from 1) roots, 2) microbial exploitation of litter, and 3) microbial exploitation of soil organic matter. They capitalized on the progressive age-related enrichment of $\delta^{13}\text{C}$ (in 2 permil increments) of each source. The $\delta^{18}\text{O}$ signature of the intermediately enriched product (litter respiration) was used to accurately resolve the intermediate source, since its' $\delta^{18}\text{O}$ signal was strongly fractionated from the others by evaporation at the soil surface. The work of Lin and his collaborators (97) represents one of the first attempts to successfully partition the components of root and microbial respiration using stable isotopes, but because the enrichment is relatively small, reliable resolution may prove difficult in the field. In general, few stable isotopic methods using natural abundance $\delta^{13}\text{C}$ have yet to be tested in-situ.

0.3 Physical Tools to Partition Microbial and Root Respiration

Non-isotopic partitioning tools such as trenching (21; 95; 92; 51) and/or other root exclusion techniques (74) are popular, and are used to identify both relative source contributions and flux magnitudes, for process study or for budgeting purposes. Carbon dioxide production from roots is calculated as the difference in surface flux between intact (roots and microbes) plots and trenched areas (microbial activity only) where roots have been excluded by severing. Root exclusion trenches are typically small ($\sim 2\text{m}^2$) plots between trees around which a square trench is excavated to sever all roots leading into the area. Trenches are lined with vapor barrier to prevent root regrowth, and to minimize lateral CO_2 gradients. The trench plot must be left for at

least one year prior to the initiation of the study to allow for sufficient recovery from a respiratory burst initiated by the presence of freshly severed roots.

Traditionally, trench plot soil respiration has been studied using surface flux methods (121), which measures the diffusion of gas from the soil surface in response to a concentration gradient as described by Fick's Law. In the ideal situation, surface flux should reflect CO_2 production, as only production should cause subsurface CO_2 concentrations to vary. In most soils, however, CO_2 storage is significant (and transient) due to the limited diffusivity of the soil matrix, which varies mostly as a function of water filled pore space (106). Recent research has shown that CO_2 production in the soil profile is often strongly decoupled from surface emissions because the depth of production and rates of transport differ from soil to soil (128) and over time as a result of fluctuations in soil water content. Using a subsurface approach, instantaneous rates of CO_2 production can be calculated using soil CO_2 concentration profiles and a simple multilayered diffusion models first outlined by deJong (41). Production of CO_2 for a given layer is calculated by flux across layer i minus input from layer $i-1$ below, from the surface to maximum sampling depth,

$$p_{\text{CO}_2i} = [D_{e_i}(\frac{C_i - C_{i-1}}{z})] - [D_{e_{i+1}}(\frac{C_{i+1} - C_i}{z})], \quad (0.3.1)$$

where p_{CO_2} is production of CO_2 , C_i and D_{e_i} are the concentration and effective diffusivity for layer i , respectively, and z represents depth. When applied to trench plot studies, root contributions can be assumed as the difference in CO_2 production between root-free and intact areas.

Depth resolution may be a major advantage for partitioning studies, because many biological processes are separated vertically. For example, it may be possible to make comparisons of microbial decomposition in new and old (labile and recalcitrant) soil

organic matter pools, or about CO_2 production from surface fine roots, and deeper tap roots. Currently available physical and isotopic partitioning methods, even in combination, would have difficulty resolving all these processes simultaneously.

A significant weakness associated with the method lies in determining soil gas diffusivity in the field. In natural temperate soils where soil water content is subject to large annual fluctuations (100% to <10%), D can vary annually by 100 or 1000 times (106). In contrast, the annual variability in C may only be a factor of 3. As a result, diffusivity is the primary numerical determinant of CO_2 flux (and production) estimates. When using a subsurface approach, diffusivity values must be tightly constrained.

0.4 Relative Advantages of Partitioning Approaches

Isotopic and physical approaches offer different spatial resolution. Trenches offer information only about the small plots being monitored, within, and immediately adjacent to, the trenched area. Replication is used to overcome this spatial limitation, sometimes with high labour cost. Isotopic techniques may offer greater spatial resolution in ideal situations where site variability is low and source differences are distinctive. At appropriate sites, adequate characterization of site variability could allow researchers to distinguish between root and microbial contributions at any point across a forest floor. In addition, error analysis models are currently available for isotopic partitioning methods (118), offering plug-in statistical support for different combinations of replication, source signature difference, and variability.

Although attractive, isotopic techniques are certainly less accessible. Continuous flow isotope ratio mass spectrometry (CF-IRMS) is becoming more routine, but the

equipment is still out of reach for many laboratories and per sample processing costs are high. Trench plot installation and sampling can be labour intensive, but the relatively simple analytical equipment required to determine CO₂ concentrations in soil gases is generally available to most researchers.

To date, natural abundance isotopic techniques are not adequately field tested, nor has anyone carried out systematic investigations of isotopic fractionations potentially associated with biology, gas transport, and gas sampling in natural systems. Given the small differences in source signatures, fractionations could affect data interpretation, and the overall statistical robustness of conclusions made using stable isotopic partitioning techniques. Trenches are widely used and give reliable data, but are under-utilized for process-based research; investigators repeatedly use the same combination of sampling methodologies to gather data for the purposes of budgeting. There are significant opportunities to develop trench techniques.

Partitioning methodology is, at the moment, integral to our curiosity about different climatic sensitivity of roots and microbes. It is impossible to directly assess the individual response of each source without being able to measure them separately.

0.5 Study Objectives

The primary objective of this study is to address a central question related to the landscape soil carbon balance: Are root and microbially-derived sources of soil CO₂ responding differently to environmental cues? In order to achieve these objectives, I seek to:

- a) Develop and test new field methodologies for measuring soil gas diffusivity values, sampling soil $\delta^{13}\text{C}$ -CO₂, and maximizing resolution (temporal and vertical) in

source partitioning studies.

b) Characterize variability and spatial patterns in tree and soil $\delta^{13}\text{C}$ as a foundation for isotopic partitioning efforts.

c) Apply in-situ natural abundance $\delta^{13}\text{C}$, and root exclusion partitioning techniques at sites in Atlantic Canada, and assess the relative utility of these methodologies in addressing this objective.

d) Quantify the in-situ effects of soil climate on soil respiration from microbial and root sources in-situ.

0.6 Outline and Inter-Relationship of Chapters

There are seven research chapters in this thesis, each of which tackle a different aspect of the core research question. These papers can be broadly divided into those which use isotopic and physical approaches to examining this question. The first six chapters form the basis for publications that are being submitted to leading journals, and the last presents results from a pilot study. Each chapter is listed below, and its relevance to the core research question is highlighted textually.

1. *Characterization of variability and spatial patterns in tree and soil $\delta^{13}\text{C}$ at research sites in eastern Canada.* This paper is the first in an attempt to apply an isotopic approach opportunistically at research sites where ongoing soil C work is currently being conducted. The chapter documents the isotopic characterization of physical tissues (no gas samples), a necessary requirement for establishing isotopic source values for soil CO_2 partitioning. To be submitted to Canadian Journal of Forest Research. Co-authors are Lisa Kellman (supervisor) and Martin Moroni (used his research sites in Newfoundland).

2. *Spatial variability of soil respired $\delta^{13}\text{CO}_2$ as measured using an improved sampling methodology.* In order to initiate a rigorous field sampling, it was necessary to devise a new method of isotopic sampling that targets deficiencies of other techniques. This paper documents laboratory and field testing of the method, and addresses isotopic fractionation effects that could be encountered during partitioning studies. Submitted to Canadian Journal of Forest Research. Co-authors are Nick Nickerson (undergraduate assistant who conducted lab tests under my direction and contributed to some writing and plotting in his spare time), Lisa Kellman (supervisor) and Martin Moroni (used his research sites in Newfoundland).

3. *Testing natural abundance partitioning of soil respired $\delta^{13}\text{CO}_2$ at forest sites in Eastern Canada.* This is the culmination of the isotopic approach to source partitioning where information obtained using Chapters 1 and 2 is applied in-situ. This study uses a root exclusion technique for testing of isotopic partitioning methodologies to quantify microbial and root contributions to total soil respiration. Results illustrate interesting root respiration dynamics that prevent straightforward isotopic partitioning, but which are of high interest to tree physiologists and to other researchers partitioning soil respiration. Submitted to Global Change Biology. Co-authors are Lisa Kellman (supervisor) and Martin Moroni (used his research sites in Newfoundland).

4. *A new method for in-situ soil gas diffusivity measurement and applications to trace gas studies.* Physical root exclusion techniques are used for respiration source partitioning, but would provide more information where subsurface methods could be applied to add depth resolution. Subsurface methods are hampered by poor characterizations of soil gas diffusivity (rates of gas transport in soil). This chapter outlines the development and testing of a fully automated field sampling device for survey

measurements of soil gas diffusivity. Submitted to Journal of Geophysical Research - Biogeosciences. Co-authors are Hugo Beltrami (derived the necessary mathematical formulae), and Lisa Kellman (supervisor).

5. *In-situ incubations by root exclusion highlight the climatic sensitivity of soil organic matter pools.* Using trench plots in conjunction with subsurface sampling, this chapter looks at the environmental response of microbial decomposition only, in native and experimentally warmed soils. Submitted to Geophysical Research Letters. Co-authors are Lisa Kellman (supervisor) and Hugo Beltrami (diffusivity formulae helped make this study possible).

6. *Soil microbes and roots respond to different environmental cues.* As the title suggests, this chapter describes a successful attempt at addressing the individual environmental controls on microbial and root activity at two sites, and within several vertically-distinct organic matter pools and rooting zones. To be submitted to Global Change Biology. Co-authors are Asfaw Bekele (contributed to final statistical work and plotting), Lisa Kellman (supervisor) and Hugo Beltrami (diffusivity formulae helped make this study possible).

7. *Pilot study testing high resolution partitioning of soil respiration.* Using the same approach as in chapter 6, this chapter describes field tests of a promising method for improving temporal resolution of partitioned respiration from ~ 1 week to ~ 1 hour. This chapter expected to form the basis for an article that will be submitted to the Journal of Geophysical Research - Biogeosciences. Lisa Kellman (supervisor) would co-author.

Chapter 1

Characterization of variability and spatial patterns in tree and soil $\delta^{13}\text{C}$ at research sites in eastern Canada

Preamble

This article forms the basis for a paper that will be submitted to the Canadian Journal of Forest Research.

Abstract

This study seeks to broadly characterize $\delta^{13}\text{C}$ variability and spatial patterns within soils and canopy tissues at five research sites in order to identify possible source signatures of root- and microbially-respired $\delta^{13}\text{CO}_2$, and to quantitatively evaluate site opportunities for potential soil respiration source partitioning. We observe consistent and predictable patterns of leaf $\delta^{13}\text{C}$ variation within trees, and a consistent offset between woody and leafy tree tissues. Patterns are similar for both hardwoods and softwoods, but overall hardwoods had canopies that were more depleted in $\delta^{13}\text{C}$.

Soil carbon $\delta^{13}\text{C}$ enrichment occurred with depth and appeared to vary according to site soil texture. Upper soil $\delta^{13}\text{C}$ values were intermediate between leaves and woody tissues, while deeper soil values suggested important contributions from more enriched tree tissues, such as persistent woody debris and possibly roots. Successful source partitioning of soil respiration requires that sources are isotopically distinct, variability is low, and that source signatures can be readily identified. The results of site isotopic characterizations suggest that source partitioning may be possible on a site-specific basis.

1.1 Introduction

Stable isotopes are widely used as tracers in terrestrial ecosystems, aboveground for research related to plant function (40), in soils to examine biogeochemistry and transport processes (46), and also in ecosystem atmospheres to differentiate between processes (47). Due to the importance of isotopic tracers in plant research, there is a large volume of literature related to $\delta^{13}\text{C}$ variations in leaves or needles. Stable isotopes are widely used as tracers in terrestrial ecosystems, aboveground for research related to plant function (40), in soils to examine biogeochemistry and transport processes (46), and also in ecosystem atmospheres to differentiate between processes (47). Due to the importance of isotopic tracers in plant research, there is a large volume of literature related to $\delta^{13}\text{C}$ variations in leaves or needles (24; 55), woody tissue and annual growth rings (54; 81; 112; 119).

Considerable aboveground variability in canopy $\delta^{13}\text{C}$ is often observed, often exceeding 5 permil in a single tree canopy (133; 20) but some sites show characteristically low variability (31). Canopy variations result from irradiance gradients and

hydraulic controls on carbon discrimination (102; 133; 94; 115; 114) in addition to the well-known $\delta^{13}\text{C}$ offsets between different tissues, with roots and wood being typically enriched relative to foliar tissues in C3 vegetation across many ecosystem types (72; 79; 161).

Many studies have also examined carbon isotope signatures in soil profiles (9; 16; 25; 15). Soils show a progressive $\delta^{13}\text{C}$ enrichment with depth, hypothesized to be the result of increasing incorporation of enriched, microbially-derived tissues (46). Depth enrichment varies with soil texture, owing to different controls on organic matter decomposition and mixing of soil carbon with different isotopic ratios (163). Mechanisms that could contribute to depth enrichment include historical land use and differences in microbial tissue incorporation (46), texture (163), and to a lesser extent CH_4 production, which is known to contribute to extreme enrichments of waterlogged sediments (64). Although there is little information about the proportion of persistent root carbon in deeper soil organic matter, they are enriched relative to aboveground carbon (72; 161) and root turnover likely contributes to the enriched isotopic signature at depth, especially at coarsely textured sites where rooting is characteristically deep (134).

There is a close relationship between aboveground vegetation $\delta^{13}\text{C}$ and soil profile $\delta^{13}\text{C}$. Knowledge of these relationships is based largely on work in contrasting C3/C4 vegetation sites, where the large difference in vegetation signatures highlight important processes. But, there have been surprisingly few studies exploring these vegetation-soil relationships under a single photosynthetic pathway to identify more subtle linkages. One recent study by Stevenson et al. (144) examines the dependence

of soil profile $\delta^{13}\text{C}$ on signatures of dominant C3 grasses. Buchmann et al. (25) documented soil and vegetation $\delta^{13}\text{C}$ values in forest (C3) systems, where high canopy variability and isotopic discrimination may provide opportunities for tracking these processes, but exploration of these soil-vegetations relationships were not the primary aim of the study. Despite a lack of direct research, the variations in forest tissue and soil $\delta^{13}\text{C}$ may provide some indications as to mechanisms responsible for soil carbon enrichment, and the contribution of certain tissues to the various soil labile and recalcitrant profile organic matter pools. These are processes that will in part determine the future stability of soil organic matter stocks in response to shifting climate and vegetation.

Advancements in our understanding of spatial patterns, species variability, and corresponding site-specific controls on isotopic discrimination greatly facilitate our ability to apply isotopic tools to improve resolution of biogeochemical processes. Soil respiration partitioning is one example of such an application. This technique aims to identify the relative contributions of plant root respiration and microbial respiration, each of which contributes in roughly equal quantities to overall soil CO_2 efflux (67). Isotopic partitioning techniques employed to date have involved labeling (21; 145; 117), C3/C4 transition approaches (132), and natural abundance partitioning methodologies (90; 57; 74; 97) that exploit small source differences relative to other isotopic techniques. In general, $\delta^{13}\text{CO}_2$ released during decomposition of organic matter reflects that of the substrate (or combination of substrates) being decomposed (53). Root respiration is slightly more depleted in $\delta^{13}\text{C}$ (97) and driven by current photosynthates within the plant, as assimilation and respiration are isotopically linked but with a time shift of several days (48). For young trees grown in

controlled conditions, foliar buds have been found to lock in the isotopic signature of current photosynthates and may be a useful aboveground indicator of potential root respiration signatures (97). Despite this potential indicator of root respiration signatures, greater complexity is indicated by other in-situ studies (86; 34; 7) that isolate rapid temporal shifts in tree $\delta^{13}\text{C}$ discrimination, which is likely also manifested in the root zone. Microbial respiration dynamics are more straightforward, and these should reflect soil organic matter being decomposed as there is no fractionation associated with this process (138). Phillips and Gregg (118) outline the requirements for successful natural abundance isotopic partitioning of soil respiration, which includes adequate source differentiation, low variability and sufficient replication.

The goal of this study is to characterize canopy $\delta^{13}\text{C}$ spatial variability in leaves/needles/ woody tissue, and soil profile $\delta^{13}\text{C}$ variability to investigate the potential for isotopic source partitioning or root and microbial soil respiration at forested sites in Eastern Canada. Working with the model of Lin et al. (97), we estimate the potential signatures of soil- and root-respired $\delta^{13}\text{CO}_2$, by characterizing soil profile $\delta^{13}\text{C}$, and $\delta^{13}\text{C}$ tissue variability in representative species at hardwood and softwood sites in eastern Canada that vary in species composition, soil texture and growing season length. Opportunities for source partitioning are explored quantitatively based upon observed source differentiation and site variability. A secondary benefit of this study is that ecosystem $\delta^{13}\text{C}$ values have not been reported for forest systems in this area; these results can provide a useful baseline for future isotopic studies in temperate, sub-boreal forests of eastern Canada.

1.2 Methods

The study was conducted at five sites in Eastern Canada which are currently being used for various carbon and soil respiration studies. The Nova Scotia sites include mature intact mixed forest (L1) on well drained sandy soils, an adjacent forest that was pre-commercially thinned in 1995 (L2) to enhance growth of hardwoods, and a 50 year old primarily softwood plantation on till-derived clay soils which formerly supported agricultural activities (P2). All are typical for Nova Scotia where forest disturbance regimes are high. The Newfoundland sites are characteristic of two important forest types in the province and sits near the geographic boundary of fir stands (NF) that grow in the south-western portion of the island, and the black spruce (NS) forests characteristic of north-central regions. Soils at all study sites are typically wet, acidic Humo-Ferric Podzols with textures ranging from clayey to sandy. Soil development has occurred mainly on coarse-textured, iron-rich, non-calcareous sediments, or materials where carbonates have been removed. Figure 1.1 shows the location of all study sites, and Table 1.1 summarizes soil textural characteristics.

We anticipated large canopy $\delta^{13}\text{C}$ variability, within individual trees and between species. As a result, we chose to carry out more extensive aboveground sampling to characterize this variability. At each study site, several trees representative of species diversity, age, and typical growth patterns were selected. Figure 1.2 shows crown sampling locations, and lists tree species represented in the study. Where little or no species diversity exists, such as at the Newfoundland sites, we chose to study replicates of the dominant species. Despite the fact the L2 site supports mixed vegetation, replicates of young fir trees were selected for isotopic sampling rather than sample widely spaced mature hardwoods, because the former are extremely dense and likely

have a more consistent spatial influence on site root respiration. In August 2004, trees were felled for the purpose of sampling so that we could obtain accurate length and width measurements, since isotopic variation within trees is known to vary according to height and branch length (102; 133). From each tree, three living branches were chosen for study from the top, middle and bottom of the tree crown. At three points along each of these branches, we sampled needles/leaves and cut samples of woody tissue with shears or a hand saw. New growth buds were sampled, and where present, samples of cones were also taken. All measurements were carefully logged, including measurements of overall canopy diameter at several heights. Table 1.2 lists tree species sampled at each site, including sampling locations within the canopy. At each aboveground sampling location, both foliar and woody tissues were sampled. Soils were sampled by digging a deep pit and sampling from a cleaned face using a clean trowel, using fine depth increments near the surface (2.5 cm) and coarser increments of 10 cm below. The absolute number of samples and depth increments differed among Nova Scotian and Newfoundland sites owing to bedrock depth and other factors. As shown in Table 1.2, approximately 150 samples were used to characterize soil $\delta^{13}\text{C}$ signatures. All samples were immediately placed on ice in coolers, and frozen upon return to the laboratory.

Laboratory processing of the samples involved oven drying at 60°C for 24 hours. Soil samples were then passed through a fine sieve where root materials were removed. Bark was removed from woody samples and needles were cut from stems before initial homogenization of these samples using mortar and pestle and liquid N_2 . All samples were then placed in separate scintillation vials with clean 4.8mm or 6.4mm stainless steel rods and rolled for >24 hours on a compact, purpose built 100-vial capacity

roller mill (139; 4). The stainless rods grind the samples to powder consistency whilst minimizing any likelihood of sample cross contamination. Subsamples of the powder were weighed and processed using a Eurovector Elemental Analyzer coupled to a GV Isoprime Continuous Flow Mass Spectrometer. $\delta^{13}\text{C}$ repeatability was determined to be better than 0.15 permil for random replicate samples of bulk samples collected in the field.

Using IsoError04.xls (118), we evaluated opportunities for potential soil respiration source partitioning, given observed isotopic variability at the site, and the likely differences in source signatures. Of particular interest was the number of sample replicates (root, microbial $\delta^{13}\text{CO}_2$ and the soil $\delta^{13}\text{CO}_2$ mixture) that would be required to determine source contributions with satisfactory confidence. This analysis will be used to indicate the feasibility of future partitioning efforts at these sites.

1.3 Results and Discussion

1.3.1 Foliar $\delta^{13}\text{C}$ distributions and variability

Foliar $\delta^{13}\text{C}$ values for each species are shown in Figure 1.3. We observed low $\delta^{13}\text{C}$ variability within the crown of each tree, especially in softwood stands. Despite the fact that most of the trees studied were mature and greater than 14 m in height, the canopy range of foliar $\delta^{13}\text{C}$ values rarely exceeded 1 permil, and was more typically 0.6 to 0.8 permil. Other researchers have reported total canopy $\delta^{13}\text{C}$ variation exceeding 4 permil in temperate systems (24; 115). Since hydraulic conductivity, water use efficiency and irradiance determines within-canopy variation (102; 133), it is understandable that variation at our sites is low, as typically wet soils dominate and growing seasons are moist owing to the maritime climate. There is, however, larger

average within-canopy range of foliar $\delta^{13}\text{C}$ at the P2 site, even among softwoods, where 3 permil is typical. This is perhaps related to the clayey soil texture at this site, which may limit water transport to roots and affect tree hydraulic efficiency despite high volumetric soil water contents.

Although within-canopy variability was low, we did observe predictable patterns in foliar $\delta^{13}\text{C}$ values with increasing height and branch length. In general, we observed $\delta^{13}\text{C}$ depletion with increasing height and branch length. Radial distance from stem and $\delta^{13}\text{C}$ values were closely coupled, as were branch length and $\delta^{13}\text{C}$. These relationships could be described using branch-specific linear regressions, with average R^2 values routinely exceeding 0.75, similar to other studies, for example Samuelson et al., 2003 (133)). As a result of these predictable patterns of variation (shown in Figure 1.4), it was possible to standardize $\delta^{13}\text{C}$ values to an arbitrary height and branch length, but owing to small within-tree variation, standardized foliar $\delta^{13}\text{C}$ values were very similar to mean values of all samples collected from each tree.

Between species, we noted that average foliar $\delta^{13}\text{C}$ in hardwoods was slightly more depleted (roughly 2 permil) than softwoods, which is a consistent trend in this dataset. Across all five study sites, foliar $\delta^{13}\text{C}$ values were remarkably similar, showing a minimum value of -31.3 permil and a maximum value of -26.2 permil in >300 samples from 16 trees. The total range of foliar values here is only 5 permil, or less than the variability in a single tree crown found by other authors in different settings (24; 115). Low system variability is a desirable site attribute for isotopic partitioning methods (118). Unfortunately, foliar buds, the newest tree tissues and those potentially most representative of current photosynthates (97), did not exhibit a distinctive isotopic signature, but rather their signature could be explained by location

within the crown. At these sites, we could not identify any aboveground tissue that would likely elucidate information about potential root respiration signatures at our sites, and at the time of our sampling which was late in the growing season.

1.3.2 Woody tissue $\delta^{13}\text{C}$ distributions and variability

As shown in Figure 1.4, woody tissue $\delta^{13}\text{C}$ followed predictable patterns, but were less universal and more tree-specific than for foliar values. Despite tree-specific patterns, linear regressions were also able to describe much of the relationship between $\delta^{13}\text{C}$ and branch height and/or length. There was a consistent, and almost universal offset between woody and foliar tissues, with the former being enriched by roughly 2.5 permil relative to adjacent leaves/needles, as shown in Figure 1.5. This offset has been observed in other studies (72; 79; 161). Woody tissues also showed slightly more within-tree variation than for foliage, particularly in hardwoods where $\delta^{13}\text{C}$ varied by as much as 2.5 permil.

1.3.3 Soil profile $\delta^{13}\text{C}$ distributions and variability

Litter layer $\delta^{13}\text{C}$ generally reflected site-averaged woody tissue values more closely than foliar values, and we observed some enrichment with depth which is consistent with other studies (16; 15). The rate at which soils were enriched appears to vary according to soil texture at the sites in Nova Scotia (Figure 1.6). We observed a mild enrichment through surface soil layers at the P2 site relative to the strong enrichment through surface soil layers at sites L1 and L2. This observation was further supported by data from additional study sites at P2, L1, and L2 not included here; more detailed profile analyses are currently being conducted at these sites. The depth enrichment at sites L1 and L2 was best described by fitting exponential regressions to the data,

while at P2 a linear regression best described the steady enrichment through depth. The Lakevale and Pomquet sites are separated by less than 15km, and share many similarities, including litter layer and deep soil $\delta^{13}\text{C}$ signature, forest composition, climate and topography, but the rate of enrichment may be closely related to differences in soil texture.

At these sites, most of the variability in the upper soil profile can be explained by mixing, if roots contribute a significant portion of the (enriched) carbon deeper in the profile. Only at two sites were soil carbon signatures more enriched than expected root values, indicating that while non-mixing related enrichment mechanisms are still important, but they may not be necessary to explain depth enrichment at P2, LV1 and NFS. Mechanisms that could contribute to differential depth enrichment include historical land use and differences in microbial tissue incorporation (46), texture (163), and to a lesser extent CH_4 production, which is known to contribute to extreme enrichments of waterlogged sediments (64). Methane can be produced (and consumed) microbially even in aerobic soils at the center of aggregates or near the water table (83; 160; 78).

From this ecosystem $\delta^{13}\text{C}$ characterization, we can also broadly speculate as to the linkages between vegetation and soil carbon. Figure 1.6 shows the relationship between average site canopy and soil profile $\delta^{13}\text{C}$ values. As expected, soil organic $\delta^{13}\text{C}$ signatures near the surface reflect a mix of woody debris and needles/leaves. Deeper soil signatures are close to, or slightly more enriched than woody aboveground tissues. Although this study does not specifically address root signatures, past work has shown that roots have signatures slightly (about 1 permil) more enriched than wood (72; 161). Estimated values of solid root $\delta^{13}\text{C}$ (not to be confused with root-derived

$\delta^{13}\text{CO}_2$) are shown in Figure 1.6. This suggests that, in addition to other mechanisms noted earlier, soil depth enrichment may result from shifts in the dominant forms of organic matter supplied to soils through depth. Litter is of course important at the surface, and at depth we may infer that some of the observed enrichment is the result of important contributions from persistent woody debris with sluggish inherent decomposition rates relative to needles (109), and also from root turnover. The importance of root turnover to soil organic matter stocks has been observed elsewhere (73; 19; 6), and has been recently addressed in the review of (123), but has not been directly linked to patterns of depth enrichment.

1.3.4 Potential opportunities for $\delta^{13}\text{C}$ based partitioning

Successful $\delta^{13}\text{C}$ partitioning of soil respiration requires that 1) isotopic differences between sources are distinct, 2) variability is low and of course that 3) one can adequately identify the source signatures (118). This study, and others that show rapid differentiation of current photosynthates (7; 34) means that 3) will be the most difficult to overcome. It may not be reasonably possible through solid tissue analysis to infer the likely signature of root respiration (97). These sites, however, do show low isotopic variability, and are in general good candidates for further partitioning investigations for this reason.

Opportunities for partitioning are summarized in Table 1.3, based on the statistical model of (118). We assume that root-respired $\delta^{13}\text{C}$ values are similar to the average canopy foliar values, and that microbially-respired signatures are equivalent to soil or litter. Table 1.3 presents the approximate replicates (both sources and mix) required to determine source proportions $\pm 10\%$ with 95 % confidence, for Foliar-Litter (F-L) sources and for Foliar-Soil (F-S) sources. Foliar-soil distinctions could

be possible with soil profile sampling, as opposed to surface flux, techniques.

Since distinctiveness of the likely root and microbial source signatures is desirable for the purposes of source partitioning, it appears that the L1 and L2 sites offer the most promise. These are sites at which $\delta^{13}\text{C}$ enrichment, or departure from signatures of aboveground tissues, happens most quickly with depth. Especially if $\delta^{13}\text{CO}_2$ respiration was to be measured using subsurface techniques (128) rather than soil CO_2 surface flux (121), one could exclude near surface soil layers (intermediate signatures) from partitioning analysis because $\delta^{13}\text{CO}_2$ diffusion occurs overwhelmingly in the upwards direction and downwards contamination from litter-respired CO_2 is unlikely. It is important to keep in mind that the isotopic differences between plant and soil $\delta^{13}\text{C}$ shown in Figure 1.5 are of course only useful for partitioning if the assumptions mentioned at the outset are true. These assumptions are a) that there is no fractionation associated with soil organic matter decomposition and that b) root respiration signatures will reflect isotopic values of other tree tissues hold.

Given a range of different CO_2 sampling strategies such as subsurface or surface flux measurements (128), it is possible to exclude certain isotopic components with intermediate signatures (especially the litter layer) from the partitioning analysis. Better yet, it may be possible to leverage the range of available physical partitioning methodologies to help identify the source signatures at small spatial scales. This could be done most easily using root exclusion (trench) plots to identify the signatures of microbial respiration, and that of the undisturbed forest floor separately. The results of this study, however, provide an indication of source isotopic differences, site-specific variability, and the likely sampling methods that might provide the best chances of success for partitioning, which is a useful basis for further study at these sites. Due

to small source differences and inherent natural variability, potential opportunities for soil respiration partitioning using $\delta^{13}\text{C}$ must be evaluated on a site-specific basis.

1.4 Conclusions

In this study, we broadly characterized the $\delta^{13}\text{C}$ signature of vegetation (foliage and wood) and soils at five research sites, to identify possible opportunities for isotopic source partitioning of root and microbial soil respiration. We observed consistent and predictable patterns of leaf and woody tissue variation within trees, and a regular offset between woody and leafy tree tissues. Patterns were similar for both hardwoods and softwoods. Soil carbon near the surface was an isotopic mixture of woody and foliar signatures, indicating that both are likely important sources at the surface. Deeper enriched soil values may suggest important contributions from relatively enriched sources, such as persistent woody debris, and organic matter derived from root turnover. Other factors that could be important are soil texture, microbial tissue incorporation and methane production at anaerobic microsites and/or near the water table.

We assume that the signature of microbial respiration reflects that of soil organic matter, since there is little or no fractionation associated with the decomposition processes. Soil organic matter $\delta^{13}\text{C}$ values were increasingly enriched through depth, but near the surface where decomposition is fastest, soil organic matter signatures showed only small variations across sites and were coupled closely with aboveground foliar and woody signatures, all of which aid in the identification of potential microbial respiration signatures. Unfortunately, at these sites we found no aboveground indicator that was clearly indicative of potential root respiration signatures. The $\delta^{13}\text{C}$ of new

tree tissues (buds) reflected their physical location within the tree rather than any unique signature reflective of current tree photosynthate that is thought to drive root respiration.

Results clearly show that in-situ, $\delta^{13}\text{C}$ -based partitioning techniques must be approached opportunistically because site-specific factors may, or may not, favor application of these techniques. Useful future studies would include coupled physical-chemical approaches to further characterize the spatial and temporal variability associated with root respired $\delta^{13}\text{C}$. More specifically, this could involve the use of root exclusion plots and isotopic gas analyses to positively identify microbial- and root-respired $\delta^{13}\text{CO}_2$ values, and to track these during the growing season to assess temporal variability. Potential partitioning opportunities may exist amongst the sites studies, which provides a strong argument to continue our investigations using coupled physical-chemical approaches and innovative $\delta^{13}\text{CO}_2$ measurement strategies.

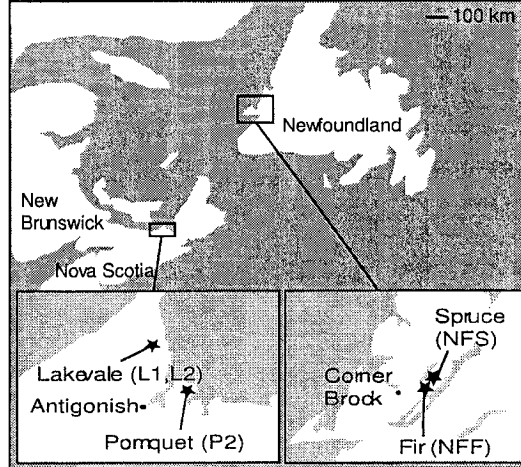


Figure 1.1: Site Location Map

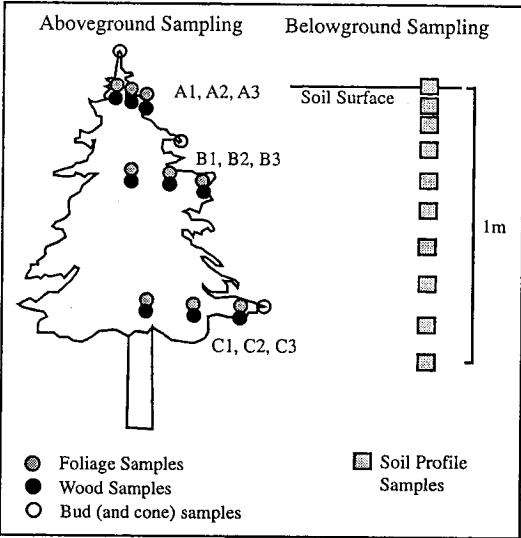


Figure 1.2: Sampling protocol for characterization of isotopic signatures.

Table 1.1: Soil pH and textural characteristics for all study sites after (28) and (84).

Site	pH	gravel %	sand %	silt %	clay %	stoniness
Pomquet (P)	4.7	21	20	39	41	-
Lakevale (L)	4.4	10	85	7	8	-
NFLD Fir (NF)	3.7	-	53	43	3	high
NFLD Spr (NS)	4.0	-	54	41	5	high

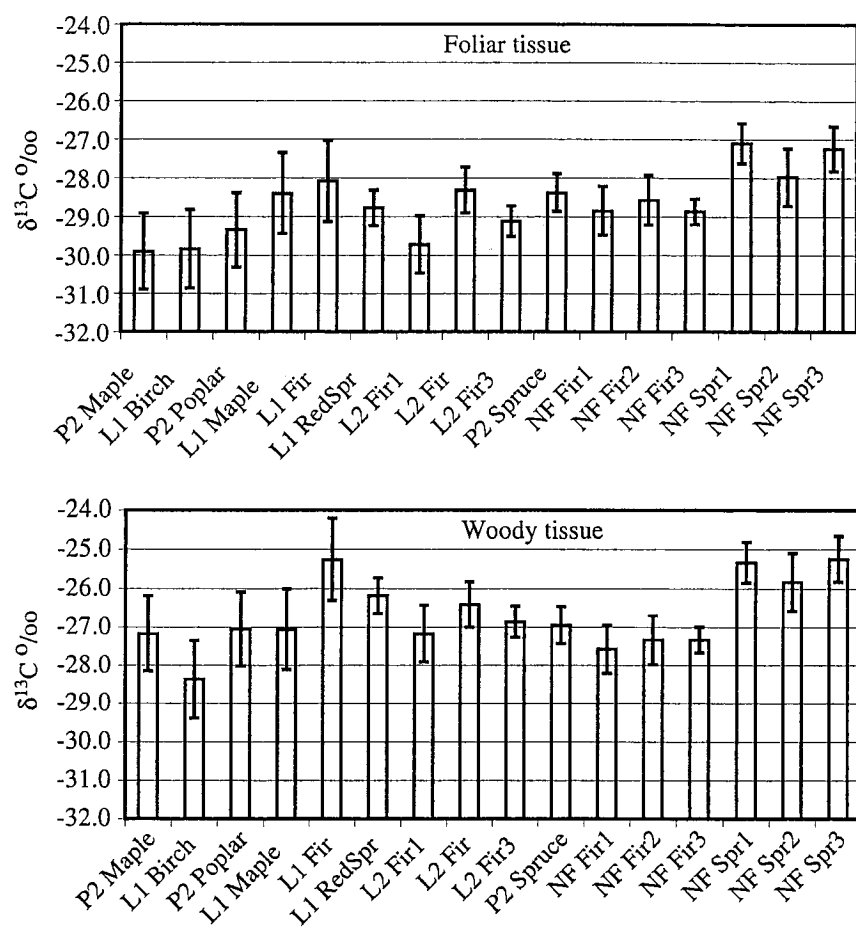


Figure 1.3: Average canopy foliar and woody $\delta^{13}\text{C}$ values and standard deviations for individual trees sampled.

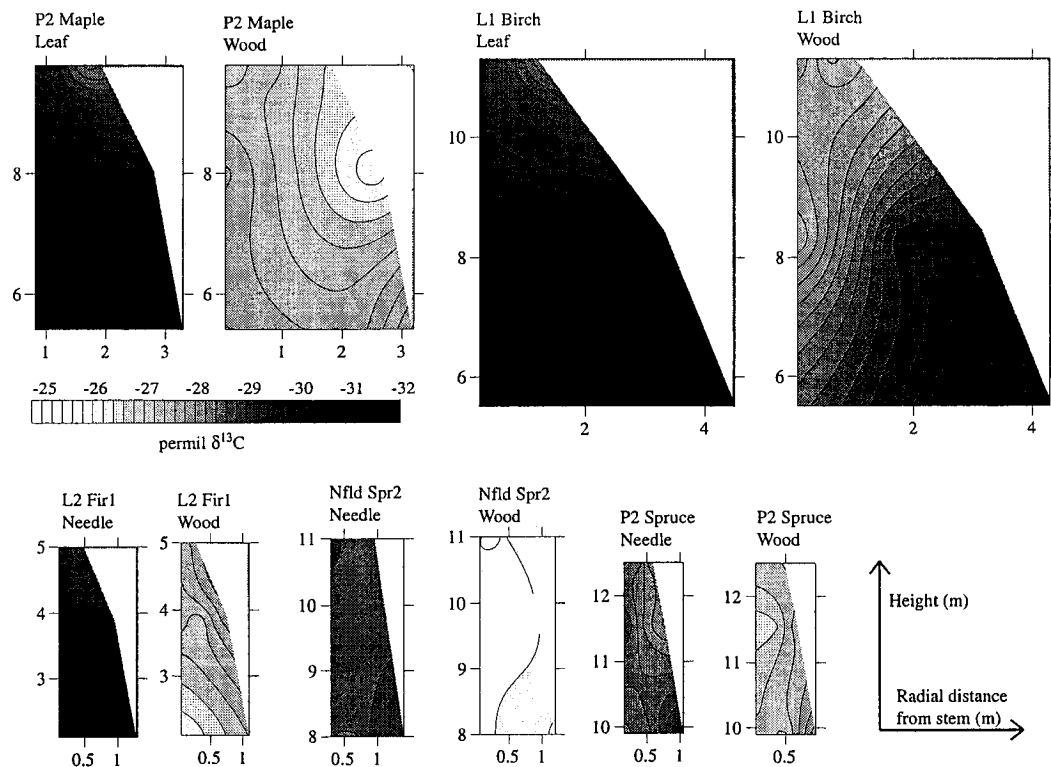


Figure 1.4: Examples of foliar and wood $\delta^{13}\text{C}$ patterns in selected tree crowns.

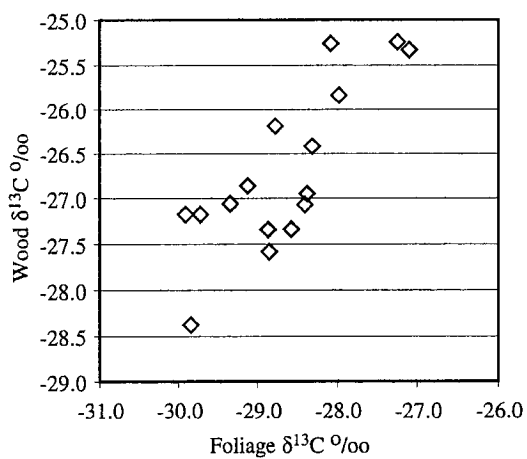


Figure 1.5: Foliage-wood $\delta^{13}\text{C}$ offset, calculated as average values for individual branches.

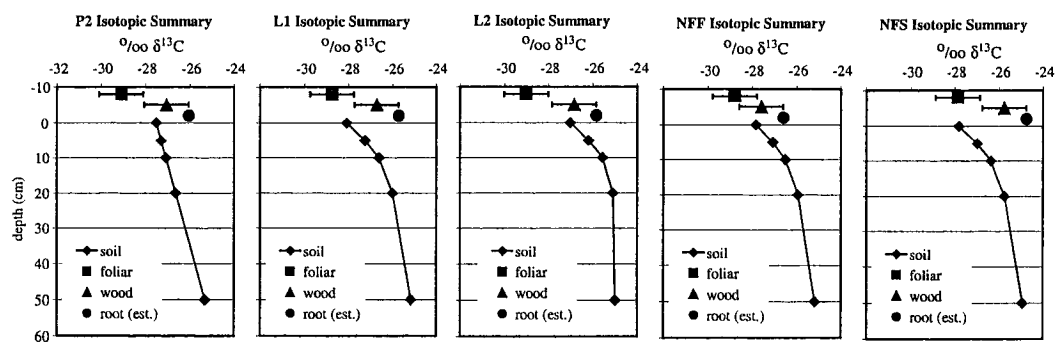


Figure 1.6: Relationship between site-averaged vegetation and smoothed (2 point weighted average) soil profile $\delta^{13}\text{C}$ signatures.

Table 1.2: Vegetation and soil sampling, showing tree characteristics and tissue (foliage, wood) sampling numbers in mature trees (mat.) and saplings (sap.). D represents the maximum canopy diameter.

Site	Nfd		Nfd		Nfd		Nfd		Nfd		L2	L2	L2	L1	L1	L1	L1	L1	L1	P2	P2
Species	Fir	Nfd	Fir	Nfd	Fir	Nfd	Fir	Nfd	Fir	Nfd	Fir	Fir	Fir	R.Spr	Maple	Birch	Fir	Maple	Fir	Maple	R.Spr
Age	Mat.	Mat.	Mat.	Mat.	Mat.	Mat.	Mat.	Mat.	Sap.	Sap.	Sap.	Sap.	Sap.	Mat.	Mat.	Mat.	Mat.	Mat.	Mat.	Mat.	Mat.
Height	14.8	15.2	15.9	11.8	13.9	13.1	6.10	6.20	6.50	12.6	14.5	13.0	10.8	11.8	13.2	13.2	10.8	11.8	13.2	13.2	13.2
D (m)	3.30	2.30	2.20	2.50	2.30	2.20	2.10	2.40	1.90	2.30	2.50	5.50	3.00	3.50	1.20	1.20	3.00	3.50	1.20	1.20	1.20
C (m)	13.6	14.2	15.8	11.1	12.8	12.10	5.00	1.65	5.40	11.6	13.0	11.3	10.2	9.80	12.5	12.5	10.2	9.80	12.5	12.5	12.5
B (m)	10.8	13.5	13.1	10.0	11.0	10.7	3.80	1.10	3.50	10.0	11.5	8.30	8.70	8.00	11.5	11.5	8.70	8.00	11.5	11.5	11.5
A (m)	5.80	12.00	10.60	8.40	8.00	9.30	2.10	0.50	1.60	8.00	9.00	5.50	5.40	5.40	9.90	9.90	5.40	5.40	9.90	9.90	9.90
Tissue n	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20
Buds	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
Cone	N	N	N	Y	Y	Y	N	N	N	Y	N	N	N	N	N	N	N	N	N	N	N
Soil n	9	9	9	9	9	9	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
Z _{max}	30	30	30	30	30	30	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100

Table 1.3: Partitioning opportunities as determined with Isoerror.xls (118).

$\delta^{13}\text{C}$ permil	P2	L1	L2	NF	NS
Foliar (F)	-29.10	-28.75	-29.03	-28.80	-27.87
Litter (L)	-27.52	-26.72	-26.85	-27.60	-27.77
Soil (S) at 20cm	-27.08	-25.72	-25.85	-26.60	-24.77
F-L difference	1.58	2.03	2.18	1.19	0.10
F-S difference 20cm	2.019	3.03	3.18	2.19	3.10
F-L req'd replicates	34	21	18	61	20
F-S req'd replicates	21	9	8	18	9

Chapter 2

Spatial variability of soil respired $\delta^{13}\text{CO}_2$ as measured using an improved sampling methodology

Preamble

This article forms the basis for a paper submitted to the Canadian Journal of Forest Research.

Abstract

Here we describe a new Keeling-free $\delta^{13}\text{CO}_2$ soil surface flux sampling methodology and laboratory trials to determine transport fractionations related to the sampling methodology. Using field tests, we also consider the importance of physical determinants on forest-floor $\delta^{13}\text{CO}_2$ flux variability relative to variability resulting from biological factors, in particular the isotopically distinct contributions from roots and microbial activity. Errors associated with the measurement technique were small and are only slightly higher than CF-IRMS analysis alone. Because the technique is also

less labor intensive than others, it can be potentially be applied to boost the effectiveness of root/microbial partitioning studies by minimizing error and maximizing the ability for replication. Fractionations are associated with this sampling technique, but can be held constant under a wide range of field conditions. In field surveys at five sites across 60m² grids, we found that variations in forest floor transport characteristics (diffusion + atmospheric invasion) were likely more important determinants of isotopic signature than would likely be associated with distinct signatures from root and microbial respiration processes. Forest floor transport variability was able to describe the magnitude of $\delta^{13}\text{CO}_2$ variability, the spatial pattern of effects, and the systematic offsets between expected and measured signatures. Because non-biologic parameters may influence forest soil $\delta^{13}\text{CO}_2$ variability, more research is needed to integrate physical considerations into biological inquiry where stable isotopes are used for fingerprinting purposes.

2.1 Introduction

Stable isotopic tracers are used in many areas of terrestrial ecosystem science to shed light on rates or fates of physical or biogeochemical processes. Stable isotopes of carbon (C) and oxygen (O) are frequently used aboveground in carbon cycling studies to separate photosynthetic assimilation and ecosystem respiration into their component fluxes (91; 23), and in opportune circumstances using dual-isotope models to distinguish between ecosystem autotrophic and heterotrophic respiration (62; 110). Belowground, laboratory studies using natural abundance $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ have been used to successfully isolate autotrophic and heterotrophic soil respiration (97). In the field, a wide variety of partitioning techniques have been applied including C3/C4

transition or amendment experiments (132), Free Air Carbon Enrichment and labeling (21; 145; 117) or using natural abundance techniques (90; 57; 74) that capitalize on small respired $\delta^{13}\text{C}$ differences between sources, ranging from 2 to 4 permil (97).

Isotopic sampling of soil surface flux is typically done using static chambers, and Keeling Plot analysis of several samples taken during a period of headspace equilibration (57; 48). The Keeling method requires 1) multiple concentration samples during the headspace equilibration period, and 2) mathematical extrapolation outside the data range to determine the isotopic value of the soil gas mixing into the chamber. Errors are associated with this method, resulting primarily from extrapolation and noisy data (116). Under excellent circumstances, these errors may fall below 0.2 permil, but may also substantially exceed this value when measured ranges of concentration are small and/or regression significance is weak (116). The Keeling plot and multiple sample methodology is not required, however, when the headspace chamber starts the equilibration period completely free of atmospheric CO_2 . For relatively small chambers, this is practical, and accomplished by purging with a CO_2 -free gas or by passing chamber air through soda lime columns prior to the measurement period. This method provides a simple, fast and effective alternative to current methods of measuring the isotopic composition of soil CO_2 surface flux, where constant attention to chamber sampling is not required during equilibration. However, adequate effort must be targeted at understanding purge and equilibration timing, chamber shape and venting, in addition to potential fractionations associated with the method.

Researchers have made assumptions that *temporal* variations in forest floor $\delta^{13}\text{C}$ were linked with biological variation, resulting from experimental site manipulations (74). In a similar way, *spatial* variability of flux signatures may reflect the distribution

of root and microbial activity in the subsurface, since these have distinctive signatures of respired $\delta^{13}\text{CO}_2$ (97). In addition, recent studies highlight relationships between tree proximity and flux magnitude (149), suggesting that isotopic signatures of soil surface flux may vary similarly across the forest floor as a result of predictable source spatial variation. There are likely other important controls on spatial variability of isotopic signatures. Variations in microbially-respired signatures may be related to exploitation of different soil organic matter pools derived from wood, roots and foliar tissues, all of which have distinct signatures (72; 161). Deep, slow cycling pools of soil organic matter are typically enriched in $\delta^{13}\text{C}$ and tend to respire heavier $\delta^{13}\text{CO}_2$ (46). Methane production and re-oxidation may also be important, as isotopically light CH_4 can be produced in the deep soil or within soil aggregates which, if oxidized in the upper soil profile, would skew soil $\delta^{13}\text{CO}_2$ towards lighter values characteristic of soil CH_4 (151).

Non-biological mechanisms also have the potential to introduce significant isotopic offsets as a result of atmospheric invasion and diffusion fractionation, both of which serve to enrich soil $\delta^{13}\text{CO}_2$ (29). The theoretical diffusion fractionation of -4.4 permil is the result of preferential diffusion of lighter ^{12}C in CO_2 (29). Invasion of isotopically enriched atmospheric air results in mixing that isotopically skews surface soil values towards enriched atmospheric values. Examples of such an invasion offset, generated using the model of Cerling (29) is shown in Figure 2.1. It demonstrates that these effects are manifested near the soil surface, and the overall magnitude of the isotopic offset and the depth to which invasion effects penetrate are both a function of soil respiration rate and gas diffusivity. It is not clear whether the spatial or temporal variability in atmospheric invasion (respiration, diffusivity) can potentially overprint

distinct biological source contributions. Grid survey sampling provides a good opportunity to explore the spatial distribution of source signatures and to assess the potential relative importance of these mechanisms as a control on soil surface flux $\delta^{13}\text{CO}_2$ variability.

Here we document the development and testing of this sampling method for $\delta^{13}\text{CO}_2$ soil surface flux measurement. We present results of laboratory tests followed by surface flux $\delta^{13}\text{CO}_2$ grid surveys at five forested sites to broadly characterize the potential importance of isotopic transport effects a) associated with the sampling method, and b) for field investigations. We demonstrate that, at these sites, variations in forest floor transport characteristics (diffusion + atmospheric invasion) are likely more important determinants of measured $\delta^{13}\text{CO}_2$ than small differences in source signature from respiring roots and soil microbes.

2.2 Methods

2.2.1 Chamber design and method development

Static flux chambers are widely used for measuring trace gas emissions from soil. Chambers are cost effective, and function under a wide range of conditions (140). Isotopic composition of gas fluxes can also be measured using flux chambers. Using a typical chamber design as a springboard, an improved method for isotopic sampling of $\delta^{13}\text{CO}_2$ has been developed for this study, which is fast, accurate, and applicable to other trace gases.

Chambers are 100 mm I.D. \times 50 mm tall, offering a relatively high surface area to volume ratio. A double-ended needle and two venting hoses are incorporated into the chamber for sampling, pressure equilibration, and purging. A strip of closed

cell foam was glued to the inside circumference of the chamber to ensure a proper seal onto the field collar, a PVC cylinder (100 mm O.D. \times 60 mm tall) with open ends. We addressed many design considerations including volume, mixing, wind protection, venting, and collar installation (121). Because soil CO₂ is typically present in relatively high concentrations and drives a strong concentration gradient outwards from the soil, a wide variety of chamber designs are functionally acceptable (121), except large chamber volumes in which mixing may become an issue. Mixing in our small chambers is rapid, helping to obtain representative headspace samples. Wind protection was provided by the foam sealing strip to ensure a tight fit with the collar because the $\delta^{13}\text{C}$ of atmospheric CO₂ is considerably different from that of soil respired CO₂ (29). Venting is critical in controlling pressure variations due to the collection of gas within the chamber (37) and a proper vent hose size is critical to ensure that there is no diffusion of atmospheric air into the chamber during the collection process. Laboratory testing revealed a vent tube of 2mm I.D., 400 mm length was adequate to ensure no back diffusion was occurring during sampling. Lastly, it is important to ensure the collar is installed without gaps or improper seals with the soil (121) to ensure that atmospheric invasion is not an issue during sampling.

A complete in-situ chamber purge with CO₂-free air is the unique characteristic of the method. A simple valve is installed at the terminus of the intake hose, which can be opened temporarily and connected to a small pump and a soda lime column drawing air from the free atmosphere. During purging, air is pushed into the chamber headspace and out the chamber pressure vent. When purging is complete the inlet valve is closed, the pump is disconnected and the headspace, because of zero initial CO₂ concentration, recruits soil CO₂ to fill the chamber. An equivalent of ten chamber

volumes of CO₂-free air is required to satisfactorily remove all CO₂ that may be trapped in the chamber headspace when the chamber and the collar are initially coupled.

2.2.2 Laboratory testing

Since the chambers recruit soil CO₂ by diffusion, they likely initiate a fractionation of ambient soil $\delta^{13}\text{CO}_2$ values. Assuming an infinite source (soil) and sink (chamber), this fractionation will be -4.4 permil, or the theoretical fractionation associated with CO₂ diffusion (29). But, given non-infinite sources and increasing equilibration time, $^{13}\text{CO}_2$ will increasingly catch up with faster moving $^{12}\text{CO}_2$, reducing the apparent theoretical fractionation. To characterize the performance of the chamber and technique in relation to these parameters, repeatability and fractionation tests were performed in laboratory columns to test the effects of equilibration time, purge time, and soil diffusivity on observed isotopic fractionation values.

The column incorporated a lower reservoir, an artificial soil composed of washed silica sand packed to 40% porosity held between metal screens, and a collar at the top of the column to which our flux chamber was be fitted. Prior to each experiment, the chamber was installed on top and the entire system (column and chamber) was flushed with approximately 10L of a CO₂/N₂ mixture from UHP gas standard tanks. Mixed batches varied slightly but were always well characterized both isotopically ($\delta^{13}\text{CO}_2$ signature near -1 permil) and in concentration (roughly 1000 ppm). This mixture was introduced into the bottom reservoir over a two minute period, ultimately exiting the top of the column-chamber system by the flux chamber vent. Following the flush with known CO₂, the chamber headspace was purged with CO₂-free N₂ at 6 l/min according to our sampling protocol. After a headspace equilibration period,

isotopic samples were drawn from the chamber and the reservoir in 10 ml evacuated Exetainers.

The first test, performed at zero percent soil moisture, was used to determine repeatability and fractionation associated with variations in headspace equilibration time. In this test, we performed repeat measurements using headspace equilibration intervals of 5, 10 and 15 minutes. The second test held equilibration time constant at 10 minutes but effects of volumetric water content was tested using volumetric water contents of 0, 10 and 30 percent. In the final lab test at zero percent soil moisture, purge times of 20, 40 and 80 seconds (equivalent to 5, 10 and 20 chamber volumes) were used to examine fractionation effects. For all tests, a minimum of five replicate tests were performed to characterize repeatability.

2.2.3 Field testing

Using the new sampling method, we sought to assess the importance of physical determinants on forest-floor $\delta^{13}\text{CO}_2$ flux variability relative to variability resulting from biological factors.

The field tests were conducted at five forested sites in Atlantic Canada, the details of which are summarized in Table 1.1. Four parallel rows of collars were installed approximately one hour prior to sampling, each 10m long with 1m collar spacing. Rows were separated by 2m, providing a sampling grid with 40 points, covering a forest floor surface area of 60 m². Collars were pushed ~40 mm into the soil at the sampling site prior to the sampling period. Despite other research suggesting collar insertion affects respiration rates (121), we found no difference in tests using a Licor LI-8100 automated flux chamber, configured to take repeat measurements at 15-30 minute intervals for 3 days post-insertion. The chamber equilibration period at LV1,

LV2 and P2 was 10 minutes whereas equilibration period at NFF and NFS sites was 20 minutes to compensate for smaller CO₂ fluxes at these sites. Samples from the headspace were collected via the needle in 10ml evacuated Exetainers and were analyzed within 24 hours to minimize the likelihood of contamination. Repeatability and microscale variability were also tested in the field, by a) repeatedly purging and sampling from the same chamber and b) installing many chambers beside one another in treeless areas where spatial variability was assumed to be minimal.

Using the model of Cerling (29), we assess the maximum potential range of isotopic effects that could be expected from variations in atmospheric invasion across our sampling grids. Originally derived for carbonate nodule experiments in old soils, Cerling combines the theoretical diffusion fractionation with a mixing model that predicts the invasion of atmospheric CO₂ into the soil surface, as a function of soil gas diffusivity and respiration rate. Since atmospheric air has a signature distinct from that of soil (roughly -8 and -25 permil, respectively), invasion of atmospheric air into the upper soil surface enriches soil $\delta^{13}\text{CO}_2$ signatures. Since diffusivity and respiration rate are implicated, they likely show variability across the soil surface.

Model parameters were collected and/or estimated from existing site data. Soil respiration rates were calculated for each sampling point from IRMS concentration data, and for LV1 they compared favorably with a Licor 8100 survey that had been conducted 10 days earlier. Spatial variability in respiration rate from the Licor and those calculated from the isotopic chambers were comparable, with standard deviations (σ) of 1.12 (n=20) and $\sigma=0.76$ (n=40), respectively. Unlike respiration rates, we lacked simultaneous collar-specific moisture data, but did have good information about the spatial distribution of volumetric soil moisture at the L1 site, collected

during bulk density analysis of soil cores in September 2004. The permanent fixed meteorological station at this site indicates that soil moisture values during this sampling was almost identical to those at the time of this study. Bulk density analyses showed a 13% average soil moisture content ($n=20$) with a standard deviation of 7% across all microtopography. These moisture values were used to calculate soil CO_2 diffusivity rates with the model of (106) using 40% total soil porosity (bulk density average). This spatial variation in diffusivity was applied to all sites under the assumption that all 5 sites could be expected to have similar variation in soil moisture content because of similar mound and hollow microtopography. The permil offsets predicted by the (29) model were averaged over the top 5 cm of soil, since this was a typical observed recruitment depth under our range of experimental laboratory conditions. The average isotopic offsets were calculated for two scenarios that illustrate the maximum (lowest site respiration, highest site diffusivity) and minimum (highest site respiration, lowest site diffusivity) potential effects of atmospheric invasion. The source and atmospheric signatures for this simulation were estimated at -27 and -9 permil, respectively.

Forest floor variability resulting from biological factors, in particular the isotopically distinct contributions from roots and microbial activity, was assessed using radial distance from trees as a proxy for the spatial distribution of root and microbial sources. Recent studies highlight relationships between tree proximity and overall flux magnitude (149), suggesting that isotopic signatures of soil surface flux may vary similarly across the forest floor as a result of predictable source spatial variation. We measured tree proximity for each grid sampling location, and also enumerated the number of trees within a radius of 1.5m.

2.2.4 Mass Spectrometry

Both field and lab gas samples were analyzed on a continuous flow stable isotope mass spectrometer (GV Instruments) and $\delta^{13}\text{C}$ values are reported as per mil variation from the $\delta^{13}\text{C}$ isotopic standard Pee Dee Belemnite:

$$\delta^{13}\text{C} = \frac{R_{\text{sample}} - R_{\text{std}}}{R_{\text{std}}} \times 1000$$

Where R_{sample} and R_{std} are the $^{13}\text{C}/^{12}\text{C}$ ratios of the sample and the standard, respectively. During analysis of both field and laboratory gas samples the mass spectrometer was found to have a error of <0.2 per mil between replicate samples, which is low for CF-IRMS *plus* sampling error. Concentrations of bulk CO_2 were determined simultaneously.

2.3 Results and Discussion

2.3.1 Laboratory Repeatability and Fractionation

Reproducibility was high for all laboratory measurements, and added only small error beyond the analytical variability error with IRMS analysis. The maximum observed standard deviation of replicate column experiments ($n=5$) was 0.31 permil, associated with shorter headspace equilibration time intervals of 5 minutes. As equilibration time increased to 10 minutes or beyond, standard deviations among replicate column experiments fell to roughly 0.20 permil regardless of soil gas diffusivity/column soil moisture content or other parameters that were manipulated during the laboratory tests. Standard deviations for all tests and experimental conditions ($n=60$) were 0.24 permil, which compares favorably with Keeling approaches, where propagation of R^2 uncertainty in surface flux chamber data results in additional errors beyond

reproducibility and IRMS precision. Our method of isotopic flux sampling proved to be reliable, and contributes only small errors beyond those associated with IRMS analysis, despite several opportunities for contamination common to all flux chamber methods, including chamber venting, chamber sealing, Exetainer purging, vacuuming and leakage before analysis. Through careful method design and laboratory practices, these opportunities for error have been minimized, and are not appreciable individually or collectively.

Fractionation associated with the sampling method varied according to headspace equilibration time, as shown in Figure 2.2. Short equilibration times maximize the fractionation associated with the method, where we observed an offset close to the theoretical -4.4 permil offset. Offsets decrease linearly with increasing equilibration time. Paired samples t-tests reveal that the observed variability in fractionation at different equilibration times cannot be explained by the repeatability error associated with the various trials. We understand this time dependent fractionation to be the product of our non-infinite isotopic source (soil) and sink (chamber) system. Headspace concentrations accumulate quickly within the chamber initially, and slow down as the soil-chamber concentration gradient weakens, which allows for increased equilibration of slow-moving $^{13}\text{CO}_2$. Headspace equilibration time is held constant for site grid surveys, so relative isotopic values within site are preserved but care must be exercised when comparing across sites. For cross-site comparisons where equilibration times must be tuned according to the magnitude of expected soil respiration values, equilibration times must be taken into account.

The degree of fractionation associated with the sampling method showed a strong apparent dependence on soil gas diffusivity, which was varied by changing the water

content of the column soil. When water was introduced into the system error was unaffected, but fractionation under 10 and 30% water contents was approximately one half of that observed under dry (0%) conditions, roughly 2 per mil as shown in Figure 2.3. These water content of 0, 10 and 30% are equivalent to soil gas diffusivities of 4.62×10^{-6} , 1.77×10^{-6} 4.56×10^{-8} m^2/s , respectively (106). This diffusivity-fractionation effect was further investigated by adding three extra sampling needles in the experimental column (0, 5 and 10 cm depth), from which we measured soil profile CO_2 concentrations. Figure 2.4 shows that for dry soils, CO_2 is recruited from deeper in the profile, whereas in wet soils, near-surface CO_2 is preferentially exploited to increase chamber concentrations, despite the lower gas-filled pore space and more limited CO_2 pool (less gas phase present). Average depth recruitment over all tests was approximately 5cm. Our three point concentration profile means that potentially we may miss localized recruitment effects near the surface, but we speculate that in wet soils, shallow recruitment serves to limit the apparent size of the source, so in effect, $^{13}\text{CO}_2$ and $^{12}\text{CO}_2$ has more opportunity for equilibration. Regardless of the cause, since fractionation was stable at approximately -2 per mil over the wide range of soil moisture/diffusivity values observed at all sites, the diffusivity-related sampling fractionation can be assumed to be invariant for the purposes of our study.

In completely dry soils, we observed trends between purge time and measured isotopic values. At 40s or longer purge times (equivalent to 10 chamber volumes), fractionations were stable at almost -4.4 permil, but during short (20s) purge times, we observed only ~ 2 per mil fractionation, or approximately half that of the 40 and 80 second purge times and theoretical diffusion fractionation. The assumed mechanism for these purge time fractionation discrepancies is the concentration gradient induced

during purging, which may result in infiltration of the CO₂-free air into the sand matrix. This effect can definitely not be explained by residual atmospheric $\delta^{13}\text{CO}_2$ contamination in the chamber, which would instead serve to further deplete signatures beyond the theoretical -4.4 permil. But, again, for the purposes of our study, purge time fractionation effects are unimportant, because purge times were always held constant in the field.

In summary, the cumulative errors associated with sampling and analysis using this method of flux sampling are small (0.2-0.31 permil), and are only modestly higher than the accepted ~ 0.2 permil error associated with CF-IRMS analysis. In addition to speed, the method may be associated with less sampling variability than Keeling plot approaches, especially where headspace concentration increases are small due to low flux rates (116). Our laboratory studies indicate that fractionations are associated with this sampling method, serving to offset the measured isotopic value from the actual $\delta^{13}\text{CO}_2$ signature in the soil. Fractionations were lower for low soil gas diffusivities (high moisture content), long equilibration times, and shorter purge times. For the purposes of field study, sampling fractionations can be held constant at roughly -2 permil with careful selection of method parameters. The low variability and speed associated with this technique boosts the effectiveness of root/microbial partitioning, because measurement variability can be kept low and many replicate $\delta^{13}\text{CO}_2$ samples can be taken in a short span of time. Aside from source signature differences, spatial replication and low variability are critical parameters in source partitioning studies (118).

2.3.2 Forest floor $\delta^{13}\text{CO}_2$ variability

Before carrying out forest floor grid sampling, repeatability was assessed by repeated purging and sampling cycles, and from flux chamber measurements in flat, treeless areas in a former agricultural field. During field method testing, isotopic flux repeatability within 1m^3 ($n=10$) was 0.33 permil, which compared favorably with laboratory tests of replicate samples at 0.2 permil.

Grid sampling of forest floor $\delta^{13}\text{CO}_2$ at all of the sites show a large degree of spatial variability, as shown in Figure 2.5 and summarized in Table 1.2. We observed slightly different average forest floor $\delta^{13}\text{CO}_2$ values at each site. Standard deviations were fairly consistent for each site at ~ 1 permil, but the total range of measured values was at least 4 permil.

Recent studies have identified relationships between tree proximity and flux magnitude (149), and we sought to investigate whether isotopically distinct contributions from root and microbial respiration may vary similarly across the forest floor. We found no apparent relationship between the total number of trees in close proximity to sampling locations for either isotopic signature or bulk respiration rate. We did, however, find weak correlations to absolute tree proximity (i.e. nearest neighbour, in metres), as shown in Figure 2.6. The observed relationships at each site were, however, opposite to what we had expected; increasing tree proximity resulted in more enriched values (Nova Scotia sites) or no change (Newfoundland sites). This suggests that root respired $\delta^{13}\text{CO}_2$ is more enriched than (Nova Scotia), or the same as (Newfoundland), microbially respired signatures.

Several factors suggest that root and microbial source contributions were not controlling the observed forest floor variability. First, weak or absent correlations between

tree proximity and isotopic signature were direct evidence that other factors are important. Secondly, the total measured $\delta^{13}\text{CO}_2$ variability at each site (>4 permil) was higher than could be attributed to distinct contributions of roots and microbes. Only small permil differences should separate root and microbial respiration, perhaps 2 permil near the surface (97) where most of the microbial respiration is generated by exploitation of fresh organic matter. Thirdly, all measured signatures were substantially more enriched than expected based on the measured range of upper soil $\delta^{13}\text{C}$ values at these sites, which range broadly from -27 to -28 permil at these sites (unpublished data), very typical of cool temperate hardwood/softwood forests. Given the small source difference between root and microbial respiration and the lack of fractionation associated with microbial decomposition (138), respired $\delta^{13}\text{CO}_2$ should be broadly similar to soil $\delta^{13}\text{C}$ values. During our sampling, however, we observed offsets of >6 permil between expected and measured forest floor $\delta^{13}\text{CO}_2$ signatures.

Cerling (29) presents a theoretical model for differentiation between respired $\delta^{13}\text{CO}_2$ and soil $\delta^{13}\text{CO}_2$ as a result of differential diffusion of $^{12}\text{CO}_2$ and $^{13}\text{CO}_2$ in the soil profile, and invasion of (relatively enriched) atmospheric air into the soil profile. At each site, we used this model to predict atmospheric invasion offset using collar-specific respiration rates and two diffusivity values (lowest, highest moisture from L1). These collar-specific effects, averaged over the top 5cm of soil, were found to be as low as 4.5 permil, or as high as 13.5 permil for low and high diffusivities, respectively. Mean site offsets of roughly 8 permil were of similar magnitude to observed offsets between sampled $\delta^{13}\text{CO}_2$ and site organic matter $\delta^{13}\text{C}$. The difference between the "maximum" and "minimum" model scenarios were larger than the maximum range of forest floor $\delta^{13}\text{CO}_2$ variability observed at our sites (4-5 permil), but

it is also unlikely that microsite conditions at each collar would serve to entirely favor either scenario. In addition, real effects are likely smaller at most sites, which are typically wetter and have lower soil gas diffusivities than LV1, the site from which moisture/diffusivity data was taken and assumed to be representative everywhere.

Although we do not have explicit information related to spatial variability of transport effects, collar-specific respiration rates can be used as a rough index of collar-specific atmospheric invasion + diffusion offsets. At each site, relationships between respiration rate and measured isotopic signature always exceeded those of signature-tree proximity, with linear R^2 values falling between 0.2 and 0.5. The observed relationships were also consistent with atmospheric invasion theory presented in (29), where low respiration rates are associated with enriched signatures and vice versa.

Atmospheric invasion shows significant theoretical variability over large areas and microtopographies, and when diffusion fractionations are also considered, the effects are of large magnitude as shown in Table 1.3. These effects vary spatially and likely also show temporal variation, which can easily serve to overprint any variability that may be associated with distribution of roots, or other biological processes. Surface flux techniques recruit soil CO_2 from the surface pool of soil gas via the concentration gradient applied by the flux chamber at the soil surface. For example, if $\delta^{13}\text{CO}_2$ signatures were measured in a forest pre- and post-harvest, the observed post-harvest differences in isotopic signature could be related to a) the exclusion of root respiration (likely favors enrichment), b) the decrease in respiration rate and increase in atmospheric invasion (also favors enrichment), c) the increase in soil moisture due to reduced evapotranspiration (favors depletion due to higher diffusivities), or d) all of the above. At our study sites, forest floor $\delta^{13}\text{CO}_2$ variability can likely be explained

wholly or in large part by isotopic offsets related to CO₂ diffusion and atmospheric invasion, as no other mechanism would likely provide an enrichment of similar magnitude or one showing such large spatial variability. Given this physical variability, it is difficult to assess the presence and magnitude of biological processes at our study sites. Researchers should be conscious of these physical effects, especially because temporal changes in respiration rate and diffusivity control the magnitude of atmospheric invasion. Respiration and diffusivity are in turn influenced by site temperature (127; 85; 122), moisture (36; 63) and other factors (35).

2.4 Conclusions

The purpose of this study was to develop and test a $\delta^{13}\text{CO}_2$ soil surface flux measurement sampling method that is rapid and avoids error inherent to Keeling approaches. This is made possible by using a high surface area to volume chamber that can be purged of atmospheric CO₂ prior to the headspace equilibration period. We present results of initial laboratory column tests, in addition to a field application in which we characterize forest floor $\delta^{13}\text{CO}_2$ variability at five sites in eastern Canada to assess the spatial dependence of respiration signature in relation to tree distribution and atmospheric invasion.

In the laboratory, errors associated with sampling and analysis were small, and are only slightly higher than CF-IRMS analysis alone. Fractionations are associated with this sampling technique, and were lower for low soil gas diffusivities (high moisture content), long equilibration times, and shorter purge times. For the purposes of field study, sampling fractionations can be held constant at roughly -2 permil with careful selection of method parameters. The low variability and speed associated with

this technique could potentially boost the effectiveness of root/microbial partitioning studies, to minimize measurement variability and maximize replication.

Results from field tests were particularly interesting. We assumed that distinct signatures of root and microbial respiration would exert strong control on forest floor $\delta^{13}\text{CO}_2$ variability. Instead, we found that forest floor transport variations (diffusion+ atmospheric invasion) were likely more important controls on isotopic signatures across the 60 m² grids used for sampling, as indicated by modeling with our site data and the approach described in Cerling (29). Forest floor transport variability was able to describe magnitude of $\delta^{13}\text{CO}_2$ variability, the spatial pattern of effects (as indicated by respiration rates), and the systematic offsets between expected and measured signatures. At all sites, physical effects strongly overprinted any biological differences. While additional biologic or non-biologic parameters may influence forest soil $\delta^{13}\text{CO}_2$ variability, our results are consistent with spatial variations in atmospheric invasion.

In conclusion, our sampling methodology described here provides a robust alternative to the Keeling approach for sampling upper-soil $\delta^{13}\text{CO}_2$ values using surface flux chambers. Researchers should be mindful of potential sampling-related fractionations associated with flux chamber techniques, and especially of atmospheric invasion/diffusion processes that serve to skew upper soil $\delta^{13}\text{CO}_2$ values from those respired by source tissues. These transport offsets may show large spatial and temporal variability as a result of the highly variable nature of respiration rate and soil gas diffusivity, which are the primary determinants of transport-related isotopic offsets. Despite the complexity and magnitude of these effects, flux chamber $\delta^{13}\text{CO}_2$ measurements can still be used to garner information about biological processes, or for

partitioning experiments. When field experiments are conducted on limited spatial scales and under constant soil conditions and where sampling depth of recruitment is well characterized, offsets will remain fixed and are easily predicted by theoretical methods. The results of this study demonstrate that, in general, more research needs to be targeted at integrating transport physics into biological inquiry where stable isotopes are used for fingerprinting purposes.

Note

Provisional Canadian and American patent applications have been filed to protect intellectual property described in this chapter.

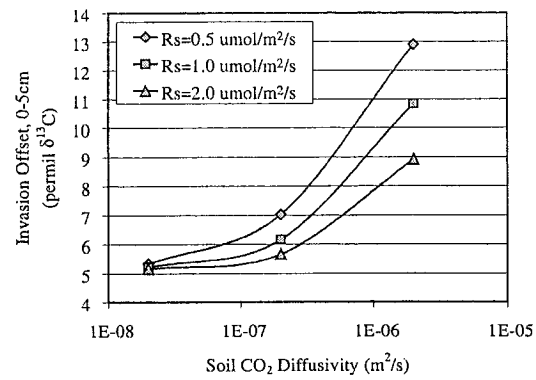


Figure 2.1: Isotopic offsets averaged over 0-5cm depth as a function of soil gas diffusivity and respiration rate.

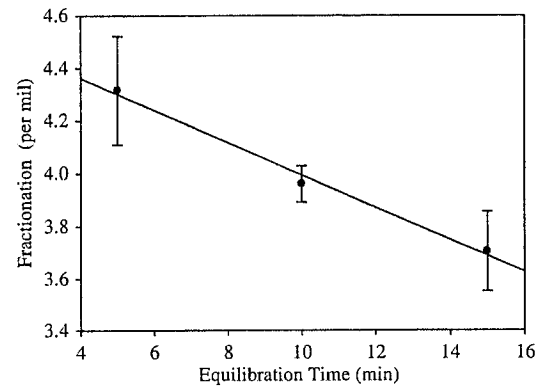


Figure 2.2: Equilibration time dependency of sampling technique in laboratory columns.

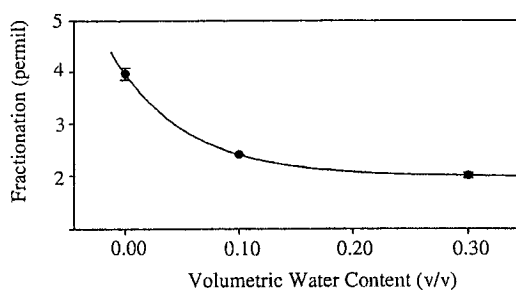


Figure 2.3: Effect of soil volumetric water content on method fractionations.

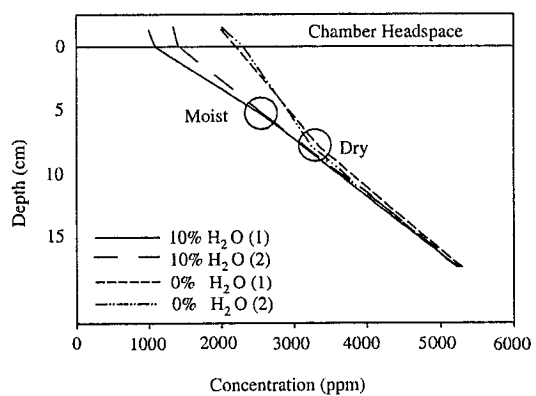


Figure 2.4: Concentration profiles at different sand column moisture contents.

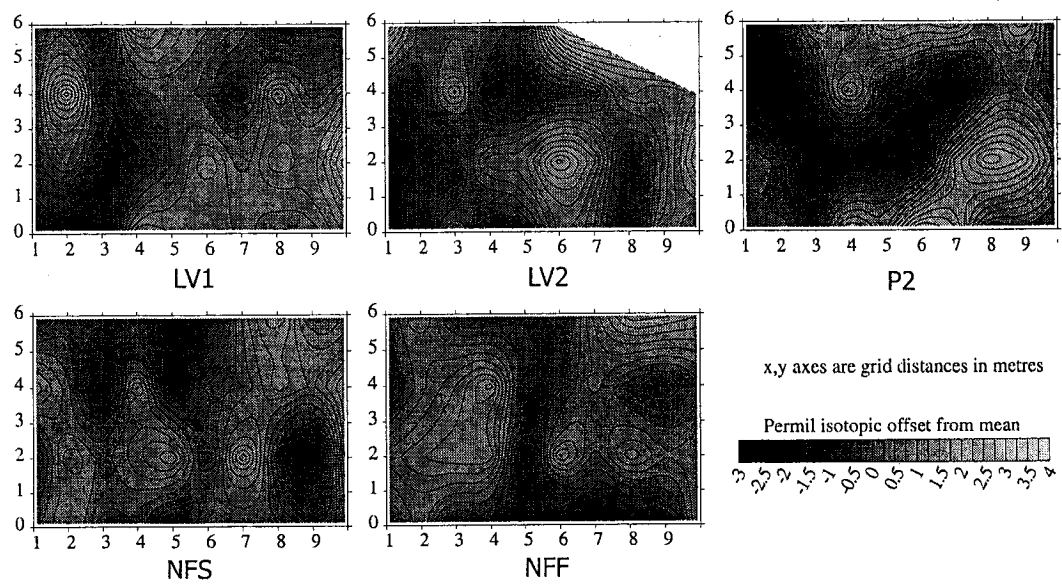


Figure 2.5: Contour plots showing relative $\delta^{13}\text{CO}_2$ values across forest floor grid sampling locations.

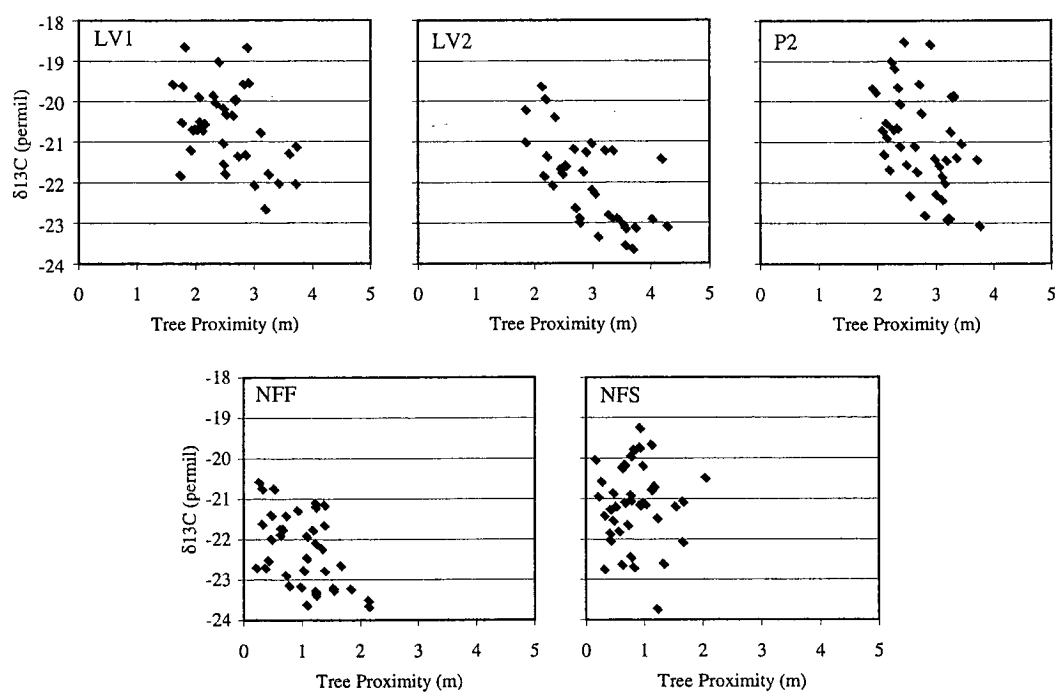


Figure 2.6: Relationship between tree proximity and measured isotopic signature for each sampling location.

Table 2.1: Site locations, soil and vegetation characteristics

Site Name	Location		Soils	Forest composition
	Lat, Lon			
LV1	Lakevale, Nova Scotia		Coarse Texture	Mixed Hardwood/Softwood
	45°45'13"N, 61°59'09"W		Well drained	Intact, 80 years old
LV2	Lakevale, Nova Scotia		Coarse Texture	HW overstory, SW understory
	45°45'13"N, 61°59'09"W		Well drained	Selective Harvest 1996
P2	Pomquet, Nova Scotia		Fine Texture	Mixed Hardwood/Softwood
	45°39'34"N, 61°50'26"W		Poorly drained	40 year regeneration
NFF	nr. Pasadena, Newfoundland		Fine Texture	Fir stand
	48°57'24"N, 57°37'17"W		Rocky, Wet	60 years or more
NFS	nr. Pasadena, Newfoundland		Fine Texture	Black Spruce stand
	49°05'42"N, 57°25'39"W		Rocky, Wet	60 years or more

Table 2.2: Average flux $\delta^{13}\text{C}$ and standard deviation (per mil), corrected for sampling fractionation

Site Name	$\delta^{13}\text{C}$	Max $\delta^{13}\text{C}$	Min $\delta^{13}\text{C}$	σ
LV1	-20.6	-19.6	-24.1	1.0
LV2	-22.1	-18.6	-22.6	1.1
P2	-21.0	-18.5	-23.0	1.2
NFF	-22.4	-20.6	-24.5	1.0
NFS	-21.0	-19.2	-23.7	1.0

Table 2.3: Initial values and results from theoretical modeling of atmospheric invasion + diffusion (I+D) fractionation variability across all forest floors under best and worst case scenarios using the model of Cerling (29).

Site Name	n	Min. Resp. $\mu\text{mol}/\text{m}^2/\text{s}$	Max. Resp. $\mu\text{mol}/\text{m}^2/\text{s}$	Min. Diffusivity m^2/s	Max. Diffusivity m^2/s	Invasion+Diffusion 0-5cm, permil	I+D Std. Dev. 0-5cm, permil
LV1	40	3.21E-08	1.99E-07	4.39E-07	2.78E-06	8.1	+/- 4.3
LV2	40	3.87E-08	1.31E-07	4.39E-07	2.78E-06	8.1	+/- 3.7
P2	40	4.09E-08	1.02E-07	4.39E-07	2.78E-06	8.2	+/- 3.4
NFF	40	3.21E-08	1.14E-07	4.39E-07	2.78E-06	8.5	+/- 3.9
NFS	40	2.83E-08	9.33E-08	4.39E-07	2.78E-06	10.5	+/- 2.3

Chapter 3

Testing natural abundance partitioning of soil respired $\delta^{13}\text{CO}_2$ at forest sites in Eastern Canada

Preamble

This article forms the basis for a paper submitted to Global Change Biology.

Abstract

Stable isotopic partitioning of soil respiration may be possible where source signatures are distinct and variability is low. Here, we assess the potential application of isotopic tools for partitioning sources of soil respiration, by using trench/intact sites to monitor $\delta^{13}\text{CO}_2$ signatures of root, microbial and overall soil respiration through the latter half of a growing season. We observed temporally stable signatures of microbially respired CO_2 , broadly reflecting isotopic signatures of litter. In contrast, root respiration varied temporally by as much as 6 permil, but the degree of total variability was regionally dependent. We found that the average $\delta^{13}\text{CO}_2$ signature of root respiration could be more enriched, or more depleted than microbial sources depending on site and

sampling day. Overall, variability and error were high relative to the difference in root and microbial source signatures, limiting partitioning opportunities to specific sites and dates. These results suggest that before embarking upon partitioning studies, researchers should carefully consider physical, methodological and biological controls on isotope dynamics.

3.1 Introduction

Soil CO₂ is generated by both microbes and roots in roughly equal quantities (67) and combined natural production from these sources exceeds anthropogenic CO₂ emissions by roughly 10 times (76). Research suggests that variability in soil autotrophic and heterotrophic respiration is driven by different environmental cues (43; 159; 93; 92), but standard soil respiration measurements do not distinguish between these sources. Although methodologically challenging, good partitioning tools may yield a better understanding of soil respiration dynamics and potential responses to changes in climate and land use.

Both (67) and (89) have recently reviewed both physical and isotopic partitioning methods, and outline the methodological challenges that can limit accessibility, and which may compromise success in some study locales.

Isotopic techniques involve labeling (21; 145; 117), or take opportunistic advantage of C3/C4 transition sites or amendments (132) where distinctive $\delta^{13}\text{C}$ values that result from the different photosynthetic pathways help to distinguish between production from each source. Natural abundance partitioning methodologies (90; 57; 74; 97) exploit smaller source differences relative to other isotopic techniques. There is no

fractionation involved in microbial respiration (46), and $\delta^{13}\text{CO}_2$ released during decomposition of organic matter reflects that of the substrate (or combination of substrates) being decomposed (53). Root respiration is slightly more depleted in $\delta^{13}\text{C}$ (97) and driven by current photosynthates within the plant, as assimilation and respiration are isotopically linked but with a time shift of several days (48). Roots respire $\delta^{13}\text{CO}_2$ that is reflective of this pool, which varies temporally due to shifts in stomatal conductance and other factors (7; 34; 82). These processes directly control the $\delta^{13}\text{C}$ signatures of respired CO_2 , while in contrast $\delta^{18}\text{O}$ values most closely reflect the depth of production, due to evaporative enrichments in pore water near the soil surface (143). Natural abundance partitioning efforts based on $\delta^{13}\text{C}$ measurements typically assume temporal stability of source signatures. This is true for belowground studies focusing on root and microbial source partitioning (90; 57; 74; 97) and also for aboveground studies where soil respiration is distinguished from aboveground respiration isotopically (110).

Physical partitioning methodologies are widely used, where roots are excluded from plots by trenching (21; 95; 92; 51). The fraction of respiration coming from plant roots is calculated as the difference in respiration between trenched (microbes only) and untrenched (microbes and roots) areas. Although it has been established from laboratory studies (30) that a "Priming effect" exists whereby root exudates affect the decomposition of soil organic matter, field studies do not conclusively show the same patterns, with some studies showing significant priming effects (66; 146) and other studies showing no effect (66). Trenches are typically a few square meters and though they reliably provide good information on small scales, they provide limited information about forest floor spatial variability. For this reason, isotopic,

and especially natural abundance, techniques are attractive because they potentially offer improved spatial resolution of respiration dynamics.

A weakness of past natural abundance isotopic partitioning efforts is the failure to consider gas transport as a potential determinant of measured isotopic values, as physical mechanisms may cause isotopic fractionation and/or mixing at the soil surface. Ambient soil $\delta^{13}\text{CO}_2$ may differ considerably from soil-respired $\delta^{13}\text{CO}_2$ due to diffusion and atmospheric invasion (29), so data may need to be shifted or offset if it is desirable to link a pool of respired CO_2 to its source. Similarly, it is important to consider the magnitude of sampling-related fractionations because chamber techniques recruit shallow soil CO_2 by diffusion, which is associated with a fractionation of 4.4 permil (29). This is the maximum possible discrimination under natural conditions given infinite sources and sinks, but in practice the observed fractionation may be smaller. For flux chambers of finite volume, the apparent fractionation decreases with increasing headspace equilibration. Soil-atmosphere diffusion fractionations of less than 4.4 permil have also been observed in ecosystem isofluxes (110; 44), and are perhaps the results of increased boundary layer thickness and decreased concentration gradients at the soil surface under still atmospheric conditions. Isotopic data can be corrected to compensate for all of these processes, but there is error inherent in establishing and applying the offsets. These effects cannot, however, be ignored. They are of large magnitude, and vary in space and time in response to respiration rate and soil gas diffusivity (29). Across broad spatial scales, it is critical to quantify the contribution of physical variability to measured isotopic values and associated uncertainty, especially if the aim is to resolve small differences in biologic variability.

Phillips and Gregg (118) outline the statistical requirements for successful natural

abundance isotopic partitioning of soil respiration, which includes adequate source differentiation, low variability and analytical error, and sufficient replication. In this study, we systematically address each of these issues in relation to a simple natural abundance $\delta^{13}\text{C}$ isotopic partitioning technique that we hope to use at research sites in Atlantic Canada where carbon studies are ongoing (128). We are hoping to identify sites that may show promise for isotopic partitioning, due to site-specific characteristics that maximize source differentiation.

Here we use trench plots to characterize autotrophic and heterotrophic $\delta^{13}\text{CO}_2$ signatures and their variability during the growing season. This is done by applying a simple isotope mass balance model to $\delta^{13}\text{CO}_2$ surface flux values from inside the trenches (microbial activity) and outside (root and microbial activity) to determine the separate signatures respired from root and microbial sources. Our results demonstrate that at these study sites, microbially-respired signatures are relatively stable over the growing season while root respiration signatures show appreciable temporal variability.

3.2 Methods

The study was conducted at five research sites in Atlantic Canada, some of which are the focus of other carbon and energy balance research (12; 127). Table 3.1 summarizes site locations and characteristics.

Root exclusion trenches are used to physically partition root- and microbially-derived soil respiration (21; 95; 92; 51). Trench plots of 2 m² were lined with vapor barrier to a depth of 50cm to prevent root regrowth and to minimize lateral CO₂ gradients. The Newfoundland sites (NFF, NFS) each contain four trenches installed

during the growing season of 2004. Sites in Nova Scotia each have a single trench plot (LV1, LV2, P2) installed during the summer of 2003. All trenches were left for a period of one year prior to the start of this study, to allow recovery from a respiratory burst initiated by the presence of freshly severed roots. Meteorological observatories exist at each site, including subsurface temperature and moisture probes, similar to that described in (12).

Soil $\delta^{13}\text{CO}_2$ surface flux was collected using 500 cm³ (10 cm i.d. x 6 cm tall) vented PVC chambers similar to those described in (77), attached to permanent collars installed on the soil surface. At the Newfoundland sites, 5 collars were installed inside and immediately outside the 4 trench plots, while in Nova Scotia, 10 collars were installed inside and outside each trenched area. A three stage procedure was used to collect soil $\delta^{13}\text{CO}_2$ surface flux. First, CO₂-free air, provided by drawing atmospheric air through soda lime columns, was pushed into the chamber via a secondary vent valve at 6 l/min. Five litres of purge air (10 times the chamber internal volume) escaped from the chamber by the pressure vent tube. This flow rate and volume was found to be sufficient for the establishment of a CO₂-free environment inside the chamber. The secondary vent valve was closed prior to the second stage, a headspace equilibration period. During equilibration, CO₂ concentrations were allowed to increase to roughly 600-700 ppm (typically 20 minutes) as a result of soil surface emissions. The third stage of measurement involved sampling the headspace within the chamber by purged and evacuated 10ml Exetainer through a double-sided needle installed in the top of the chamber. Keeling plot analysis and multiple samples were not necessary (57; 48), since all of the $\delta^{13}\text{CO}_2$ in the chamber at the end of the equilibration period was derived from the soil.

All samples were analyzed within 48 hours using a GV Isoprime CF-IRMS and Multiflow gas bench. Analytical and sampling errors were cumulatively determined to be less than 0.2 permil and 1 percent for $\delta^{13}\text{C}$ and CO_2 concentrations, respectively. Respiration rate was calculated using chamber concentration increases per unit area per unit time. Initial tests showed that flux magnitudes measured with our isotopic sampling methodology compared favorably with those of a Licor LI-8100 automated surface flux chamber.

We determined methodology-related fractionations using laboratory tests with soil columns purged with known δ^{13} -signature CO_2 . In-situ isotopic offsets due to diffusion and atmospheric invasion were estimated using the model of Cerling (29). Model input parameters included measured respiration rate and soil gas diffusivity, calculated using TDR soil moisture values (available only at LV1, LV2, P2) and the model of McCarthy and Johnson (103). Since diffusion+invasion effects are greatest near the surface, we averaged the depth-specific offsets over the depths from which CO_2 is recruited using our sampling methodology. We observed an average 5 cm depth recruitment in laboratory column concentration profiles under a wide range of volumetric water contents. Errors inherent in determining the offset are important, as they represent the uncertainty associated with gas transport effects and their potential contribution to observed isotopic variability. For this reason, these errors must be included in source partitioning error analyses.

The corrected isotopic flux values collected from inside and outside the trench were used to infer source signatures. Trench plot $\delta^{13}\text{CO}_2$ respiration was used to indicate microbial values, due to lack of root activity in this area. Outside the trench, both root

and microbial sources contribute, but with simultaneous measurement of flux magnitudes (and by difference, the relative respiratory contribution of root and microbial sources), a simple mixing model can be used to infer the source $\delta^{13}\text{CO}_2$ signature of the root component. Figure 3.1 shows this approach schematically. Temporal variability in source signatures was determined by repeated measurements through the growing season.

Aboveground and soil profile $\delta^{13}\text{C}$ values were also used as background information for this study, especially to identify the potential signatures of microbial and root respiration. The isotopic characterizations of each site are presented elsewhere (130).

3.3 Results and Discussion

3.3.1 Isotopic offsets due to physical factors

Figure 3.2 shows the range of isotopic offsets associated with atmospheric invasion and diffusion, given observed respiration rates, gas diffusivities, and recruitment depths. The cumulative magnitude of these effects was typically +6 permil for trenched and untrenched areas. All sites showed similar average values. Although we observed minor temporal variability in atmospheric invasion+diffusion, these were typically small in magnitude (0 to 1 permil) owing to the coincident opposing influence of decreasing respiration rates and increasing soil water contents (lower diffusivity) as the growing season progressed. Due to low seasonal variability, a single offset value was applied to each site. An additional fractionation of -2 permil was associated with our sampling methodology, less than the theoretical -4.4 permil associated with diffusion, due to the large concentration increases in our small chambers that maximized soil-chamber equilibration over short (20 minute) sampling timescales. Net

offsets (physical, methodological) were typically about +4 permil. Table 3.2 shows the magnitude of net offsets for each site, and observed temporal variability due to changes in respiration rate and gas diffusivity.

3.3.2 Respired $\delta^{13}\text{CO}_2$ relative to ecosystem values

A thorough characterization of foliage, wood and soils was carried out prior to this partitioning study, and is documented elsewhere (130). We observed consistent patterns of leaf and woody tissue variation within trees, regular offsets between woody and leafy tree tissues, and surface soil carbon signatures reflective of a mixture of woody and foliar signatures. Deeper soil values suggested important contributions from relatively enriched sources, possibly roots, or strong depth enrichment by other mechanisms (46). In this study, we expected to find microbially-respired signatures reflective of soil $\delta^{13}\text{C}$ values, since there is no fractionation associated with the decomposition process (138). Furthermore, signatures of respired $\delta^{13}\text{CO}_2$ might indicate whether surface or deep soils are more active in the decomposition process. Root respired $\delta^{13}\text{CO}_2$ is more complicated. It is known to reflect current photosynthates in the tree (97) but in-situ studies (86; 34; 7) have isolated rapid temporal shifts in tree $\delta^{13}\text{C}$ discrimination and it is unknown whether these temporal shifts can also be manifested in the root respired CO_2 signatures.

Figure 3.3 shows the relationships between ecosystem $\delta^{13}\text{C}$ values and corrected surface flux $\delta^{13}\text{CO}_2$, averaged for the sampling period. There is a broad correlation between microbially-respired signatures and those of current vegetation foliage and woody tissues, both of which provide the feedstock for current respiration at the surface of the litter layer. There was little evidence that deeper soils contributed in any meaningful way to microbially respired $\delta^{13}\text{CO}_2$, which is consistent with the results

of other studies (128). Somewhat surprisingly, there were no consistent trends for root respired signatures. At LV1 and P2, root respiration was on average isotopically heavier than the microbial source, while at other sites such as LV2 and NFS, the opposite was true. Average differences in source signatures were small, typically only 2 permil.

Errors related to spatial variability and reproducibility were low for each sampling period. Source signature standard deviations rarely exceeded 1 permil among the 5-10 surface flux replicates, taken from inside and outside the trenches. This spatial variation was often as low 0.5 permil inside the trenches themselves, where respiration dynamics are simpler. The error bars plotted in Figure 3.4 represent the range of temporal variability encountered, which are discussed in the following section.

3.3.3 Temporal variability in $\delta^{13}\text{CO}_2$ source signatures

The isotopic signature of microbially respired CO_2 was stable in time, and observed fluctuations were within measurement and spatial variability-related error. As shown in Figure 3.4, however, root respiration signatures varied widely throughout the sampling period on a site-specific basis. There was significant observed variation at the Nova Scotia sites despite similar species composition. Depleted $\delta^{13}\text{CO}_2$ values of root respiration were generally favored at the beginning and end of the sampling period which corresponded roughly to August and October, respectively. At all of the sites in Nova Scotia, we observed a trend towards very enriched values late in the growing season, occurring slightly earlier at the P2 site. These swings in root respiration were of high magnitude, and on occasion, exceeded the average site source difference by several times. There was no additional error or spatial variability associated with quantification of the root source during these periods, suggesting that this is a real

effect, and not an artifact of an increase in spatial variability.

There are several possible explanations for temporary root respired $\delta^{13}\text{CO}_2$ enrichment. Wind pumping, rapid and large scale atmospheric pressure fluctuations or rain events can entrain large volumes of enriched atmospheric air into the soil profile, and could be manifested as an apparent enrichment. However, root exclusion sites should have shown similar trends and do not. An alternative explanation could be that tree $\delta^{13}\text{C}$ signatures change in response to moisture stress or transpirational demand. This is consistent with variability in photosynthate $\delta^{13}\text{C}$ documented in other studies (86; 34; 7).

Similar root respiration enrichment trends were not observed in Newfoundland soils, which show characteristically smaller variations in soil matric potentials due to cooler, wetter climate. In Nova Scotian soils, moisture varies markedly through the season, as shown by trends in soil water matric potentials plotted in Figure 3.5. These data suggest that the enrichment (over 6 permil) of root respiration observed at the fine textured P2 site could be related to moisture stress, because it occurs when soil matric potentials are very high. At LV sites, however, high matric potentials are not achieved despite large annual variability in soil volumetric water content, which reached a low of 10% during the sampling period roughly correspond to the period of root respiration enrichment. At all sites, the timing of soil volumetric water content minima, seasonal temperature maxima, and root signature enrichment trends were all coincident, indicating that several factors related to transpirational demand could be implicated. While this mechanism is suggested as one that may explain the marked periods of enrichment in root respiration isotopic signatures, it may also explain more subtle variability in root respiration signature through the rest of the growing season.

The observed temporal variability in root respired $\delta^{13}\text{CO}_2$ at these sites points to both advantages and disadvantages in attempts to partition root and microbial contributions to respiration using this approach. The main disadvantage is that source signatures must be characterized continuously by this technique rather than once at the outset of a study. The exception is at sites where it is likely or where it has been determined that root respiration will not vary in signature according to hydraulic or other effects. Temporal variability did, however, hold positive implications for partitioning, as it pushed source signatures farther apart at certain times of year, potentially improving opportunities, albeit temporarily. For researchers interested in plant carbon allocation, the ability to capture isotopic signatures of root respiration offers a potentially interesting tool that could be combined with aboveground techniques.

These observations also have implications for aboveground partitioning studies, and provide one possible mechanism to explain the observations of Knohl et al. (86), who noted short term isotopic variations in soil contributions to ecosystem respiration. Due to the temporal variability of the root respired $\delta^{13}\text{CO}_2$ at this site, soil respiration signatures would have to be characterized continually to identify soil isoflux signatures (110), especially since root respiration signatures may well exhibit shorter term variations than observed here.

3.3.4 Belowground source partitioning opportunities

As mentioned earlier, and as outlined by Phillips and Gregg (118), the error involved in determining source proportions in a single stable isotope model depends primarily on two factors; the degree of source isotopic differentiation and the error/variability inherent in source and mix $\delta^{13}\text{CO}_2$ measurements. We define a "realistic" partitioning

opportunity as one that would require fewer than 20 samples of each source and the resulting mix, to distinguish between root and microbial sources $\pm 10\%$ with 95% confidence intervals.

The number of required replicate samples of each source, and the gas mixture are calculated here using IsoError04.xls (118). Input values for statistical modeling are shown in Table 3.3. Total random errors associated with microsite spatial variability, CF-IRMS and isotopic offset estimates due to invasion+diffusion are expressed as the Root Mean Square (RMS) of all errors rather than a cumulative sum, since all are unlikely to occur in the same direction. Because they are difficult to determine accurately, errors associated with invasion + diffusion corrections were estimated at 1 permil (about 15 % of the correction offsets). It is necessary in the partitioning error analysis to account for isotopic uncertainty that could be caused by non-biological factors. Atmospheric invasion + diffusion effects are influenced by respiration rate and soil gas diffusivity, both of which show large characteristic variation across forest floors.

Table 3.4 lists partitioning opportunities in terms of sampling replicates needed to partition with good statistical certainty. We see only three real partitioning opportunities, all at the sites in Nova Scotia during temporary root respiration $\delta^{13}\text{CO}_2$ excursions towards enriched values. During most of the growing season, source differences are too small relative to natural variability and errors/uncertainties to allow for adequate partitioning.

3.4 Conclusions

Stable isotopic partitioning of soil respiration may be possible where source signatures are distinct and variability is low (118). In this study, we assessed the potential application of isotopic tools for partitioning sources of soil respiration, by using trench/intact sites to measure $\delta^{13}\text{CO}_2$ signatures of root, microbial and overall soil respiration. We monitored these signatures through the latter half of a growing season, and documented variability in source signatures. Despite easily available statistical tools to support partitioning efforts (118) and prior use of stable isotopes in field partitioning studies (74), this study represents the first attempt to systematically monitor source signatures, spatial and temporal variability, and errors/variability associated with physical transport effects in the field.

We observed signatures of microbial respiration that were temporally stable, and reflected the signature of tree/litter signatures. In contrast, root respiration varied temporally by as much as 6 permil. The degree of total variability was, however, highly regionally dependent, and was observed at all sites in Nova Scotia but not in Newfoundland. The average $\delta^{13}\text{CO}_2$ signature of root respiration was site-specific and was more enriched (2 sites), more depleted (2 sites) or approximately the same as microbial respiration (1 site). This may be related to transpirational demand, a known control on $\delta^{13}\text{C}$ discrimination in tree photosynthates.

Using observed errors and variability, we assessed real partitioning opportunities using the error analysis model of Phillips and Gregg (118). We calculated required sampling replicates of both sources and the gas mixture, given $\pm 10\%$ partitioning with 95% confidence intervals. Overall, variability was high relative to the difference in root and microbial source signatures, so actual partitioning opportunities were

limited to soils in which root signatures showed temporary enrichments that helped maximize source differentiation. However, in order to capitalize on this temporal variability in root respiration, signatures would have to be monitored continuously for use in respiration source partitioning, for both belowground and aboveground applications. While unattractive for source partitioning, continuous monitoring of root signatures may have applications for other process studies, in particular when coupled with aboveground photosynthate $\delta^{13}\text{C}$ monitoring, to help unravel the metabolic sources and controls on root respiration.

Overall, results suggest that isotopic dynamics in root and microbial sources are highly site specific and temporally variable. There are implications here for belowground partitioning studies, and also for aboveground ecosystem partitioning strategies that identify soil contributions based on isotopic fingerprint. More work has to be done in relation to physical fractionations associated with atmospheric invasion, diffusion, and sampling methodologies. The cumulative magnitude of these effects far outstrips natural $\delta^{13}\text{C}$ differences in source signature. Important research contributions can be made in this area, specifically to quantify the uncertainty/spatial and temporal variability associated with these mechanisms. This information would be applicable to future isotopic studies at the soil surface, in addition to aboveground isoflux studies.

Table 3.1: Site locations, soil and vegetation characteristics

Site Name	Location	Soils	Forest composition
LV1	Lakevale, Nova Scotia 45°45'13"N, 61°59'09"W	Coarse Texture Well drained	Mixed Hardwood/Softwood Intact, 80 years old
LV2	Lakevale, Nova Scotia 45°45'13"N, 61°59'09"W	Coarse Texture Well drained	HW overstory, SW understory Selective Harvest 1996
P2	Pomquet, Nova Scotia 45°39'34"N, 61°50'26"W	Fine Texture Poorly drained	Mixed Hardwood/Softwood 40 year regeneration
NFF	nr. Pasadena, Newfoundland 48°57'24"N, 57°37'17"W	Fine Texture Rocky, Wet	Fir stand 60 years or more
NFS	nr. Pasadena, Newfoundland 49°05'42"N, 57°25'39"W	Fine Texture Rocky, Wet	Black Spruce stand 60 years or more

Table 3.2: Summary of $\delta^{13}\text{C}$ transport offsets and variability for the measurement period.

Site	Trench Headspace increase (ppm)	Intact (ppm)	Trench Net Offset (permil)	Intact Net Offset (permil)	Trench Offset Variability* (permil)	Intact Offset Variability* (permil)
LV1	741	656	4	4	0.5	0.2
LV2	884	628	4	4	0.4	0.2
P2	722	417	4	4	0.7	0.1
NFF	940	655	4	4	1.0	0.9
NFS	970	579	4	4	0.6	0.9

*due only to changes in respiration rate

Table 3.3: Root and microbial source differences during the sampling period. Natural variability and estimates of error associated with transport offsets are also shown as standard deviations from the seasonal mean. Letters a through d represent different measurement sorties for each site.

Site	Source Difference (permil $\delta^{13}\text{C}$)				Reproducibility Error (permil $\delta^{13}\text{C}$) Nat. Variab+IRMS	Offset Correction Error (permil $\delta^{13}\text{C}$) Modeling
	a	b	c	d		
LV1	1.32	2.12	4.53	1.33	0.71	0.80
LV2	1.21	2.67	5.58	2.34	1.04	0.75
P2	1.53	6.05	2.01	2.64	0.83	0.83
NFF	2.49	0.17	0.60	na	1.05	0.80
NFS	1.11	0.04	0.21	na	1.02	0.80

Table 3.4: Source partitioning opportunities analysis using IsoError04.xls (118). Letters a through d represent different measurement sorties for each site.

Site	Replicates required (sources, mix)			
	a	b	c	d
LV1	>100	62	9	>100
LV2	>100	51	12	68
P2	>100	9	>80	47
NFF	64	>100	>100	na
NFS	>100	>100	>100	na

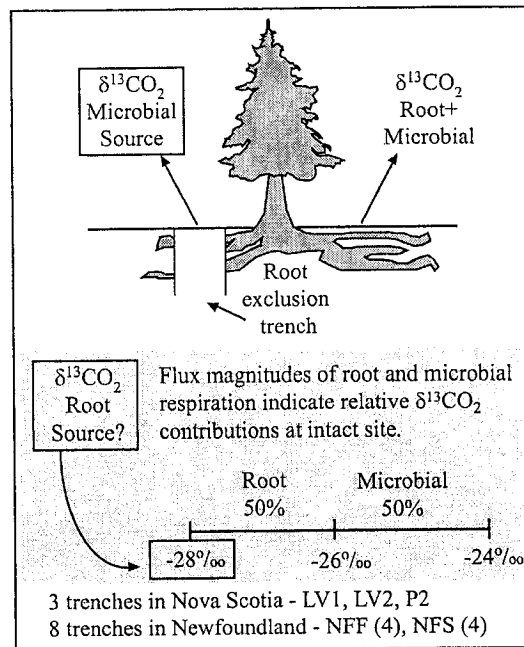


Figure 3.1: Methodological approach, whereby trench plots were leveraged to obtain information about isotopic signatures of microbial and root respiration.

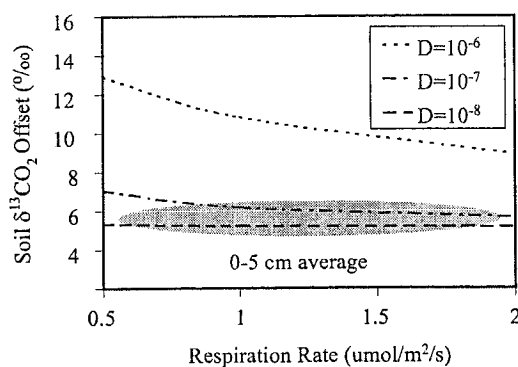


Figure 3.2: Transport offsets as a function of respiration rate and diffusivity for the top 5cm of soil.

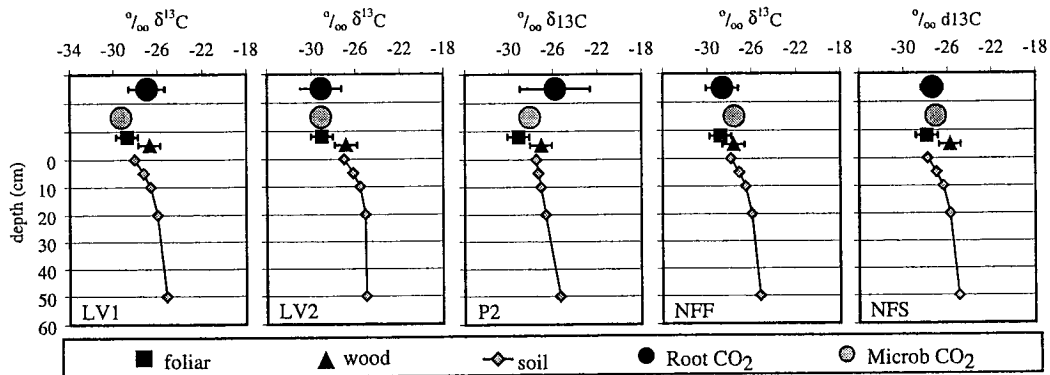


Figure 3.3: Corrected isotopic signatures of respired $\delta^{13}\text{CO}_2$ as measured using our root exclusion-based monitoring approach, relative to ecosystem vegetation and soil $\delta^{13}\text{C}$ values for each site.

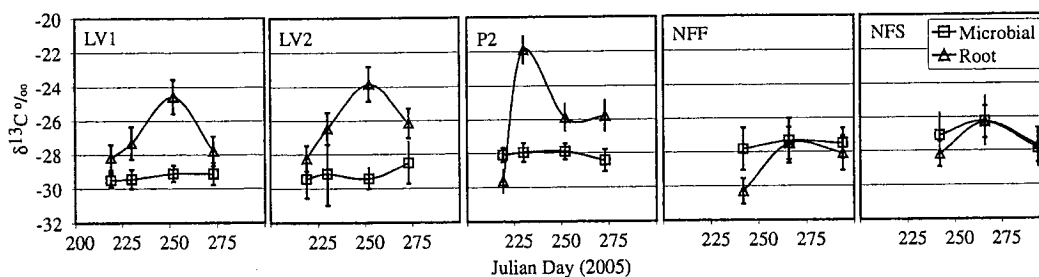


Figure 3.4: Temporal variability of root and microbial respiration signatures through the sampling period.

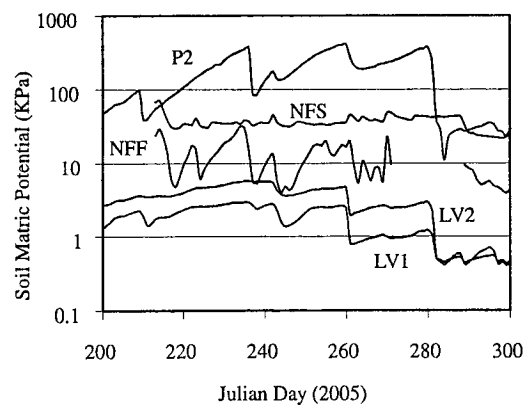


Figure 3.5: Soil matric potentials through the growing season at study sites in Nova Scotia (LV1, LV2, P2) and Newfoundland (NFF, NFS).

Chapter 4

A new method for in-situ soil gas diffusivity measurement and applications to trace gas studies

Preamble

This article forms the basis for a paper submitted to the Journal of Geophysical Research - Biogeosciences.

Abstract

Subsurface approaches to soil CO₂ monitoring are becoming increasingly popular for process studies in terrestrial carbon research. When used in conjunction with a diffusion model to determine CO₂ production, subsurface methods require good estimates of effective soil gas diffusivity (D_e). Outlined here is a novel membrane probe and continuous flow system for in-situ soil gas diffusivity measurements. Laboratory tests confirm performance across the range of CO₂ diffusivities found in natural soils. Field tests were performed across a range of soil moisture contents by artificially hydrating eight soils over a period of three to seven days. These soils were representative

of a range of textural classes in Eastern Nova Scotia, Canada. The absolute values of diffusivity, and also the rate at which diffusivity decreased with increasing soil moisture content was typically quite different from model predictions. When used in conjunction with subsurface trace gas studies, the robust site-specific diffusivity measurements described here can improve the reliability of CO₂ production estimates, to facilitate production comparisons between depths and across sites.

4.1 Introduction

In temperate forests, soil respiration accounts for approximately half of total forest respiration and is regarded as the least well understood component of landscape carbon balance (157). Many research efforts have been aimed at determining the physical controls on total soil respiration (127; 92), and to that end, a wide variety of methods have been employed. The large-scale eddy covariance towers that are in operation at many key research sites are not able to resolve CO₂ dynamics at the soil level and surface based CO₂ measurements are required. Subsurface approaches to soil CO₂ monitoring are becoming increasingly popular (45; 68; 71; 128; 165) and while substantially more equipment intensive than surface flux measurements and limited in terms of spatial coverage and averaging, they offer significant advantages for resolving soil gas processes. Advantages include excellent vertical resolution of CO₂ dynamics in the layered soil system, and the ability to estimate instantaneous CO₂ production at specific depths. Such soil CO₂ production estimates are helping to clarify temperature controls on soil respiration processes (127; 45). This is especially true for shorter time scales when measured surface fluxes may often lag well behind actual CO₂ production due to CO₂ storage in the soil profile (71).

When used in conjunction with a diffusion model to determine instantaneous CO₂ production (41; 58; 27), subsurface methods require good estimates of effective soil gas diffusivity (D_e). Concentration profiles of natural or injected ²²²Rn (96) or other tracers (158) have been used to determine diffusivity in the field, but many researchers defer to empirically-derived approximations such as the Millington model (106; 103) or improved models that require soil -specific input parameters (108; 107). Unfortunately, diffusivity models tend to perform better in some soils than in others. And, more importantly in cases where diffusivity fluctuates regularly, this parameter can control output from the gas production model because modeled diffusivity values may change by an order of magnitude or more during the course of a week, whereas the relative variation in trace gas concentrations is much smaller (128). There are several approaches that allow for laboratory testing of intact soil cores collected in the field (45). These have the advantage that soil gas diffusivity can be determined on a relatively fine scale that would be difficult to measure with ²²²Rn concentration profiles, and in highly organic substrates such as soil litter that are not clearly dealt with in diffusivity model approximations. There remains, however, the potential for changes to soil physical properties (e.g. soil aggregation, compaction etc) that could have a large influence on resulting values. It would be desirable to directly evaluate soil gas diffusivity in the field, as this would minimize potential problems with alteration to diffusivity in extracted soil cores and allows for field conditions to be monitored in-situ at the time of soil gas measurement.

Some field methods allow assessment of D_e at the soil surface (105). In waterlogged sediments, a method has been used to determine gas diffusivity based on diffusion of

a tracer away from a membrane of known diffusivity (126). Similar waterproof gas-permeable diffusion membranes have been applied to soil gas studies (65; 70), but they have been used primarily for gas sampling purposes rather than to determine soil gas diffusivity. In these studies, samples have been taken either 1) by pulling soil air through the microporous surface, or 2) by sampling from a volume of air that has been allowed to equilibrate across the membrane. The latter diffusion sampler is well suited to tracer-based gas diffusivity methods if constructed in a continuous flow loop and coupled to a gas detector.

This paper outlines the development of a membrane probe and continuous flow system for in-situ soil gas diffusivity measurements. This method is designed to determine soil gas diffusivity using diffusion of CO_2 across a permeable membrane. The system is also capable of continuous high temporal resolution soil CO_2 monitoring with minimal subsurface impacts. Laboratory tests will be presented in addition to soil diffusivity-moisture curves that were obtained from in-situ measurements at eight sites during periods of artificially induced soil hydration. We assumed that diffusivity-moisture curves would be highly site specific, resulting from the range of individual soil characteristics including soil porosity, aggregation, structure, compaction, organic content and especially macropores created by frost, soil organisms or root activity.

4.2 Material and methods

The following section outlines the mathematical theory and the development of the diffusion probe and related components. Methodology related to laboratory testing and field tests of continuous CO_2 monitoring and soil gas diffusivity testing are also described.

4.2.1 Diffusion probe theory

In a homogeneous semi-infinite, source-free half-space the CO_2 gas concentration, C , at depth z due to a time varying concentration at the boundary is governed by the one-dimensional unsteady gas diffusion equation, which is analogous to the response of the ground to sudden changes in temperature [e.g., (10; 11)]. The concentration anomaly at depth z , $C(z, t)$, due to a step change in surface temperature is obtained from the forward model (33):

$$C(z, t) = C_0 \operatorname{erfc}\left(\frac{z}{2\sqrt{Dt}}\right), \quad (4.2.1)$$

where erfc is the complementary error function, D is the gas diffusivity, t is time, z is depth (positive downwards) and C_0 is the concentration at some initial time. At $z = 0$, the rate of change in concentration or loss of diffusing substance from a semi-infinite medium is given by:

$$\left(\frac{DdC}{dz}\right)_{z=0} = \frac{DC_0}{\sqrt{\pi Dt}}. \quad (4.2.2)$$

The first term in Eq. 2 is Fick's first law and equivalent to mass of diffusive gas flux per unit area per time, the amount of mass crossing a given surface area over a given time interval (MF) is given by integrating the right side of equation (2) with respect to t , provided that the concentration C_0 does not change,

$$MF = \int_0^t \frac{DC_0}{\sqrt{\pi Dt}} dt \quad (4.2.3)$$

For known concentration gradients, time intervals and surface areas, diffusivity can be estimated from the following rearrangement of equation 3,

$$D = \frac{\pi}{t} \left[\frac{MF}{2C_0} \right]^2 \quad (4.2.4)$$

Equation 4 can be used to solve for one-dimensional diffusion of a tracer away from (or to) a membrane where an instantaneous step change in concentration has occurred on one side of its surface, provided that the following conditions are met: 1) One-dimensional diffusion is a reasonable approach given the length to width ratio of the membrane surface, and the shape of the area outside the membrane in which soil gas concentrations are altered by the measurement technique. 2) The diffusive fluxes initiated by the step change in concentration are not of sufficient magnitude to change the concentration of the porous medium C_0 in equation 1. That is, for soil testing, soil concentrations must be well buffered against any concentration changes introduced by the technique. 3) In order to be used in the assessment of soil gas diffusivity, the membrane must have a diffusivity coefficient equal to or less than that of the surrounding soil. If the membrane limits the rate of diffusion, equation 4 can be used to solve for the diffusivity of the membrane surface.

The resulting measurement actually represents the "effective diffusivity coefficient", the sum of all soil transport processes occurring during measurement. The probe's membrane surface and internal volume are subject to the same pressure fluctuations as in surrounding soil that may be induced by factors such as wind pumping, atmospheric pressure changes or temperature fluctuations. These fluctuations are typically considered to be insignificant and diffusion the dominant soil transport mechanism (141; 71), however, based on this theory the diffusion probe would integrate diffusivity, permeability and all driving forces that can be described by basic diffusion/mass flow theory.

4.2.2 Physical description of diffusion probe and related components

The most important component of a diffusion probe is the membrane, which must be able to withstand field conditions (i.e. resistant to tearing, waterproof etc) and maximize rates of gas diffusion. In membrane construction, this is accomplished by carefully controlling pore sizes, which are larger than gas molecules of interest but smaller than liquid water molecules (other fluids have different capillarity characteristics). Pore size is relatively fixed among commonly available waterproof-breathable membrane materials but pore counts per unit area vary significantly. A variety of commonly available membranes were tested for diffusive potential in an apparatus consisting of two cylinders of known volume separated by the membrane being tested. Stable CO₂ concentrations were maintained on one side of a membrane of known surface area and CO₂-free air was circulated at a known rate through the other cylinder and into a Licor LI-7000 IRGA that continuously monitored CO₂ concentration. The gaseous diffusivity coefficient was calculated using the mass fluxes across the membrane once a steady state concentration gradient was attained. In general, smaller concentration gradients indicated greater membrane diffusivity. A DuPont spun polyolefin membrane commonly used in building construction was chosen. It was highly tear resistant, waterproof (holds better than 3.5 m water column) and had high rates of diffusivity (CO₂ diffusivity coefficient of $\sim 8.5 \times 10^{-6} \text{ m}^2/\text{s}$), exceeding soil gas transport rates under most normal soil conditions present in Eastern Canada.

Two variations of diffusion probe were built for this study, one for “permanent” deployment, and a “direct push” probe that can moved between sites easily. Both use 19 mm O.D. machined PVC carriers approximately 125 mm long with four slits

along its length, offset from one another by 90° but connected hydraulically so that there are two distinct flow loops on each side of the probe. These flow loops share PVC inlet and outlet tubes that lead to the soil surface. The membrane is bonded to the carrier and covered with a tight-fitting fine stainless steel mesh cylinder for protection and to maintain internal volume against the pressure of the surrounding soil. Both inlet and outlet tubes have Luer Lock valves at their ends.

Both types of probes are installed by drilling a hole into the soil of slightly smaller diameter than the probe, and pushing in the probe. The permanent probes require that the hole be sealed with a mixture of soil and bentonite. Aside from a drive tip, the direct push probes incorporate a 50 cm long extension on the back end of the probe with a fattened section to self-seal the probe in the drilled installation hole. The direct push probes can be hammered in and removed by pulling on the extension, while the permanent probes can only be removed by digging. Both permanent and direct push probes can be installed via the surface (vertical installation) or a pit (horizontal installation). The permanent probes are simple to fabricate and smaller, making them more convenient for lab work. When deployed in the field, they are allowed to settle for 1-2 weeks before making measurements, while the direct push probes can be used immediately as a result of superior hole sealing characteristics.

As shown in Figure 4.1, the diffusion probe operates in conjunction with a simple closed loop flow system. A small rotary vane pump (Clark Solutions) moves gas through the loop between the probe and infrared gas analyzer (LICOR LI-820 with 5 cm optical path). Using two small valves, flow can be diverted through a soda lime column just upstream from the gas analyzer for autozero functions and to scrub CO_2 from the loop. A Campbell Scientific CR10X-2M datalogger controls all valve and

pump functions in addition to logging LI-820 data and performing all final diffusivity calculations. The entire system is housed in a waterproof field portable box. Figure 4.1 shows a general layout of the diffusivity system components, closed loop gas flows and electrical connections. To minimize pressure gradients in the system that could induce mass flow across the membrane surface, relatively large tubing and internal orifices were chosen in order to maintain a slow loop flow speed. Simulations of pressure differences across the membrane surface as a function of gas linear velocity were performed for areas within the probe before final design and pump selection to minimize trans-membrane mass flows, and to ensure that they would be insignificant relative to diffusive flux across the membrane under normal conditions. Similarly, any components of the system (primarily the valves and column) that exert limitations on flow were placed immediately upstream of the pump so that vacuum would not be present within the probe body.

Measurement cycles are controlled automatically by the datalogger, and can generally be divided into three parts; concentration, scrub, and recovery. During the first (concentration) part of the measurement period, the logger uses the probe as a reservoir of air that has been allowed to equilibrate to soil conditions (65). Where the system is left connected to the probe between measurements, the probe is already equilibrated with soil gas concentrations, which is recorded as C_0 . In cases where permanent probes are surveyed occasionally, they are sealed from exchange with atmospheric air during non-sampling periods. When the IRGA and associated tubing are connected to the buried probes and flow is initiated, air in the IRGA is displaced and the IRGA "sees" only the CO_2 that was resident in the probe for approximately 30 seconds before mixing starts. The maximum value attained during this period is

recorded as C_0 in the datalogger memory.

The next parts of measurement cycle involve scrubbing and re-equilibration. Circulating air is directed through the valves and into the soda lime column, scrubbing CO_2 from the loop. Once loop concentrations are negligible, a normal flow pattern is resumed and the datalogger monitors the recovery of the loop concentrations to the ambient soil concentrations at 1 hz. The run is terminated before full equilibration occurs, usually at 15 minutes, which typically corresponds to approximately 25 per cent re-equilibration at normal field diffusivity values. Full re-equilibration is not required for calculations, but greater levels of re-equilibration increase accuracy. The typical 15-minute measurement interval is a reasonable compromise between measurement speed and accuracy, especially when doing survey measurements. The final diffusivity value is calculated based on mass flux of CO_2 into the probe over the measurement period, calculated by the change in concentration, loop volume and membrane surface area. This value is written to the datalogger output file.

4.2.3 Laboratory tests

Several basic laboratory tests were designed to test the general performance of the diffusion probe system. Firstly, experiments were used to determine the constants of membrane area and internal volume of the probe and system to satisfy the requirements of equation 4, which requires measurements of CO_2 mass flux, calculated using the change in concentration in a given volume through a given membrane area. Membrane area was calculated from design drawings and the internal volume of the closed loop was assessed by covering the membrane with impermeable plastic, scrubbing CO_2 from the flow loop, and measuring the dilution of a very small slug volume of injected CO_2 gas. This dilution test was repeated each time changes were made to

the system.

The first six probes constructed were then tested in media of known diffusivity to determine performance of the system. This test was designed to determine whether a probe-specific calibration factor would be required due to small differences in assembly or materials. Tests were carried out on six probes in two mediums of known CO_2 diffusivity, atmospheric air ($1.6 \times 10^{-5} \text{ m}^2/\text{s}$ at 25°C) and ultrapure water bubbled with 1000ppm CO_2 for ~ 24 hrs ($1.6 \times 10^{-9} \text{ m}^2/\text{s}$ at 25°C). The experiment was repeated four times for each probe in each media.

Further tests using packed columns were conducted to test the diffusivity system. Plastic ABS cylinders 10 cm I.D. and 20 cm long were packed with pre-wetted sand to achieve 22.5 per cent and 35 per cent volumetric water contents. A stainless steel screen at each end contained the column and the probe was inserted before packing through a hole in the side of the column. Small reservoirs in the end of the column were fitted with inlet and outlet valves through which standard gases of two separate concentrations (2238 ppm and 0 ppm CO_2) could be introduced to develop a gradient across the column. Inlet and outlet flows were carefully balanced and measured using an Alltech DFC-HR low flow chromatography flowmeter. Static diffusivities were calculated using the mass CO_2 increase in the CO_2 -free carrier gas per cross sectional column area as the gas flowed through the reservoir. Diffusivity was measured using the probe after purging the column with the standard gas solution. Modeled values were also generated using a modified Millington relationship (103) using the bulk density and moisture information. All measurements were conducted within hours of packing the columns to minimize any drying effects.

4.2.4 Field tests

During the summer of 2004, diffusivity-moisture curves were established at eight sites for both the organic matter - mineral interface and at 25 cm depth at eight research sites where subsurface concentrations are being monitored to establish subsurface CO₂ production rates. Table 4.1 provides details of the site characteristics.

The direct push diffusivity probes were installed horizontally at the given depths from a dug pit, with the membrane area directly under the center of an artificial “rain” disk of 1m² (r=57 cm) sitting ~20 cm above the soil surface. The underside of this disk was fitted with a 15m coil of PEX tubing with 0.5 mm holes every 5 linear cm. When water was pumped from our 220 liter reservoir into the tubing, the result was an evenly distributed wetting of the soil. A Campbell Scientific CR10X-2M datalogger was used to 1) monitor two CS615 soil moisture probes installed at diffusivity measurement depths, 2) to switch the pump for a given interval at the start of every hour and to 3) cue the diffusivity systems to measure at the end of each hour. The cumulative depth of precipitation over time is shown in Figure 4.2. Soil moisture information was recorded every five minutes, and diffusivity every hour. The entire experiment lasted approximately 40 hours at each site, and was repeated several times at one site (P2) to completely saturate the soil. Calculations suggest that under most field test conditions, each probe’s radial distance of influence was between 3 and 5 cm. This large radial distance suggests that potential small imperfect contact points between soil and membrane would not affect the soil diffusivity estimates.

4.3 Results and Discussion

4.3.1 Laboratory tests

Variation in maximum diffusivity rates between probes was negligible (standard deviation of $0.2 \text{ m}^2/\text{s}$) relative to the large range of diffusivities measured (5 orders of magnitude difference). The absolute error was estimated at approximately 5 per cent for all probes. The tests carried out in free air were limited by the membrane CO_2 diffusivity (approximately $8.5 \times 10^{-6} \text{ m}^2/\text{s}$). In most soils, it is unlikely that the membrane will limit diffusion, but in the unlikely event that measured soil diffusivities are equal to the membrane diffusivity, results will have to be discarded. Figure 4.3 summarizes performance across a normal range of soil diffusivity values. Assuming a linear relationship between measured and real diffusivities, calibration tests other than those conducted in water and air are not strictly required, as soil CO_2 diffusivity values generally fall somewhere between these extremes. But, our column tests using sieved silica sand at two different levels of hydration help to confirm 1) linearity across the measurement range and 2) correspondence with known or theoretical values.

4.3.2 Field tests

Results of field hydration tests show that as Millington and other models predict, diffusivity values fall as soil moisture content rises, giving the diffusivity-moisture curve a negative slope. Table 4.2 shows the diffusivity-moisture constants from linear regression relationships developed at sites where diffusivity values did not approach those of saturated conditions.

Soils in proximity to one another (all LV sites, all P sites and both CC sites) tended to have roughly similar slopes to the diffusivity-moisture relationship, except where

sites have been freshly disturbed, such as the LV3 and P3 clearcut sites. In contrast, organic-mineral (OM) interface and deeper (25 cm) diffusivity-moisture curves were not predictably different from one another. In fact the diffusivity values at the OM interface were quite similar to deeper values, although the hydrologic conditions were different, with the OM interface tending to be either drier (LV) or wetter (P,CC) than deeper soils.

During field wetting experiments, we were successful in fully saturating only one soil. At P2, the reservoir was filled three times (over 600 l total) to verify that saturated diffusivity values generally agreed with the known diffusivity of pure water. Figure 4.4 shows the results of testing at this site, along with data from the diffusivity model of (103). Only at field capacity and saturation do the measured and modeled values agree. In general the measured diffusivity values are much higher and drop precipitously as saturation is approached. This suggests that the most of the diffusive transport occurs via a well drained network of macropores. Where soils did become nearly or fully saturated, a third order polynomial (i.e. Figure 4.4) provided the best fit. In general, the linear fits are robust (average r^2 of over 0.80, $n=14$) but valid only to some point just below saturation, before the precipitous drop in diffusivity towards diffusion through pure water.

The absolute values of diffusivity and rate at which diffusivity decreased was typically quite different from that predicted by the modified Millington model. Regular light rains kept soils at field capacity all summer, and along with topographic position and drainage, determined the starting moisture value for these field experiments. As summarized in Table 4.3, field capacity diffusivity values were higher than modeled values for the LV soils, whereas for most other soils the model over-predicted rates

of diffusive transport. The model performance was worse at greater initial moisture contents. We did note a hysteresis effect, where somewhat different diffusivity values were associated with wetting and drying, by analyzing data from the period after the reservoir had emptied. But, soil drying occurs so slowly that we were unable to include this as part of our tests.

Further investigations of the discrepancy between measured and modeled values for all soils revealed that the model error factors (measured/modeled results) could be plotted as a function of soil water content, as shown in Figure 4.5. Across all site soils, the McCarthy/Millington model (103) mildly overestimated at small moisture contents, and moderately underestimated diffusivity at high moisture contents. Laboratory testing revealed excellent correspondence in packed sand columns, where pore size and shape was well controlled. In natural settings, however, soil particles are aggregated and distributed heterogeneously. A simple conceptual model may explain the discrepancy between measured and modeled values: Small pores are not well recruited for diffusion because they are plugged with organic matter, cemented into aggregates, filled with capillary water and/or unconnected to one another (high tortuosity). Instead, the network of well-connected and well-drained larger pores in the inter-aggregate space is preferentially recruited for diffusion. The influence of capillary water is smaller in large pores, so that gas diffusivity values do not fall precipitously until near saturation. This macropore network likely remains relatively open as a result of root turnover, seasonal frost, and activity of soil organisms.

4.4 Conclusions

A new portable membrane probe and system to rapidly measure CO₂ diffusivity in the field has been tested successfully in the laboratory, and applied to quantification of diffusivity rates as a function of moisture content at eight field sites. Diffusivity-moisture curves at field sites were highly significant and where soils weren't brought to full saturation, they were best described by a linear fit. At high soil moisture contents, we observed a strong departure from this linear behavior, with very rapid decreases in soil gas diffusivity near saturation. In these circumstances, site-specific polynomial relationships provided the best overall fit across the measured soil volumetric water contents.

In the laboratory, measured, modeled and known rates of diffusivity agreed well with one another, but in the field, the diffusivity model over- or under-predicted diffusivity by up to two orders of magnitude. Field values were in agreement only at soil saturation, when diffusive transport takes place mainly through the water phase. Across all sites, the discrepancies between measured and modeled results at moderate and low moisture contents were found to depend on the soil moisture content itself. One possible explanation for this behavior in field soils is the preferential recruitment of large pores for diffusive transport.

Ideally, when subsurface concentration measurements are being used to calculate trace gas production, diffusivity should be measured at the time of sampling. Where this is not convenient, the next best approach would be to exploit moisture data and calculate diffusivity based on a measured diffusivity-moisture curve for the specific soil and depth in question. The arbitrary use of a diffusivity model that isn't specifically developed for the site in question should be regarded as the least appealing approach,

and model output should be regarded only as a rough estimate.

When used in conjunction with subsurface trace gas studies, the robust site-specific diffusivity measurements described here can help to improve the reliability of production estimates, to facilitate production comparisons across sites and between depths. This will help improve results from all types of subsurface trace gas studies, whether used for the purposes of budgeting or to elucidate information on soil processes.

Note

Provisional Canadian and American patent applications have been filed to protect intellectual property described in this chapter.

Table 4.1: Site characteristic including aboveground vegetation, and soil textural information after (28) and (84).

Site	Vegetation Type	Max Porosity %	Sand %	Silt/Clay %
LV1	Forest	~ 45	85	15
LV2	Selective Cut	~ 45	85	15
LV3	Clear Cut	~ 45	85	15
CCF	Field	~ 45	20	80
CCW	Forest	~ 45	20	80
P1	Field	~ 50	20	80
P2	Forest	~ 50	20	80
P3	Clear Cut	~ 50	20	80

Table 4.2: Constants and regression significance for diffusivity-moisture curves at all sites, both for mineral (25cm) and OM-interface depths. Where data is not available (na), polynomial fits were applied.

Site	Depth (cm)	Linear Slope	Linear Intercept	Linear r^2
LV1	OM interface	-1.1823	0.7506	0.85
	25	-1.2028	0.8260	0.89
LV2	OM interface	-1.8450	0.8077	0.87
	25	-0.9267	0.7522	0.71
LV3	OM interface	-9.3390	3.4206	0.98
	25	-1.8346	0.9599	0.74
CCF	OM interface	-0.5666	0.4464	0.46
	25	-4.7419	1.4387	0.72
CCW	OM interface	-1.9489	0.9988	0.73
	25	-7.0188	1.7716	0.92
P1	OM interface	na	na	na
	25	-1.6574	0.9015	0.90
P2	OM interface	na	na	na
	25	-0.4232	0.3873	0.84
P3	OM interface	-28.3110	5.0238	0.69
	25	-40.3300	5.9479	0.97

Table 4.3: Initial soil volumetric water contents at the start of rain experiments (field conditions), and corresponding soil CO₂ diffusivity values (both measured and modeled).

Site Ratio	Moisture v/v %	Meas. D_e $m^2/s \times 10^{-6}$	Mod. D_e $m^2/s \times 10^{-6}$	Error Factor
LV1	39.6	0.355	0.0040	89.23
LV2	30.0	0.497	0.1403	3.54
LV3	25.2	1.1856	0.4303	2.76
CCF	21.4	0.453	0.7693	0.59
CCW	15.0	0.344	1.5760	0.22
P1	38.8	0.255	0.0542	4.71
P2	24.5	0.286	0.7100	0.40
P3	11.9	1.241	2.5160	0.49

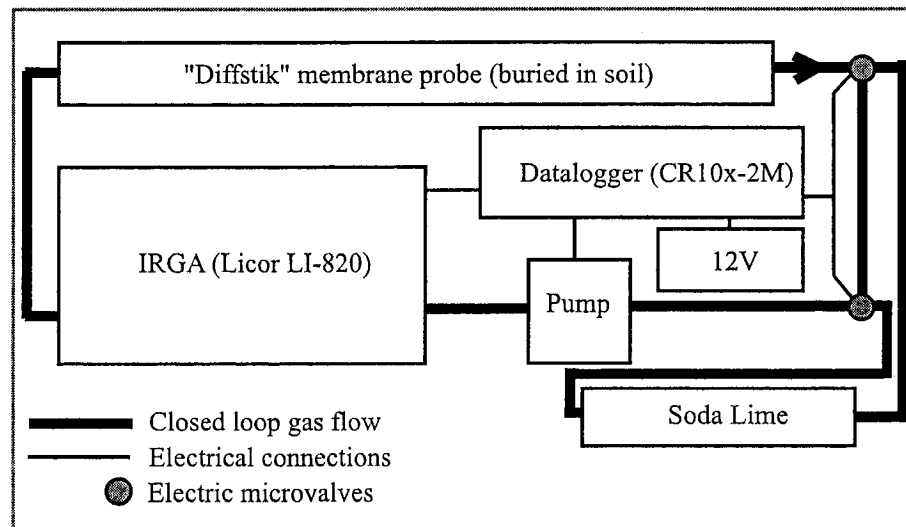


Figure 4.1: Schematic of diffusivity system, showing components, electrical connections and closed loop gas flow (clockwise direction).

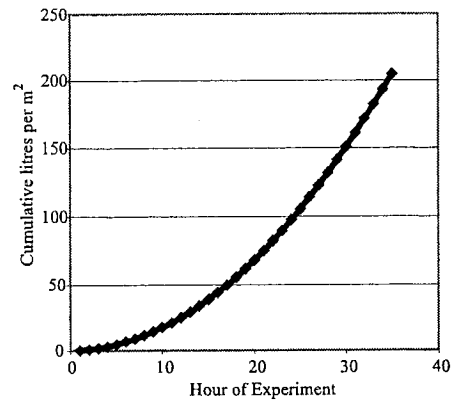


Figure 4.2: Cumulative artificial rain rate for field diffusivity testing.

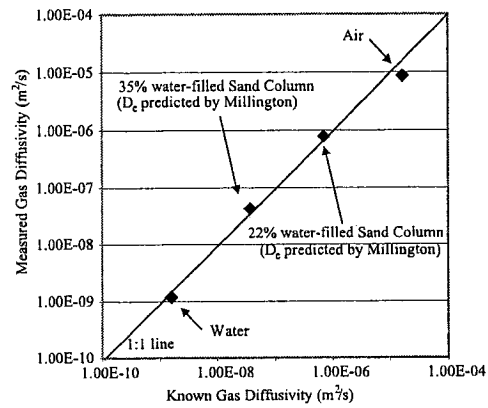


Figure 4.3: Measured diffusivities plotted against theoretical diffusivity for various testing media; pure water, pure air (both have known diffusivities) and sand columns of different moisture content (diffusivity predicted by modified Millington of (103)).

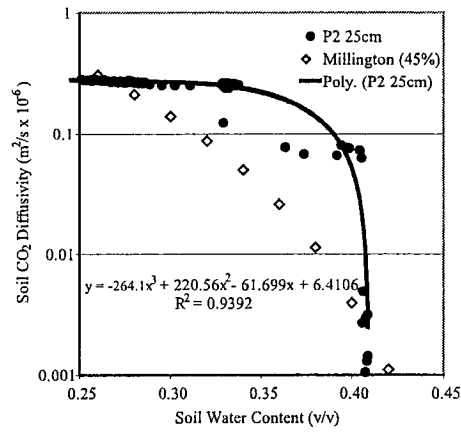


Figure 4.4: Diffusivity-moisture curve for P2, the only site that was fully saturated during the course of field testing. Open squares show modified Millington model diffusivity estimates (103) for the same soil (total porosity was estimated at 43).

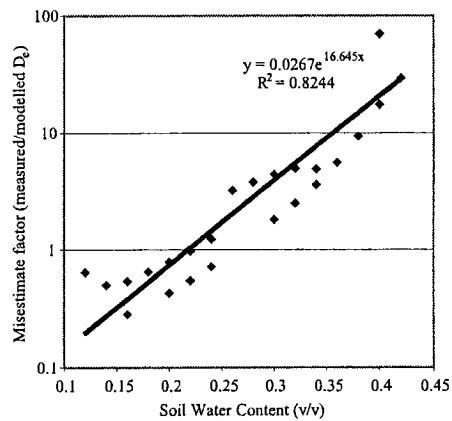


Figure 4.5: Diffusivity error factors (measured/modelled D) as a function of soil volumetric water content, for mineral soils (25cm depth) at all six sites where test data were fit linearly.

Chapter 5

In-situ incubations by root exclusion highlight the climatic sensitivity of soil organic matter pools

Preamble

This article forms the basis for a paper submitted to the Geophysical Research Letters.

Abstract

We use root exclusion plots, subsurface gas sampling and in-situ diffusivity measurements to quantify rates of decomposition at climatically different sites, and in vertically distinct soil organic matter (SOM) pools. Site thermal regimes are different by $\sim 2.5^\circ\text{K}$, which is approximately the expected rise in surface temperature by 2050, providing a simulation of soil responses to future warming. At the warm site, soil organic matter activity, or "apparent quality" has not systematically declined after two years, despite a lack of new organic matter inputs and calculated SOM losses of roughly 20% at 0cm and 0.12% at 35 cm. Although decomposition-temperature

response was similar at different depths, every gram of soil C at 35 cm was more than 100 times less active in decomposition than surface soil, indicating high apparent recalcitrance, and/or the importance of limiting physical conditions. At depth, physical protection of SOM could match or even override the importance of quality and temperature in determining the future stability of deeper, resistant pools.

5.1 Introduction

Evaluating soil organic matter (SOM) responses to perturbations in the physical and biological environment are critical to understanding how the terrestrial-atmospheric carbon balance may shift in the face of climate change. Increases in primary production are expected to increase SOM inputs, however this will be offset by increases in the rates of SOM decomposition due to elevated soil temperatures (35; 127; 85; 122). Whether the balance will lie in a net gain or loss of soil carbon is a topic of current debate (87; 60; 154; 85; 52), especially the response of different soil pools to changes in soil temperature over the long term.

A variety of techniques are used to assess SOM decomposition as a function of temperature. Modeling studies have provided useful estimates of long term SOM turnover processes (153; 13) and contradictory results may be a result of assumptions about how different pools of SOM respond to temperature over time. Studies which assume a single soil carbon pool and uniform turnover rate (60) are unlikely to produce results consistent with those which assume multiple pools with different turnover times (87). Incubation experiments have also provided critical evidence (56; 124; 99), but are often conducted under non-native conditions which may explain differences in

predicted temperature response (1). In addition, the perturbation of soils in such experiments may alter soil structural properties that might otherwise play an important role in the temperature response (104; 135).

A recurrent theme in this debate is the accurate representation of natural processes and conditions, pointing to a need for further clarification of temperature-decomposition processes in-situ, where soil physical properties and environment can be preserved. Current methods alone are unlikely to resolve debates and in-situ approaches are valuable, because in addition to SOM quality, there are a suite of physical factors that contribute to the actual response of SOM to climate, vegetation and land use changes (35).

Under field conditions, it is methodologically difficult to resolve and/or quantify natural decomposition rates among different carbon pools, but isotopic labeling techniques have provided good recent information (148) in natural soils. A simple alternative is to examine rates of natural decomposition at different depths in the soil profile. Since organic matter is on average older and more resistant to decomposition deep in soil profiles, depth can be used as a proxy for organic matter resistance. This depth proxy is not strictly analogous to chemical recalcitrance, but also includes the influence of physical mechanisms that protect organic matter from decomposition, as outlined in (35). The basic methodology exists whereby we can examine depth-specific decomposition rates (127), but this has not been applied in a "field incubation" using root-free soils where CO_2 is generated solely from microbial activity.

This subsurface sampling approach can be hampered by issues surrounding the magnitude of gas diffusivity values used in method calculations. Carbon dioxide production at each depth is assumed to be the difference between the flux across soil

layers. Flux (F) for each layer is determined from Fick's Law in one dimension.

$$F = -D \frac{\partial C}{\partial z}, \quad (5.1.1)$$

where D is the diffusivity ($m^2 s^{-1}$), C is the CO_2 concentration (gm^{-3}) and z is depth (m). In natural temperate soils where soil water content is subject to large annual fluctuations (100% to <10%), D can vary annually by 100 or 1000 times (106). In contrast, the annual variability in C may only be a factor of 3. As a result, diffusivity is the primary numerical determinant of CO_2 flux (and production) estimates. When using a subsurface approach, diffusivity values must be tightly constrained.

Using a newly developed in-situ diffusivity measurement system and a subsurface sampling approach, we are examining the temperature response of organic matter pools that should be functionally distinct by virtue of depth and organic matter properties. Site thermal regimes are different by ~ 2.5 degrees K, which is approximately the expected rise in global surface temperature by 2050 (76). We seek to identify potential changes in organic matter dynamics that reflect a shift from a steady source of new organic matter to one which has been both devoid of fresh inputs, and over the same period subjected to elevated soil temperatures. The unique characteristic of this study is that we examine decomposition-temperature relationships among different organic matter pools in-situ, ensuring minimal soil physical and microclimatic disturbance.

5.2 Methods

The study was conducted in Lakevale, Nova Scotia, Canada, in sandy soils supporting temperate hardwood/softwood forest, a 40 ha portion of which was clearcut in 2002. We have one study site within the clearcut (WARM) and another in the intact

forest (CTRL). Harvesting has provided a step change in soil temperature and carbon biogeochemistry. The WARM site was left relatively free of slash, and regrowth has been curtailed by regular weeding. Fresh C inputs since harvesting are negligible, though some material may be blown in from adjacent areas.

We use root exclusion techniques at the intact CTRL site to mimic the absence of autotrophic activity of the WARM soils, allowing us to compare natural decomposition dynamics at both sites (21; 95; 92; 51). A 2 m² square trench, lined with vapor barrier to a depth of 50cm to prevent root regrowth, and to minimize lateral CO₂ gradients, was installed during summer of 2003 at the CTRL site. It was left for one year prior to this study, to allow recovery from a respiratory burst initiated by the presence of freshly severed roots.

Subsurface CO₂ samples were drawn from 50 cm long subsurface gas equilibration tubes, installed horizontally from a temporary pit. Subsurface samples were determined using a LI-7000 gas analyzer configured (continuous flow) and soil CO₂ surface fluxes were measured using a Licor LI-8100 survey system. Both sites are equipped with above and below ground meteorological instrumentation (12). Soil carbon profiles were determined by EA-IRMS and ranged from 3.5-1.5% and 5.5-2.6% at the CTRL and WARM sites, respectively.

This study is made possible by direct in-situ measurements of soil CO₂ diffusivity. We established a unique diffusivity-moisture curve for each site across a wide range of soil moisture contents, using automated in-situ diffusivity measurements and artificially-induced soil hydration (129).

In order to comment on in-situ heterotrophic C cycling within different and functionally distinct soil carbon pools, we calculated heterotrophic subsurface CO₂ production for two depths (0 and 35 cm) using the multilayered diffusion model approach (128). The depths selected maximize vertical separation, and act as proxies for different pools of soil carbon; a resistant pool at depth and a fresh, labile pool at the surface. Typically, pools are identified instead on the basis of physical or chemical fractionation (14; 104), but we assume vertical differences are due primarily to organic matter quality, although limitation by physical factors may also be important. We assess differences quantitatively by representing microbial CO₂ production at a standardized temperature, per unit mass of soil carbon, per unit time, to pinpoint the actual activity of carbon in the different depth pools.

5.3 Results and Discussion

5.3.1 Methodological validation

To test the accuracy of our diffusivity values, we used them along with shallow subsurface concentrations to derive theoretical surface fluxes, which were compared against known measured values (Licor 8100). We also generated diffusivity values using the (103) model. Results are presented in Figure 5.1, which shows the good overall correspondence between measured and theoretical fluxes when diffusivity-moisture curve values are used. Modeled diffusivity values resulted in underestimates of surface flux, routinely by 10x at the disturbed WARM site. This site is also typically wetter than the CTRL site (season average volumetric water content of 0.3 vs. 0.2, respectively), due to decreased post-harvest evapotranspiration. We have observed similar issues surrounding universal applicability of modeled values elsewhere (129), perhaps the

result of a well-drained macropore network that is preferentially recruited for gas transport in some soils.

5.3.2 Temperature response in SOM pools

We plotted the temperature dependence of SOM decomposition for different depths, as shown in Fig. 2. There was no apparent difference in the temperature response of surface or deep pools at either site. Surface soil layers did, however, produce >100x more CO₂ than deeper layers despite minor variations in soil C content through depth at both sites. This suggests that the quality of C is different between depths and that magnitudes of SOM are not driving the observed differences. These results reinforce the fact that this vertical approach is successful in isolating functionally different SOM pools.

5.3.3 Respiratory indicators of apparent pool quality

The decomposition rate per unit kg of soil C, normalized for soil temperature (10 *deg* C) is shown in Fig. 3. This index of “apparent quality” allows comparison of the activity of each gram of soil organic matter C, in shallow and deep pools, and sites where thermal regimes may differ. The apparent quality of CTRL and WARM deep layers are comparable, and every gram of soil organic matter is again >100 times less active in decomposition than at the surface. The low apparent quality of the deep soil layers indicates high recalcitrance, and/or the importance of limiting physical conditions at this depth. While we would expect the apparent quality of SOM to decline over time at the WARM site (higher respiration, no fresh C inputs), to date we do not observe detectable differences. At this site, organic matter may be protected to some degree by the higher soil moisture contents.

Similarities in quality between sites results in sustained SOM consumption that is 30-50% higher at the WARM site due to elevated soil temperatures, as shown in Figures 5.3c and 5.3d which plot the observed decomposition rates during the study period, normalized for C content. The site thermal regimes are different by ~ 2.5 deg K during the growing season, which is approximately the expected rise in surface temperature by 2050 (76), providing an interesting analogue to future climate. At the WARM site, calculated post-harvest losses of SOM (rates vs. stocks) are approximately 10%/yr at 0 cm and 0.06%/yr at 35 cm, and to date show no apparent increase in resistance which would help slow C losses. Relative to the ephemeral surface layer, the deep pool is well buffered against losses due to the high resistance/low "apparent quality" of this layer and/or limiting physical conditions. Despite deceptively small SOM loss rates at depth, losses of this magnitude are still significant on large scales; if equivalent (0.06%/yr) losses were expected in other soils as a consequence of global climate change and were unmatched by an increase in gross primary productivity, they would approach 1 PgC/yr, roughly two thirds the annual anthropogenic C emissions of the United States.

Here, we observed decomposition rates in the deep soil profile that are many times ($> 100\times$) slower than at the surface, even after correcting for differences in carbon content. Incubation studies such as (52) have shown far smaller differences (3-4x) between surface and deep soil decomposition rates. This suggests that physical mechanisms of protection (35) could match or even override the importance of carbon quality in determining future stability of deep, resistant carbon pools. In situ, many factors such as texture, aggregation/occlusion, soil moisture, oxygen availability and compaction act to limit "potential" respiration rates observed by incubation.

In addition, temperature-covariant soil parameters may obscure the pure temperature response. For example, early season saturation strongly inhibits soil respiration at these sites, but when temperatures rise, soils dry, pores become aerobic, and high soil gas diffusivities help replenish consumed oxygen. Together, these boost decomposition rates to "normal" values over short time and temperature intervals. Although in-situ pool decomposition studies must be approached and interpreted carefully, they should assume an important role in questions pertaining to future soil organic carbon stability, providing contrast and context to the decomposition "potentials" and climatic responses observed during laboratory incubation. They can also be used to highlight important limitations of the soil physical environment that, because of burial depth, preferentially act on resistant pools.

5.4 Conclusions

The goals of this study were to isolate decomposition rates and temperature response in vertically distinct, root free soils at sites differing climatically. The novelty of this study is associated with the in-situ, physically-based subsurface approach, which resolves decomposition dynamics in vertically and functionally distinct SOM pools. This approach is critically dependent on accurate in-situ diffusivity values, which make inter-site comparisons possible.

Deep soil layers (35 cm) produced approximately 100 times less CO_2 than surface (0 cm) layers, suggesting that organic matter quality differs between these layers/pools. There was no substantive difference in the decomposition-temperature response of surface or deep pools at either site. The "apparent quality" of surface and deep pools, or decomposition rate normalized for temperature and carbon stocks,

was also comparable between sites. At the WARM site, two years of +2.5 *deg* K growing season temperatures, high sustained respiration rates and lack of fresh C inputs have not yet had a measurable impact on SOM resistance, which would help to slow losses.

Relative to surface, which has likely lost 20% of soil carbon due to warmer site temperatures and lack of inputs, deceptively small SOM loss rates at depth are significant when considered on large scales. Decomposition rates in the deep soil profile were sluggish (100x lower than at the surface), and far slower than deep soil respiration rates measured by incubation, suggesting that physical mechanisms of protection could match or even override the importance of quality in determining the future stability of deep, resistant carbon pools at our study sites.

Although covariant factors can make the interpretation of data more complicated, in-situ studies provide the only hope of capturing "true" decomposition-climate responses. In-situ and laboratory research should be conducted in tandem to indicate whether measured, or "potential" laboratory responses are in fact observed in the field, and the extent to which additional physical factors influence organic matter pool stability.

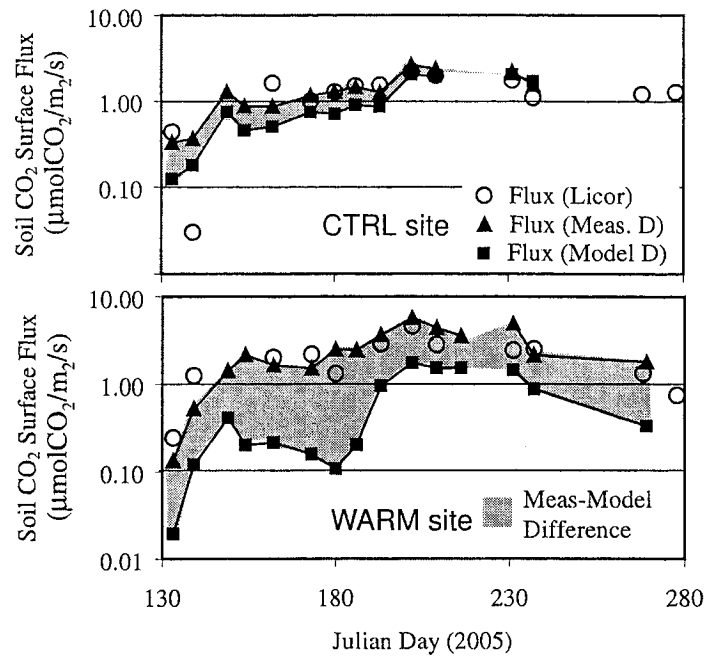


Figure 5.1: Soil surface fluxes from decomposition, as measured (open circles), and calculated from subsurface concentrations using measured and modeled diffusivities.

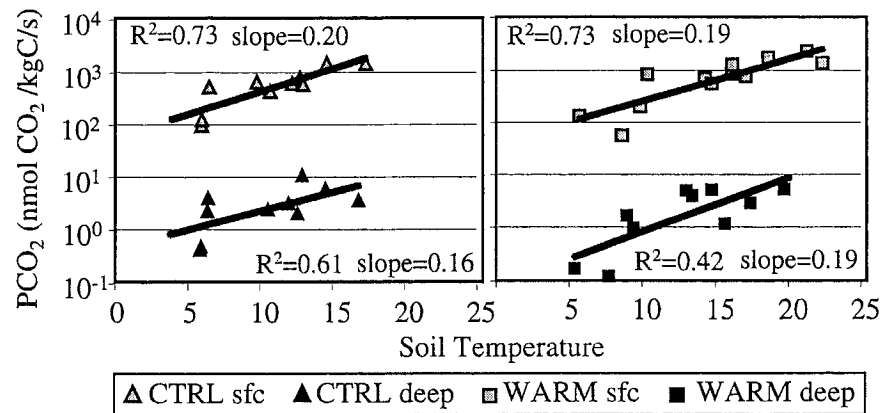


Figure 5.2: Relationships between soil CO₂ production and soil temperature at two depths.

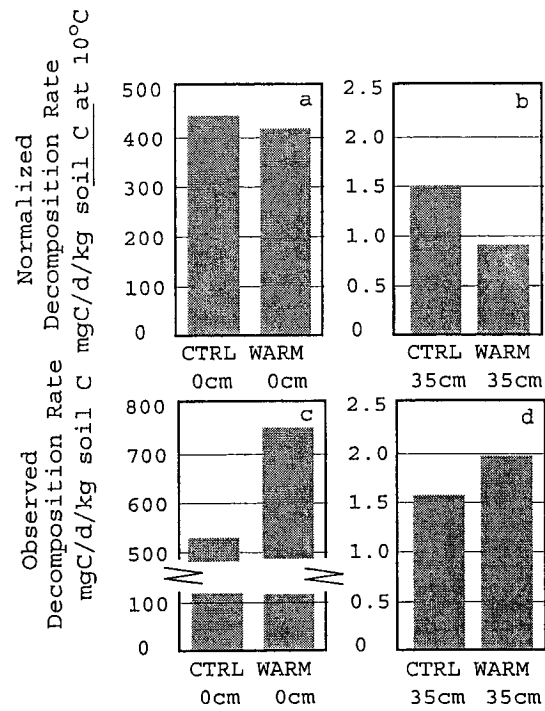


Figure 5.3: Decomposition rate standardized for soil C content and temperature (a,b), showing the “apparent quality” of SOM, and actual field decomposition rates (c,d) per unit mass soil C.

Chapter 6

Soil microbes and roots respond to different environmental cues

Preamble

This article forms the basis for a paper that will be submitted to the Global Change Biology.

Abstract

Despite widespread research efforts, aspects of soil respiration dynamics need further clarification, in particular the individual responses of microbes and roots to climatic cues. Root respiration constitutes roughly half of total soil CO₂ efflux but climatic constraints on the process are largely unknown. This in-situ study seeks to apply a new combination of existing techniques to measure rates of CO₂ production and climatic response from a) 5 vertically distinct pools of soil organic matter along a continuum ranging from labile to recalcitrant, and b) roots in 3 soil layers. We hope to answer a simple question: Do roots and soil microbes respond to different climatic cues? Both microbial and root respiration activity was highest between the surface

and 10cm, accounting for roughly 90% of profile production. We also observed surprisingly large microbial contributions from >50cm depth. Microbes and roots were both sensitive to temperature but displayed unique behavior; microbial decomposition rates continually increased with increasing temperature, and in contrast, root respiration rate response diminished with increasing temperatures. This suggests that soil microbial respiration could become proportionately more important in a warmer world. Root activity was more universally related to temperature across study sites, while microbial responses were distinctly site-specific. Neither roots nor microbes showed strong sensitivity to soil moisture, except for deeper microbial sources. This unique behavior, combined with our limited understanding of root processes, highlights the importance of partitioning methodologies in soil carbon research.

6.1 Introduction

Soil respiration represents the largest land-atmosphere flux in the global carbon cycle. Carbon dioxide is produced naturally in soils through microbial decomposition and by plant roots, which contribute in roughly equal quantities based on vegetation type (67). Natural soil respiration rates exceed all anthropogenic emissions by approximately ten times, and significant carbon reservoirs in soil relative to the atmosphere (76) mean that small but widespread increases in respiration rates have the capacity to profoundly affect atmospheric CO₂ concentrations over both short and long time intervals.

It has been widely established that soil respiration is sensitive to temperature (35; 127; 85; 122), so that increased rates of emission in a warmer world could act as a positive greenhouse feedback. Increases in soil emissions, however, will be party or

fully offset at the landscape level by increases in aboveground primary productivity, though the likely balance of these processes remains a topic of debate (87; 60; 154; 85; 52). The role of moisture may confound anticipate trends, as soil respiration is inhibited by high soil moisture contents in many ecosystems (35), and higher soil moisture values are forecast for certain regions (76) in response to an intensification of the hydrologic cycle.

In addition to temperature and moisture, there are other physical determinants on soil respiration that may change in response to climate. These secondary determinants of soil respiration rates are summarized by Davidson and Janssens (35), and include factors such as soil texture, aggregation and gas diffusivity rates. Additional geophysical parameters such as soil thermal conductivity, heat capacity and heat content (127) may also play important roles. The relative importance of these secondary determinants is not well understood, and these could either offset or enhance the primary control of temperature on respiration.

Methodologies that offer improved resolution of biological soil respiration dynamics are helping to clarify the individual climatic responses of root and soil microbial sources. Traditionally, soil respiration approaches have examined total soil CO₂ efflux (121) and this information forms the basis for our current understanding of controls on soil respiration (136). Much of the observed variability in temperature and moisture dependence from surface flux studies could potentially be the result of unique climatic sensitivities of contributing sources. Researchers are addressing this issue using field and laboratory techniques to separate flux into its components (respiration partitioning). Two recent reviews of respiration partitioning methods are provided by Hanson (67) and Kuzyakov (89). A variety of isotopic tools have been applied

(137; 49; 21; 145; 117; 97; 132), and at appropriate sites, provide resolution of relative microbial/root contributions and/or turnover time. Non-isotopic partitioning tools such as trenching (21; 95; 92; 51) and/or other root exclusion techniques such as girdling (74) are used to identify both relative source contributions and flux magnitudes, for process study or for budgeting purposes.

Despite widespread partitioning efforts, there is still little information about the relative climatic sensitivities of root and soil microbial processes. Root respiration, in particular, is poorly characterized though it constitutes approximately half of total soil emissions (67). Information points to the fact that roots and microbes respond to different microclimatic and biological factors, from studies involving seasonal fine root turnover and dynamics (18; 75), isotopic information about the coupling of root respiration to photosynthesis and aboveground processes (74), and partitioning studies elucidating the individual seasonal and temperature-specific dynamics of these processes (159; 92; 22). These and other studies provide some evidence as to climatic determinants on these processes, but the potential responses of root respiration rates to climate change are still poorly characterized. Biochemical controls on root respiration are complex (5) and research in this area, particularly in-situ studies on mature, natural ecosystems, is sorely needed.

The goal of the study is simply to determine whether controls on root and microbial activity are indeed different. In order to accomplish this goal, we measure relative rates and climatic dependence of root and microbial respiration. By coupling a subsurface approach (127) with root exclusion studies (21; 95; 92; 51) and in-situ soil gas diffusivity measurements (129), we are able to measure rates of CO₂ production resulting from root and microbial respiration at distinct depths. Vertical

resolution allows us to monitor decomposition in several distinct pools of soil organic matter along a continuum ranging from labile (surface) to resistant (deep), due to a combination of chemical recalcitrance and limitations imposed by the physical environment (35). We can also monitor root respiration rates in two or more distinct soil layers. We use this information in conjunction with meteorological data to assess the climatic dependence of each respiration source.

We demonstrate that in-situ, the root contribution is substantial, shows different temperature response relative to soil organic matter decomposition in either labile or resistant pools. Both are relatively unaffected by soil moisture at our sites.

6.2 Methods

The study was conducted at research sites in proximity to Antigonish, Nova Scotia, Canada where other carbon balance initiatives are ongoing. The Lakevale sites (L1 and L2) have coarse soil textures and support temperate hardwood/softwood forest. The L1 site is undisturbed, and has been free of harvesting or other management for at least 80 years. The L2 site was pre-commercially thinned in the mid 1990s, the result being a canopy composed of mature hardwoods, and a dense understory of fir saplings. The third study site (P2) in Pomquet, Nova Scotia, also supports mixed hardwood/softwood forest but has very fine soil texture.

A root exclusion trench plot (21; 95; 92; 51) of 2 m² square was dug at each site during the summer of 2003. Trenches were lined with vapor barrier to a depth of 50cm to prevent root regrowth, and to minimize lateral CO₂ gradients. This study was conducted during the growing season of 2005, and the two years post trenching has allowed recovery from a respiratory burst initiated by the presence of freshly

severed roots.

Subsurface CO₂ samples were drawn from 50 cm long subsurface gas equilibration tubes, fabricated from 0.5" I.D. perforated PVC tubing and covered with water-proof/breathable membranes. Sampling tubes were installed by drilling horizontally from a temporary pit. Soil CO₂ concentrations were determined using a LI-7000 gas analyzer configured in a continuous flow arrangement and soil CO₂ surface fluxes were measured using a Licor LI-8100 survey system. Both sites are equipped with above and below ground meteorological instrumentation (12).

Carbon dioxide production profiles were calculated for adjacent trench and untrenched areas using the approach first outlined by (41). Production for a given layer is calculated by flux across layer i minus input from layer $i-1$ below, from the surface to maximum sampling depth,

$$p_{CO_2i} = [D_{e_i}(\frac{C_i - C_{i-1}}{z})] - [D_{e_{i+1}}(\frac{C_{i+1} - C_i}{z})], \quad (6.2.1)$$

where p_{CO_2} is production of CO₂, C_i and D_{e_i} are the concentration and effective diffusivity for layer i , respectively, and z represents depth. Root and microbial contributions to the production profiles were assumed to be the difference in production between root-free and intact areas. The primary assumption of trenching experimentation is that trenched and adjacent areas are identical. Some natural variation between these areas is acceptable and unavoidable. Where we observed differences between trenched and untrenched plots that exceeded 50% in basal fluxes of CO₂ into the bottom of our experimental profile, data was discarded.

. The vertical resolution offered by the subsurface approach allows us to comment on decomposition rates in distinct soil organic matter pools, and on root respiration in distinct soil layers. For soil organic matter, depth acts as a proxy for different pools

of soil carbon; labile pools near the soil surface, and a gradient towards increasingly recalcitrant pools at depth. Typically, pools are identified on the basis of physical or chemical fractionation (14; 104), but we assume that vertical differences in microbial respiration are due primarily to organic matter quality, in combination with limitation by physical factors such as inhibition by high moisture contents may also be important (35).

This subsurface sampling approach can be hampered by poorly- constrained gas diffusivity values used in method calculations. In natural temperate soils where soil water content is subject to large annual fluctuations (from 100% to <10%), effective soil gas diffusivities (D_e) can vary annually by 100 times or more (106). The annual variability in profile concentrations is much smaller (3-4 times) and as a result, seasonal variation in diffusivity values can become the primary numerical determinant of CO₂ production.

We use direct in-situ measurements of soil CO₂ diffusivity to established a unique diffusivity-moisture curve for each site across a wide range of soil moisture contents, using automated in-situ diffusivity measurements and artificially-induced soil hydration (129). Data presented here uses these diffusivity-moisture curves, which were found to be superior to model-generated (103) values in their ability to predict overall rates of soil respiration.

The response of root and microbial respiration to volumetric soil water content and soil temperature was evaluated using the Proc Reg procedure of SAS (SAS Institute, 2004). Several regression models were evaluated to derive empirical relationships between respiration and volumetric soil water content and soil temperature to determine which of these two factors control soil respiration within these forest soils. The

adjusted R^2 was used to judge the goodness of fit of the regression models and determine the relative contribution of each of these two environmental factors and to assess the adequacy of simple versus quadratic regression models. The adjusted R^2 takes into account the number of predictors in the model and is useful for comparing different regression models (167). All statistical significance was judged at the 5% probability level.

6.3 Results and Discussion

6.3.1 Concentration Profiles and Validation

Concentration profile measurements at L2 suggested that respiration dynamics differ between intact and trenched areas. As shown in Figure 6.1, basal (>50cm depth) CO_2 flux was appreciably different between adjacent plots. Since this natural variability may affect the entire depth profile, we excluded the L2 site from our analysis. Other potential problems associated with trenching experiments are rarely discussed in the literature but may include, for example, the development of lateral gradients of CO_2 in the absence of vapor barrier linings (92). The solutions to are a) critical examination of available methodologies, b) better replication, c) validation using concentration profiles, pre-trenching measurements or other, and of course d) retroactive data exclusion as done here. The last is certainly the least attractive option.

Figure 6.2 shows the total soil profile CO_2 production as calculated from concentration gradients using measured and modeled diffusivities, and measured soil CO_2 surface fluxes. Measured diffusivity values result in reasonable production estimates, which agree with surface fluxes and fall along a 1:1 line when plotted. In contrast, when modeled values (103) were used for calculation, CO_2 production was grossly

overestimated. This is not likely due to model-specific problems, but to model application in varied field settings, and in soils with different characteristics than those of sand-filled laboratory columns for which the Millington-based model was developed. Soil diffusivity values were generally lower than predicted by the model despite depressed volumetric water contents, suggesting that aggregation and other structural controls in natural soils may reduce the availability of interconnected pore space for diffusion even in relatively dry soils.

6.3.2 Relative contribution of root and microbial respiration

The depth-specific respiration rates for L1 and P2 are shown in Figure 6.3. For both microbial and root sources, the most active zone is near the surface. The top 10 cm of soil is responsible for approximately 90% of profile CO₂ production. Microbial respiration tapers off with depth more slowly at the coarse textured L1 site than it does at the fine textured P2 site. Root activity was approximately constant through the profile below 10 cm depth, with deeper layers contributing only about 5% of that observed nearer the surface. Basal respiration from underneath trenched areas (>50 cm) was a surprisingly large component of total soil respiration, and was similar in magnitude to production between 10 and 50 cm. Although microbial respiration drops off sharply beneath surface layers, the cumulative effect of deeper layers is important at these sites.

Vertical resolution of processes allow us to comment on decomposition rates in distinct soil organic matter pools, and on root respiration in distinct soil layers. As shown in Figure 6.3, respiration at 10-35 cm is only about 5% of values in the 0-10 cm layer. The difference in decomposition rate grows to over 100 times if the surface layer is further subdivided so that decomposition per unit depth in the upper

2.5 cm layer is compared to that in the 10-35 cm layer. The gradient of resistance is steepest in this 0-10 cm surface layer, below which there are relatively smaller changes through the balance of the profile. Vertical resolution of rooting processes may capture the activity of different rooting types since fine roots tend to be concentrated higher in the profile than tap roots. We do not, however, have information on rooting distribution, so conclusions related to actual distributions of root types are speculative. Less speculative, however, are the clear depth linkages between root and microbial activity. Their relative zones of activity suggest important interplay between these processes, with roots concentrated at depths where they can maximize uptake of freshly mineralized nutrients.

During the growing season, relative root and microbial contributions to respiration were approximately 1.75:1 at L1 and 2.33:1 at P2. Although (67) cites approximately equal contributions on average, there is a large range of variability. Our observations are similar to those of Vogel et al. (159) in black spruce forests of Alaska, where sites with slow microbial decomposition rates had increased rates of root respiration. As shown in Figure 6.3, the P2 site has rates of microbial respiration that are much lower than those of L1 (seasonal averages of 0.55 vs. 0.80 $\mu\text{molCO}_2/\text{m}^2/\text{s}$, respectively). If this is a universal trend among forests, it may be due to increased tree allocations to fine roots, maximizing nutrient scavenging where mineralization rates are slow relative to potential photosynthesis. Large observed rates of root respiration may also be due to the "priming effect" (88) whereby microbial activity is stimulated by the presence of roots. It is uncertain whether this is important here; although the priming effect has been identified in the laboratory, results in field studies are mixed, with some attaching high importance to this process (146) while others find that it

is relatively unimportant (145) or site-specific (66).

The temporal variability of microbial and root CO₂ production is shown in Figure 6.4, relative to total profile respiration and measured surface fluxes. At P2, they mirror one another closely through the growing season whereas at L1 we observed more complex interplay between root and microbial respiration. The marked drop in root respiration rates at L1 early in the growing season corresponds to the period when leaves first appeared on trees, and not to any specific environmental condition (site temperatures and/or precipitation) that persisted during this period. We did not observe a similar drop in root respiration at P2, and because the sites are removed from one another by only ~10 km, climatic differences are not likely implicated in the site-specific root behavior.

6.3.3 Temperature and moisture response in SOM pools

We observed site-specific microbial dynamics in the different soil organic matter pools through the profile. Within the shallow depth (0-10 cm), microbial respiration increased with increasing temperature but only at L1. The response was well described by a quadratic regression model ($R^2_{\text{adj}}=0.78$, $p = 0.0009$; Figure 6.5). At depth, a significant linear relationship between soil temperature and microbial respiration was observed ($R^2_{\text{adj}}=0.78$, $p = 0.018$) but only for the P2 site.

Moisture did not have a significant effect on microbial respiration, although regression significance increased with depth. Moisture always had an inhibitory effect on microbial decomposition of soil organic matter, where high soil moisture contents limit decomposition by decreasing microbial accessibility to soil organic matter feedstocks and oxygen (35).

6.3.4 Temperature and moisture response in root respiration

Root respiration significantly increased as a quadratic function of temperature for the shallower depth; and for the deeper depth, root respiration decreased with increasing temperature (Figure 6.6). The responses to temperature were similar at the two sites within the 0-10 cm depth with higher root respiration at L1 than P2. This pattern is reversed below the 10 cm depth with P2 showing relatively greater root respiration than L1. Although the contribution of root respiration from the deeper depth was small, the observed significant relationship with temperature is worth noting. Similar to the results for microbial respiration, moisture did not have a significant effect on root respiration anywhere in the profile. The lack of response of respiration to moisture at these sites may be attributed to the fact that these sites are not typically moisture limited, and soil moisture only varies within a narrow range. Rather, respiration responds readily to moisture in tropical environments and where there are pronounced seasonal wet-dry cycles (69).

6.3.5 Total soil profile respiration

Having assessed the individual climatic controls on root and microbial respiration, we chose to combine the source data to observe the overall (total soil respiration) climatic response, as a contrast to the source-specific data. Figure 6.7 shows profile respiration (roots+microbes) vs. soil temperature and the significant linear relationships that can be plotted through each series. Although the combined relationships are reasonable, they demonstrate the importance of separating the individual responses of roots and microbes to changes in soil temperature. Microbes showed large increases

in activity with increasing temperature, while root respiration response to temperature decreased as temperature increased. This suggests that other factors may limit plant productivity and/or plant allocations to roots during the hotter parts of the growing season. As a result, microbial decomposition may be a proportionately more important determinant of total soil respiration as regional temperatures rise. In a total profile approach, these opposing strengths of root and soil respiration tend to offset one another, but in relation to climate warming, the partitioned data lends increased value to the numerous studies targeting the temperature dependence of decomposition (35; 127; 85; 122). However, the response will clearly be very complex as climate warming will also lead to changes in species composition, productivity, and plant carbon allocations. Partitioning techniques will be important for future research to resolve changes in individual ecosystem processes.

6.4 Conclusions

Using a combination of in-situ methods, we measured respiration in several vertically-distinct soil organic matter pools, and from roots in different soil layers. The most active zone for soil microbes was near the surface where labile organic matter proportions are high. Root respiration activity was also highest between the surface and 10 cm. Microbes and roots were both sensitive to temperature but displayed unique behavior; microbial decomposition rates were site specific but generally increased with increasing temperatures, and in contrast, root respiration rates reached a plateau of activity above 15° C. This means that that microbial respiration may be a proportionately more important determinant of total soil respiration at warmer temperatures. Neither roots or microbes showed strong sensitivity to soil moisture,

except for deeper microbial sources.

In practical terms, the results of this study suggest that a) total soil respiration can give reasonable information on climatic response of soil respiration, b) partitioning may not add significance to regression relationships, but adds detail about the individual behavior and potential climatic responses of root and soil microbial sources and c) available physical partitioning techniques have the ability to resolve many processes when used creatively in tandem with other techniques.

The study is a spatially-limited microsite approach that provides detailed information on small-scale ecosystem processes. To further characterize root respiration dynamics relative to those of soil organic matter decomposition, a concerted effort must be made to develop accessible partitioning techniques and to apply them broadly.

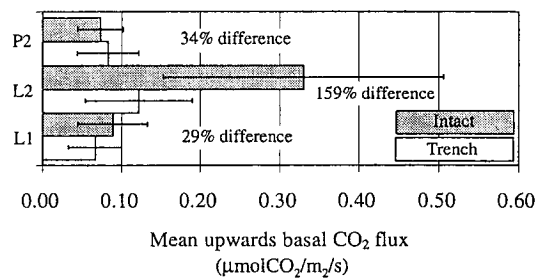


Figure 6.1: Basal CO₂ fluxes at 50cm at all trench and adjacent intact areas.

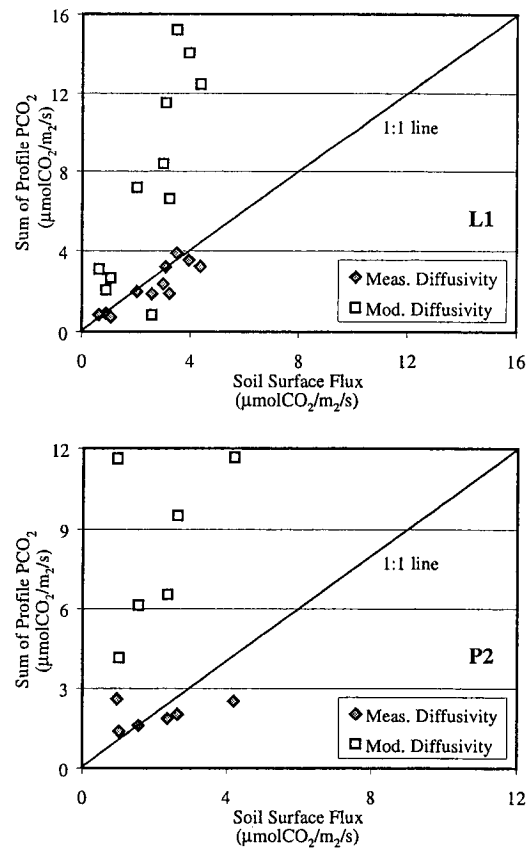


Figure 6.2: Validation of measured diffusivity data.

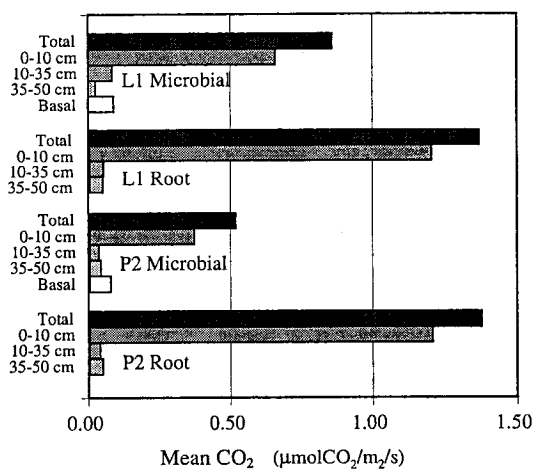


Figure 6.3: Calculated respiration rates for root and microbial sources, for different soil layers at L1 and P2.

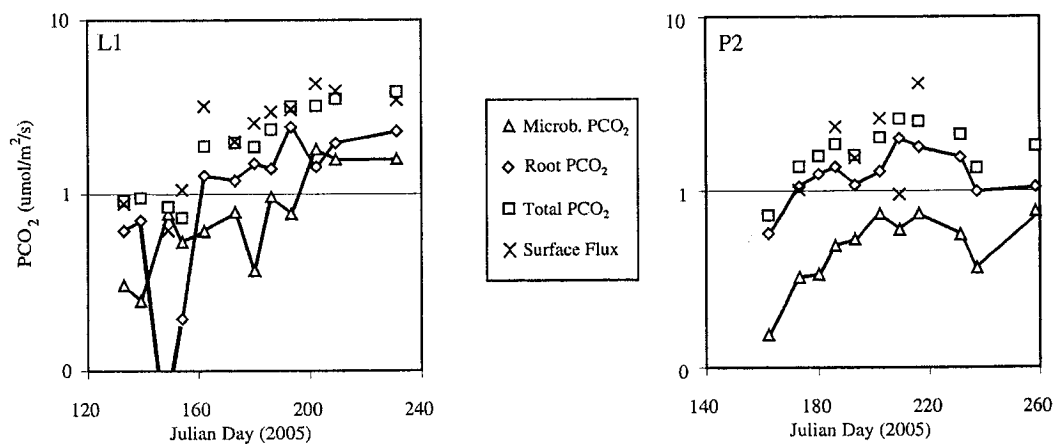


Figure 6.4: Calculated respiration rates for root and microbial sources showing temporal variability of source contributions. PCO₂ denotes production of CO₂.

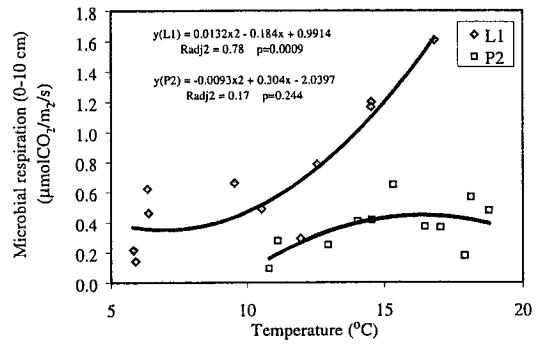


Figure 6.5: Microbial respiration (0-10cm) vs. soil temperature.

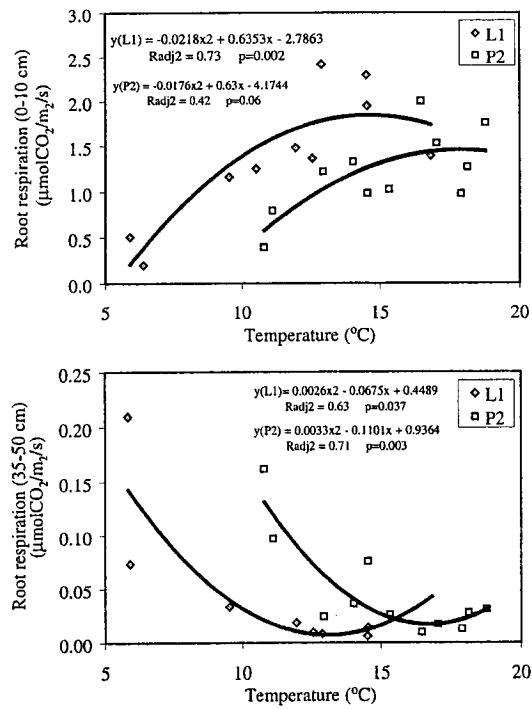


Figure 6.6: Root respiration (0-10cm and 35-50cm) vs. soil temperature.

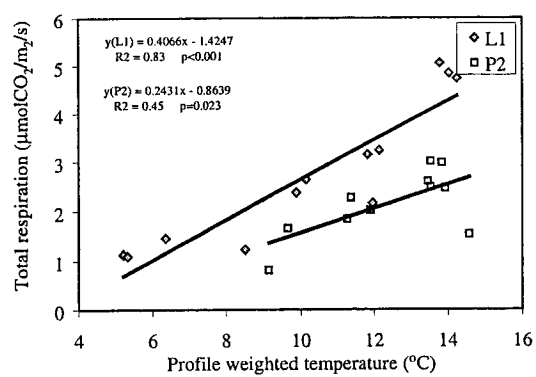


Figure 6.7: Total profile respiration (roots+microbes) vs. soil temperature.

Chapter 7

Pilot study testing high resolution partitioning of soil respiration

Preamble

This article will one day for the basis for a paper that will be submitted to the Journal of Geophysical Research - Biogeosciences.

Abstract

This chapter reports on a pilot study in which a new methodology was tested for partitioning soil profile CO₂ production at high temporal resolution. The method uses a datalogger to monitor a mobile array of HVAC CO₂ probes buried at different depths, in root exclusion and intact plots. The technique was tested three times during the growing season of 2004 at a site near Antigonish, Nova Scotia, Canada. Values of partitioned CO₂ production from root and microbial sources were calculated using a multilayer diffusion model, with soil diffusivity values obtained from in-situ diffusivity measurements. Results suggest that the method is successful, that diurnal variability is significant, and that roots and microbes show characteristically different

diurnal response patterns at different points in the growing season.

7.1 Introduction

New methodologies and/or combinations of existing approaches are important for addressing key questions in soil carbon dynamics. Hanson (67) identifies “resolution” as the key need of terrestrial scientists. Although Hanson was referring specifically to resolution of individual biological processes, improvements in technical precision, accuracy and measurement speed are equally important. Following closely on the approach outlined in Chapter 6, I report here on a pilot study to improve the temporal resolution of partitioned soil CO₂ production measurements.

In several recent field experiments, Vaisala GMP222 CO₂ probes originally designed for heating/ventilation/air conditioning monitoring have been plied to use in the soil environment (80; 150). These probes are capable of high-precision infrared-based measurements, and are available in several concentration ranges. Previous studies have used the probes to assess probe durability and the magnitude of variability in subsurface CO₂ production over a range of temporal scales. To date, the probes have not been used in field manipulations such as root exclusion, where they could replace the typical protocol of manual sampling and subsequent lab analysis. More importantly, when applied to root exclusion experiments, the individual diurnal dynamics of root and microbial CO₂ production could be examined in detail.

During the growing season of 2004, the array of Vaisala GMP222 probes were rotated between sites L1,L2 and P2, allowing collection of high temporal resolution data of partitioned resolution. Results suggest that the method is successful, that diurnal variability is significant, and that roots and microbes show characteristically

different diurnal response patterns at different points in the growing season.

7.2 Methods

High resolution sampling was undertaken at the trenched sites between May and November, 2004. The sampling rotation is shown in Figure 7.1. The trench was installed during the summer of 2003, and lined with vapor barrier. High frequency sampling was done using eight modified Vaisala GMP 222 probes attached to 0-5V OEM linearization modules (Vaisala), and a Campbell Scientific CR23X datalogger. The probes were installed in permanently-installed PVC tubes extending from above-ground to four different depths. Fine depth intervals were chosen for installation, 0, 2.5, 7.5, and 22.5 cm. The probes sat at the bottom of the carrier tube, where a neoprene seal held it tightly, preventing exchange up the tube with atmospheric air. On the bottom of each tube an orifice covered with a waterproof/ breathable membrane allowed gas exchange with the soil. Because the probes themselves were not buried directly in the soil, they were protected from direct contact with wet soils, and could also be removed easily for other applications.

One data series (5 minute intervals) was collected from each site over a ~ 10 day period in spring, summer and fall (Figure 7.1). As with other field experiments at these sites, meteorological and soil temperature/moisture data was available from permanent observatories at each site (12).

Carbon dioxide production profiles were calculated for adjacent trench and untrenched areas using the approach first outlined by (41). Production for a given layer is calculated by flux across layer i minus input from layer $i-1$ below, from the surface

to maximum sampling depth,

$$p_{CO_2i} = [D_{e_i}(\frac{C_i - C_{i-1}}{z})] - [D_{e_{i+1}}(\frac{C_{i+1} - C_i}{z})], \quad (7.2.1)$$

where p_{CO_2} is production of CO_2 , C_i and D_{e_i} are the concentration and effective diffusivity for layer i , respectively, and z represents depth. Root and microbial contributions to the production profiles were assumed to be the difference in production between root-free and intact areas. Diffusivity values for the model were taken from diffusivity-moisture relationships that were carefully established for each site using a new in-situ diffusion measurement system (129).

For the purposes of this short application note, soil profile root respiration and soil respiration are represented by the maximum interval of production for each source (just below the surface), and not profile totals. As we assume that the interval of maximum production should give the clearest indication of dynamic behavior characteristic of each source of soil CO_2 .

7.3 Results and Discussion

The bulk of microbial CO_2 production was found to lie near the surface, where fresh litter is available. Root CO_2 production was most prominent in the most heavily rooted zone, near the top of the mineral soil. Although the L2 profile was excluded from other analyses in Chapter 6, this site still appears to give adequate results for testing this methodology for the very shallow depths being studied here.

Figure 7.2 shows the partitioned data for the sampling period. In general, spring-time root respiration was high and tapered off at the end of the growing season. Diurnal variability in respiration was significant relative to seasonal variability. Daily

peaks in root and microbial respiration appeared to be offset from one another. Root respiration may show more than one daily peak, especially at the L2 site (summer).

7.4 Conclusions

This method provides a promising approach for future studies. The primary limitation to this type of study is the cost of each Vaisala GMP probe relative to their durability. Two probes were irreversibly damaged during the summer, one from condensation damage and the other from being dropped when moving the array. The probes could be protected better if installed in a container that would sit on the soil surface, where air could be circulated to and from a membrane probe buried in the ground. Another (relatively delightful) drawback of this method is the enormous dataset generated which requires significant processing time.

Once depth information is extracted carefully from this dataset or from future studies, the result will be a complete set of diurnally-resolved, vertically-separated microbial and root pools. These should give interesting information about the contrasting dynamics of soil and root CO₂ production.

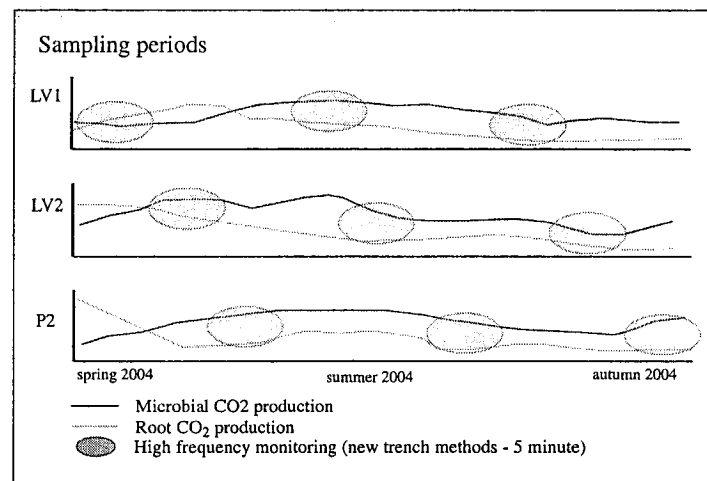


Figure 7.1: Conceptual diagram showing sampling rotation among sites for pilot study (no real data shown here).

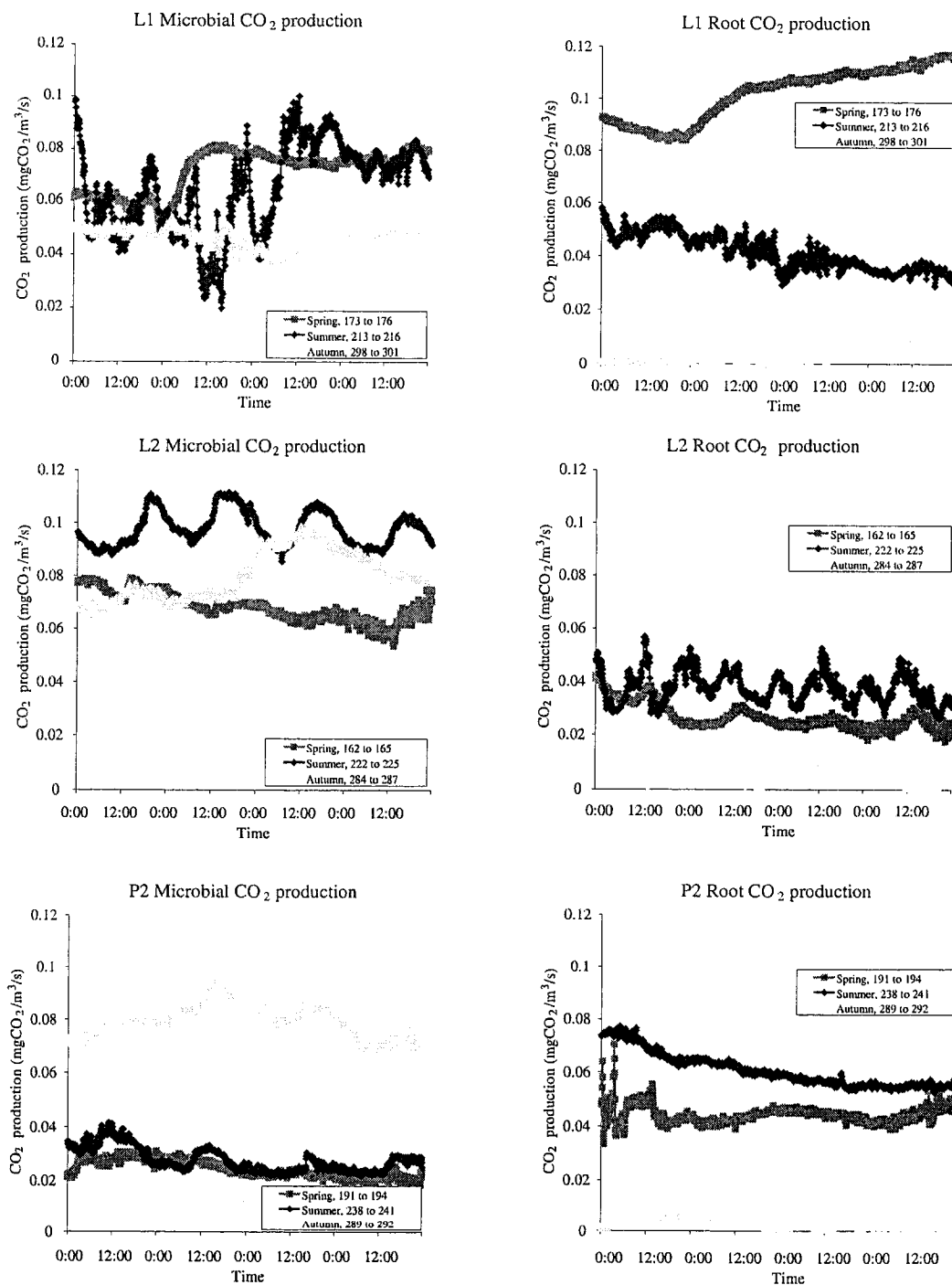


Figure 7.2: High resolution records of shallow CO₂ production from root and microbial sources.

Chapter 8

Conclusions

This thesis evaluated new and existing measurement methodologies for soil respiration source partitioning studies, in order to address a central research question: Do roots and soil microbes respond to different environmental cues?

Isotopic approaches to soil respiration partitioning

Isotopic characterizations of trees and soils at several study sites in Atlantic Canada provided the foundation for further isotopic source partitioning studies in this thesis. There were consistent and predictable patterns of leaf $\delta^{13}\text{C}$ variation within trees, and a consistent offset between woody and leafy tree tissues. Patterns were similar for both hardwoods and softwoods, but overall hardwoods had canopies that were more depleted in $\delta^{13}\text{C}$. Soil carbon $\delta^{13}\text{C}$ enrichment occurred with depth and appeared to vary according to site soil texture. Upper soil $\delta^{13}\text{C}$ values were intermediate between leaves and woody tissues, while deeper soil values suggested important contributions from more enriched tree tissues, such as persistent woody debris and possibly roots. Successful source partitioning of soil respiration requires that sources are isotopically distinct, variability is low, and that source signatures can be readily identified. The

results of site isotopic characterizations suggest that source partitioning may be possible on a site-specific basis.

A new technique for isotopic sampling of soil surface fluxes was developed to monitor isotopic signatures of soil CO₂. This approach offers advantages in sampling speed with potential reductions in isotopic error, which is important for distinguishing small differences in isotopic signature. Testing was carried out in laboratory columns, and in the field at sites where isotopic soil respiration source partitioning was potentially promising. Fractionations are associated with this sampling technique, but can be held constant under a wide range of field conditions. In field surveys at five sites across 80m² grids, variations in forest floor transport characteristics (diffusion + atmospheric invasion) were high, and possibly more important determinants of isotopic signature than would likely be associated with distinct signatures from root and microbial respiration processes. Forest floor transport variability was able to describe the magnitude of $\delta^{13}\text{CO}_2$ variability, the spatial pattern of effects, and the systematic offsets between expected and measured signatures. Because non-biologic parameters may influence forest soil $\delta^{13}\text{CO}_2$ variability, more research is needed to integrate physical considerations into biological inquiry where stable isotopes are used for fingerprinting purposes. Patent protection for the sampling method has been filed in the United States, including a proven approach to integrate the required hardware and software modifications into commercially-available soil surface flux sampling equipment.

The potential application of isotopic tools for partitioning sources of soil respiration was assessed by leveraging trench/intact sites to monitor $\delta^{13}\text{CO}_2$ signatures of root, microbial and overall soil respiration through the latter half of a growing season.

This is among the first systematic tests of natural abundance isotopic partitioning technique efficacy in the field. Signatures of microbially respired CO_2 were temporally stable and broadly reflected isotopic signatures of litter. In contrast, root respiration varied temporally by as much as 6 permil, but the degree of total variability was regionally dependent. Average $\delta^{13}\text{CO}_2$ signature of root respiration could be more enriched, or more depleted than microbial sources depending on site and sampling day. Overall, variability and error were high relative to the difference in root and microbial source signatures, limiting partitioning opportunities to specific sites and dates. The observed temporal variability in root respired $\delta^{13}\text{CO}_2$ was a major disadvantage for soil partitioning studies, and also holds implications for aboveground studies using ecosystem isofluxes to isolate soil respiration.

Physical approaches to soil respiration partitioning

When used in conjunction with a diffusion model to determine CO_2 production, subsurface methods require good estimates of effective soil gas diffusivity (D_e). There is, however, no method to practically measure gas diffusivities in-situ and most researchers defer to empirical models for diffusivity estimates. A new method, probe and fully automated system for soil diffusivity sampling was developed here, in addition to field testing with automated soil saturation experiments to establish diffusivity-moisture curves for soils at field sites. Laboratory testing established the performance of the system. In the field, absolute values of diffusivity, and also the rate at which diffusivity decreased with increasing soil moisture content was typically quite different from model predictions. When used in conjunction with subsurface trace gas studies, the robust site-specific diffusivity measurements improved the reliability of

CO₂ production estimates, thus facilitating production comparisons between depths and across sites. Patent protection for the method, system and probe is pending in Canada and the United States.

The diffusivity measurement system was used with a subsurface approach at root exclusion plots to measure rates of microbial decomposition through the soil profile at sites with contrasting climates. This is the first study to measure natural decomposition in different soil organic matter pools using a physically-based approach. Previous successful attempts have used radioisotopes, which are less accessible to most researchers. At the warm site, soil organic matter activity, or "apparent quality" had not systematically declined during the two years of warming, despite a lack of new organic matter inputs and calculated soil organic matter losses of roughly 20% at 0cm and 0.12% at 35cm. Although decomposition-temperature response was similar at different depths, every gram of soil C at 35cm was more than 100 times less active in decomposition than surface soil, indicating high apparent recalcitrance, and/or the importance of limiting physical conditions. At depth, physical protection of soil organic matter could match or even override the importance of quality and temperature in determining the future stability of deeper, recalcitrant pools.

To specifically target the primary objective of this thesis, the physically-based approach is also extended to other research sites, and to include measurements of root respiration. Rates of CO₂ production and climatic response are established a) in 5 vertically distinct pools of soil organic matter along a continuum ranging from labile to recalcitrant, and b) from roots in 3 soil layers. Both microbial and root respiration activity was highest between the surface and 10cm, accounting for roughly 90% of profile production. There were surprisingly large microbial contributions from >50cm

depth. Microbes and roots were both sensitive to temperature but displayed unique behavior; microbial decomposition rates increased in an exponential manner, and in contrast, roots respiration rates followed near logarithmic rates of increase, reaching a plateau of activity above 15° C. This suggests that soil microbial respiration could become proportionately more important in a warmer world. Root activity was more universally related to temperature across study sites, while microbial responses were distinctly site-specific. Neither roots nor microbes showed strong sensitivity to soil moisture, except for deeper microbial sources.

As a further extension of physically-based subsurface partitioning techniques, root exclusion plots were instrumented with CO₂ probes to continuously monitor rates of root and microbial respiration. Preliminary results suggest that the approach is successful, that diurnal variability is significant, and that roots and microbes show characteristically different diurnal response patterns at different points in the growing season. Physical techniques hold significant promise to improve resolution of CO₂ production dynamics in natural soils.

Our knowledge of soil-related processes is almost entirely based on studies that aren't able to resolve the contributing processes. Past in-situ methods are not able to distinguish CO₂ source OR to resolve the contributions of different variables, such as the different qualities of organic matter exploited by microbes. Total soil respiration, the historical measurement standard, gave statistically reasonable information on overall climatic response due to the opposing responses of soil microbes and roots. But, since soil microbes and plants show complex response to site climate, species composition and other factors, the complimentary relationship will not hold in the future. Results suggest that soil microbial activity may become proportionately more

important as a determinant of carbon balance. Past methods lack important detail, and the added resolution of newer methods must form the basis for projections of soil respiration under climate change and changing land use scenarios.

Chapter 9

Future Work

Partitioning of soil respiration will be an important component of many future investigations. To best address research questions, further work must be done to bring measurement methodologies, and especially field approaches, to a more sophisticated level. In-situ approaches are particularly valuable, but must be planned carefully to satisfy the needs for experimental control. At some point, a standard approach that will be accessible to many researchers may emerge. This would allow quantitative relationships to be compared across many regions, benefitting process research, and the parameterization of process models.

Resolution has been identified as a critical gap in our knowledge about soil respiration, although this challenge is slowly being met by this study and others. The next challenge will be one of in-situ experimentation, by using new techniques and resolution to get realistic and targeted results about soil respiration quickly. Rather than straightforward monitoring approaches, researchers can take advantage of contrasting field site conditions, of space-for-time substitutions. In particular, more researchers should strongly consider manipulative field experiments to obtain information. For example, in-situ soil heating experiments have been attempted successfully within

trenched plots, but not plot-scale ecosystem heating experiments, or soil moisture control by tile drainage, air sparging or other. Well constructed field experiments often become classics because of the initiative and creativity of the scientists involved.

Bibliography

- [1] Agren, G.I., and E. Bosatta. 2002. Reconciling differences in predictions of temperature response of soil organic matter. *Soil Biology and Biochemistry*, **34**: 129-132.
- [2] Amador, J.A., Y. Wang, M.C. Savin, and J.H. Gorres. 2000. Fine-scale spatial variability of physical and biological soil properties in Kingston, Rhode Island. *Geoderma*, **98**: 83-94.
- [3] Andrews, J.A., and W.H. Schlesinger. 2001. Soil CO₂ dynamics, acidification and chemical weathering in a temperate forest with experimental CO₂ enrichment. *Global Biogeochemical Cycles*, **15**: 149-162.
- [4] Arnold, S.L., and J.S. Schepers. 2004. A simple roller-mill grinding procedure for plant and soil samples. *Communications in Soil Science and Plant Analysis*, **35**: 537-545.
- [5] Atkin, O.K., D. Bruhn, V.M. Hurry, and M.G. Tjoelker. 2005. The hot and the cold: unravelling the variable response of plant respiration to temperature. *Functional Plant Biology*, **32**: 87-105.
- [6] Balesdent, J., and M. Balabane. 1996. Major contribution of roots to soil carbon storage inferred from maize cultivated soils. *Soil Biology and Biochemistry*, **28**: 1261-1263.

- [7] Barbour, M.M., J.E. Hunt, R.J. Dungan, M.H. Turnbull, G.W. Brailsford, G.D. Farquhar, and D. Whitehead. 2005. Variation in the degree of coupling between delta C-13 of phloem sap and ecosystem respiration in two mature *Nothofagus* forests. *New Phytologist*, **166**: 497-512.
- [8] Batjes, N.H. 1996. Total carbon and nitrogen in the soils of the world. *European Journal of Soil Science*, **47**: 151-163.
- [9] Bekele, A., and W.H. Hudnall. 2003. Stable carbon isotope study of the prairie-forest transition soil in Louisiana. *Soil Science*, **168**: 783-792.
- [10] Beltrami, H., and L. Kellman. 2003. An examination of short- and long-term air-ground temperature coupling. *Global and Planetary Change*, **38**: 291-303.
- [11] Beltrami, H. Climate from Borehole Data: Energy Fluxes and Temperatures since 1500. *Geophys. Res. Lett.* **29**, 2111, 10.1029/2002GL015702, 2002.
- [12] Beltrami, H. 2001. On the relationship between ground temperature histories and meteorological records: A report on the Pomquet station. *Global Planetary Change*, **29**: 327-352.
- [13] Bernoux, M., C.C. Cerri, C. Neill, and J.F.L. de Moraes. 1998. The use of stable carbon isotopes for estimating soil organic matter turnover rates. *Geoderma*, **82**: 43-58.
- [14] Bhupinderpal, S., M.J. Hedley, and S. Saggar. 2005. Characterization of recently C-14 pulse-labelled carbon from roots by fractionation of soil organic matter. *European Journal of Soil Science*, **56**: 329-341.
- [15] Bird, M.I., A.R. Chivas, and J. Head. 1996. A latitudinal gradient in carbon turnover times in forest soils. *Nature*, **381**: 143-146.

- [16] Bird, M.I., H. Santruckova, A. Arneth, S. Grigoriev, G. Gleixner, Y.N. Kalaschnikov, J. Lloyd, and E.D. Schulze. 2002. Soil carbon inventories and carbon-13 on a latitude transect in Siberia. *Tellus Series B-Chemical and Physical Meteorology*, **54**: 631-641.
- [17] Bird, M., O. Kracht, D. Derrien, and Y. Zhou. 2003. The effect of soil texture and roots on the stable carbon isotope composition of soil organic carbon. *Australian Journal of Soil Research*, **41**: 77-94.
- [18] Block, R.M.A., K.C.J. Rees, and J.D. Knight. 2006. A review of fine root dynamics in *Populus* plantations. *Agroforestry Systems*, **67**: 73-84.
- [19] Bolinder, M.A., D.A. Angers, M. Giroux, and M.R. Laverdiere. 1999. Estimating C inputs retained as soil organic matter from corn (*Zea Mays* L.). *Plant and Soil*, **215**: 85-91.
- [20] Bonal, D., D. Sabatier, P. Montpied, D. Tremeaux, and J.M. Guehl. 2000. Interspecific variability of delta C-13 among trees in rainforests of French Guiana: functional groups and canopy integration. *Oecologia*, **124**: 454-468.
- [21] Bond-Lamberty, B., C.K. Wang, and S.T. Gower. 2004. Contribution of root respiration to soil surface CO₂ flux in a boreal black spruce chronosequence. *Tree Physiology*, **24**: 1387-1395.
- [22] Boone, R.D., K.J. Nadelhoffer, J.D. Canary, and J.P. Kaye. 1998. Roots exert a strong influence on the temperature sensitivity of soil respiration. *Nature*, **396**: 570-572.
- [23] Bowling, D.R., P.P. Tans, and R.K. Monson. 2001. Partitioning net ecosystem carbon exchange with isotopic fluxes of CO₂. *Global Change Biology*, **7**: 127-145.

- [24] Brendel, O., L. Handley, and H. Griffiths. 2003. The delta C-13 of Scots pine (*Pinus sylvestris* L.) needles: spatial and temporal variations. *Annals of Forest Science*, **60**: 97-104.
- [25] Buchmann, N., W.Y. Kao, and J. Ehleringer. 1997. Influence of stand structure on carbon-13 of vegetation, soils, and canopy air within deciduous and evergreen forests in Utah, United States. *Oecologia*, **110**: 109-119.
- [26] Buchmann, N., T.M. Hinckley, and J.R. Ehleringer. 1998. Carbon isotope dynamics in *Abies amabilis* stands in the Cascades. *Canadian Journal of Forest Research-Revue Canadienne De Recherche Forestiere*, **28**: 808-819.
- [27] Burton, D.L., and E.G. Beauchamp. 1994. Profile N₂O and CO₂ concentrations in a soil subject to freezing. *Soil Science Society of America Journal*, **58**: 115-122.
- [28] Cann, D.B. and J.D. Hilchey. 1954. Soil Survey of Antigonish County, Agriculture Canada.
- [29] Cerling, T.E., D.K. Solomon, J. Quade, and J.R. Bowman. 1991. On the Isotopic Composition of Carbon in Soil Carbon-Dioxide. *Geochimica Et Cosmochimica Acta*, **55**: 3403-3405.
- [30] Cheng, W.X., D.W. Johnson, and S.L. Fu. 2003. Rhizosphere effects on decomposition: Controls of plant species, phenology, and fertilization. *Soil Science Society of America Journal*, **67**: 1418-1427.
- [31] Chevillat, V.S., R.T.W. Siegwolf, S. Pepin, and C. Korner. 2005. Tissue-specific variation of delta C-13 in mature canopy trees in a temperate forest in central Europe. **6**: 519-534.
- [32] Craig, H. 1961. Isotopic variations in meteoric waters. *Science*, **133**: 1702-1703.

- [33] Crank, J. (1975), The mathematics of diffusion, Oxford University Press, Toronto.
- [34] Damesin, C., C. Barbaroux, D. Berveiller, C. Lelarge, M. Chaves, C. Maguas, R. Maia, and J.Y. Pontailler. 2005. The carbon isotope composition of CO₂ respired by trunks: comparison of four sampling methods. *Rapid Communications in Mass Spectrometry*, **19**: 369-374.
- [35] Davidson, E.A., and I. Janssens. 2006. Temperature sensitivity of soil carbon decomposition and feedbacks to climate change. *Nature*, **440**: 165-173.
- [36] Davidson, E.A., L.V. Verchot, J.H. Cattanio, I.L. Ackerman, and J.E.M. Carvalho. 2000. Effects of soil water content on soil respiration in forests and cattle pastures of eastern Amazonia. *Biogeochemistry*, **48**: 53-69.
- [37] Davidson, E.A., K. Savage, L.V. Verchot, and R. Navarro. 2002. Minimizing artifacts and biases in chamber-based measurements of soil respiration. **113**: 21-37.
- [38] Davidson, E.A., E. Belk, and R.D. Boone. 1998. Soil water content and temperature as independent or confounded factors controlling soil respiration in a temperate mixed hardwood forest. *Global Change Biology*, **4**: 217-227.
- [39] Davis, A.A., M.H. Stolt, and J.E. Compton. 2004. Spatial distribution of soil carbon in southern new England hardwood forest landscapes. *Soil Science Society of America Journal*, **68**: 895-903.
- [40] Dawson, T.E., S. Mambelli, A.H. Plamboeck, P.H. Templer, and K.P. Tu. 2002. Stableisotopes in plant ecology. *Annual Review of Ecology and Systematics*, **33**: 507-559.
- [41] de Jong, E.a.H.J.V.S. 1972. Calculation of soil respiration and activity from CO₂ profiles in the soil. *Soil Science*, **113**: 328-333.

- [42] DeGaetano, A.T., D.S. Wilks, and M. McKay. 1996. A physically based model of soil freezing in humid climates using air temperature and snow cover data. *Journal of Applied Meteorology*, **35**: 1009-1027.
- [43] Dilustro, J.J., B. Collins, L. Duncan, and C. Crawford. 2005. Moisture and soil texture effects on Soil CO₂ efflux components in southeastern mixed pine forests. *Forest Ecology and Management*, **204**: 85-95.
- [44] Dorr, H., and K.O. Munnich. 1987. Annual Variation in Soil Respiration in Selected Areas of the Temperate Zone. *Tellus Series B-Chemical and Physical Meteorology*, **39**: 114-121.
- [45] Drewitt, G.B., T.A. Black, and R.S. Jassal. 2005. Using measurements of soil CO₂ efflux and concentrations to infer the depth distribution of CO₂ production in a forest soil. *Canadian Journal of Soil Science*, **85**: 213-221.
- [46] Ehleringer, J.R., N. Buchmann, and L.B. Flanagan. 2000. Carbon isotope ratios in belowground carbon cycle processes. *Ecological Applications*, **10**: 412-422.
- [47] Ehleringer, J.R., D.R. Bowling, L.B. Flanagan, J. Fessenden, B. Helliker, L.A. Martinelli, and J.P. Ometto. 2002. Stable isotopes and carbon cycle processes in forests and grasslands. *Plant Biology*, **4**: 181-189.
- [48] Ekblad, A., and P. Hogberg. 2001. Natural abundance of C-13 in CO₂ respired from forest soils reveals speed of link between tree photosynthesis and root respiration. *Oecologia*, **127**: 305-308.
- [49] Ekblad, A., B. Bostrom, A. Holm, and D. Comstedt. 2005. Forest soil respiration rate and delta C-13 is regulated by recent above ground weather conditions. *Oecologia*, **143**: 136-142.

- [50] Enoki, T., H. Kawaguchi, and G. Iwatsubo. 1996. Topographic variations of soil properties and stand structure in a *Pinus thunbergii* plantation. *Ecological Research*, **11**: 299-309.
- [51] Epron, D., L. Farque, E. Lucot, and P.M. Badot. 1999. Soil CO₂ efflux in a beech forest: the contribution of root respiration. *Annals of Forest Science*, **56**: 289-295.
- [52] Fang, C., P. Smith, J.B. Moncrieff, and J.U. Smith. 2005. Similar response of labile and resistant soil organic matter pools to changes in temperature. *Nature*, **433**: 57-59.
- [53] Fernandez, I., N. Mahieu, and G. Cadisch. 2003. Carbon isotopic fractionation during decomposition of plant materials of different quality. *Global Biogeochemical Cycles*, **17**: Art. No. 1075.
- [54] Ferrio, J.P., and J. Voltas. 2005. Carbon and oxygen isotope ratios in wood constituents of *Pinus halepensis* as indicators of precipitation, temperature and vapour pressure deficit. *Tellus Series B-Chemical and Physical Meteorology*, **57**: 164-173.
- [55] Fessenden, J.E., and J.R. Ehleringer. 2002. Age-related variations in delta C-13 of ecosystem respiration across a coniferous forest chronosequence in the Pacific Northwest. *Tree Physiology*, **22**: 159-167.
- [56] Fierer, N., J.M. Craine, K. McLauchlan, and J.P. Schimel. 2005. Litter quality and the temperature sensitivity of decomposition. *Ecology*, **86**: 320-326.
- [57] Formanek, P., and P. Ambus. 2004. Assessing the use of delta C-13 natural abundance in separation of root and microbial respiration in a Danish beech (*Fagus sylvatica* L.) forest. *Rapid Communications in Mass Spectrometry*, **18**: 897-902.

- [58] Freijer, J.I. 1994. Calibration of Jointed Tube Model for the Gas-Diffusion Coefficient in Soils. *Soil Science Society of America Journal*, **58**: 1067-1076.
- [59] Gessler, A., S. Schrempp, A. Matzarakis, H. Mayer, H. Rennenberg, and M.A. Adams. 2001. Radiation modifies the effect of water availability on the carbon isotope composition of beech (*Fagus sylvatica*). *New Phytologist*, **150**: 653-664.
- [60] Giardina, C.P., and M.G. Ryan. 2000. Evidence that decomposition rates of organic carbon in mineral soil do not vary with temperature. *Nature*, **404**: 858-861.
- [61] Gregorich, E.G., P. Rochette, A.J. VandenBygaart, and D.A. Angers. 2005. Greenhouse gas contributions of agricultural soils and potential mitigation practices in Eastern Canada. *Soil and Tillage Research*, **83**: 53-72.
- [62] Griffis, T.J., T.A. Black, D. Gaumont-Guay, G.B. Drewitt, Z. Nesic, A.G. Barr, K. Morgenstern, and N. Kljun. 2004. Seasonal variation and partitioning of ecosystem respiration in a southern boreal aspen forest. *Agricultural and Forest Meteorology*, **125**: 207-223.
- [63] Groffman, P.M., and J.M. Tiedje. 1991. Relationships between Denitrification, CO₂ Production and Air-Filled Porosity in Soils of Different Texture and Drainage. *Soil Biology and Biochemistry*, **23**: 299-302.
- [64] Gu, B.H., C.L. Schelske, and D.A. Hodell. 2004. Extreme C-13 enrichments in a shallow hypereutrophic lake: Implications for carbon cycling. **49**: 1152-1159.
- [65] Gut, A., A. Blatter, M. Fahrni, B.E. Lehmann, A. Neftel, and T. Staffelbach. 1998. A new membrane tube technique (METT) for continuous gas measurements in soils. *Plant and Soil*, **198**: 79-88.

- [66] Hamer, U., and B. Marschner. 2005. Priming effects in different soil types induced by fructose, alanine, oxalic acid and catechol additions. *Soil Biology and Biochemistry*, **37**: 445-454.
- [67] Hanson, P.J., N.T. Edwards, C.T. Garten, and J.A. Andrews. 2000. Separating root and soil microbial contributions to soil respiration: A review of methods and observations. *Biogeochemistry*, **48**: 115-146.
- [68] Hashimoto, S., and M. Suzuki. 2002. Vertical distributions of carbon dioxide diffusion coefficients and production rates in forest soils. *Soil Science Society of America Journal*, **66**: 1151-1158.
- [69] Hashimoto, S., N. Tanaka, M. Suzuki, A. Inoue, Takizawa, H. Kosaka, I. Tanaka, K. Tantasirin, and N. C. Tangtham. 2004. Soil respiration and soil CO₂ concentration in a tropical forest. *Journal of Forest Research*, **9**: 75-79.
- [70] Hirsch, A.I., S.E. Trumbore, and M.L. Goulden. 2002. Direct measurement of the deep soil respiration accompanying seasonal thawing of a boreal forest soil. *Journal of Geophysical Research-Atmospheres*, **108**: 10.1029/2001JD000921.
- [71] Hirsch, A.I., S.E. Trumbore, and M.L. Goulden. 2004. The surface CO₂ gradient and pore-space storage flux in a high porosity litter layer. *Tellus Series B-Chemical and Physical Meteorology*, **56**: 312-321.
- [72] Hobbie, E.A., and R.A. Werner. 2004. Intramolecular, compound-specific, and bulk carbon isotope patterns in C-3 and C-4 plants: a review and synthesis. *New Phytologist*, **161**: 371-385.
- [73] Hobbie, E.A., M.G. Johnson, P.T. Rygielwicz, D.T. Tingey, and D.M. Olszyk. 2004. Isotopic estimates of new carbon inputs into litter and soils in a four-year climate change experiment with Douglas-fir. *Plant and Soil*, **259**: 331-343.

- [74] Hogberg, P., A. Nordgren, N. Buchmann, A.F.S. Taylor, A. Ekblad, M.N. Hogberg, G. Nyberg, M. Ottosson-Lofvenius, and D.J. Read. 2001. Large-scale forest girdling shows that current photosynthesis drives soil respiration. *Nature*, **411**: 789-792.
- [75] Hogberg, P., A. Nordgren, and G.I. Agren. 2002. Carbon allocation between tree root growth and root respiration in boreal pine forest. *Oecologia*, **132**: 579-581.
- [76] Houghton, R.A., M. Jenkins, and J.J.e. Ephraums. 2001. *Climate Change 2001: The Scientific Basis: Contribution of Working Group I to the third assessment report of the Intergovernmental Panel on Climate Change*. Cambridge University Press, New York: 881.
- [77] Hutchinson, G.L., and A.R. Mosier. 1981. Improved soil cover method for field measurement of nitrous oxide fluxes. *Soil Science Society of America Journal*, **45**: 311-316.
- [78] Jackel, U., S. Schnell, and R. Conrad. 2001. Effect of moisture, texture and aggregate size of paddy soil on production and consumption of CH₄. *Soil Biology and Biochemistry*, **33**: 965-971.
- [79] Jaggi, M., M. Saurer, J. Fuhrer, and R. Siegwolf. 2002. The relationship between the stable carbon isotope composition of needle bulk material, starch, and tree rings in *Picea abies*. *Oecologia*, **131**: 325-332.
- [80] Jassal, R.S., T.A. Black, G.B. Drewitt, M.D. Novak, D. Gaumont-Guay, and Z. Nesic (2004). A model of the production and transport of CO₂ in soil: predicting soil CO₂ concentrations and CO₂ efflux from a forest floor. *Agricultural and Forest Meteorology*, **124**: 219-236.
- [81] Kagawa, A., D. Naito, A. Sugimoto, and T.C. Maximov. 2003. Effects of spatial and temporal variability in soil moisture on widths and delta C-13 values of

- eastern Siberian tree rings. *Journal of Geophysical Research-Atmospheres*, **108**: 10.1029/2002JD003019.
- [82] Keitel, C., M.A. Adams, T. Holst, A. Matzarakis, H. Mayer, H. Rennenberg, and A. Gessler. 2003. Carbon and oxygen isotope composition of organic compounds in the phloem sap provides a short-term measure for stomatal conductance of European beech (*Fagus sylvatica* L.). *Plant Cell and Environment*, **26**: 1157-1168.
 - [83] Khahil, M.I., and E.M. Baggs. 2005. CH₄ oxidation and N₂O emissions at varied soil water-filled pore spaces and headspace CH₄ concentrations. **37**: 1785-1794.
 - [84] Kirby, G.E. 1988. *Soils of the Pasadena-Deer Lake area, Newfoundland*, Agriculture Canada.
 - [85] Kirschbaum, M.U.F. 2000. Will changes in soil organic carbon act as a positive or negative feedback on global warming? *Biogeochemistry*, **48**: 21-51.
 - [86] Knohl, A., R.A. Werner, W.A. Brand, and N. Buchmann. 2005. Short-term variations in delta C-13 of ecosystem respiration reveals link between assimilation and respiration in a deciduous forest. *Oecologia*, **142**: 70-82.
 - [87] Knorr, W., I.C. Prentice, J.I. House, and E.A. Holland. 2005. Long-term sensitivity of soil carbon turnover to warming. *Nature*, **433**: 298-301.
 - [88] Kuzyakov, Y. 2002. Review: Factors affecting rhizosphere priming effects. *Journal of Plant Nutrition and Soil Science-Zeitschrift Fur Pflanzenernahrung Und Bodenkunde*, **165**: 382-396.
 - [89] Kuzyakov, Y., and A.A. Larionova, Kuzyakov, Y., and A.A. Larionova. 2005. Root and rhizomicrobial respiration: A review of approaches to estimate respiration by autotrophic and heterotrophic organisms in soil. *Journal of Plant*

Nutrition and Soil Science-Zeitschrift Fur Pflanzenernahrung Und Bodenkunde, **168**: 503-520.

- [90] Kuzyakov, Y., Kuzyakov, Y. 2005. Theoretical background for partitioning of root and rhizomicrobial respiration by delta C-13 of microbial biomass. *European Journal of Soil Biology*, **41**: 1-9.
- [91] Lai, C.T., J.R. Ehleringer, A.J. Schauer, P.P. Tans, D.Y. Hollinger, K.T. Paw U, J.W. Munger, and S.C. Wofsy. 2005. Canopy-scale delta 1(3)C of photosynthetic and respiratory CO₂ fluxes: observations in forest biomes across the United States. *Global Change Biology*, **11**: 633-643.
- [92] Lavigne, M.B., R. Boutin, R.J. Foster, G. Goodine, P.Y. Bernier and G. Robitaille. 2003. Soil respiration responses to temperature are controlled more by roots than by decomposition in balsam fir ecosystems. *Canadian Journal of Forest Research-Revue Canadienne De Recherche Forestiere*, **33**: 1744-1753.
- [93] Lavigne, M.B., R.J. Foster, and G. Goodine. 2004. Seasonal and annual changes in soil respiration in relation to soil temperature, water potential and trenching. *Tree Physiology*, **24**: 415-424.
- [94] Le Roux, X., T. Bariac, H. Sinoquet, B. Genty, C. Piel, A. Mariotti, C. Girardin, and P. Richard. 2001. Spatial distribution of leaf water-use efficiency and carbon isotope discrimination within an isolated tree crown. **24**: 1021-1032.
- [95] Lee, M.S., K. Nakane, T. Nakatsubo, and H. Koizumi. 2003. Seasonal changes in the contribution of root respiration to total soil respiration in a cool-temperate deciduous forest. *Plant and Soil*, **255**: 311-318.
- [96] Lehmann, B.E., M. Lehmann, A. Neftel, and S.V. Tarakanov. 2000. Radon-222 monitoring of soil diffusivity. *Geophysical Research Letters*, **27**: 3917-3920.

- [97] Lin, G.H., J.R. Ehleringer, P.T. Rygielwicz, M.G. Johnson, and D.T. Tingey. 1999. Elevated CO₂ and temperature impacts on different components of soil CO₂ efflux in Douglas-fir terracosms. *Global Change Biology*, **5**: 157-168.
- [98] Loader, N.J., I. Robertson, and D. McCarroll. 2003. Comparison of stable carbon isotope ratios in the whole wood, cellulose and lignin of oak tree-rings. *Palaeogeography Palaeoclimatology Palaeoecology*, **196**: 395-407.
- [99] Lomander, A., T. Katterer, and O. Andren. 1998. Modelling the effects of temperature and moisture on CO₂ evolution from top- and subsoil using a multi-compartment approach. *Soil Biology and Biochemistry*, **30**: 2023-2030.
- [100] Lopez, B., S. Sabate, and C.A. Gracia. 2001. Vertical distribution of fine root density, length density, area index and mean diameter in a *Quercus ilex* forest. *Tree Physiology*, **21**: 555-560.
- [101] Magnusson, T. 1989. A Method for Equilibration Chamber Sampling and Gas-Chromatographic Analysis of the Soil Atmosphere. *Plant and Soil*, **120**: 39-47.
- [102] Martinez-Vilalta, J., A. Sala, and J. Pinol. 2004. The hydraulic architecture of Pinaceae - a review. *Plant Ecology*, **171**: 3-13.
- [103] McCarthy, K.A., and R.L. Johnson. 1995. Measurement of Trichloroethylene Diffusion as a Function of Moisture-Content in Sections of Gravity-Drained Soil Columns. *Journal of Environmental Quality*, **24**: 49-55.
- [104] McInerney, M., and T. Bolger. 2000. Temperature, wetting cycles and soil texture effects on carbon and nitrogen dynamics in stabilized earthworm casts. *Soil Biology and Biochemistry*, **32**: 335-349.
- [105] McIntyre, D.S., and J.R. Philip. 1964. A field method for measurement of gas diffusion into soils. *Aust. Journal of Soil Respiration*, **2**: 133-45.

- [106] Millington, R.J. 1959. Gas diffusion in porous media. *Science*, **130**: 100-102.
- [107] Moldrup, P., T. Olesen, T. Komatsu, P. Schjonning, and D.E. Rolston. 2001. Tortuosity, diffusivity, and permeability in the soil liquid and gaseous phases. *Soil Science Society of America Journal*, **65**: 613-623.
- [108] Moldrup, P., T. Olesen, S. Yoshikawa, T. Komatsu, and D.E. Rolston. 2004. Three-porosity model for predicting the gas diffusion coefficient in undisturbed soil. *Soil Science Society of America Journal*, **68**: 750-759.
- [109] Moore, T.R., J.A. Trofymow, M. Siltanen, and C. Prescott. 2005. Patterns of decomposition and carbon, nitrogen, and phosphorus dynamics of litter in upland forest and peatland sites in central Canada. *Canadian Journal of Forest Research-Revue Canadienne De Recherche Forestiere*, **35**: 133-142.
- [110] Mortazavi, B., and J.P. Chanton. 2002. Carbon isotopic discrimination and control of nighttime canopy δ O-18- CO_2 in a pine forest in the southeastern United States. *Global Biogeochemical Cycles*, **16**: 10.1029/2000GB001390.
- [111] Mortazavi, B., J.L. Prater, and J.P. Chanton. 2004. A field-based method for simultaneous measurements of the δ O-18 and δ C-13 of soil CO_2 efflux. *Biogeosciences*, **1**: 1-9.
- [112] Nakatsuka, T., K. Ohnishi, T. Hara, A. Sumida, D. Mitsuishi, N. Kurita, and S. Uemura. 2004. Oxygen and carbon isotopic ratios of tree-ring cellulose in a conifer-hardwood mixed forest in northern Japan. *Geochemical Journal*, **38**: 77-88.
- [113] O'Leary, M.H. 1988. Carbon Isotopes in Photosynthesis, *BioScience* **38**: 328-336.

- [114] Panek, J.A., and R.H. Waring. 1995. Carbon-Isotope Variation in Douglas-Fir Foliage - Improving the Delta-C-13-Climate Relationship. *Tree Physiology*, **15**: 657-663.
- [115] Panek, J.A. 1996. Correlations between stable carbon-isotope abundance and hydraulic conductivity in Douglas-fir across a climate gradient in Oregon, USA. **16**: 747-755.
- [116] Pataki, D.E., J.R. Ehleringer, L.B. Flanagan, D. Yakir, D.R. Bowling, C.J. Still, N. Buchmann, J.O. Kaplan, and J.A. Berry. 2003. The application and interpretation of Keeling plots in terrestrial carbon cycle research. *Global Biogeochemical Cycles*, **17**: Art. No. 1022.
- [117] Pendall, E., S. Del Grosso, J.Y. King, D.R. LeCain, D.G. Milchunas, J.A. Morgan, A.R. Mosier, D.S. Ojima, W.A. Parton, P.P. Tans, and J.W.C. White. 2003. Elevated atmospheric CO₂ effects and soil water feedbacks on soil respiration components in a Colorado grassland. *Global Biogeochemical Cycles*, **17**: Art. No. 1046.
- [118] Phillips, D.L., and J.W. Gregg. 2001. Uncertainty in source partitioning using stable isotopes. *Oecologia*, **127**: 171-179.
- [119] Porte, A., and D. Loustau. 2001. Seasonal and interannual variations in carbon isotope discrimination in a maritime pine (*Pinus pinaster*) stand assessed from the isotopic composition of cellulose in annual rings. *Tree Physiology*, **21**: 861-868.
- [120] Powlson, D. 2005. Climatology - Will soil amplify climate change? *Nature*, **433**: 204-205.
- [121] Pumpanen, J., P. Kolari, H. Ilvesniemi, K. Minkinen, T. Vesala, S. Niinisto, A. Lohila, T. Larmola, M. Morero, M. Pihlatie, I. Janssens, J.C. Yuste, J.M.

- Grunzweig, S. Reth, J.A. Subke, K. Savage, W. Kutsch, G. Ostreng, W. Ziegler, P. Anthoni, A. Lindroth, and P. Hari. 2004. Comparison of different chamber techniques for measuring soil CO₂ efflux. *Agricultural and Forest Meteorology*, **123**: 159-176.
- [122] Raich, J.W., and W.H. Schlesinger. 1992. The Global Carbon-Dioxide Flux in Soil Respiration and Its Relationship to Vegetation and Climate. *Tellus Series B-Chemical and Physical Meteorology*, **44**: 81-99.
- [Raich *et al.* 1990] Raich, J. W., R. D. Bowden, and P. A. Steudler. 1990. Comparison of two static chamber methods for determining carbon dioxide efflux from forest soils. *Soil Sci. Soc. of Am. J.* **54**: 1754-1757.
- [123] Rasse, D.P., C. Rumpel, and M.F. Dignac. 2005. Is soil carbon mostly root carbon? Mechanisms for a specific stabilisation. *Plant and Soil*, **269**: 341-356.
- [124] Reichstein, M., F. Bednorz, G. Broll, and T. Katterer. 2000. Temperature dependence of carbon mineralisation: conclusions from a long-term incubation of subalpine soil samples. *Soil Biology and Biochemistry*, **32**: 947-958.
- [125] Reichstein, M., J.A. Subke, A.C. Angeli, and J.D. Tenhunen. 2005. Does the temperature sensitivity of decomposition of soil organic matter depend upon water content, soil horizon, or incubation time? *Global Change Biology*, **11**: 1754-1767.
- [126] Revsbech, N.P., L.P. Nielsen, and N.B. Ramsing. 1998. A novel microsensor for determination of apparent diffusivity in sediments. *Limnology and Oceanography*, **43**: 986-992.
- [127] Risk, D., L. Kellman, and H. Beltrami. 2002. Carbon dioxide in soil profiles: Production and temperature dependency. *Geophysical Research Letters*, **29**: Art. No. 1087.

- [128] Risk, D., H. Beltrami, and L. Kellman. 2002. Soil CO₂ production and surface flux at four climate observatories in eastern Canada. *Global Biogeochemical Cycles*, **16**: Art. No. 1122.
- [129] Risk, D., H. Beltrami and L. Kellman, A new method for in-situ soil gas diffusivity measurement and applications to tracegas studies, *submitted to J. Geophys. Res.*, 2006.
- [130] Risk, D., Kellman, L. and M. Moroni, Characterization of variability and spatial patterns in tree and soil $\delta^{13}\text{C}$ at research sites in eastern Canada, Submitted to *Global Biogeochemical Cycles*, 2006.
- [131] Risk, D., N. Nickerson, L. Kellman, and M. Moroni, Spatial variability of soil respired $\delta^{13}\text{CO}_2$ as measured using an improved sampling methodology, Submitted to *Canadian Journal of Forest Research*, 2006.
- [132] Rochette, P., D.A. Angers, and L.B. Flanagan. 1999. Maize residue decomposition measurement using soil surface carbon dioxide fluxes and natural abundance of carbon-13. *Soil Science Society of America Journal*, **63**: 1385-1396.
- [133] Samuelson, L.J., P.C. McLemore, and G.L. Somers. 2003. Relationship between foliar delta C-13 and hydraulic pathway length in *Pinus palustris*. *Forest Science*, **49**: 790-798.
- [134] Schenk, H.J., and R.B. Jackson. 2005. Mapping the global distribution of deep roots in relation to climate and soil characteristics. *Geoderma*, **126**: 129-140.
- [135] Schjonning, P., I.K. Thomsen, J.P. Moberg, H. de Jonge, K. Kristensen, and B.T. Christensen. 1999. Turnover of organic matter in differently textured soils - I. Physical characteristics of structurally disturbed and intact soils. *Geoderma*, **89**: 177-198.

- [136] Schlesinger, W.H., and J.A. Andrews. 2000. Soil respiration and the global carbon cycle. *Biogeochemistry*, **48**: 7-20.
- [137] Schuur, E.A.G., and S.E. Trumbore. 2006. Partitioning sources of soil respiration in boreal black spruce forest using radiocarbon. *Global Change Biology*, **12**: 165-176.
- [138] Schweizer, M., J. Fear, and G. Cadisch. 1999. Isotopic (C-13) fractionation during plant residue decomposition and its implications for soil organic matter studies. *Rapid Communications in Mass Spectrometry*, **13**: 1284-1290.
- [139] Smith, J.L. and Myung, H.U. 2005. Rapid procedures for preparing soil and KCl extracts for ^{15}N analysis. *Communications in Soil Science and Plant Analysis* **21**: 2173-2179.
- [140] Smith, K.A., P.E. Thomson, H. Clayton, I.P. McTaggart, and F. Conen. 1998. Effects of temperature, water content and nitrogen fertilisation on emissions of nitrous oxide by soils. **32**: 3301-3309.
- [141] Sommerfeld, R.A., A.R. Mosier, and R.C. Musselman. 1993. CO_2 , CH_4 and N_2O Flux through a Wyoming Snowpack and Implications for Global Budgets. *Nature*, **361**: 140-142.
- [142] Steinmann, K.T.W., R. Siegwolf, M. Saurer, and C. Korner. 2004. Carbon fluxes to the soil in a mature temperate forest assessed by C-13 isotope tracing. *Oecologia*, **141**: 489-501.
- [143] Stern, L., W.T. Baisden, and R. Amundson. 1999. Processes controlling the oxygen isotope ratio of soil CO_2 : Analytic and numerical modeling. *Geochimica Et Cosmochimica Acta*, **63**: 799-814.

- [144] Stevenson, B.A., E.F. Kelly, E.V. McDonald, and A.J. Busacca. 2005. The stable carbon isotope composition of soil organic carbon and pedogenic carbonates along a bioclimatic gradient in the Palouse region, Washington State, USA. *Geoderma*, **124**: 37-47.
- [145] Subke, J.A., V. Hahn, G. Battipaglia, S. Linder, N. Buchmann, and M.F. Cotrufo. 2004. Feedback interactions between needle litter decomposition and rhizosphere activity. *Oecologia*, **139**: 551-559.
- [146] Sulzman, E.W., J.B. Brant, R.D. Bowden, and K. Lajtha. 2005. Contribution of aboveground litter, belowground litter, and rhizosphere respiration to total soil CO₂ efflux in an old growth coniferous forest. *Biogeochemistry*, **73**: 231-256.
- [147] Swanston, C.W., M.S. Torn, P.J. Hanson, J.R. Southon, C.T. Garten, E.M. Hanlon, and L. Ganio. 2005. Initial characterization of processes of soil carbon stabilization using forest stand-level radiocarbon enrichment. *Geoderma*, **128**: 52-62.
- [148] Taneva, L., J.S. Phippen, W.H. Schlesinger and M.A. Gonzalez-Meler. The turnover of carbon pools contributing to soil CO₂ and soil respiration in a temperate forest exposed to elevated CO₂ concentration. *Global Change Biology*, *in press*.
- [149] Tang, J.W., and D.D. Baldocchi. 2005. Spatial-temporal variation in soil respiration in an oak-grass savanna ecosystem in California and its partitioning into autotrophic and heterotrophic components. *Biogeochemistry*, **73**: 183-207.
- [150] Tang, J.W., D.D. Baldocchi, Y. Qi, and L.K. Xu (2003). Assessing soil CO₂ efflux using continuous measurements of CO₂ profiles in soils with small solid-state sensors. *Agricultural and Forest Meteorology*, **118**: 207-220.

- [151] Teh, Y.A., W.L. Silver, and M.E. Conrad. 2005. Oxygen effects on methane production and oxidation in humid tropical forest soils. *Global Change Biology*, **11**: 1283-1297.
- [152] Tierney, G.L., T.J. Fahey, P.M. Groffman, J.P. Hardy, R.D. Fitzhugh, C.T. Driscoll, and J.B. Yavitt. 2003. Environmental control of fine root dynamics in a northern hardwood forest. *Global Change Biology*, **9**: 670-679.
- [153] Torn, M.S., P.M. Vitousek, and S.E. Trumbore. 2005. The influence of nutrient availability on soil organic matter turnover estimated by incubations and radiocarbon modeling. *Ecosystems*, **8**: 352-372.
- [154] Trumbore, S.E., O.A. Chadwick, and R. Amundson. 1996. Rapid exchange between soil carbon and atmospheric carbon dioxide driven by temperature change. *Science*, **272**: 393-396.
- [155] Trumbore, S. 2000. Age of soil organic matter and soil respiration: Radiocarbon constraints on belowground C dynamics. *Ecological Applications*, **10**: 399-411.
- [156] Trumbore, S. 2006. Carbon respired by terrestrial ecosystems - recent progress and challenges. *Global Change Biology*, **12**: 141-153.
- [157] Valentini, R., G. Matteucci, A.J. Dolman, E.D. Schulze, C. Rebmann, E.J. Moors, A. Granier, P. Gross, N.O. Jensen, K. Pilegaard, A. Lindroth, A. Grelle, C. Bernhofer, T. Grunwald, M. Aubinet, R. Ceulemans, A.S. Kowalski, T. Vesala, U. Rannik, P. Berbigier, D. Loustau, J. Guomundsson, H. Thorgeirsson, A. Ibrom, K. Morgenstern, R. Clement, J. Moncrieff, L. Montagnani, S. Minerbi, and P.G. Jarvis. 2000. Respiration as the main determinant of carbon balance in European forests. *Nature*, **404**: 861-865.
- [158] van Bochove, E., N. Bertrand, and J. Caron. 1998. In situ estimation of the

- gaseous nitrous oxide diffusion coefficient in a sandy loam soil. *Soil Science Society of America Journal*, **62**: 1178-1184.
- [159] Vogel, J.G., D.W. Valentine, and R.W. Ruess. 2005. Soil and root respiration in mature Alaskan black spruce forests that vary in soil organic matter decomposition rates. *Canadian Journal of Forest Research-Revue Canadienne De Recherche Forestiere*, **35**: 161-174.
- [160] Von Arnold, K., P. Weslien, M. Nilsson, B.H. Svensson, and L. Klemedtsson. 2005. Fluxes of CO₂, CH₄ and N₂O from drained coniferous forests on organic soils. *Soil Biology and Biochemistry*, **210**: 239-254.
- [161] Vonfischer, J.C., and L.L. Tieszen. 1995. Carbon-Isotope Characterization of Vegetation and Soil Organic-Matter in Subtropical Forests in Luquillo, Puerto-Rico. *Biotropica*, **27**: 138-148.
- [162] Walcroft, A.S., W.B. Silvester, J.C. Grace, S.D. Carson, and R.H. Waring. 1996. Effects of branch length on carbon isotope discrimination in *Pinus radiata*. *Tree Physiology*, **16**: 281-286.
- [163] Wynn, J.G., M.I. Bird, and V.N.L. Wong. 2005. Rayleigh distillation and the depth profile of C-13/C-12 ratios of soil organic carbon from soils of disparate texture in Iron Range National Park, Far North Queensland, Australia. *Geochimica Et Cosmochimica Acta*, **69**: 1961-1973.
- [164] Yakir, D., and L.D.L. Sternberg. 2000. The use of stable isotopes to study ecosystem gas exchange. *Oecologia*, **123**: 297-311.
- [165] Yoshikawa, S., and S. Hasegawa. 2000. Diurnal and seasonal changes in CO₂ concentration and flux in an Andisol and simulation based on changes in CO₂ production rate and gas diffusivity. *Jarq-Japan Agricultural Research Quarterly*, **34**: 1-13.

- [166] Yuste, J.C., M. Nagy, I.A. Janssens, A. Carrara, and R. Ceulemans. 2005. Soil respiration in a mixed temperate forest and its contribution to total ecosystem respiration. *Tree Physiology*, **25**: 609-619.
- [167] Zar, J.H. 1999. *Biostatistical analysis*. Prentice Hall, Upper Saddle River, N.J.