CARBON IN RIPARIAN SUBSURFACE ECOSYSTEMS: SOURCES, LABILITY, AND SPATIAL PATTERNS

A Dissertation

Presented to the Faculty of the Graduate School

of Cornell University

in Partial Fulfillment of the Requirements for the Degree of

Doctor of Philosophy

by

Noel Paul Gurwick

January 2007

CARBON IN RIPARIAN SUBSURFACE ECOSYSTEMS: SOURCES, LABILITY, AND SPATIAL PATTERNS

Noel Paul Gurwick, Ph.D.

Cornell University 2007

Numerous studies suggest that denitrification in riparian zones removes nitrogen from groundwater as it moves from terrestrial to aquatic ecosystems. However, removal rates vary widely among sites complicating the incorporation of riparian zones into models of nitrogen movement across landscapes. Because denitrification in the riparian subsurface is often limited by the supply of microbially-available carbon, explaining how and why carbon supply varies among riparian zones using mappable landscape attributes holds practical and theoretical appeal. First principles suggest three carbon sources for subsurface microbes: (1) dissolved organic carbon leached from surface soils; (2) deep plant roots; and (3) buried, carbon-rich soil horizons deposited long ago. Working in Rhode Island USA at riparian zones mapped as outwash and alluvium, I investigated the relative importance of different carbon sources to 3 meters depth.

Field and laboratory experiments showed that both roots and buried horizons can supply carbon in the shallow subsurface (40-75 cm), but that buried horizons dominate below 75 cm. Radiocarbon dates and results from ingrowth cores showed that roots 40-75 centimeters deep grow and decompose on decadal time scales and form patches of organic matter that may influence nitrogen removal from groundwater. However, in both alluvial and outwash profiles, most roots below 80 cm are relics (usually > 140

years old) and therefore do not act as direct carbon conduits between the surface and deep subsurface. Laboratory incubations of buried soils from many sites demonstrated that high rates of carbon mineralization associated with these soils are common. In-situ groundwater incubations and ^{14}C dating demonstrated that metabolism of ancient carbon constitutes at least 31% of total carbon mineralization >2 meters below the surface at some sites.

My results suggest that: (1) the depth of the biologically active zone extends as deep as buried horizons; (2) on outwash and alluvium the riparian surface and subsurface are largely decoupled on time scales of months to years; (3) functional classifications of riparian zones intended to support management need to include buried horizons and recognize the limited influence of surface vegetation on subsurface biogeochemistry over short time frames.

BIOGRAPHICAL SKETCH

Noel Gurwick began his foray into research science in 1981, as a participant in the Juneau Icefield Research Program, studying field glaciology and geomorphology. While an undergraduate at Brown University, he spent a summer in Dr. Pat Lohman's paleoecology lab at the Woods Hole Oceanographic Institution, studying relationships between evolution and development. Following his growing interest in ecology, he worked in Dr. Mark Bertness' lab studying community ecology in salt marshes and rocky beaches. Seeking experience in applied ecology, he conducted his undergraduate thesis research on behavior and management of the apple maggot fly, under the guidance of Dr. Ronald Prokopy at the University of Massachusetts and Dr. Jonathan Waage at Brown. In 1987, he graduated Magna Cum Laude, receiving a Bachelors of Science with honors in biology.

After completing his BS, Noel taught high school and middle school science, and then traveled in Sub-Saharan Africa, visiting schools and development projects along the way. Returning to the U.S. in 1990, he began work as "Research Translator" at the Waquoit Bay National Estuarine Research Reserve, on Cape Cod, Massachusetts, where he worked with ecosystem researchers and local, regional, and state agencies on coastal zone policy.

In 1998 he completed an MS in natural resource policy and management at Cornell University, with Drs. Barbara Knuth and Barbara Bedford. His MS thesis research concerned key stakeholders' perspectives on wetlands regulation, property value, and landowner rights in New York State, and the implications of these data for regulatory agencies and policy makers.

He began his Ph.D. in 1998 working with Dr. Peter Groffman at the Institute of Ecosystem Studies on nutrient cycling in riparian zones. Noel now works at the Jasper Ridge Global Change Experiment at Stanford University and the Carnegie Institution Department of Global Ecology, where he maintains his long-time interest in ecosystem science and its links to public policy.

ACKNOWLEDGMENTS

My committee members, Peter Groffman, Barbara Bedford, Joe Yavitt, and Tim Fahey, all contributed insights, critiques, suggestions, laboratory resources, and encouragement throughout my time at Cornell. They supported my quests for funding and equipment, without which I could not have pursued much of my research, and they have provided me with invaluable models of creative, collaborative research and mentoring.

The research I describe here was supported by a wide variety of sources. They include awards to Noel Gurwick from the EPA STAR Fellowship Program, the Theresa Heinz Scholars for Environmental Research, the Cornell Center for the Environment, and the Cornell NSF-sponsored programs in biogeochemistry and environmental change (RTG) and biogeochemistry and Biocomplexity (IGERT). Other sources of financial support included a USDA-NRI grant to Art Gold (University of Rhode Island) and Peter Groffman, funds from the Andrew W. Mellon Foundation to the Institute of Ecosystem Studies for the investigation of ecosystem processes and landscape heterogeneity, and in-kind support to Noel Gurwick from the National Ocean Sciences Accelerator Mass Spectrometry facility in Woods Hole, Massachusetts.

Sandy Tartowski, who coordinated the biogeochemistry program at Cornell University, influenced my development as a scientist in more ways than I can list here. Her door was always open, often late at night, and her passion for ecosystem science was contagious. She created numerous opportunities for graduate students and I benefited tremendously from them.

In Woods Hole, Dan McCorkle went far beyond the call, setting up a vacuum line that would enable me to strip carbon dioxide from groundwater and working with me to develop a reliable protocol. Since 2003, he has given his time and expertise with radiocarbon generously and cheerfully.

For discussions and advice about measuring root turnover, characterizing root anatomy, and using radiocarbon dating to measure root turnover, pertinent particularly to investigations described in chapter 4, I thank Geri Tierney, Ruth Yanai, and Dominic Paolilo. Janice Theis and Chris Jones in the Department of Soil Science at Cornell University provided unfettered access to their microscope and imaging facility, which was also critical for my investigations of root biology.

A number of individuals in Rhode Island helped immensely with this research. Mike and Polly Hutchison and Barbara Shays opened their homes to me. David Smith, Scott Nixon, and Steve Granger at the Graduate School of Oceanography provided a variety of laboratory facilities. Alison Roberts generously granted access to microscope facilities and worked with me in my attempts to develop effective root staining protocols. Carl Sawyer gave me space to store my field sampling equipment as well as essential help building prototypes and, eventually, functional versions of soil sampling tools. Pete Seitz-Rundlett worked as my field assistant in Fall-Winter 2002 and I am grateful for his unwavering attention to detail and hard work in the field at all hours of day or night, often in freezing temperatures. The data presented in chapter 3 could not have been collected without his help. The entire riparian research group in Rhode Island (Art Gold, Adam Rosenblatt, Kelly Addy, Mark Stolt, Gary Blazejewski, and Q Kellogg) helped in many ways, introducing me to field-based study of riparian soils and groundwater. I especially thank Q Kellogg and Art Gold

for their help in the experiments described in chapter 4, and Gary Blazejewski and Mark Stolt for collecting soil samples that formed the basis of investigations described in chapter 2. Art Gold also gave me access to his office and computer while he was on sabbatic leave and shared his many interesting observations about riparian zone classification, regulation, and management. During the field campaigns, Mike and Polly Hutchinson, Barbara, Rebecca, and Jordan Shays, and Pam Rubinoff provided lodging and friendly homes in Rhode Island, resources without which I could not have completed this research.

A number of undergraduate students at Cornell University assisted with this research: Amanda Senft, Jill Schondebare, Esther Pullen, Krista Guererro, Abraham Parker, Cristen Mendoza, Josh Hunn, Ashley Wilson, and Jamecia Finnie. I have been grateful for their skills, time, dedication, humor, and enthusiasm. Interacting with them has been a pleasure and contributed to my own thinking and enjoyment of the research and my time at Cornell.

My graduate student and post-doc colleagues gave me feedback on ideas and presentations, critiqued and edited proposals, taught me a variety of laboratory techniques, and contributed labor freely and cheerfully. They also provided encouragement, good humor, stimulating ideas, and wonderful friendship throughout my Ph.D. program. For this I thank, among others, Alison Aldous, Kathy Bailey, Peter Weishampel, Carmen Chapin, Kurt Smemo, Jason Demers, Kathy Crowley, Lynn Vacarro, Dana Warren, Steve Thomas, Sam Simkin, Rich Phillips, Gretchen Gettel, Melanie Fisk, and Erik Lilleskov. Several close friends provided advice, support, and companionship at times when I was immersed in my studies; for their

help I thank Ana Cordova, Rich Schwartz, Anna Kukekova, Bob Stuart, and Ashleigh Imus.

Last, I thank my parents, Dora Haddad Gurwick and Paul Gurwick, for giving me the foundation that enabled me to get this far; and Gerald Anders, for stepping in when they could not.

TABLE OF CONTENTS

BIOGRAPHICAL SKETCH iii
ACKNOWLEDGMENTS v
TABLE OF CONTENTS ix
LIST OF FIGURES xi
LIST OF TABLES xiii

CHAPTER ONE: POTENTIAL CONTRIBUTIONS OF LIKELY CARBON SOURCES IN THE RIPARIAN SUBSURFACE AND THEIR RELEVANCE FOR NITROGEN CYCLING 1
Landscape-scale nitrogen fluxes 1
Roots as a C source in the riparian subsurface 10
Ancient carbon 14
Coupling of surface and subsurface ecosystems 16
Thesis outline 18
Literature Cited 20

CHAPTER TWO: MICROBIALLY-AVAILABLE CARBON IN BURIED SOILS 27
Introduction 27
Methods and site descriptions 33
Results 36
Discussion 48
Literature Cited 57

CHAPTER THREE: MINERALIZATION OF ANCIENT CARBON IN THE SUBSURFACE OF RIPARIAN FORESTS 63
Introduction 63
Methods and site descriptions 68
Results 76
Discussion 100
Literature Cited 117

CHAPTER FOUR: CARBON SUPPLY TO RIPARIAN SURFACE AND SUBSURFACE SOILS VIA ROOTS 123
Introduction 123
Methods 126
Results 135
Discussion 146
Literature Cited 158

CHAPTER FIVE: SYNTHESIS: RIPARIAN RIFTS IN THE SPACE-TIME CONTINUUM 163
Introduction 163
Legacy effects in riparian zones and functional classifications 165

Surface-subsurface connections 168
Hot spots at multiple scales 170
Future research directions 170
Literature Cited 174

APPENDIX A. CHARACTERISTICS AND ^{14}C-AGES OF ROOTS FROM THE RIPARIAN SUBSURFACE 178

LIST OF FIGURES

Figure 1.1. Possible sources of carbon to the riparian subsurface....9
Figure 2.1. CO_2 accumulation during incubations of 18 riparian soil horizons (A, Ab, B, C, A/C, C/A) collected in September 2003. 37
Figure 2.2. Carbon mineralization versus depth of riparian forest soil horizons during anaerobic incubations amended with KNO_3 39
Figure 2.3. Microbial biomass versus depth in 21 riparian soil horizons (O, A, Ab, B, C, A/C, C/A) collected in September 2003. 41
Figure 2.4. Carbon mineralization versus microbial biomass in 21 riparian soil horizons (O, A, Ab, B, C, A/C, C/A) collected in September 2003.....42
Figure 2.5. Variation in carbon mineralization among samples from six riparian forest soils, in response to amendments with deionized water (DI), nitrate (N), and dissolved organic carbon (DOC). 43
Figure 2.6. (a) Percent C and (b) percent N v. carbon mineralization. 46
Figure 2.7. Spatial patterns of (a) delta ^{15}N and (b) delta ^{13}C of soil organic matter from surface and subsurface riparian soils. 47
Figure 3.1. Calcium and magnesium concentrations in ambient groundwater from August-September 2003, by site....77
Figure 3.2. Concentration gradients of dissolved inorganic carbon (DIC) in ambient groundwater. 82
Figure 3.3. ^{14}C signatures of dissolved inorganic carbon (DIC) in ambient groundwater sampled in 2002 and 2003.....84
Figure 3.4. Relationships between depth, ^{13}C-DIC, and ^{14}C-DIC in ambient groundwater in 2002 and 2003 at (a) site A and (b) site C.....87
Figure 3.5. Relationships between alkalinity, ^{13}C-DIC, and ^{14}C-DIC in ambient groundwater in 2003, at each of four sites. 89
Figure 3.6. Correlation between [DIC] and ^{14}C-DIC in ambient groundwater, by site and year. 90
Figure 3.7. ^{14}C-DIC of groundwater in ambient samples and samples following in-situ incubation at alluvial (C) and outwash (A,B) sites. 91
Figure 3.8. ^{14}C-DIC of groundwater following in-situ incubation vs depth at alluvial (C) and outwash (A,B) sites. 94
Figure 3.9. Production of dissolved inorganic carbon (DIC) during in-situ groundwater incubations. 95
Figure 3.10. ^{14}C-DIC signatures (calculated) of mineralized C for each incubation site....96
Figure 3.11. Relationship between the contribution of: (1) C mineralization during the incubation to total DIC in the incubated sample; and (2) ancient carbon to C mineralization 97
Figure 3.12. Relationship between calculated radiocarbon signatures of C mineralized during the in-situ incubations and C mineralization rate....99
Figure 3.13. ^{14}C-calibrated ages of mineralized C for each incubation with pre-modern estimated ^{14}C signatures 103
Figure 4.1. Root biomass from soil cores at site B (outwash).....136
Figure 4.2. Frequency distribution of root biomass 50-100 cm at site B. 137

Figure 4.3. Root biomass with depth in three cores at each of three sites. 138
Figure 4.4. Mean root biomass below 50 cm at three sites (3 cores per site). 139
Figure 4.5. Root biomass accumulated in ingrowth cores over 12, 20, 24, and 31 months. 141
Figure 4.6. Mass in ingrowth cores within the top 30 cm of the soil column. 142
Figure 4.7. Radiocarbon signatures of individual roots (a); and inferred ages (b) with depth. 144
Figure A.1. Root sample 1. 178
Figure A.2. Root sample 2. 178
Figure A.3. Root sample 3. 179
Figure A.4. Root sample 4. 179
Figure A.5. Root sample 5. 180
Figure A.6. Root sample 6. 180
Figure A.7. Root sample 7. 181
Figure A.8. Root sample 8. 181
Figure A.9. Root sample 9. 182
Figure A.10. Root sample 10. 182
Figure A.11. Root sample 11. 183
Figure A.12. Root sample 12. 183
Figure A.13. Root sample 13. 184
Figure A.14. Root sample 14. 184
Figure A.15. Root sample 15. 185
Figure A.16. Root sample 16. 185
Figure A.17. Root sample 17. 186
Figure A.18. Root sample 18. 186

LIST OF TABLES

Table 2.1. Stream and soil characteristics at sites where we sampled buried soil horizons (PD=poorly drained, VPD=very poorly drained, SPD=somewhat poorly drained). 34
Table 2.2. Pearson correlation coefficients showing relationships among variables characterizing soil microbial activity, chemistry, and location. 45
Table 3.1. Concentrations (mg L^{-1}) of elements in ambient groundwater sampled in August-Sept 2003, ... 78
Table 3.2. Anion and dissolved oxygen (D.O.) concentrations (mg L^{-1}) and groundwater temperature at sites and wells used for in-situ incubation experiments in 2002. 80
Table 3.3. Alkalinity, pH, specific conductance, and temperature of ambient groundwater in 2003, by site and depth. 81
Table 3.4. Isotopic signatures for samples with premodern ^{14}C signatures. 85
Table 3.5. Isotopic signatures for samples with modern ^{14}C signatures. 86
Table 3.6. Minimum ages of mineralized SOM required to account for ^{14}C-DIC signatures of ambient and incubated groundwater samples. 93
Table 3.7. Minimum ages of mineralized SOM required to account for calculated ^{14}C-DIC signatures. 102
Table 3.8. Estimated changes in ^{13}C- and ^{14}C-DIC of riparian groundwater from dissolution of carbonate minerals associated with a doubling of [calcium + magnesium] in groundwater 106
Table 3.9. Estimated changes in ^{13}C- and ^{14}C-DIC of riparian groundwater from dissolution of carbonate minerals associated with varying increases in the [calcium + magnesium] 260 cm deep at site C 107
Table 4.1. Number of root samples analyzed for ^{14}C signatures by AMS by diameter class and site. 133
Table 4.2. Radiocarbon signatures of roots. 145

CHAPTER ONE: POTENTIAL CONTRIBUTIONS OF LIKELY CARBON SOURCES IN THE RIPARIAN SUBSURFACE AND THEIR RELEVANCE FOR NITROGEN CYCLING

This dissertation concerns the confluence of three veins of research in ecosystem biology and biogeochemistry: (1) controls on landscape-scale nitrogen fluxes; (2) distribution and turnover of deep roots; and (3) chemistry and bioavailability of ancient carbon. In this chapter I articulate the theoretical and applied motivation behind my research with respect to each of these perspectives, and explain how they converge in my investigations of carbon cycling in the riparian subsurface.

Landscape-scale nitrogen fluxes

Nitrogen (N) additions to terrestrial ecosystems have increased rapidly since the 1950s, and 10-40% of this N reaches coastal waters (NRC 2000; Boyer et al. 2002). Over time, the proportion of anthropogenic N reaching the terrestrial-aquatic interface may rise. In the United States, N fluxes to coastal waters are expected to increase unless consumption of meat, which drives fertilizer use, decreases or agricultural practices change (Howarth et al. 2002). Even without increased N loads to terrestrial ecosystems, N fluxes to coastal waters could increase because terrestrial ecosystems can become saturated and "leaky" after receiving chronic N additions (Aber et al. 1989, 1998; Van Breeman et al. 2002).

The most common form of N transported across the landscape is nitrate, which enters ecosystems as fertilizer and, more commonly, from nitrification in aerobic soils. Two attributes account for nitrate's high mobility. First, because nitrate carries a negative charge it is not attracted to soil particles, which tend also to carry a negative charge. Second, nitrate is highly soluble and therefore easily transported to groundwater.

Indeed, nitrate is the most commonly detected groundwater pollutant in the U.S. (U.S. EPA 1990).

Intensive nitrogen inputs to terrestrial ecosystems cause diverse problems for human health and environmental conservation in upland forests and downgradient ecosystems (Vitousek et al. 1997; Carpenter et al. 1998). In temperate coastal waters, acute problems resulting from eutrophication include reductions in biodiversity, increased frequency of nuisance algal blooms, mass death of fish and shellfish resulting from anoxic conditions, and long-term declines in coastal marine fisheries (Vitousek et al. 1997; NRC 2000; Rabalais et al. 2002).

The strong link between N enrichment of terrestrial ecosystems and eutrophication of coastal waters points to two needs: (1) the ability to predict nitrogen fluxes to coastal waters as N-additions to terrestrial ecosystems change; and (2) the development of effective strategies to manage landscape-scale N fluxes. Several approaches are converging to achieve these objectives. One set of studies has documented relationships between N inputs and N outputs at large spatial scales. A second group of investigations has focused intensively on N cycling and N removal in specific landscape elements, notably riparian zones. In combination, these investigations are building towards a mechanistic understanding of where N removal occurs in landscapes.

Considerable effort has been directed towards comparing patterns of N fluxes at large scales. In sixteen watersheds in the northeastern U.S., the ratio of N inputs to N outputs ranged from 10% to 40%, with most of the variation at higher N input rates (Boyer et al. 2002, Caraco et al. 2003). Comparisons of larger watersheds globally

have yielded similar results (Howarth et al. 1996). Johnson et al. (1997) included land use and surficial geology in their analysis of relationships between watershed characteristics and stream chemistry for 62 subcatchments within the Saginaw Bay watershed, Michigan. They found landscape characteristics in a 100 m buffer strip around the streams explained stream chemistry as well as landscape characteristics of entire subcatchments. Although seasonal patterns can vary (e.g., Johnson et al. 1997), nitrogen exports from watersheds often correlate with watershed characteristics such as land cover patterns. The percent of a watershed in agricultural or developed land can account for a substantial proportion of variation in N fluxes among watersheds (Johnson et al. 1997; Jones et al. 2001; Strayer et al. 2003; Weller et al. 2003).

Riparian zones have often been identified as hot spots of N removal because: (1) much of the water and associated nutrients moving from terrestrial to aquatic ecosystems passes through these landscape positions (Haycock et al. 1993); and (2) empirical studies suggest that denitrification rates in wetlands, including riparian zones, are high. Although the use of varying methods and site characteristics hampers a systematic synthesis of existing studies (Groffman 1994 but see Martin et al. 1999), many researchers have observed a decline in nitrate concentrations in shallow groundwater across short distances near streams (Clement et al. 2002 and references therein). These studies leave open the possibility that changes in nitrate concentration reflect dilution or measurements from different flow paths (Altman and Parizek 1993; Schnabel 1993; Pinay et al. 1998), and some studies have found substantial nitrate entering streams despite intact riparian zones along the stream channel (Kemp and Dodds 2001). In aggregate, however, studies of nitrate concentrations in riparian groundwater suggest that substantial nitrate removal occurs in shallow groundwater as it moves towards streams. Fewer studies have measured directly denitrification rates

in riparian groundwater, and these results are consistent with the general inference from the concentration gradient approach (Simmons et al. 1992; Pinay et al. 1995, Hill 1996, 2000; Hedin et al.1998; Tobias et al. 2001).

Syntheses of riparian zone N retention also indicate variability in N removal rates both within and among sites (Hanson et al. 1994; Gold et al. 2001; Johnston et al. 2001). This observation raises questions about which hydrogeologic features underlie this variation and whether differences in riparian zone N sink strength among types account for variation in N exports among watersheds (e.g., Jordan et al. 1997). Answering these questions requires additional study and understanding of subsurface flow, where considerable N transport and N removal occurs (Martin et al., 1999).

Considering these lines of research together highlights the insights and limitations associated with each one. Theoretical and empirical research on nitrogen cycling in riparian zones has established that substantial N removal from groundwater often occurs in these small-scale landscape features. At the landscape scale, these findings imply that: (1) N exports from landscapes should increase where changes in land use or climate remove hydrologic connections between riparian zones and terrestrial landscapes; and (2) landscapes with a higher percentage of intact riparian zones should have lower N export: N import ratios than landscapes with fewer intact riparian zones. However, aside from Jordan et al.'s (1997) highly suggestive comparison of piedmont and coastal plain watersheds, no study has yet shown empirically that N removal in riparian zones actually influences N fluxes at large scales.

In addition, models that lack riparian zones as explicit landscape elements show that landscape attributes other than riparian zone abundance exert strong influence on

watershed N exports. Statistical models relating watershed characteristics to N export have helped establish the relative importance of N sources such as agricultural fertilizer and sewage within the landscape. These models therefore allow us to estimate the types and directions of change likely to occur following large alterations in land use (Strayer et al. 2003). However, they lack even a coarse process-oriented treatment of N sinks. Without a mechanistic basis, models relating landscape characteristics to N exports have limited ability to assess the magnitude of shifts in N export in response to continued increases in N additions, or to N inputs in combination with new patterns of precipitation or disturbance.

These two lines of research lead to different, though not mutually exclusive, management implications. By identifying the relative importance of N sources, large-scale watershed analyses suggest where source reduction would be most effective. Research establishing riparian zones as strong N sinks suggests that protecting these landscape elements ("hot spots") will reduce N exports from landscapes. These different implications underscore the need to reconcile studies at different scales.

A central objective of linking studies at large and small scales is to determine whether, as Jordan et al. (1997) suggested, the extent of intact riparian zone explains variation in N exports if we control for N imports. If N removal at the soil-stream interface plays a large role in landscape-scale N fluxes, then statistical models that predict N exports solely as a function of N inputs are strongly disconnected from an important mechanism regulating landscape-scale N fluxes, increasing the chance that they will fail as these driving variables move outside their current ranges. Including factors such as riparian zone abundance or type in landscape N retention models might

explain some of the considerable variation in N export : N import ratios that remains after considering variables such as land cover type.

Ideally, we seek a mechanistic understanding of N fluxes through catchments, but incorporating N sinks such as riparian zones into landscape-scale N flux models presents a formidable challenge, particularly because so far we have failed to ascertain the distance from a stream over which landscape patterns influence streamwater chemistry (Gergel et al. 2002). Alternative approaches may be more tractable and likely to yield useful results in a shorter time frame.

One line of research that begins to link studies of riparian zone N cycling with investigations of landscape N flux builds on observed variation in N removal among riparian zones and considers how and why riparian zone N removal capacity varies with hydrogeologic setting (e.g., Pinay et al. 1995, 2002; Hill 1996; Jordan et al. 1997; Devito et al. 2000; Merrill 2001). This approach is potentially powerful because it seeks to explain variation in N sinks at the scale of recognizable functional landscape units. A focus on distal controls on denitrification facilitates the task of scaling up site-level processes to landscapes (Merrill 2001). Organizing riparian zone ecosystem processes according to a typology of sites has theoretical appeal because features which arise from hydrogeologic processes operating over large spatial and long temporal scales (e.g., soil texture, land surface slope, surficial geology, landscape position), in turn influence variables that control denitrification rates on small spatial and short temporal scales (e.g., organic matter distribution, groundwater residence time, water table depth).

A summary of findings from studies linking geomorphology and nitrogen cycling in riparian zones illustrates the approaches used and provides a point of departure for future research. Merrill (2001) found differences in denitrification, nitrification, and N mineralization among five mountain riparian ecosystem types in the Tahoe Basin differing in steepness and vegetation. In a comparison of nitrogen cycling among a levee, a riverbed, and a backwater, Johnston et al. (2001) concluded that biogeochemical variations among these wetlands resulted primarily from hydrologic zonation associated with geomorphic features. Focusing on soil texture, Pinay et al. (1995) and Groffman and Tiedje (1989) found greater denitrification rates in loamy compared to sandy riparian soils. Muller et al. (1980) and Groffman et al. (1991) found that denitrification potential correlated with wetland soil pH; because pH often reflects surficial geology, these data also suggest a link between hydrogeologic setting and denitrification potential. Rosenblatt et al. (2001) measured site attributes believed to correlate with denitrification and developed a geomorphic typology of forested riparian zones in Rhode Island relating denitrification potential to mapped soil characteristics. In a comparison of coastal plain and piedmont landscapes, Jordan et al. (1997) argued that lower N retention in piedmont watersheds resulted partially from deeper flow paths in the piedmont that delivered groundwater directly to streams.

Relationships between landscape-scale denitrification and geomorphology occur because site and landscape attributes influence C distribution and hydrology. Proximal controls on denitrification are simple: nitrate supply, anoxic conditions, and a supply of microbially-available carbon. Many studies have found the rate of denitrification to be limited by relatively low concentrations of nitrate in groundwater (see Groffman 1994 and references therein). However, because we are concerned with the capacity of riparian subsoils to remove nitrogen from groundwater, we

assume nitrate is present in excess. Of the remaining two variables (oxygen and carbon), oxygen levels should drop in areas of high microbial activity, which we expect to occur where available C is abundant. Thus, at small scales regulation of denitrification reduces to a single factor: supply of microbially-available C. To provide information that is both useful for management and relevant to landscape scale processes, we must identify site characteristics that: (1) create, or are associated with high concentrations of microbially-available C; and (2) lead to long contact times between nitrate-bearing groundwater and zones of high C supply.

First principles suggest three possible C sources for subsurface microbes: (1) dissolved organic carbon (DOC) leached from surface soils rich in available C; (2) plant roots growing through subsoils; and (3) buried, C-rich soil horizons formed by erosion and deposition in alluvial landscapes (Figure 1.1). Qualls and Haines (1991) found that DOC leached through the forest floor in a deciduous forest was recalcitrant; Siemens et al. (2003) and McCarty and Bremner (1992) also concluded that DOC leached from surface soils into groundwater was recalcitrant. Further, in mesocosm experiments conducted using subsoils from forested riparian zones in Rhode Island, DOC additions failed to stimulate denitrification (Jacinthe et al., 1998). Finally, a limited set of laboratory incubations in which I added DOC to riparian subsoils also suggested DOC was unlikely to fuel denitrification in riparian groundwater ecosystems. I therefore focused my research on tree roots and buried horizons as potential sources of microbially-available C in the subsurface and interpreted these results with respect to site and landscape level processes.

The mix of C sources may vary depending upon hydrogeologic setting, and this relationship connects my studies of C supply to landscape scale N cycling. Although

there are many ways to distinguish among hydrogeologic settings, those based on mapped feature have particular promise for applying results at large spatial scales. At a coarse scale, Rhode Island's riparian soils can be classified based on surficial geology as glacial till, glaciofluvial deposits, or alluvium, and have been mapped according to these categories (Wright and Dautter 1988; Rosenblatt et al. 2001). The later two categories (glaciofluvial, alluvium) occur on very low surface slopes (<2%) and are frequently dominated by *Acer rubrum* with some *Quercus alba* and an understory of *Clethra.spp* and *Vaccinium.spp*. A critical difference between these landscape settings is that in the subsurface, riparian soil profiles of alluvial origin characteristically include buried C horizons whereas soil profiles in sites of glaciofluvial origin do not. If buried horizons constitute an important source of microbially-available C, then C supply likely differs between glaciofluvial and alluvial sites.

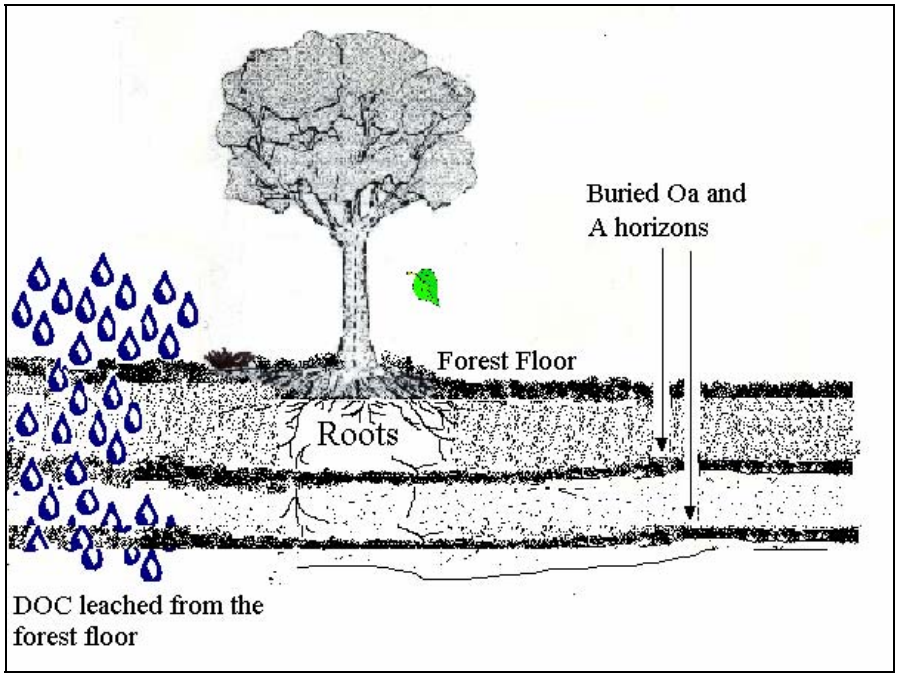


Figure 1.1. Possible sources of carbon to the riparian subsurface.

The mix of surface-derived C and ancient C supporting subsurface microbial activity may control both sustainability of riparian N sinks in the long term and maximum rates of denitrification they can support in the short term. Subsurface labile C derived from recent C fixation is likely replenished on annual time scales, and rates of denitrification that can be supported by this source may be limited by rates of C fixation and belowground NPP. Subsurface labile C derived from ancient, buried soils is not replenished; large quantities of C reside in these buried soils and they can potentially support high rates of denitrification. However, not all organic carbon is readily metabolized by microbes. Small organic molecules that are easily degraded contrast large molecules with complex geometries that resist microbial attack.

Roots as a C source in the riparian subsurface

Carbon (C) fixation aboveground and its subsequent transfer to soil ecosystems profoundly influence belowground processes including denitrification. A large body of research indicates that roots are an important source of labile C belowground and hence drive belowground processes (Bottner 1988; Qualls and Haines 1991; Coles 1997; Grayston 1997; Hogberg et al. 2001). Fine roots turn over rapidly, so C shuttled belowground becomes available to subsurface microbes (Hendrick and Pregitzer 1992), and root-derived carbon accounts for 70-80% of total soil respiration across a wide range of forests (Bowden et al. 1993 — reference therein). In short, the importance of root-derived C to microbial activity in surface soils of forests and grasslands has been established beyond doubt.

Because root biomass decreases exponentially with depth (Jackson et al. 1996), most research on root structure and function has focused in the upper 40 cm of the soil profile (e.g., Baker et al. 2001). However, Stone and Kalisz (1991) documented that

roots penetrate far below the surface in a number of ecosystems, and several studies have demonstrated that plants maintain functional roots many meters beneath the soil surface. Nepstadt et al. (1994) estimated that half the closed-canopy forests of the Brazilian Amazon depend upon deep roots to maintain green canopies during the dry season, and that roots below 1 meter contained more carbon than above-ground biomass. They further estimated that as much as 15% of carbon below 1 m decomposed on annual to decadal timescales. Dawson (1993), in studies of Acer saccharum, demonstrated that functional deep roots have ecosystem consequences in mesic as well as semi-arid ecosystems.

Investigations of plants' physiological adaptations to wetland soils have yielded a thorough understanding of how plant roots survive in saturated, oxygen-poor environments (Armstrong 1979; Bedford et al. 1991; Colmer 2003), but research on distributions, dynamics, and ecosystem consequences of roots in wetland soils has lagged behind. Despite physiological adaptations of wetland plants to saturated soils, a number of studies suggest that root biomass decreases below the water table, and in hollows compared to hummocks (Lieffers and Rothwell 1986; Day and Megonigal 1993; Jones et al. 1996; Burke and Chambers 2003). Nevertheless, wetland plant roots have been reported growing at depths well below the water table (e.g., Saarinen, 1996). In fact, wetland plants may more efficiently maintain root systems in continuously flooded sites than in sites with highly fluctuating water tables because the latter requires marked changes in root structure and physiology (Burke and Chambers 2003). A comparison of root biomass among four communities in the Great Dismal Swamp found the highest biomass in the least flooded site but the second-highest biomass at the site with the longest duration of soil saturation (Powell and Day 1991). In addition, a number of studies have measured root production in wetland soils (e.g.,

Shaver and Billings 1975; Symbula and Day 1988; Aerts et al. 1989; Powell and Day 1991; Megonigal and Day 1992; Conlin and Lieffers 1993; Jones et al. 2000; Weltzin et al. 2000; Baker et al. 2001; Moore et al. 2002; Burke and Chambers 2003). However, as with most terrestrial ecosystem research, these studies have focused on surface soils.

Wetland plant roots may drive microbial activity in saturated soils, but evidence regarding biogeochemical consequences of root-derived C in saturated soils and sediments is scarce especially at depth. Schade et al. (2001) demonstrated that belowground C inputs from shrubs support denitrification beneath gravel bars in desert stream ecosystems, but the plants' influence appeared to diminish below 20 cm depth. Gold et al. (1998) and Jacinthe et al. (1998) addressed the potential influence of roots on microbial processes a meter below the surface in northeastern riparian forests. They showed that hot spots of microbial activity occur in association with C-rich microsites apparently derived from roots, but they did not measure root biomass or estimate root turnover. From the point of view of landscape-scale N fluxes, data on root distributions and dynamics are necessary to estimate quantitatively the amount of microbial activity that root-derived C can support, and hence the levels of anthropogenically-derived N that could be removed in riparian zones.

My first objective was to describe root distributions in riparian subsoils. Areas of high root density in forest soils are often patchily distributed, as are hot spots of denitrification (e.g., Parkin, 1987). To estimate the capacity of riparian zones as N sinks, we must be primarily concerned with the abundance and distribution of hot spots rather than with the mean value of denitrification. Therefore, I measured root

biomass at 10 cm intervals between 50 and 100 cm depth from cores taken at 3 m intervals along transects through poorly-drained (PD) soil.

My second objective was to explain variation in root biomass among samples in order to improve predictive capacity of where high concentrations of root biomass and associated hot spots of microbial activity in the subsurface are likely to occur. In addition to soil depth, a likely determinant of root activity is soil texture. It is also possible that roots in the subsurface originate from tap roots that may form most frequently near the main trunk of a tree or shrub. In sum, I evaluated relationships between root biomass and depth from the surface, depth in relation to water table, distance to nearest tree, and soil texture.

My third objective was to estimate root production in the riparian subsurface. Burke and Chambers (2003) estimated root production in a bottomland hardwood forest at 1.0 - 1.2 Mg ha^{-1} yr^{-1} using root screens, and turnover of 12-40%. Powell and Day (1991) reported root production up to 3.6 Mg ha^{-1} yr^{-1} in a cedar dominated site in the Great Dismal Swamp. Baker et al. (2001) reported fine root production of 0.9 Mg ha^{-1} yr^{-1} in a poorly-drained soil in a mixed-oak floodplain. Working in forested wetlands along low-order streams in Alabama, Jones et al. (1996) estimated fine root net primary production (NPP) in the upper 50 cm of soil between 1.90 and 4.55 Mg ha^{-1} yr^{-1}. I used two independent methods to measure root production and turnover: ingrowth cores and radiocarbon dating. To estimate the contribution of root production to denitrification I conducted this study in a riparian zone characterized by soils of glaciofluvial origin, where I could eliminate the influence of buried C-rich horizons on subsurface C cycling.

Ancient carbon

Previous research on nitrogen cycling in riparian zones has suggested that buried channel deposits may provide a C source for denitrifying bacteria (Devito et al. 2000; Hill et al. 2000). Organic rich soil horizons beneath carbon poor horizons in floodplains have been reported previously by geomorphologists (e.g., Magilligan 1985). Blazejewski (2003) demonstrated that buried, C-rich soil horizons are ubiquitous within ten meters of low-order streams on shallowly-sloping, glaciated landscapes in New England, USA, raising the possibility that buried channel deposits are a common C source fueling denitrification across large regions. However, no published measurements of microbial C availability in these soils exist, and preliminary work revealed that carbon in these buried soils has a mean ^{14}C age of >4,000 ybp at 83-93 cm and >14,000 ybp at 300 cm.

Different perspectives lead to contrasting expectations about the lability of C associated with buried channel deposits and its potential role in supporting denitrification. Most forest soils research would lead to the expectation that C in buried horizons is recalcitrant. If belowground microbial activity is C limited, then microbially-available C should be quickly metabolized. Hence, soil C thousands of years old would be considered resistant to microbial decay. However, buried channel deposits probably formed during high-energy, episodic flood events, and the associated carbon may have been removed quickly from the microbially active surface. Further, although we cannot reconstruct the hydrology of these sites with precision, it is possible that this ancient C was underwater for much of the time since burial. The speed of burial and possible extent of saturation since that time could have greatly retarded decay of labile C pools. Finally, studies in geomicrobiology have shown conclusively that microbes can metabolize C sources that would never be

considered labile from an ecological perspective. For example, Petsch et al. (2001) discovered prokaryotes living on 365 million year old black shale using shale macromolecular compounds as their sole source of organic carbon. The question remains whether C mineralization rates associated with buried channel deposits are sufficient to be relevant to ecosystem processes. Relatively low rates may have a marked influence on denitrification in groundwater at sites with long residence times.

The overall objective of this section of my dissertation research was to ascertain the extent to which C associated with ubiquitous buried soil layers in alluvial landscapes is microbially-available. Within this broad objective, I tested two hypotheses with implications for management.

1) Lability of C associated with buried horizons would decrease with depth. This follows from the principle of superposition (i.e., strata on top necessarily were deposited after strata underneath), which implies that deeper horizons are older and have therefore had more time for labile C pools to be metabolized.

2) Sites with buried horizons would have higher C-availability than sites without buried horizons.

If C lability of buried soils decreases with depth, then the extent of denitrification expected should also be less for deeper than shallower flow paths. Although it would be unrealistic to obtain detailed hydrologic information for numerous catchments, understanding how C availability varies with depth nevertheless might allow a qualitative analysis of N removal and N transport through riparian zones.

Establishing the extent to which C availability varies between hydrogeologically distinct riparian zones could facilitate incorporation of riparian zone biogeochemistry into landscape-scale nitrogen flux models. The challenge in linking site-based and landscape-based analyses is how to move across scales. One way to meet this challenge might be to estimate the percentage of alluvial vs. glaciofluvial riparian zone in a catchment and to shift the expected N removal of each catchment accordingly (Rosenblatt et al. 2001).

Coupling of surface and subsurface ecosystems

Evaluating the relative importance of these two C sources to subsurface microbial activity enabled me to address the extent to which surface and subsurface ecosystems are linked and to what extent they are decoupled. If microbial processes in the subsurface are fueled mainly by plant roots, then subsurface processes and surface processes are strongly linked. This also means that questions about vegetation type and associated rooting depths and rates of root production are relevant to subsurface processes. In contrast, if subsurface microbial activity is supported mainly by buried channel deposits, then surface and subsurface ecosystems are decoupled and vegetation management may have only indirect influence, e.g., through bank stabilization.

To evaluate the extent of coupling between the surface and subsurface in riparian zones, I used two approaches. First, I compared variability of surface and subsurface processes among four riparian sites in Rhode Island. I drew on previous data comparing subsurface processes among four sites and showing high variability in potential denitrification rates. I also extracted soil cores from 3 of these sites and

measured C-mineralization rates in the laboratory. At each site I measured litterfall and soil respiration as indicators of aboveground C cycling.

Second, I measured the ^{14}C signature of dissolved inorganic carbon (DIC) in groundwater from three depths at the same four riparian sites, two alluvial and two glaciofluvial. Because soils in these landscapes have low carbonate contents (P. Groffman, W. Wright, personal communication), DIC must originate from microbial degradation of soil organic matter. Hence, the ^{14}C signal of DIC in groundwater should provide an integrative measure of the age of SOM used by microbes along the flow path. If surface and subsurface processes are strongly coupled, then groundwater DIC should have a ^{14}C signal reflecting recent surface processes. The weaker the coupling between surface and subsurface processes, the more the ^{14}C DIC signal will depart from the current atmospheric signal.

There are strong functional differences between these C sources (buried horizons and roots). Where buried horizons occur, they contain large C stores. However, most of the carbon in these buried horizons is old (4,000 – 14,000 ybp, Gurwick et al. 2002) and not replenished frequently. In contrast, plant root C pools are relatively small but are considerably younger and likely to be renewed on time scales less than ten years (Powell and Day 1991; Ruess et al. 1996, Gaudinski et al. 2000, Tierney and Fahey 2002).

If microbial activity in the riparian subsurface relies primarily on buried channel deposits, then a management focus on aboveground vegetation as a direct control on water quality maintenance may lack a scientific basis. Indirectly, bank stabilization as well as numerous unrelated ecosystem functions may justify vegetation-focused

management recommendations. Further, C supply associated with buried soils is less likely to exhibit strong seasonal fluctuations and hence more likely to support denitrification throughout the year.

Evidence that microbially-available C associated with buried channel deposits supports denitrification in shallow groundwater is ironic in two respects. First, it suggests that we are relying on old, presettlement C deposits to minimize environmental damage associated with recent anthropogenic acceleration of N fixation. Second, the potential degradation of old C associated with northern peatlands has engendered concern because of their spatial extent and potential contribution to the global atmospheric CO_2 and CH_4 pools. In the subsurface, buried channel deposits have a relatively small spatial extent and therefore comprise a relatively small fraction of the terrestrial C pool. In this case, benefits of N removal from groundwater may outweigh negative impacts of mineralizing ancient C.

Thesis outline

In chapter two I present data from laboratory experiments designed to assess C availability in riparian subsoils. These experiments enabled me to evaluate potential C availability of buried horizons and to compare C availability in sites with and without these alluvially-derived subsurface features. I use a simple modeling approach to estimate the potential denitrification supported by buried horizons in the riparian subsurface.

Chapter three focuses on in-situ measurements designed to evaluate the relative importance of these two C sources. Unlike lab incubations of buried soils, logistics and costs constrained my ability to assess variation within and among sites using this

approach. In-situ measurements described here therefore provide a small number of more definitive measurements to complement the more extensive measurements discussed in chapters two and four.

In chapter four I present my evaluation of root distributions and carbon supply. Most of this research focused on one site characterized by soils on outwash, but I also include data from two alluvial sites. I present results about root biomass, production, and age, and I use this data to develop a conceptual of how the relative importance of roots varies among three depth strata in riparian zones, from surface soils to the deep subsurface. As with my investigations of buried horizons in chapter 2, I use a simple modeling approach to estimate the potential denitrification supported by root production in the riparian subsurface.

Chapter five provides a synthesis of the main points in chapters two through four and articulates the primary insights gained by considering these investigations as a whole. I draw together results and insights gained from laboratory incubations of numerous buried horizons, in-situ studies of carbon mineralization and the age of respired carbon, and measurements of root biomass, production, and age. In this chapter, I also identify several promising directions for future research.

Literature Cited

Aber, J., W. McDowell, K. Nadelhoffer, A. Magill, G. Berntson, M. Kamakea, S. McNulty, W. Currie, L. Rustad and I. Fernandez (1998). "Nitrogen saturation in temperate forest ecosystems - Hypotheses revisited." Bioscience **48**(11): 921-934.

Aber, J. D., K. J. Nadelhoffer, P. Steudler and J. M. Melillo (1989). "Nitrogen saturation in northern forest ecosystems." BioScience **39**(6): 378-386.

Addy, K. L., A. J. Gold, P. M. Groffman and P. A. Jacinthe (1999). "Ground water nitrate removal in subsoil of forested and mowed riparian buffer zones." Journal of Environmental Quality **28**(3): 962-970.

Aerts, R., F. Berendse, N. M. Klerk and C. Bakker (1989). "Root production and root turnover in two dominant species of wet heathlands." Oecologia **81**(3): 374-378.

Altman, S.J. and R. R. Parizek (1993). Evaluation of Nitrate Removal from Groundwater in the Riparian Zone. Riparian Ecosystems in the Humid US, Atlanta, GA - Sheraton, National Association of Conservation Districts.

Armstrong, W. (1979). "Aeration in Higher Plants." Advances in Botanical Research **7**: 226-332.

Backeus, I. (1990). "Production and depth distribution of fine roots in a boreal open bog." Annales botanici fennici **27**: 261-265.

Baker III, T.T., W. H. Conner, B. G. Lockaby, J. A. Stanturf and M. K. Burke (2001). "Fine Root Productivity and Dynamics on a Forested Floodplain in South Carolina." Soil Science Society of America Journal **65**(2): 545-556.

Bedford, B.L., D.R. Bouldin and B.D. Beliveau (1991). "Net Oxygen and Carbon-Dioxide Balances in Solutions Bathing Roots of Wetland Plants." Journal of Ecology **79**(4): 943-959.

Bottner, P., Z. Sallih and B. G. (1988). "Root activity and carbon metabolism in soils." Biology and Fertility of Soils **7**(71): 71-78.

Bowden, R.D., K.J. Nadelhoffer, R.D. Boone, J.M. Melillo and J.B. Garrison (1993). "Contributions of Aboveground Litter, Belowground Litter, and Root Respiration to Total Soil Respiration in a Temperature Mixed Hardwood Forest." Canadian Journal of Forest Research **23**(7): 1402-1407.

Boyer, E.W., C.L. Goodale, N. A. Jaworski and R.W. Howarth (2002). "Anthropogenic nitrogen sources and relationships to riverine nitrogen export in the northeastern USA." Biogeochemistry **57**(1): 137-169.

Burke, M.K. and J. Chambers (2003). "Root dynamics in bottomland hardwood forests of the Southeastern United States Coastal Plain." Plant and Soil **250**(1): 141-153.

Caraco, N.F., J.J. Cole, G.E. Likens, G.M. Lovett and K.C. Weathers (2003). "Variation in NO_3^- Export from Flowing Waters of Vastly Different Sizes: Does One Model Fit All?" Ecosystems **6**(4): 344-352.

Carpenter, S.R., N.F. Caraco, D.L. Correll, R.W. Howarth, A.N. Sharpley and V.H. Smith (1998). "Nonpoint pollution of surface waters with phosphorous and nitrogen." Ecological Applications **8**(3): 559-568.

Clement, J.C., G. Pinay and P. Marmonier (2002). "Seasonal dynamics of denitrification along topohydrosequences in three different riparian wetlands." Journal of Environmental Quality **31**(3): 1025-1037.

Coles, J.R. (1997). Understanding the mechanisms controlling carbon dioxide and methane production in peat. Ithaca, NY, Cornell University**:** 159.

Colmer, T.D. (2003). "Long-distance transport of gases in plants: a perspective on internal aeration and radial oxygen loss from roots." Plant Cell and Environment **26**(1): 17-36.

Conlin, T.S.S. and V.J. Lieffers (1993). "Seasonal Growth of Black Spruce and Tamarack Roots in an Alberta Peatland." Canadian Journal of Botany **71**(2): 359-360.

Dawson, T.E. (1993). "Hydraulic lift and water use by plants: Implications for water balance, performance and plant-plant interactions." Oecologia **95**(4): 565-574.

Devito, K. J., D. Fitzgerald, A. R. Hill and R. Aravena (2000). "Nitrate dynamics in relation to lithology and hydrologic flow path in a river riparian zone." Journal of Environmental Quality **29**(4): 1075-1084.

Gaudinski, J. B., S. E. Trumbore, E. A. Davidson and S. H. Zheng (2000). "Soil carbon cycling in a temperate forest: radiocarbon-based estimates of residence times, sequestration rates and partitioning of fluxes." Biogeochemistry **51**(1): 33-69.

Gergel, S. E., M. G. Turner, J. R. Miller, J. M. Melack and E. H. Stanley (2002). "Landscape indicators of human impacts to riverine systems." Aquatic Sciences **64**(2): 118-128.

Gold, A. J., P. M. Groffman, K. Addy, D. Q. Kellogg, M. Stolt and A. E. Rosenblatt (2001). "Landscape attributes as controls on ground water nitrate removal capacity of riparian zones." Journal of the American Water Resources Association **37**(6): 1457-1464.

Gold, A. J., P. A. Jacinthe, P. M. Groffman, W. R. Wright and R. H. Puffer (1998). "Patchiness in groundwater nitrate removal in a riparian forest." Journal of Environmental Quality **27**(1): 146-155.

Grayston, S. J., D. Vaughan and D. Jones (1997). "Rhizosphere carbon flow in trees, in comparison with annual plants: The importance of root exudation and its impact on microbial activity and nutrient availability." Applied Soil Ecology **5**(1): 29-56.

Groffman, P. M. (1994). "Denitrification in Freshwater Wetlands." Current Topics in Wetland Biogeochemistry **1**: 15-35.

Groffman, P. M. and J. M. Tiedje (1989). "Denitrification in North Temperate Forest Soils: Spatial and Temporal Patterns at the Landscape and Seasonal Scales." Siol Biol. Biochem. **21**(5): 613-620.

Hanson, G. C., P. M. Groffman and A. J. Gold (1994). "Denitrification in Riparian Wetlands Receiving High and Low Groundwater Nitrate Inputs." Journal of Environmental Quality **23**(5): 917-922.

Haycock, N. E., G. Pinay and C. Walker (1993). "Nitrogen Retention in River Corridors: European Perspectives." Ambio **22**(6): 340-346.

Hedin, L. O., J. C. von Fischer, N. E. Ostrom, B. P. Kennedy, M. G. Brown and G. P. Robertson (1998). "Thermodynamic constraints on nitrogen transformations and other biogeochemical processes at soil-stream interfaces." Ecology **79**(2): 684-703.

Hendrick, R. L. and K. S. Pregitzer (1992). "The Demography of Fine Roots in a Northern Hardwood Forest." Ecology **73**(3): 1094-1104.

Hill, A. R. (1996). "Nitrate removal in stream riparian zones." Journal of Environmental Quality **25**(4): 743-754.

Hill, A. R., K. J. Devito, S. Campagnolo and K. Sanmugadas (2000). "Subsurface denitrification in a forest riparian zone: Interactions between hydrology and supplies of nitrate and organic carbon." Biogeochemistry **51**(2): 193-223.

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**(14 June): 789-791.

Howarth, R. W., G. Billen, D. Swaney, A. Townsend, N. Jaworski, K. Lajtha, J. A. Downing, R. Elmgren, N. Caraco, T. Jordan*, et al.* (1996). "Regional nitrogen budgets and riverine N & P fluxes for the drainages to the North Atlantic Ocean." Biogeochemistry **35**(1): 75-139.

Howarth, R. W., E. W. Boyer, W. J. Pabich and J. N. Galloway (2002). "Nitrogen use in the United States from 1961-2000 and potential future trends." Ambio **31**(2): 88-96.

Jacinthe, P.-A., P. M. Groffman, A. J. Gold and A. Mosier (1998). "Patchiness in microbial nitrogen transformations in groundwater in a riparian forest." Journal of Environmental Quality **27**(1): 156-164.

Jackson, R. B., J. Canadell, J. R. Ehleringer, H. A. Mooney, O. E. Sala and E. D. Schulze (1996). "A global analysis of root distributions for terrestrial biomes." Oecologia **108**(3): 389-411.

Johnson, L. B., C. Richards, G. E. Host and J. W. Arthur (1997). "Landscape influences on water chemistry in Midwestern stream ecosystems." Freshwater Biology **37**(1): 193-208.

Johnston, C. A., S. D. Bridgham and J. P. Schubauer-Berigan (2001). "Nutrient Dynamics in Relation to Geomorphology of Riverine Wetlands." Soil Science Society Of America Journal **65**: 557-577.

Jones, K. B., A. C. Neale, M. S. Nash, R. D. Van Remortel, J. D. Wickham, K. H. Ritters and R. V. O'Neill (2001). "Predicting nutrient and sediment loadings to streams from landscape metrics: A multiple watershed study from the United States Mid-Atlantic Region." Landscape Ecology **16**(4): 301-312.

Jordan, T. E., D. L. Correll and D. E. Weller (1993). "Nutrient Interception by a Riparian Forest Receiving Inputs from Adjacent Cropland." Journal of Environmental Quality **22**(3): 467-473.

Jordan, T. E., D. L. Correll and D. E. Weller (1997). "Relating nutrient discharges from watersheds to land use and streamflow variability." Water Resources Research **33**(11): 2579-2590.

Kemp, M. J. and W. K. Dodds (2001). "Spatial and temporal patterns of nitrogen concentrations in pristine and agriculturally-influenced prairie streams." Biogeochemistry **53**(2): pp. 125-141.

Lieffers, V. J. and R. L. Rothwell (1986). "Effects of Depth of Water-Table and Substrate-Temperature On Root and Top Growth of Picea-Mariana and Larix-Laricina Seedlings." Canadian Journal of Forest Research **16**(6): 1201-1206.

Magilligan, F. J. (1985). "Historical floodplain sedimentation in the Gelena River Basin, Wisconsin and Illinois." Annals Association American Geographers **75**: 583-594.

Malanson, G. P. (1993). Riparian Landscapes. New York, Cambridge University Press.

Martin, T. L., N. K. Kaushik, J. T. Trevors and H. R. Whiteley (1999). "Review: Denitrification in temperate climate riparian zones." Water Air and Soil Pollution **111**(1-4): 171-186.

McClain, M. E., J. E. Richey and T. P. Pimentel (1994). "Groundwater Nitrogen Dynamics At the Terrestrial-Lotic Interface of a Small Catchment in the Central Amazon Basin." Biogeochemistry **27**(2): 113-127.

Megonigal, J. P. and F. P. Day (1992). "Effects of Flooding On Root and Shoot Production of Bald Cypress in Large Experimental Enclosures." Ecology **73**(4): 1182-1193.

Merrill, A. G. (2001). Variation in Structure and Nitrogen Dynamics of Mountain Riparian Zones. Berkeley, CA, University of California**:** 311.

Moore, T. R., J. L. Bubier, S. E. Frolking, P. M. Lafleur and N. T. Roulet (2002). "Plant biomass and production and CO2 exchange in an ombrotrophic bog." Journal of Ecology **90**(1): 25-36.

Nepstad, D. C., C. R. De Carvalho, E. A. Davidson, H. Jipp-Peter, P. A. Lefebvre, G. H. Negreiros, E. D. Da Silva, T. A. Stone, S. E. Trumbore and S. Vieira (1994). "The role of deep roots in the hydrological and carbon cycles of amazonian forests and pastures." Nature **372**(6507): 666-669.

Parkin, T. B. (1987). "Soil microsites as a source of denitrification variability." Soil Science Society of America Journal **51**: 1194-1199.

Peterjohn, W. T. and D. L. Correll (1984). "Nutrient Dynamics in an Agricultural Watershed: Observations on the Role of a Riparian Forest." Ecology **65**(5): 1466-1475.

Petsch, S. T., T. I. Eglinton and K. J. Edwards (2001). "C-14-dead living biomass: Evidence for microbial assimilation of ancient organic carbon during share weathering." Science **292**(5519): 1127-1131.

Pinay, G., J. C. Clement and R. J. Naiman (2002). "Basic principles and ecological consequences of changing water regimes on nitrogen cycling in fluvial systems." Environmental Management **30**(4): 481-491.

Pinay, G., C. Ruffinoni and A. Fabre (1995). "Nitrogen Cycling in Two Riparian Forest Soils Under Different Geomorphic Conditions." Biogeochemistry **30**: 9-29.

Pinay, G., C. Ruffinoni, S. Wondzell and F. Gazelle (1998). "Change in groundwater nitrate concentration in a large river floodplain: denitrification, uptake, or mixing?" Journal of the North American Benthological Society **17**(2): 179-189.

Powell, S. W. and F. P. Day (1991). "Root Production in Four Communities in the Great Dismal Swamp." American Journal of Botany **78**(2): 288-297.

Qualls, R. G. and B. L. Haines (1991). "Fluxes of dissolved organic nutrients and humic substances in a deciduous forest." Ecology **72**(1): 254-266.

Rabalais, N. N., R. E. Turner and W. J. Wiseman Jr (2002). "Gulf of Mexico Hypoxia, a.k.a. "The Dead Zone"." Annual Review of Ecology and Systematics **33**: 235-263.

Rosenblatt, A. E., A. J. Gold, M. H. Stolt, P. M. Groffman and D. Q. Kellogg (2001). "Identifying riparian sinks for watershed nitrate using soil surveys." Journal of Environmental Quality **30**(5): 1596-1604.

Ruess, R. W., K. Van Cleve, J. Yarie and L. A. Viereck (1996). "Contributions of fine root production and turnover to the carbon and nitrogen cycling in taiga forests of the Alaskan interior." Canadian Journal of Forest Research **26**(8): 1326-1336.

Saarinen, T. (1996). "Biomass and production of two vascular plants in a boreal mesotrophic fen." Canadian Journal of Botany **74**: 934-938.

Schade, J. D., S. G. Fisher, N. B. Grimm and J. A. Seddon (2001). "The influence of a riparian shrub on nitrogen cycling in a Sonoran Desert stream." Ecology **82**(12): 3363-3376.

Schnabel, R. R., J. B. Urban and W. J. Gburek (1993). "Hydrologic Controls on Nitrate, Sulfate, and Chloride Concentrations." Journal of Environmental Quality **22**: 589-596.

Shaver, G. R. and W. D. Billings (1975). "Root Production and Root Turnover in a Wet Tundra Ecosystem, Barrow, Alaska." Ecology **56**(2): 401-409.

Siemens, J., M. Haas and M. Kaupenjohann (2003). "Dissolved organic matter induced denitrification in subsoils and aquifers?" Geoderma **113**(3-4): 253-271.

Simmons, R. C., A. J. Gold and P. M. Groffman (1992). "Nitrate Dynamics in Riparian Forests - Groundwater Studies." Journal of Environmental Quality **21**(4): 659-665.

Stone, E. L. and P. J. Kalisz (1991). "On the maximum extent of roots." Forest Ecology and Management **46**: 59-102.

Strayer, D. L., R. E. Beighley, L. C. Thompson, S. Brooks, C. Nilsson, G. Pinay and R. J. Naiman (2003). "Effects of land cover on stream ecosystems: Roles of empirical models and scaling issues." Ecosystems **6**(5): 407-423.

Symbula, M. and F. P. Day (1988). "Evaluation of two methods for estimating belowground production in a freshwater swamp forest." American Midland Naturalist **120**(2): 405-450.

Tierney, G. L. and T. J. Fahey (2002). "Fine root turnover in a northern hardwood forest: a direct comparison of the radiocarbon and minirhizotron methods." Canadian Journal of Forest Research **32**(9): 1692-1697.

Tobias, C. R., S. A. Macko, I. C. Anderson, E. A. Canuel and J. W. Harvey (2001). "Tracking the fate of a high concentration groundwater nitrate plume through a fringing marsh: A combined groundwater tracer and in situ isotope enrichment study." Limnology and Oceanography **46**(8): 1977-1989.

Van Breemen, N., E. W. Boyer, C. L. Goodale, N. A. Jaworski, K. Paustian, S. P. Seitzinger, K. Lajtha, B. Mayer, D. Van Dam, R. W. Howarth*, et al.* (2002). "Where did all the nitrogen go? Fate of nitrogen inputs to large watersheds in the northeastern USA." Biogeochemistry **57**(1): 267-293.

Vitousek, P. M., J. D. Aber, R. W. Howarth, G. E. Likens, P. A. Matson, D. D. Schindler, W. H. Schlesinger and D. G. Tilman (1997). "Human alteration of the global nitrogen cycle: sources and consequences." Ecological Applications **7**(3): 737-750.

Weller, D. E., T. E. Jordan, D. L. Correll and Z. J. Liu (2003). "Effects of land-use change on nutrient discharges from the Patuxent River watershed." Estuaries **26**(2A): 244-266.

Weltzin, J. F., J. Pastor, C. Harth, S. D. Bridgham, K. Updegraff and C. T. Chapin (2000). "Response of bog and fen plant communities to warming and water-table manipulations." Ecology **81**(12): 3464-3478.

Wright, W. R. and E. H. Dautter (1988). Soils of the Rhode Island Landscape, University of Rhode Island Agricultural Experiment Station.

CHAPTER TWO: MICROBIALLY-AVAILABLE CARBON IN BURIED SOILS

Introduction

Intensive nitrogen (N) inputs to terrestrial ecosystems cause diverse problems for human health and environmental conservation in upland forests and down-gradient ecosystems (Vitousek et al. 1997; Carpenter et al. 1998). Acute problems associated with coastal eutrophication include reduced biodiversity, increased frequency of nuisance algal blooms, mass death of fish and shellfish resulting from anoxic conditions, and long-term declines in coastal marine fisheries (Vitousek et al. 1997; NRC 2000).

Comprehensive solutions to address N pollution problems should include both source reduction (Wigington et al. 2003) and management of N sinks (Driscoll et al. 2003) to maximize removal of N inputs before they reach downstream ecosystems. Diffuse N inputs such as N deposition are likely intercepted broadly across the landscape in surface soils, but high N loads from agricultural operations and septic effluent can bypass the surface plant-soil system and enter shallow aquifers. Nitrate removal from shallow groundwater is thought to occur primarily in specific landscape elements, and riparian zones sit atop the list of N removal hot spots in the landscape (Hill 1996).

The view that riparian zones efficiently remove N from shallow groundwater at the terrestrial-aquatic interface grew from studies showing declines in NO_3^- concentrations along transects perpendicular to streams, sometimes over distances of only a few meters (Lowrance et al. 1984, Peterjohn and Correll, 1984, Jacobs and Gilliam 1985). Over the past two decades, numerous studies — most of them focused on surface riparian soils — have revealed high variability in N removal among riparian zones,

inhibiting widespread adoption and evaluation of riparian zones as a solution to N pollution problems (Groffman et al. 1992, 1996, Hill 1996, Lowrance et al. 1997, Martin et al. 1999, Burt et al. 2002).

There have been several attempts to organize the abundant and variable data on riparian zone NO_3^- removal functions. A number of studies have asked whether N removal varies predictably with vegetation type, notably grass vs. trees and evergreen vs. deciduous trees (Sweeney 1992, Haycock and Pinay 1993). A strong influence of vegetation type on N removal would have been useful both for prediction, because it is easily identified and mapped, and for management because it can be manipulated, particularly in the context of riparian restoration. Although some studies found differences in N removal between grass dominated and tree-dominated sites, no regular pattern has emerged across studies (Correll 1997, Verchot et al. 1997, Addy et al. 1999, Sabater et al. 2003).

A second set of studies have proposed hydrogeology as a mechanistic framework for explaining variation in N removal among riparian zones in both natural and human-manipulated landscapes, and for developing functional riparian classifications (Jordan et al. 1997, Devito et al. 2000, Puckett 2004, Vidon and Hill 2004, Kellogg et al. 2005). For N removal to occur, flow paths of N-rich groundwater must intersect zones of active plant uptake and/or anaerobic conditions and microbially-available organic carbon or other electron donors. Frequently, high levels of labile C lead to high rates of microbial respiration, which draw down oxygen levels. Landscape features associated with flow paths that meet these criteria are more likely than others to harbor riparian zones with high rates of N removal.

In a number of instances, hydrogeology has proven to be an effective framework for explaining variation in riparian zone N removal. Jordan et al. (1997) invoked hydrogeologic control of groundwater:riparian zone interaction to explain differences in streamwater chemistry between coastal plain and piedmont physiographic provinces in the Chesapeake Bay drainage basin. Vidon and Hill (2004) proposed riparian zone functional classifications based on local features such as land surface slope and hydraulic conductivity that influence the interaction of upland-derived groundwater with areas of rapid denitrification. They demonstrated that low N removal rates can occur at sites where steep slopes create narrow floodplains, and impermeable sediments force N-rich groundwater to the surface (seeps), leaving little chance for re-infiltration before it reaches the stream channel (Vidon and Hill 2004). Gold et al. (2001) proposed classifying riparian zones in the glaciated northeastern United States according to mapped surficial geology (till vs. outwash vs. alluvium) associated with landform evolution. They suggested that: (1) groundwater moving across riparian zones on glacial till would often be forced to the surface, hence bypassing the intersection with high-organic matter, low-oxygen environments; and that (2) groundwater moving through glacial outwash or alluvium would move through the subsurface, creating opportunities for long residence times and interactions with organic matter. The proposition that subsurface flow is conducive to high rates of N removal is consistent with observations of low but significant rates of N removal in the riparian subsurface, although many studies have invoked low levels of available C as a fundamental constraint on denitrification at depth in soil profiles (Trudell et al. 1986, Slater and Capone 1987, Smith and Duff 1988, Francis et al. 1989, Obenhuber and Lowrance 1991, Yeomans et al. 1992, Starr and Gillham 1993, Hill 1996, Clement et al. 2002, Puckett 2004).

Confidence in these classification systems increases to the extent that they reflect our understanding of how carbon availability and anaerobic conditions vary with different geologic materials along the upland-riparian-stream continuum. This understanding has been hampered by a tendency to focus on surface soils and near-surface processes, but information on structure in subsurface soils and sediments has begun to emerge (Gold et al. 2001, Vidon and Hill 2004, Blazejewski et al. 2005). As the picture of subsurface structure has developed, it has yielded insight about factors that regulate the occurrence and lability of carbon deposits in the subsurface and mechanisms responsible for denitrification in the parts of riparian zones where most shallow groundwater flow occurs.

In particular, two types of organic matter have been observed and shown capable of supporting denitrification in the riparian subsurface in glaciated landscapes: (1) micro-scale patches ("hot spots") derived from plant material (Parkin 1987); and (2) buried soil lenses and horizons (Fustec et al. 1991, Haycock and Pinay 1993). Jacinthe et al. (1998) and Gold et al. (1998) demonstrated that root-derived micro-scale patches in C-poor glacial outwash account for the bulk of denitrification occurring in subsurface sediments in Rhode Island, and studies of riparian zones in southeastern Ontario have also reported patches of organic matter within coarse-textured mineral soils. The Ontario group also reported buried soil horizons in 4 of 5 riparian study sites and demonstrated marked increases in denitrification associated with buried horizons and channel deposits near streams (Devito et al. 2000, Hill and Cardaci 2004, Hill et al. 2004). Finally, Well et al. (2005) described fluvisols in Germany containing dark humic colors, woody debris, and buried peat deposits, with some profiles showing increased percent organic matter and denitrification capacity between 100 and 300 cm.

The impact of subsurface organic matter on landscape-scale N transport depends upon its lability, frequency, and spatial distribution.

While the occurrence of micro-scale patches will be driven by subsurface root dynamics, buried channel deposits appear to be ubiquitous subsurface features in the riparian landscape. Blazejewski (2002) surveyed 22 riparian zones of 1^{st}-4^{th} order streams in Rhode Island and found >280 buried horizons within 50 cm of the surface and 66 between 100-200 cm of the surface. Frequently, these horizons occupied positions in the soil profile adjacent to highly conductive sands and gravels creating opportunities at multiple depths for groundwater flow paths to intersect biologically active zones with a supply of electron donors. Surprisingly, buried horizons were not necessarily more common in soils classified as "alluvial" than in those classified as derived from glacial outwash. Understanding how the relative importance of C-sources varies across sites will contribute to understanding and perhaps predicting spatial variability in landscape-scale N fluxes.

The very limited number of studies on microbial activity in buried horizons contrasts the ubiquity of these soils in riparian zones, at least in Rhode Island, and critical questions therefore remain unanswered. First, it is important to determine if microbial activity — and hence the potential to support denitrification — is a general feature associated with buried soils, or an idiosyncratic feature of the Ontario soils described by Hill and Cardaci (2004). The spatial pattern of denitrification reported by Kellogg et al. (2005) suggested that buried horizons in Rhode Island also contain microbially-available C but that assumption remained to be tested.

Second, are buried horizons more or less similar in terms of microbial activity? Do shallower (and therefore younger) buried horizons support higher levels of microbial activity than those deeper in the profile? If these localized features act as hot spots of carbon mineralization and denitrification within riparian zones, then the distribution of those hot spots with respect to groundwater flow paths strongly influences their effect on landscape-scale N removal. In addition, aboveground components of ecosystems may interact more with relatively shallow buried horizons than with those deeper in the soil profile, e.g., by penetration of live roots.

Third, does microbial activity in buried horizons vary predictably with soil horizon type or soil chemistry? We expect soil horizons with more carbon (A, Ab) to have more microbially-available C and higher levels of microbial activity than C/A, A/C, or B horizons. We also expect organic matter in older buried horizons to be more decomposed compared to younger, shallower horizons. If carbon associated with buried horizons becomes more decomposed and less labile with age (and hence depth), then deeper horizons should also: (1) be more humified and therefore have lower C:N ratios; (2) have enriched $^{13}C/^{12}C$ and $^{15}N/^{14}N$ ratios; and (3) have less microbially-available C than shallower horizons.

Fourth, how sustainable is carbon mineralization associated with buried soil horizons? Radiocarbon dating showed these buried soils to be thousands of years old (Blazejewski et al. 2005), and those deeper in the soil profile are clearly older than those nearer the surface. The persistence of this organic matter over millennia raises questions about controls on its decomposition. If decomposition has been impeded by a paucity of electron acceptors, then sharp increases in electron acceptor abundance might lead to relatively rapid depletion of these deep C pools.

In this study, our objectives were to: (1) determine the extent to which C in buried horizons in Rhode Island is microbially available; (2) identify spatial patterns of carbon mineralization associated with buried horizons; (3) evaluate likely relationships between soil horizon types, chemical characteristics and carbon mineralization in buried horizons; and (4) determine whether microbial activity in buried horizons is limited by a paucity of electron acceptors.

To accomplish these objectives, we sampled and characterized a wide range of buried horizons from a variety of soil profiles in coastal, glaciated watersheds in southern New England. Lability was assessed by measuring CO_2 production in laboratory incubations and microbial biomass. Chemical characterization included percent C, percent N, and $^{13}C/^{12}C$ and $^{15}N/^{14}N$ ratios.

Methods and site descriptions

Site descriptions

We collected soil samples from riparian forests along fourteen stream reaches in the Pawcatuck Watershed, Rhode Island, USA. Surficial geology reflects the region's recent glacial history, with glacial outwash and/or alluvial deposits characterizing all sites in this study. *Acer rubrum* dominated the overstory vegetation at all sites; common understory species included sweet pepperbush (*Clethra alnifolia*), and highbush blueberry (*Vaccinium corymbosum*). Soil drainage classes ranged from very poorly drained (VPD) to somewhat poorly drained (SPD), and sampling occurred between 0.5 and 31 meters from the stream edge (Table 2.1). At four sites for which we had temporal records of water table depth, the summer minimum ranged from 46-98 cm below the surface, and the dormant season maximum ranged from 12 cm below

the surface to 13 cm above the surface, with a greater range at sites in alluvial compared to outwash settings (Kellogg et al. 2005).

Table 2.1. Stream and soil characteristics at sites where we sampled buried soil horizons (PD=poorly drained, VPD=very poorly drained, SPD=somewhat poorly drained). Sites are described in more detail in Blazejewski et al. (2005) and Kellogg et al. (2005).

Site name	Stream order	Soil drainage class	Distance from stream (m)
Burlingame	1st	PD	0.5
Carolina Fish Hatchery	1st	PD	3
Liberty Lane	1st	VPD	2
Yagoo Pond	1st	VPD	5
Meadow Brook	2nd	PD	10
Peckham	2nd	VPD	0.5
Beaver River 1	3rd	PD	10
Parris Brook	3rd	PD	31
Beaver River 2	3rd	VPD	5
Beaver River 3	3rd	VPD	20
Beaver River 4	3rd	VPD	20
Wood River 1	4th	PD	1
Wood River 2	4th	PD	10
Wood River 3	4th	SPD	0.5

Sample collection

Soil samples were collected using a standard bucket auger, separated into different horizons in the field, and transported to the laboratory in a cooler. We stored them at 4^{o}C until they were either dried or incubated.

Laboratory incubations

We incubated between 2 g (for Oa horizons) and 40 g (for C horizons) of soil anaerobically in mason jars, varying the amount of soil inversely with visual estimates of C abundance. Because our objective was to assess carbon availability under conditions of enhanced N loads, we also added 20 mg NO_3-N per kg of soil to each

jar, in 10 mls of deionized water. Jars were sequentially evacuated and flushed with N_2 gas at least three times and then brought to atmospheric pressure using a water trap. We measured the CO_2 concentration in the headspace of each jar after 1, 7, 14, and 28 days on a Varian 3400X gas chromatograph equipped with a thermal conductivity detector.

To assess the influence of nitrate availability and DOC from forest floor leachate on microbial activity in the subsurface, we incubated three replicate jars of five soil samples. For each soil, one jar received only deionized water (DI), one received DI with nitrate, and one received filtrate from forest floor shaken in DI for one hour and passed through a GF/F filter.

After concluding the incubation studies, we destructively sampled each jar to obtain a sample-specific measurement of total soil mass. We oven-dried the entire sample in each jar at 60 deg C for a minimum of three days and weighed it.

Soil chemistry

We measured soil carbon and nitrogen content, ^{13}C, and ^{15}N on a subset of samples used for our incubation experiments. We removed most roots, particulate organic matter, and rocks, and then ground samples to a fine powder using a freezer mill. Samples were analyzed at the Cornell Stable Isotope Laboratory on a Finnigan MAT Delta Plus stable isotope ratio mass spectrometer running in continuous flow mode.

Microbial biomass

We measured microbial biomass using chloroform fumigation extraction (Paul et al. 1999). We fumigated one sample of each soil in a sealed dessicator for 24 hours. We

then extracted it and a paired sample with 0.5 M K_2SO_4 and filtered resulting extracts through pre-ashed GF/F filters. Filtrate was analyzed for dissolved organic carbon (DOC) on a Shimadzu TOC-5050 analyzer at McGill University using high temperature combustion with a platinum catalyst at 680 deg C and an IRGA, with up to 6 analytical replicates per sample. We calculated microbial biomass C using a value of 0.35 for Kec.

Data analysis

For data analysis, we used JMP IN 5.1 and SAS 9.1.3 (SAS Institute, Cary, NC). We tested for differences among sample groups using 1-way ANOVAs. We used regression and correlation analyses to explain relationships between soil chemistry and microbial activity.

Results

Carbon mineralization

Carbon dioxide accumulated steadily over the 28-day incubations (Figure 2.1). While Figure 2.1 shows only data from samples collected in September 2003, we observed a similar pattern in incubations from other sample dates. For most soil samples, carbon mineralization rates were similar at the beginning and end of the incubation, diminishing only slightly. Soils with high carbon mineralization rates (A and O horizons) exhibited a different temporal pattern of carbon mineralization. Carbon mineralization rates associated with these soils declined between 200-400 hours and then increased, while carbon mineralization rates associated with Ab and C-poor soils exhibited a slow decline over the course of the study (Figure 2.1).

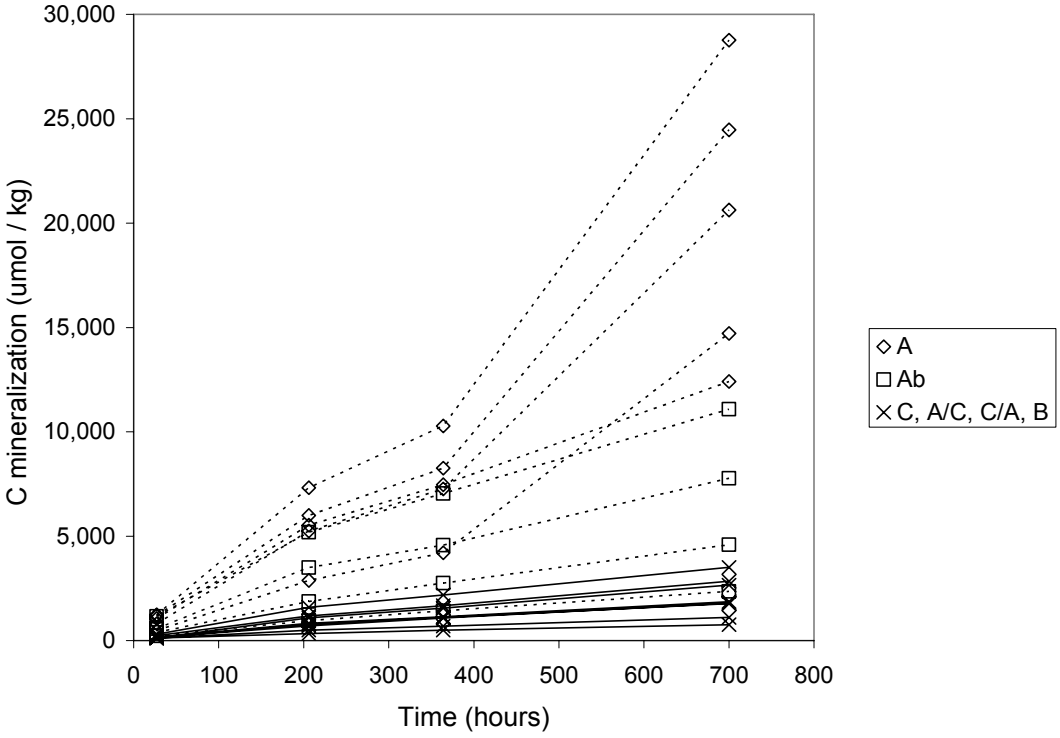


Figure 2.1. CO_2 accumulation during incubations of 18 riparian soil horizons (A, Ab, B, C, A/C, C/A) collected in September 2003. Samples from Oi and Oa horizons (not shown) mineralized 150,000 – 360,000 um CO_2 / kg by day 700.

Soil depth and horizon type

Apart from surface O horizons, which had carbon mineralization rates much higher than other soils, we found no relationship (ANOVA, $p>0.5$) between carbon mineralization rate and soil depth in the samples taken in September 2003 (Figure 2.2a). There were strong differences among soil horizons (ANOVA, $p< 0.001$). Irrespective of depth, A and Ab horizons had consistently higher rates than C, A/C, C/A, and B horizons. Ab horizons, which are overlain by B or C horizons, generally yielded lower carbon mineralization rates than deep A horizons (Figure 2.2a).

To further explore relationships between soil characteristics and carbon mineralization in the riparian subsurface, we combined data from samples taken in September 2003

with data from samples collected previously (Figure 2.2b). An ANOVA run on the entire data set with carbon mineralization as the dependent variable and depth, horizon type, horizon thickness and experiment (i.e., sample batch) as independent variables yielded an R^2 of 0.52 with overall model $p < 0.001$ (n=56). Soil horizon was a strong predictor ($p<0.005$) of mineralization, and depth was marginally significant ($p=0.06$). In an ANOVA excluding surface O horizons, which have exceptionally high activity, horizon remained a key predictor ($p<0.0001$), but depth was no longer significant ($p>0.50$). Overall model R^2 was 0.50, $p<0.001$, n=52.

Focusing on those horizons most likely to fuel microbial activity in the subsurface, i.e., A, Ab, and buried O horizons and lenses, we ran an ANOVA excluding C, A/C, C/A, and B horizons, which have exceptionally low activity, as well as the surface O horizons. This analysis (overall $R^2=0.5$, $p<0.001$) also showed a relationship between carbon mineralization and horizon type but not depth (Figure 2.2c, $p<0.001$, n=36). Sample batch was significant in some of these analyses, likely due to the different mix of horizons represented in the different batches of samples.

Microbial biomass

Microbial biomass measured on fresh samples mirrored the relationship of carbon mineralization to soil horizon. We found greatest biomass in O horizons, intermediate levels in A and Ab horizons, and lowest levels in C and C/A horizons (Figure 2.3). Below the surface O horizons, microbial biomass did not vary significantly with soil depth ($p >0.2$). We found a strong correlation between microbial biomass and carbon mineralization rate (Figure 2.4, $r=0.96$, $p < 0.0001$, n=21) for nontransformed data.

Response to amendments

Carbon mineralization declined in response to nitrate amendments (p=0.05, 2-tailed paired T-test). However, these differences were small compared with variation in carbon mineralization owing to innate characteristics of six soil horizons varying in depth and site of origin (Figure 2.5). DOC amendments did not influence carbon mineralization (p > 0.3, 2-tailed paired T-test).

(a)

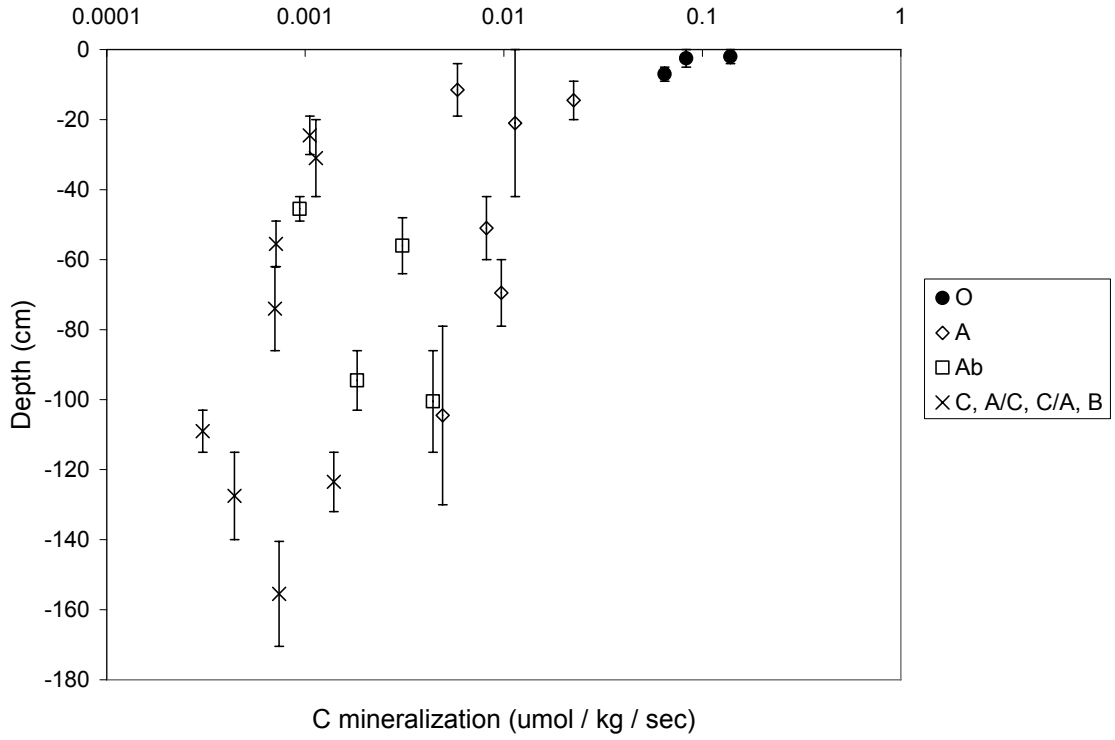
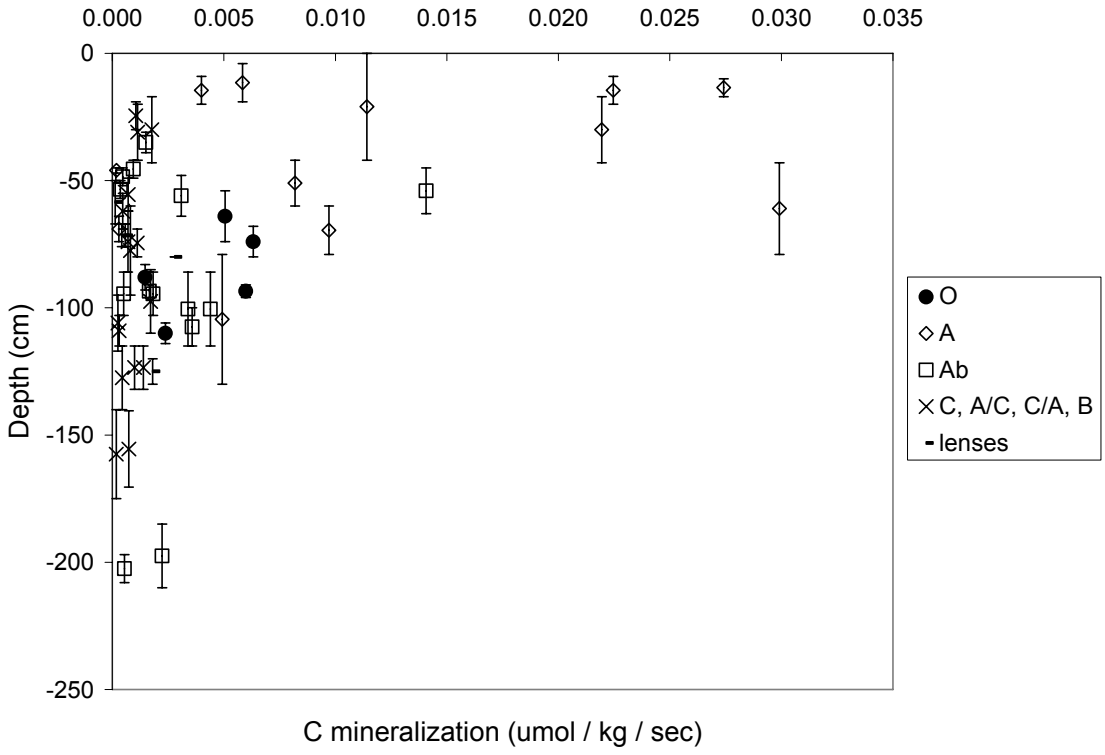


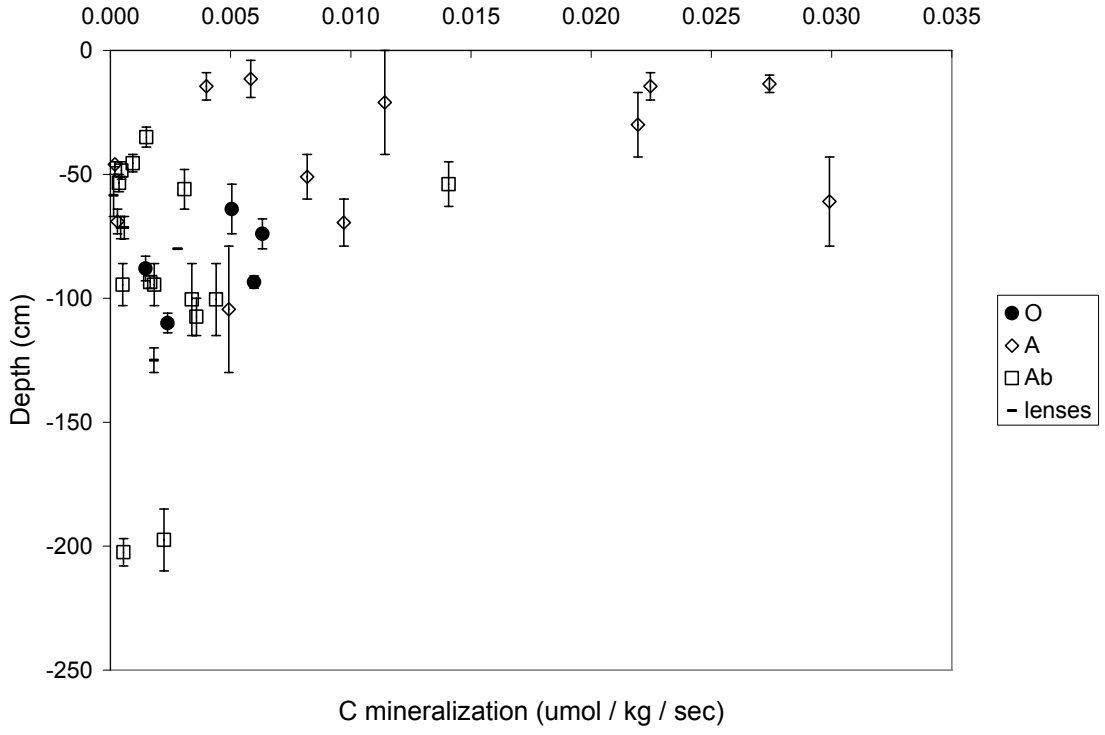
Figure 2.2. Carbon mineralization versus depth of riparian forest soil horizons during anaerobic incubations amended with KNO_3: (a) O, A. Ab, C, A/C, C/A and B horizons from three riparian forest sites sampled in September 2003. Vertical bars indicate the thickness of each soil horizon, not measurement error. Note ln scale on x-axis. (b) Data synthesized from three incubation experiments including soils from 10 sites. Surface O horizons had carbon mineralization rates 0.06 – 0.14 umol / kg / sec (data not shown). (c) Data from three incubation experiments but including samples from A, Ab, buried O horizons, and lenses only.

Figure 2.2. (Continued).

(b)



(c)



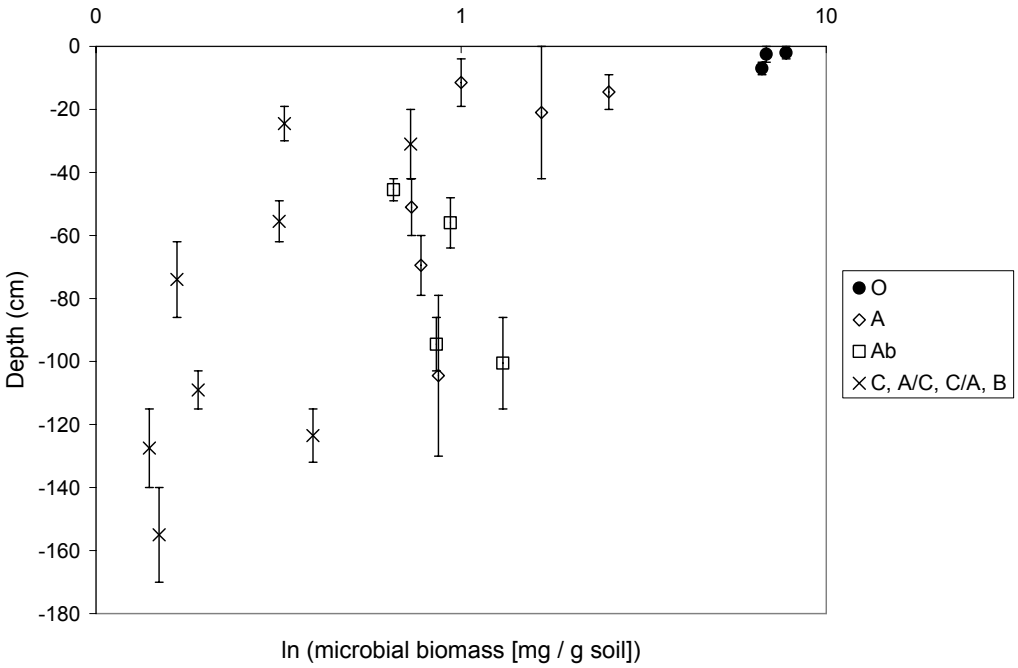


Figure 2.3. Microbial biomass versus depth in 21 riparian soil horizons (O, A, Ab, B, C, A/C, C/A) collected in September 2003.

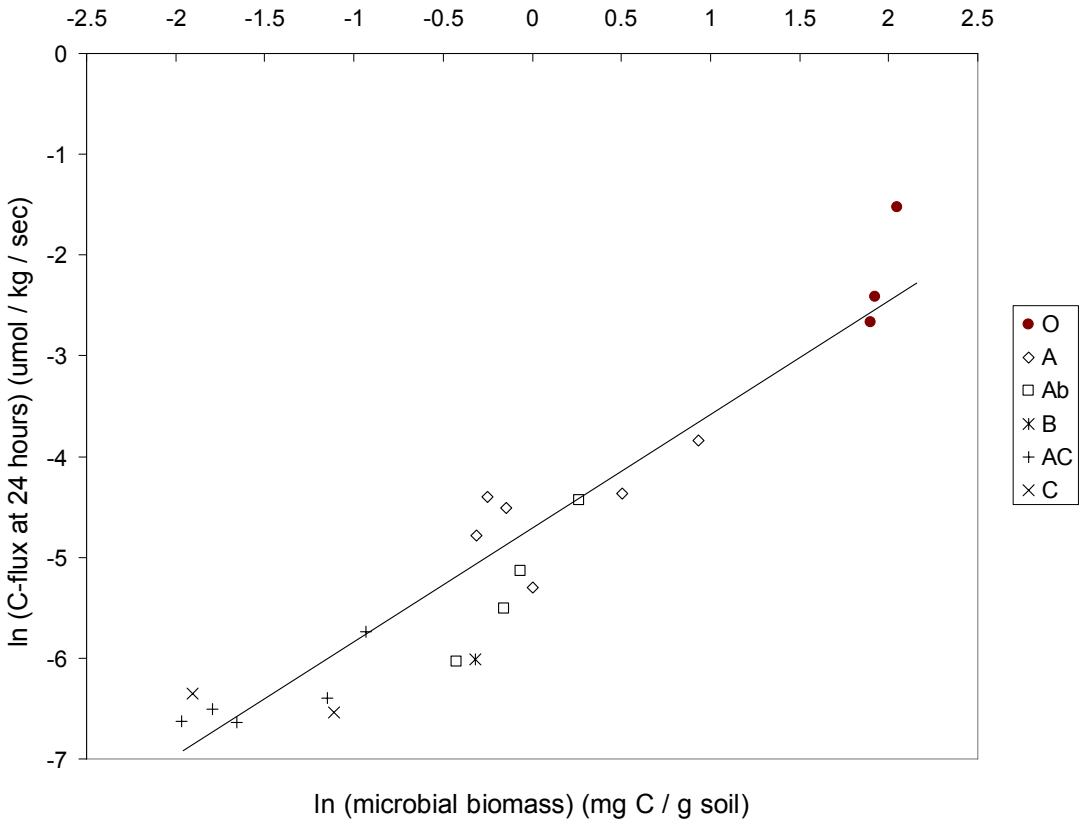


Figure 2.4. Carbon mineralization versus microbial biomass in 21 riparian soil horizons (O, A, Ab, B, C, A/C, C/A) collected in September 2003.

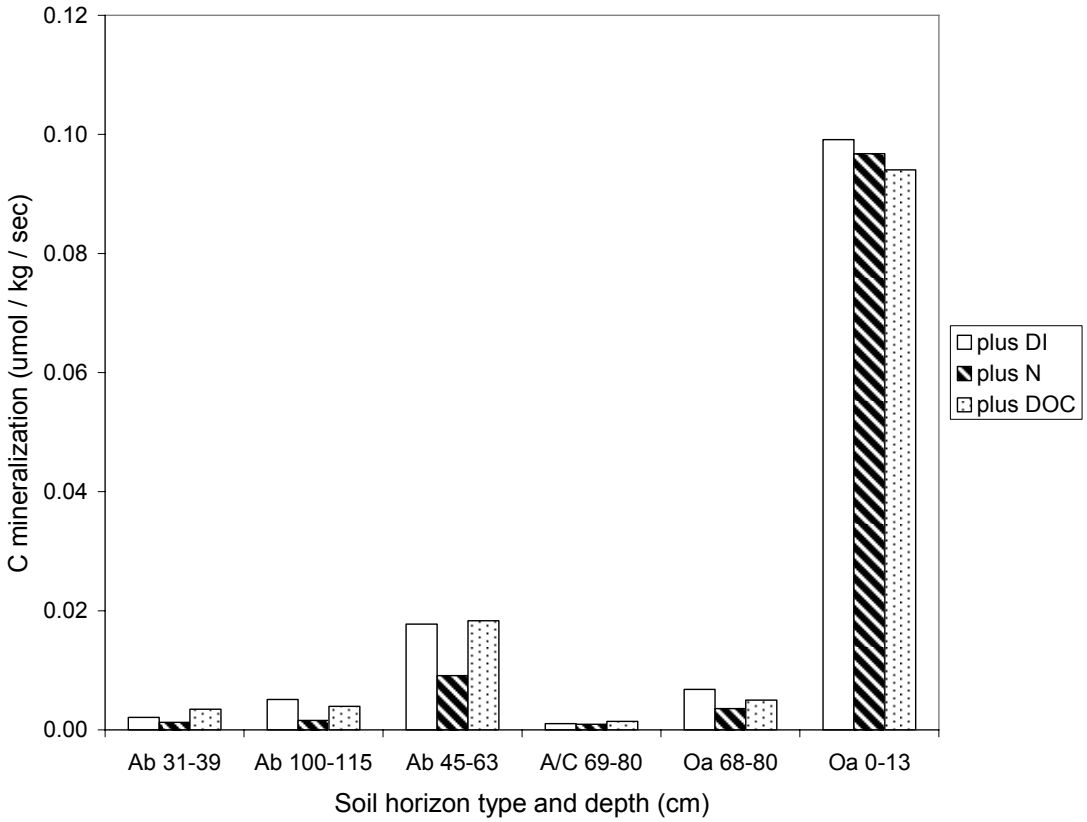


Figure 2.5. Variation in carbon mineralization among samples from six riparian forest soils, in response to amendments with deionized water (DI), nitrate (N), and dissolved organic carbon (DOC).

Soil C and N

Soil carbon in buried horizons we studied ranged from <1% in lenses 270-310 cm beneath the soil surface to 37% in a buried O horizon 54-74 cm deep. Soil nitrogen ranged from 0.1% in lenses to 1.25% in an A/C horizon 115-132 cm deep.

Soil carbon (%) and nitrogen (%) were both highly correlated with carbon mineralization rate but not with depth (Table 2.2, Figure 2.6). ANOVA models yielded R^2 values of 0.68 and 0.85 for soil carbon and nitrogen content respectively ($p<0.0001$, $n=22$). The relationship between carbon content and carbon mineralization depended strongly on two samples with greater than 30% carbon (Figure 2.6a);

excluding these two data points, the relationship remained significant ($p<0.05$) with $R^2=0.3$. Together, soil carbon and nitrogen explained 90 percent of the variation in carbon mineralization rates in these incubations ($p<0.0001$, $n=22$). Soil C/N ratio ($p>0.35$) did not show a significant relationship with carbon mineralization rate.

^{15}N and ^{13}C of soil organic matter

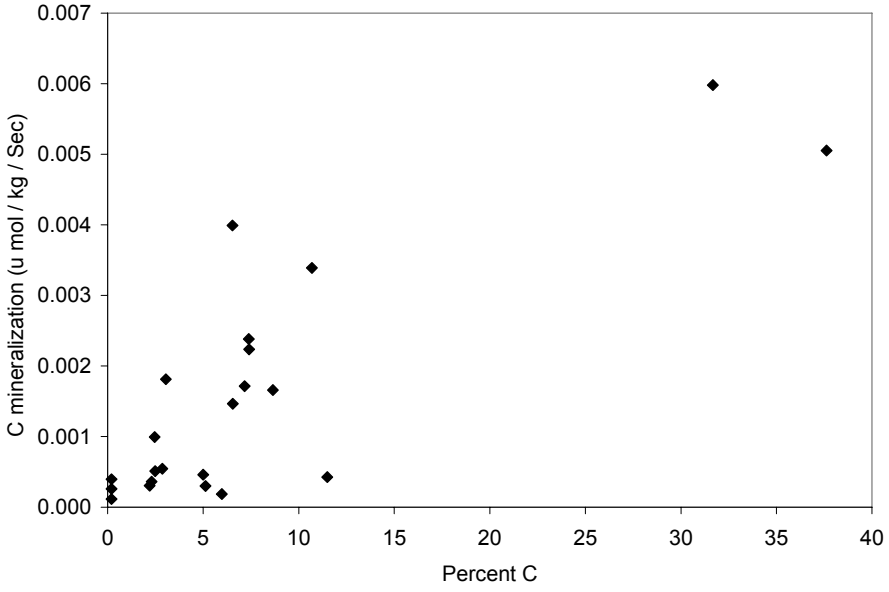
Soil ^{15}N and ^{13}C generally declined with depth (Table 2.2, Figure 2.7). A linear regression of depth vs ^{15}N yielded $R^2=0.41$, $p<0.01$ and a polynomial regression yielded $R^2=0.70$ $p<0.0001$. The difference between these models is explained by the apparent plateau of ^{15}N near -1 per mil at ~1 meter (Figure 2.7a). Excluding deeper horizons from the analysis yielded $R^2=0.71$, $p<0.0001$ ($n=14$). ANOVA suggests that horizon type explains much of the variation in ^{15}N. In a model including both depth and horizon type, horizon type ($p<0.05$) had a significant effect and depth ($p=0.17$) did not.

The relationship between ^{13}C and depth (Figure 2.7b) appeared to be strong to a depth of 150 cm; the small number of horizons included in this data set from greater depths preclude the ability to analyze that relationship further. Excluding points deeper than 150 cm yielded an $R^2=0.65$, $p<0.001$. Carbon mineralization rate was moderately correlated with the ^{13}C ratio but not at all with the ^{15}N ratio (Table 2.2). Carbon mineralization rate was also modestly related to the ^{13}C ratio (Table 2.2), with more enriched soil organic matter (SOM) associated with higher carbon mineralization rates.

Table 2.2. Pearson correlation coefficients showing relationships among variables characterizing soil microbial activity, chemistry, and location.

Variable	C-min		%C		%N		C/N		delta 15N		delta 13C		Depth		Distance to stream	
	r	p	r	p	r	p	r	p	r	p	r	p	r	p	r	p
C-min	1															
%C	0.74	<.0005	1													
%N	0.92	<.0001	0.94	<.0001	1											
C/N	-.25	0.34	0.27	0.3	0.0002	0.99	1									
delta ^{15}N	0.03	0.9	0.06	0.8	0.13	0.6	0.22	0.4	1							
delta ^{13}C	0.4	0.09	0.42	0.08	0.5	0.04	-.03	0.9	0.62	.009	1					
Depth	-.14	0.49	-.27	0.26	-.3	0.25	-.24	0.37	-.64	.007	-.36	.13	1			
Distance to stream	-.20	0.39	-.39	0.09	-.33	0.2	-.39	0.13	-.18	0.49	-.16	.53	.22	.33	1	

(a)



(b)

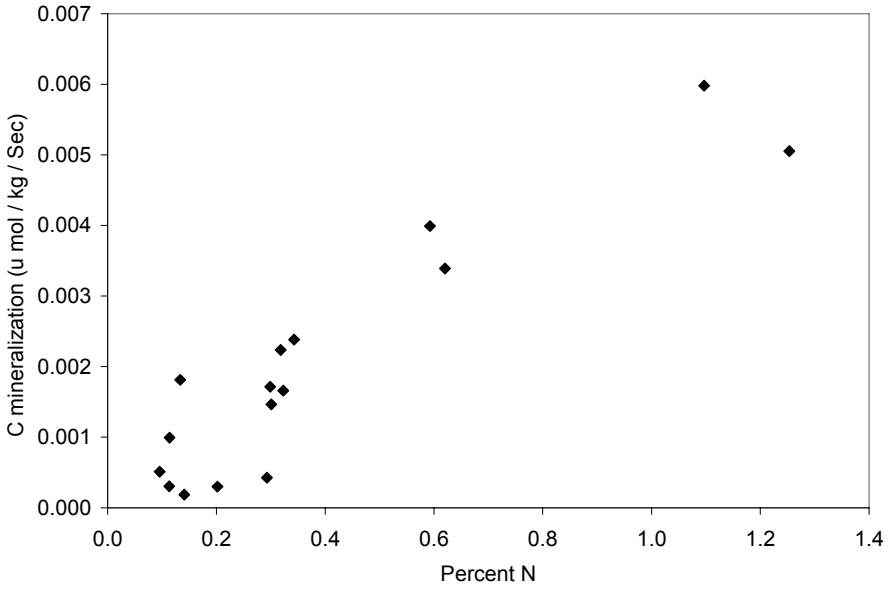


Figure 2.6. (a) Percent C and (b) percent N v. carbon mineralization.

(a)

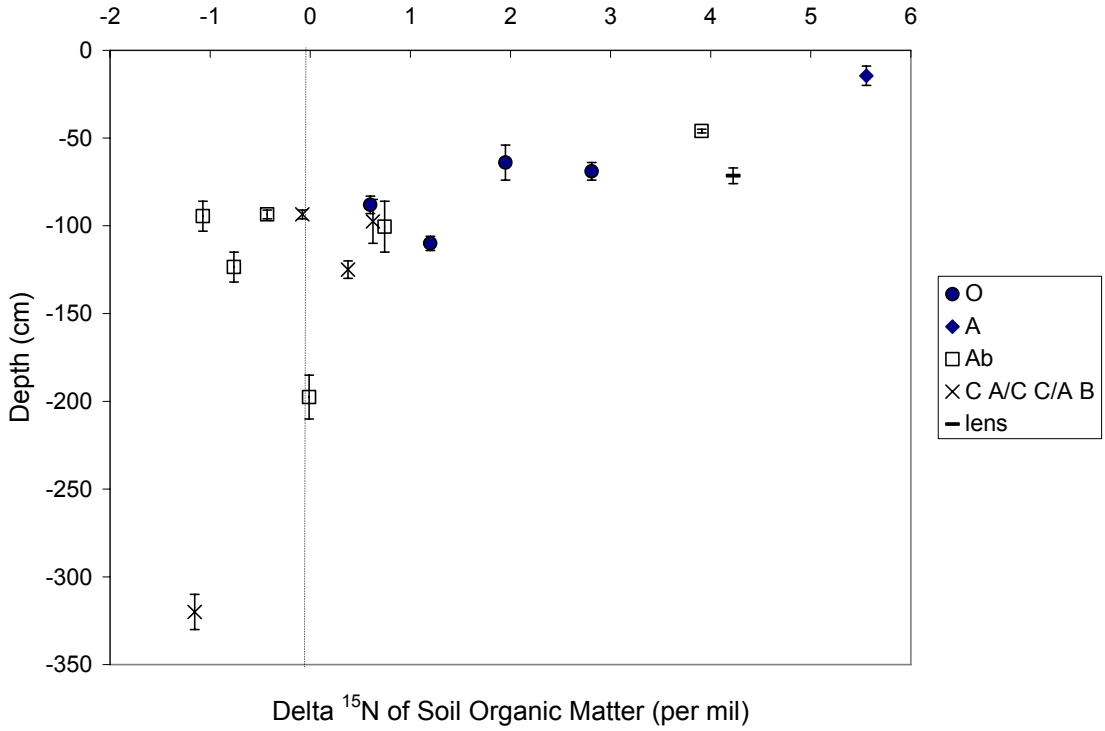
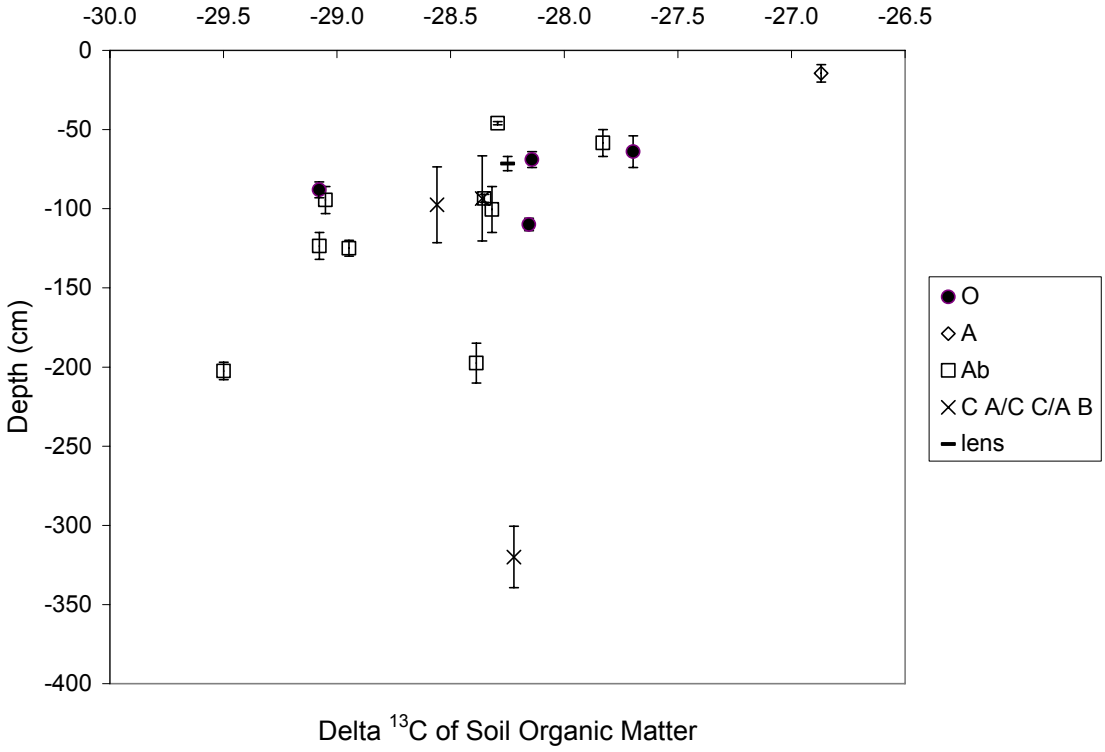


Figure 2.7. Spatial patterns of (a) delta ^{15}N and (b) delta ^{13}C of soil organic matter from surface and subsurface riparian soils.

Figure 2.7. (Continued).

(b)



Discussion

Is microbially-available C a general feature of buried horizons in riparian zones?

We found substantial amounts of microbially-available C in buried horizons in the riparian subsurface indicated both by anaerobic laboratory incubations (Figure 2.2) and by measurements of microbial biomass on fresh soil (Figure 2.3). Measurements of microbial biomass can include inactive as well as active microbes, and in-lab incubations offer microbes the opportunity to respond to conditions not normally encountered in the field. The consistent response in both measurements (Figure 2.4) suggests that our assays reflect the distribution of microbially-available C and associated microbial activity in buried horizons in shallow groundwater beneath riparian zones in Rhode Island.

Our results support the emerging view that microbially-available C is a general feature of buried horizons in riparian zones. The range of carbon content of buried horizons we studied in Rhode Island (0.2 – 37% C, Figure 2.6a) is wider than that reported by Hill et al. (2004) for buried horizons in Ontario (5-21% organic matter). The buried horizons we studied are also thousands of years old, whereas those described by Hill et al. (2004) appear to post-date European settlement. The carbon mineralization rates we measured closely resemble those reported by Hill and Cardaci (2004). We conclude that buried horizons that vary substantially in location, organic matter content, and age harbor microbially-available C in quantities relevant to ecosystem processes.

Controls on carbon mineralization in buried horizons

Carbon mineralization and microbial biomass varied systematically with horizon type but not with depth, suggesting that C availability in these buried horizons may be determined ultimately by abundance and quality of SOM at the time of horizon formation or burial, rather than by duration since burial. The pattern of carbon mineralization with respect to horizon type is consistent with observed correlations between soil carbon and nitrogen content and carbon mineralization (Figure 2.6, Table 2.2), relationships also noted in many previous studies of proximate controls on carbon mineralization (Paul and Clark 1996). It is plausible that plant community composition at these sites has varied over time, with some plants having more recalcitrant litter than others. Alternatively, current stocks of microbially-available C in buried horizons may reflect physical conditions (e.g., flood strength, topography) that controlled organic matter accumulation at the time of burial, independent of changes in plant communities over time. Similarly, SOM in some buried horizons may have begun to decompose before transport and burial while SOM in others may

have been buried while relatively fresh. The absence of strong relationships between horizon depth and C availability in the subsurface also suggests that subsurface biogeochemistry may be largely disconnected from the influence of surface ecosystem processes, which we would expect to diminish with depth.

Spatial patterns of ^{13}C and ^{15}N in buried horizons

The fact that ^{13}C and ^{15}N ratios tended to decrease with depth in the soil profile is somewhat surprising, as ^{13}C and ^{15}N ratios in SOM tend to become enriched with depth (e.g., Nadelhoffer and Fry 1988, Hogberg 1997). However, most stable isotope profiles have been restricted to the top 10 cm, and data for riparian soils and for deep soil profiles are rare.

Theoretically, older, more highly-processed SOM should be depleted in ^{13}C because: (1) those C forms considered most labile, such as carbohydrates and cellulose, tend to be enriched in ^{13}C relative to presumably more recalcitrant forms such as lipids and lignin (Kramer et al. 2003); and (2) microbial respiration does not appear to fractionate against ^{13}C (Lin and Ehleringer 1997). The modest correlation between carbon mineralization and ^{13}C ratio (Table 2.2) is consistent with the hypothesis that ^{13}C reflects C availability in buried horizons, but ^{13}C was equally correlated with soil carbon content (Table 2.2), which may be the more important driver of carbon mineralization. The observation that both high and low carbon mineralization rates were associated with SOM-^{13}C > -28.3 suggests that enriched ^{13}C in these buried horizons reflects more than one aspect of SOM decomposition and stabilization in the riparian subsurface.

The correlation between depth and ^{15}N-SOM is much stronger than the correlation between depth and ^{13}C-SOM, and unlike ^{13}C signatures, ^{15}N signatures are not correlated with carbon mineralization ($r=0.03$, $p>0.8$, Table 2.2). This may reflect a decoupling of C and N processing in buried horizons, more rapid cycling of N compared to C, or greater fractionation associated with microbial processing of N compared to C. To achieve this profile requires a combination of preferential: (1) removal of ^{14}N in the surface; (2) addition of ^{15}N in the surface; (3) removal of ^{15}N at depth; and (4) addition of ^{14}N at depth. Of these possibilities, preferential ^{14}N removal at the surface seems most likely (and is commonly observed) but by itself is insufficient to explain the observed gradient because horizons currently occupying positions at depth formerly sat at the surface.

Overall, the pattern of declining ^{13}C and ^{15}N with depth that we observed may reflect SOM processing over long time scales not observed in surface soils; differential loss of lighter isotopes associated with leaching below the water table; or differences in stable isotope signatures among soil horizons at the time of burial. The contrast between these spatial patterns and those typically reported for terrestrial surface soil profiles underscores the need for caution when interpreting patterns of stable isotope ratios in SOM and raises questions about the relative importance of physical processes (e.g., leaching, stabilization) and biotic activity in controlling both SOM chemistry and lability.

The biologically active zone

While rates of carbon mineralization in surface O horizons were an order of magnitude higher than in other soils we studied, these data and similar studies (e.g., Hill et al. 2004) call into question the view that the biologically active zone is restricted to

surface soils, just as data on biotic activity in deep upland profiles led soil scientists to include the C horizon in the concept of soil (Richter and Markowitz 1995), and as data on root distributions have led ecosystem scientists to extend the concept of rooting depth to tens of meters in seasonally dry forests (e.g., Nepstad et al. 1994, Trumbore et al. 1995). In all three cases, low rates of biological activity on a per-volume basis have important consequences for ecosystem fluxes when summed over relevant parts of the soil profile.

In cases where soil texture limits the movement of shallow groundwater through the subsurface (e.g., Wigington et al. 1993), restricting the effective biologically active zone to surface soils may be the most reasonable definition for estimating riparian zone influences on cross-system elemental budgets. However, even comparatively low levels of microbial activity in the subsurface can have profound influences on landscape-scale N fluxes. Previous studies have demonstrated low but non-zero rates of denitrification in subsoils beneath agricultural fields (Castle et al. 1998, Richards et al. 1999) and in sandy aquifers (Trudell et al. 1986, DeSimone and Howes 1996). In near-stream subsurface soils such as those in Rhode Island and much of the glaciated northeastern United States, where substantial groundwater flow and associated element transport occurs, the biologically active zone needs to include buried C-rich soil horizons and may need to include areas where micro-scale organic matter patches occur in a low-C matrix.

Hydrologic bypass of the biologically active zone

Considerable concern has emerged about the extent to which water may bypass the biologically active region of riparian zones, either by flowing over the surface or along deep flow paths (Bohlke and Denver, 1995; Vidon and Hill 2004). Such

disconnections do appear to occur, as illustrated by sites in Oregon, USA, where most of the water in the creek reaches the channel via overland flow (Wigington et al. 1993). Gold et al. (2001) invoke a similar model to suggest that fine-grained sediments in glacial till create seeps, again leading to overland flow and eliminating the potential for subsurface denitrification.

Our data about microbially-available C in the subsurface suggests that deep flow paths may not always negate the potential for riparian processing of upland-derived NO_3^-, and in-situ measurements of denitrification at four intensively-studied sites (Kellogg et al. 2005) are consistent with this view. Given that microbially-available C associated with buried horizons and lenses did not diminish systematically with depth (Figure 2.2c, Figure 2.3), that we found buried horizons in 23 of 24 riparian zones surveyed, that 30% of these horizons occurred deeper than 50 cm, and that 18% occurred deeper than 1 meter, there appears to be an abundance of opportunities for N-rich groundwater moving with the top 2 m of the soil profile to intersect biologically active zones in these landscapes.

Buried horizons >2 m deep are more likely to occur on 3rd-4th order streams than along the 1st – 2nd order streams that were the focus on this study. Of 484 buried horizons and lenses that we identified, only 13 occurred at depths > 2 meters. The deepest samples in our experiment came from ~2 m beneath the surface and these few samples had relatively low rates of carbon mineralization compared to samples from shallower depths. Further sampling on higher-order streams would be required to determine whether relatively low C-availability is a general feature of these deeper soils. Nevertheless, if these horizons occur within the hyporheic zone, then the potential for interaction with water previously delivered to the channel via lower-order streams

remains very high. The combined roles of C availability and denitrification in the riparian zone and hyporheic zone have been explored very little and remain a promising area for future research at the terrestrial-aquatic interface.

Persistence of buried horizons

It is logical to expect that buried horizons persist in riparian soil profiles due to a paucity of electron acceptors in these saturated, generally anaerobic ecosystems. This expectation carries the corollary that an increase in electron acceptor abundance, e.g. NO_3^-, might accelerate the decomposition of buried horizons, thus limiting their longevity. Two observations strongly suggest this is not the case. First, dissolved oxygen concentrations measured in groundwater in riparian zones at our sites are nearly always < 2 mg/l and often <1 mg/l but they are not zero (Simmons et al. 1992, Nelson et al. 1995, Addy et al. 1999, Kellogg et al. 2005). Second, amending soils from buried horizons with nitrate under anaerobic conditions had no influence on carbon mineralization rates (Figure 2.5). If anything, carbon mineralization declined in response to N additions, a result consistent with the explanation that N limited microbial growth but not respiration (Bengston and Bengston 2005). We conclude that decomposition rates of buried horizons are unlikely to increase dramatically as N loads in groundwater increase.

Implications for riparian zone evaluation and classification

To determine just how much upland and stream-derived NO_3^- is exposed to, and processed by, buried horizons in riparian soils will require coupled studies of hydrology and microbiology in the riparian and hyporheic zones, but current findings support the view that many riparian zones function as strong N sinks in the landscape. They apply particularly to riparian zones with surficial geology characterized by

outwash and alluvium — a common occurrence in formerly glaciated settings — and should be incorporated into hydrogeologic riparian classifications.

So far, most frameworks for classifying riparian zones according to N removal capacity have focused on either vegetation or hydrogeomorphology. These typologies have generally conceptualized the biologically active zone — and associated denitrification potential — as relatively shallow, restricted primarily to surface soils based on the view that low C content limits microbial activity in the subsurface. Data from this study and others (e.g., Hill et al. 2004, Well et al. 2005) demonstrate that in some landscapes buried A and O horizons occur frequently (particularly at the riparian-stream interface), often harbor labile C, and therefore extend the depth of the biologically active zone well below the surface. These features of the riparian soil profile dramatically influence the interaction of N-rich groundwater and microbial activity and need to be incorporated into hydrogeologic classifications of riparian zones.

More broadly, efforts to classify and evaluate riparian zones should consider historical dynamics of stream and riparian zone evolution that influence the formation of buried horizons (Nanson and Taylor 1995). These variables will need to be included along with the vegetation and hydrogeologic factors that dominate current classification and evaluation schemes. Some of the most useful classification schemes advanced previously have enabled us to target particular landscapes that have much greater capacity for riparian denitrification than others (e.g., coastal plain vs. piedmont, Jordan et al. 1997). Revised classification schemes that incorporate an historical understanding of stream and riparian zone evolution, including the presence of buried

horizons, should move us towards this practical goal and would be useful for watershed management programs to address N pollution problems.

Literature Cited

Addy, K. L., A. J. Gold, P. M. Groffman and P. A. Jacinthe (1999). "Ground water nitrate removal in subsoil of forested and mowed riparian buffer zones." Journal of Environmental Quality 28(3): 962-970.

Bengtson, P. and G. Bengtsson (2005). "Bacterial immobilization and remineralization of N at different growth rates and N concentrations." FEMS Microbiology Ecology 54(1): 13-19.

Blazejewski, G. A. (2002). Carbon in riparian zone subsoils: Morphology and spatial distribution. Dept of Natural Resources Science. Kingston, RI, University of Rhode Island.

Blazejewski, G. A., M. H. Stolt, A. J. Gold and P. M. Groffman (2005). "Macro- and Micromorphology of Subsurface Carbon in Riparian Zone Soils." Soil Science Society Of America Journal 69(July-August): 1320-1329.

Bohlke, J. K. and J. M. Denver (1995). "Combined use of ground- water dating, chemical, and isotopic analyses to resolve the history and fate of nitrate contamination in two agricultural watersheds, atlantic coastal Plain, Maryland." Water Resources Research 31(9): 2319-2339.

Burt, T. P., G. Pinay, F. E. Matheson, N. E. Haycock, A. Butturini, J. C. Clement, S. Danielescu, D. J. Dowrick, M. M. Hefting, A. Hillbricht-Ilkowska and V. Maitre (2002). "Water table fluctuations in the riparian zone: comparative results from a pan-European experiment." Journal of Hydrology 265(1-4): 129-148.

Carpenter, S. R., N. F. Caraco, D. L. Correll, R. W. Howarth, A. N. Sharpley and V. H. Smith (1998). "Nonpoint pollution of surface waters with phosphorous and nitrogen." Ecological Applications 8(3): 559-568.

Castle, K., J. R. M. Arah and A. J. A. Vinten (1998). "Denitrification in intact subsoil cores." Biology and Fertility of Soils 28(1): 12-18.

Clement, J. C., G. Pinay and P. Marmonier (2002). "Seasonal dynamics of denitrification along topohydrosequences in three different riparian wetlands." Journal of Environmental Quality 31(3): 1025-1037.

Correll, D. L. (1997). Buffer zones and water quality protection: general principles. Buffer Zones: Their Processes and Potential in Water Protection. N. Haycock, T. Burt, K. Goulding and G. Pinay, Quest Environmental.

DeSimone, L. A. and B. L. Howes (1996). "Denitrification and nitrogen transport in a coastal aquifer receiving wastewater discharge." Environmental Science & Technology 30(4): 1152-1162.

Devito, K. J., D. Fitzgerald, A. R. Hill and R. Aravena (2000). "Nitrate dynamics in relation to lithology and hydrologic flow path in a river riparian zone." Journal of Environmental Quality 29(4): 1075-1084.

Driscoll, C. T., D. Whitall, J. D. Aber, E. W. Boyer, M. Castro, C. Cronan, C. L. Goodale, P. M. Groffman, C. Hopkinson, K. Lambert, J. R. Lawrence and S. Ollinger (2003). "Nitrogen Pollution in the Northeastern United States: Sources, Effects, and Management Options." BioScience 53(4): 357-374.

Francis, A. J., J. M. Slater and C. J. Dodge (1989). "Denitrification in Deep Subsurface Sediments." Geomicrobiology Journal 7(1-2): 103-116.

Fustec, E., A. Mariotti, X. Grillo and J. Sajus (1991). "Nitrate Removal by Denitrification in Alluvial Ground-Water - Role of a Former Channel." Journal of Hydrology 123(3-4): 337-354.

Gold, A. J., P. M. Groffman, K. Addy, D. Q. Kellogg, M. Stolt and A. E. Rosenblatt (2001). "Landscape attributes as controls on ground water nitrate removal capacity of riparian zones." Journal of the American Water Resources Association 37(6): 1457-1464.

Gold, A. J., P. A. Jacinthe, P. M. Groffman, W. R. Wright and R. H. Puffer (1998). "Patchiness in groundwater nitrate removal in a riparian forest." Journal of Environmental Quality 27(1): 146-155.

Groffman, P. M., A. J. Gold and R. C. Simmons (1992). "Nitrate Dynamics in Riparian Forests Microbial Studies." Journal of Environmental Quality 21(4): 666-671.

Groffman, P. M., G. Howard, A. J. Gold and W. M. Nelson (1996). "Microbial nitrate processing in shallow groundwater in a riparian forest." Journal of Environmental Quality 25: 1309-1316.

Haycock, N. E. and G. Pinay (1993). "Groundwater Nitrate Dynamics in Grass and Poplar Vegetated Riparian Buffer Strips During the Winter." Journal of Environmental Quality 22(2): 273-278.

Hill, A. R. (1996). "Nitrate removal in stream riparian zones." Journal of Environmental Quality 25(4): 743-754.

Hill, A. R. and M. Cardaci (2004). "Denitrification and Organic Carbon Availability in Riparian Wetland Soils and Subsurface Sediments." Soil Science Society Of America Journal 68(1): 320-325.

Hill, A. R., P. G. F. Vidon and J. Langat (2004). "Denitrification Potential in Relation to Lithology in Five Headwater Riparian Zones." Journal Of Environmental Quality 33(3): 911-919.

Hogberg, P. (1997). “Tansley review No 95 - N-15 natural abundance in soil-plant systems.” New Phytologist 137(2): 179-203.

Jacinthe, P.-A., P. M. Groffman, A. J. Gold and A. Mosier (1998). “Patchiness in microbial nitrogen transformations in groundwater in a riparian forest.” Journal of Environmental Quality 27(1): 156-164.

Jacobs, T. C. and J. W. Gilliam (1985). “Riparian losses of nitrate from agricultural drainage waters.” Journal of Environmental Quality 14(4): 472-478.

Jordan, T. E., D. L. Correll and D. E. Weller (1997). “Relating nutrient discharges from watersheds to land use and streamflow variability.” Water Resources Research 33(11): 2579-2590.

Kellogg, D. Q., A. J. Gold, P. M. Groffman, K. Addy, M. H. Stolt and G. Blazejewski (2005). “In Situ Ground Water Denitrification in Stratified, Permeable Soils Underlying Riparian Wetlands.” Journal of Environmental Quality 34(2): 524-533.

Kramer, M. G., P. Sollins, R. S. Sletten and P. K. Swart (2003). “N isotope fractionation and measures of organic matter alteration during decomposition.” Ecology 84(8): 2021-2025.

Lin, G. and J. R. Ehleringer (1997). “Carbon Isotopic Fractionation Does Not Occur during Dark Respiration in C3 and C4 Plants.” Plant Physiology 114(1): 391-394.

Lowrance, R., L. S. Altier, J. D. Newbold, R. R. Schnabel, P. M. Groffman, J. M. Denver, D. L. Correll, J. W. Gilliam, J. L. Robinson, R. B. Brinsfield, K. W. Staver, W. Lucas and A. H. Todd (1997). “Water Quality Functions of Riparian Forest Buffers in Chesapeake Bay Watersheds.” Environmental Management 21(5): 687-712.

Lowrance, R., R. Todd, J. Fail, O. Hendrickson, R. Leonard and L. Asmussen (1984). “Riparian Forests as Nutrient Filters in Agricultural Watersheds.” BioScience 34(6): 374-377.

Martin, T. L., N. K. Kaushik, J. T. Trevors and H. R. Whiteley (1999). “Review: Denitrification in temperate climate riparian zones.” Water Air and Soil Pollution 111(1-4): 171-186.

Nadelhoffer, K. J. and B. Fry (1988). “Controls on natural nitrogen-15 and carbon-13 abundances in forest soil organic matter.” Soil Science Society of America Journal 52: 1633-1640.

Nanson, G. C., M. Barbetti and G. Taylor (1995). "River stabilization due to changing climate and vegetation during the late Quaternary in western Tasmania, Australia." Geomorphology 13: 145-158.

National Research Council (2000). Clean Coastal Waters: Understanding and Reducing the Effects of Nutrient Pollution. Washington, D.C., National Academy Press.

Nelson, W. M., A. J. Gold and P. M. Groffman (1995). "Spatial and Temporal Variation in Groundwater Nitrate Removal in a Riparian Forest." Journal of Environmental Quality 24(4): 691-699.

Nepstad, D. C., C. R. De Carvalho, E. A. Davidson, H. Jipp-Peter, P. A. Lefebvre, G. H. Negreiros, E. D. Da Silva, T. A. Stone, S. E. Trumbore and S. Vieira (1994). "The role of deep roots in the hydrological and carbon cycles of amazonian forests and pastures." Nature 372(6507): 666-669.

Obenhuber, D. C. and R. Lowrance (1991). "Reduction of Nitrate in Aquifer Microcosms by Carbon Additions." Journal of Environmental Quality 20(1): 255-258.

Parkin, T. B. (1987). "Soil microsites as a source of denitrification variability." Soil Science Society of America Journal 51: 1194-1199.

Paul, E. A. and F. E. Clark (1996). Soil Microbiology and Biochemistry. San Diego, Academic Press.

Paul, E. A., D. Harris, M. J. Klug and R. W. Ruess (1999). The determination of microbial biomass. Standard soil methods for long term ecological research. G. P. Robertson, D. C. Coleman, C. S. Bledsoe and P. Sollis. New York, Oxford University Press: 291-317.

Peterjohn, W. T. and D. L. Correll (1984). "Nutrient Dynamics in an Agricultural Watershed: Observations on the Role of a Riparian Forest." Ecology 65(5): 1466-1475.

Puckett, L. J. (2004). "Hydrogeologic controls on the transport and fate of nitrate in ground water beneath riparian buffer zones: results from thirteen studies across the United States." Water Science and Technology 49(3): 47-53.

Richards, J. E. and C. P. Webster (1999). "Denitrification in the subsoil of the Broadbalk Continuous Wheat Experiment." Soil Biology & Biochemistry 31(5): 747-755.

Richter, D. D. and D. Markewitz (1995). "How deep is soil?" Bioscience 45(9): 600-609.

Sabater, S., A. Butturini, J. C. Clement, T. Burt, D. Dowrick, M. Hefting, V. Maitre, G. Pinay, C. Postolache, M. Rzepecki and F. Sabater (2003). "Nitrogen removal by riparian buffers along a European climatic gradient: Patterns and factors of variation." Ecosystems 6(1): 20-30.

Simmons, R. C., A. J. Gold and P. M. Groffman (1992). "Nitrate Dynamics in Riparian Forests - Groundwater Studies." Journal of Environmental Quality 21(4): 659-665.

Slater, J. M. and D. G. Capone (1987). "Denitrification in Aquifer Soil and Nearshore Marine-Sediments Influenced by Groundwater Nitrate." Applied and Environmental Microbiology 53(6): 1292-1297.

Smith, R. L. and J. H. Duff (1988). "Denitrification in a Sand and Gravel Aquifer." Applied and Environmental Microbiology 54(5): 1071-1078.

Starr, R. C. and R. W. Gillham (1993). "Denitrification and Organic-Carbon Availability in 2 Aquifers." Ground Water 31(6): 934-947.

Sweeney, B. W. (1992). "Streamside Forests and the Physical, Chemical, and Trophic Characteristics of Piedmont Streams in Eastern North-America." Water Science and Technology 26(12): 2653-2673.

Trudell, M. R., R. W. Gillham and J. A. Cherry (1986). "An in-situ study of the occurrence and rate of denitrification in a shallow unconfined sand aquifer." Journal of Hydrology 83: 251-268.

Trumbore, S. E., E. A. Davidson, P. B. Decamargo, D. C. Nepstad and L. A. Martinelli (1995). "Belowground Cycling of Carbon in Forests and Pastures of Eastern Amazonia." Global Biogeochemical Cycles 9(4): 515-528.

Verchot, L. V., E. C. Franklin and J. W. Gilliam (1997). "Nitrogen cycling in piedmont vegetated filter zones .2. Subsurface nitrate removal." Journal of Environmental Quality 26(2): 337-347.

Vidon, P. G. F. and A. R. Hill (2004). "Landscape controls on nitrate removal in stream riparian zones." Water Resources Research 40: W03201.

Vitousek, P. M., J. D. Aber, R. W. Howarth, G. E. Likens, P. A. Matson, D. D. Schindler, W. H. Schlesinger and D. G. Tilman (1997). "Human alteration of the global nitrogen cycle: sources and consequences." Ecological Applications 7(3): 737-750.

Well, R., H. Hoper, O. Mehranfar and K. Meyer (2005). "Denitrification in the saturated zone of hydromorphic soils—laboratory measurement, regulating factors and stochastic modeling." Soil Biology and Biochemistry 37(10): 1822-1836.

Wigington Jr, P. J., S. M. Griffith, J. A. Field, J. E. Baham, W. R. Horwath, J. Owen, J. H. Davis, S. C. Rain and J. J. Steiner (2003). "Nitrate removal effectiveness of a riparian buffer along a small agricultural stream in western Oregon." Journal of Environmental Quality 32: 162-170.

Yeomans, J. C., J. M. Bremner and G. W. McCarty (1992). "Denitrification Capacity and Denitrification Potential of Subsurface Soils." Communications in Soil Science and Plant Analysis 23(9-10): 919-927.

CHAPTER THREE: MINERALIZATION OF ANCIENT CARBON IN THE SUBSURFACE OF RIPARIAN FORESTS

Introduction

Over the past two decades, numerous studies — most of them focused on surface soils — have revealed high variability in nitrogen (N) removal among riparian zones. While these areas have generally been considered "hot spots" of N removal at the terrestrial-aquatic interface (Groffman et al. 1992, 1996, Hill 1996, Lowrance et al. 1997, Martin et al. 1999, Burt et al. 2002, Kellogg et al. 2005), this high and largely unexplained variability has inhibited widespread adoption and evaluation of riparian zones as a solution to N pollution problems. Conceptual frameworks to understand and explain this variation would therefore have great theoretical and practical benefit. Progress on this front has occurred mainly through analyses of the hydrogeologic settings in which riparian zones sit (Jordan et al. 1997, Gold et al. 2001, Hill et al. 2004, Vidon and Hill 2004).

One critical feature that distinguishes some riparian zones from others is the depth and character of soil and sediment through which shallow groundwater flows. Most work on riparian zone N removal has been done at sites with shallow aquicludes that force substantial contact between N-rich groundwater and carbon-rich surface soils (Hill 1996, Martin et al. 1999). However, copious amounts of groundwater flow through riparian zones with conductive sediments well beneath the soil surface, where denitrification is usually C limited (Parkin and Meisinger 1989; Bradley et al. 1992; Groffman et al. 1992; McCarty and Bremner 1992; Yeomans et al. 1992; Starr and Gillham 1993). A more thorough understanding of C supply in the subsurface and how it varies among riparian zones in different landscapes could therefore advance the development of functional riparian classifications (Gurwick et al., in prep).

Although soil organic matter content in the subsurface can be low in riparian zones on glaciated landscapes, it is generally not zero. Jacinthe et al. (1998) and Gold et al. (1998) documented the presence of C-rich microsites in the subsurface of riparian zones on glacial outwash in Rhode Island. In some cases, and particularly in riparian zones where surficial geology has been shaped by alluvial processes, the subsurface contains buried soil lenses and horizons. These buried soils, which are ubiquitous in alluvial riparian zones, can be thousands of years old and yet appear to harbor microbially-available C that can support ecosystem-relevant rates of denitrification (Fustec et al. 1989; Haycock and Pinay 1993; Devito et al. 2000; Blazejewski 2002; Hill and Cardaci 2004; Well et al. 2005; Gurwick et al. in prep).

The proposition that microbial activity at rates relevant to contemporary element budgets depends upon buried horizons is somewhat at odds with well-accepted models of ecosystem C dynamics. Litter usually decomposes on the time scale of years (Moore et al. 1999) and the proportion that remains has generally been considered recalcitrant (i.e., unavailable for use by microbes either because it is inherently difficult to metabolize or because it has become stabilized by association with minerals) (Minderman 1968; Sollins et al. 1996; Six et al. 2002). Forest girdling experiments have demonstrated that most CO_2 associated with microbial respiration in soil derives from recent photosynthate, C fixed days to months before being respired (Hogberg et al. 2001). In the Amazon River, which carries a vast load of SOM, the substantial CO_2 flux to the atmosphere also appears to consist primarily of recently-fixed C (Mayorga et al. 2005). The perspective that recently-fixed organic C is labile and older organic C is recalcitrant is reflected in models of soil carbon cycling such as CENTURY that typically recognize several soil carbon pools, characterized by different turnover times (years to decades) (Parton et al. 1987).

In contrast to most perspectives from ecosystem science, geomicrobiologists have embraced the idea that microbes use a wide variety of C sources. Buckau et al. (2000) found mineralization of Miocene organic carbon at ~100 m depth in groundwater. D'Hondt et al. (2004) reported abundant and metabolically diverse microbial communities using organic C as the principle electron donor as deep as 0.5 km beneath the ocean floor, and it is difficult to imagine how modern organic C could be transported that far beneath the sea floor. Sulfate reducers in Cretaceous rock formations appear to use components of organic-rich shale as electron donors (Krumholz et al. 2002). Lipids isolated from microbes in the city of Halle/Salle (Germany) had highly depleted ^{14}C signatures, indicating a substantial fossil C contribution to those microbial compounds (Rethemeyer et al. 2004). Petsch et al. (2001, 2003) showed that a population of prokaryotes use black shale as their sole organic carbon source, and that microbial communities growing on black shale include anaerobes.

When, if ever, do microbes growing on old or ancient organic matter significantly influence biogeochemical cycles in either natural or human-perturbed ecosystems? In theory, the most profound way in which old carbon might impact contemporary ecosystems is via the potential decomposition of SOM in peatlands and permafrost in the face of climate change (Davidson and Janssens 2006). If inundation and freezing diminish or stop microbial degradation of labile SOM without fundamentally altering its character, then drying and thawing could lead to rapid decomposition and a dramatic increase in atmospheric CO_2. Empirical research in several ecosystems suggests that old C may already be fueling microbial activity, which regulates many elemental fluxes. In Siberian lakes, methanogenesis depends upon Pleistocene-age C (Zimov et al. 1997). Methane produced in the subsurface of terrestrial ecosystems is

derived from both modern and older SOM (Hackley et al. 1999), and methane production in groundwater has been associated with Wisconsinan-age particulate organic C fragments (Parkin and Simpkins 1995). In peatlands, where old soil carbon occurs in enormous supply, microbial respiration sometimes depends mainly upon recently-fixed C (Chanton et al. 1995; Chasar et al. 2000) and sometimes reflects a mix of modern and pre-1950 C pools (Dioumaeva et al. 2003). Between 6-10% of microbial lipids in a petroleum-contaminated Georgia salt marsh are comprised of ancient C (Wakeham et al. 2006). Bacterial assimilation of organic matter in the Hudson River reflects utilization of up to 25% C older than 24,000 ybp (McCallister et al. 2004). It appears that metabolism of old C may be more common than previously thought, but the locations where this metabolism occurs and the circumstances under which the use of ancient C influences contemporary biogeochemical fluxes have not yet been well defined.

Metabolism of old C may be particularly important where it is coupled to other elemental cycles, especially nitrogen (N). Rates of C mineralization that are of little consequence to ecosystem C budgets can support rates of denitrification (anaerobic microbial reduction of nitrate (NO_3^-) to N gases) sufficient to significantly decrease N transfer across the terrestrial-aquatic interface (Starr and Gillham 1993). Carbon availability in shale formations in aquifers 10-15 m beneath the land surface has been shown capable of supporting substantial denitrification if advective flow can supply NO_3^- to those environments (McMahon et al. 1999). Similarly, dramatic decreases in N across riparian zones can be supported by relatively low rates of C supply relative to typical C and N fluxes into and out of ecosystems.

Evidence that buried horizons and channel deposits in riparian zones support denitrification and that C associated with them is microbially-available (Hill et al. 2000; Well et al.; Gurwick et al. in prep) raises two questions relevant for evaluating riparian zone classifications and for elucidating our understanding of the role that old C plays in regulating the N cycle. First, from the perspective of C age, does old SOM in buried horizons directly support microbial activity, or does it act as a magnet for young C in the subsurface? Roots proliferate in OM-rich areas (Eissenstat and Caldwell 1988; Bilbrough and Caldwell 1995) and presumably root sloughing and exudation is correspondingly important where root production is highest. In addition, DOC leached from surface soils may adhere to particles of stabilized organic matter in the subsurface (Dunnivant et al. 1992). Thus, old C may facilitate the formation of young C hot spots in the subsurface without itself being metabolized.

Second, from the perspective of controls on landscape-scale N fluxes and riparian zone classification, do different C sources fuel denitrification in different types of riparian zones? In addition to buried horizons, C may be supplied to the riparian subsurface via deep roots or via DOC leached from surface soils. Riparian zones in alluvial geomorphic settings have a high incidence of buried horizons relative to riparian zones on glacial outwash (Kellogg et al. 2005). This framework implies that the mix of C sources in the subsurface varies among riparian zone types.

In this study, our objectives were to determine: (1) whether old C directly supports microbial activity in buried horizons in the riparian subsurface; (2) whether the mix of C sources that support microbial activity in the riparian subsurface differs between riparian zones in outwash and alluvial landscape settings; (3) how the mix of C

sources fueling microbial activity varies with depth in the subsurface; and (4) *in situ* rates of C mineralization in the subsurface.

Methods and site descriptions

We addressed our objectives by measuring the radiocarbon signature of dissolved inorganic carbon (DIC) in the subsurface of riparian zones in alluvial and outwash settings and by conducting in-situ groundwater incubation experiments to estimate the ^{14}C signature of C actually mineralized at different depths in the riparian subsurface. The radiocarbon signature of DIC produced at specific locations in the subsurface should reflect the mean age of C sources being metabolized, enabling us to distinguish the age of SOC being metabolized from the age of the bulk SOC pool.

Site descriptions and instrumentation

We sampled groundwater at four riparian zones in two hydrogeologic settings on low (1^{st} - 3^{rd}) order streams in the Pawcatuck River watershed, Rhode Island. All sites had low (<3%) surface slopes and vegetation dominated by approximately 80 year old *Acer rubrum.* Two sites (A and B) were characterized by glacial outwash and two (C and D) by alluvium, distinguished primarily by the high frequency of buried horizons in soils at the alluvial sites (Kellogg et al. 2005). The alluvial sites were characterized by entisols of moderate pH (5.3 – 6.5) while the outwash sites were more acidic (pH 5.0 – 5.8) and dominated by either histosols (site A) or inceptisols (site B). All soils had very low concentrations of carbonate minerals. Groundwater dissolved oxygen at these sites generally ranges from 2 – 6 mg L^{-1} at outwash sites; it is almost always <2 and often <1 mg L^{-1} at alluvial sites. Site designations here follow nomenclature used by Kellogg et al. (2005).

Buried horizons at the alluvial sites were thousands of years old and the oldest were consistent with the region's glacial history. Radiocarbon dating of buried C-rich lenses 300-350 cm beneath the surface at site C yielded a calibrated age of 16,267-17,047 ybp, consistent with the glacial retreat from the northeastern U.S. At site D, calibrated ^{14}C-ages were obtained on two fractions of a buried horizon 83-93 cm beneath the surface. All recognizable organic matter fragments were separated from the rest of the soil (humic material). The C associated with the humic material was fixed 10,654 +/- 70 ybp, whereas the organic matter fragments from this horizon, including roots, formed 4,730 +/- 40 ybp.

At each site, we had previously installed 3 mini-piezometers at each of 3 target depths: 65 cm, 150 cm, and 300 cm (total 9 piezometers per site). Piezometers were made of narrow gas-impermeable tubing (0.8 cm o.d.) and had been installed with a slide hammer, greatly minimizing disruption to the surrounding sediments. We sampled from piezometers at all sites and depths to characterize ambient ^{14}C in summer 2003 and used a subset of sites and depths for in-situ incubations and ambient measurements in Nov-Dec 2002. Sites and piezometer installation are described in more detail in Kellogg et al. (2005).

In-situ incubations ("push-pull") and groundwater sampling

To measure DIC production and the ^{14}C signature of DIC produced in-situ, we employed a modified "push-pull" technique, previously used at these sites to measure denitrification (Istok et al. 1997; Addy et al. 2002). Because this method is time and labor intensive, and because costs associated with ^{14}C analysis are high, we chose a subset of sites and wells for these experiments. We selected the two sites (A and C) where the highest denitrification rates had previously been measured (Kellogg et al.

2005) and conducted incubations in two wells at each of the three depths at those sites. In addition, we conducted incubations in two shallow (65 cm) wells at a second outwash site where we had also conducted intensive measurements of root biomass (Gurwick et al. in prep, chapter 4) and where several other groundwater denitrification studies have been conducted over the past decade (Groffman et al. 1992; Simmons et al. 1992; Nelson et al. 1995).

We performed in-situ groundwater incubations in Nov-Dec 2002. We collected 20 L of groundwater from each target mini-piezometer at least one day in advance of our experiment. No more than two hours before returning to the field, we transferred this groundwater to a 20-liter carboy fitted with a cap containing three ports. We added KNO_3 (32 mg N L^{-1}) to ensure that microbial activity would not be NO_3^- limited, and potassium bromide (KBr, 32 mg Br^- L^{-1}) as a conservative tracer to estimate dilution during the incubation. We bubbled helium through one port attached to a tube that reached to the bottom of the carboy and had a sparge stone on the end. A second port, open to the head space of the carboy was routed through a LiCor 6200 IRGA. Bubbling continued until the CO_2 concentration of the gas leaving the carboy dropped below 10 ppm (and often below 2 ppm), at which point we capped the ports on the carboy for immediate transport to the field.

To collect ambient groundwater samples before starting incubations, we connected a peristaltic pump to the mini-piezometer. To ensure the sampled groundwater had not been in contact with the atmosphere via the piezometer, we first pumped at least 500 mL of groundwater and discarded it. We then collected a sample and measured dissolved oxygen concentration and temperature. Continuing to pump slowly, we collected samples for: bromide concentration and DIC chemistry (HDPE bottle,

unfiltered); NO_3^- concentration (HDPE bottles, filtered with 0.45 uM filters); carbon dioxide (125 mL pre-flushed and evacuated serum bottle); and $^{14}C\text{-}CO_2$ (1 Liter amber glass bottle, pre-flushed and evacuated). We collected multiple samples of each type and collected samples for Br^- and NO_3^- concentration before and after collecting samples for $^{14}C\text{-}CO_2$ analysis.

Before introducing the amended, degassed groundwater back into the ground, we bubbled it with helium for five minutes in the field to eliminate CO_2 that might have leaked in during transport. We pumped 500 mL of groundwater from the carboy into a beaker to measure dissolved oxygen and temperature. To verify starting conditions in the experiment, we collected samples from the carboy to measure Br^- and NO_3^- concentrations and DIC chemistry. Using the pump, we reintroduced the amended, degassed groundwater into the well at a maximum rate of 13 liters per hour. We discontinued pumping as soon as we observed air bubbles in the tube, at which point we reversed the pump direction for ten seconds and stopped. We then took a final sample from the carboy to characterize initial chemistry and sealed the well. Incubations lasted between 5 and 50 hours depending mainly upon the rate at which tracers had been observed to move out of the recovery zone at each site. We collected samples of incubated groundwater with the same methods used to collect ambient groundwater samples before starting the incubation.

Collecting groundwater samples for ^{14}C-DIC signatures

To sample groundwater for ^{14}C-DIC in association with our in-situ incubations, we fitted 1-liter amber glass bottles with suba-seal stoppers, flushed them with ultra-pure helium for 5 minutes, and evacuated each one for 25 minutes. Our objective was to create a CO_2-free atmosphere with sufficient vacuum to allow us to collect 400-800

mL of groundwater. To collect samples for ^{14}C-DIC analysis, we attached a sampling tube to the piezometer, threaded it through the peristaltic pump, and attached a non-coring needle to the end of the tube. We pumped 0.5 L of water from the well and then inserted the needle through the stopper. To minimize intrusion of air through any possible leaks, we secured the bottle beneath the surface of a water-filled bucket throughout this operation.

We collected additional ambient groundwater samples from all three depths at all four sites in August-September, 2003. We used the same methods as we did to collect ambient groundwater samples in advance of our incubation experiments, with the following exceptions. Groundwater samples were collected by overfilling 500-mL glass bottles, pouring out ~10 mL, adding 100 µL of $HgCl_2$ to inhibit microbial activity, injecting helium into the ~10 mL headspace to displace atmospheric air, and immediately sealing the bottle with a ground glass stopper. We also collected an additional sample, stored in a full, screw-cap HDPE bottle on ice, that we used to measure pH and alkalinity within 10 hours of sample collection.

^{14}C-AMS analysis

DIC was extracted from groundwater samples by acidifying groundwater to pH<2 to convert all DIC to CO_2, attaching sample bottles to a vacuum line with an in-line recirculating pump, and bubbling the samples for 30-60 minutes. Trials with samples of known DIC concentration verified that we trapped all DIC within this time interval. We separated CO_2 from H_2O and noncondensible gases using cryotraps and measured CO_2 content by expanding the gas into a known volume and measuring the resulting pressure and temperature. Gas samples were stored in flame-sealed glass tubes until

analysis. Radiocarbon analysis was performed at National Ocean Sciences Accelerator Mass Spectrometer (NOSAMS) laboratory.

Groundwater chemistry

For ambient groundwater samples collected in 2003 we measured pH, alkalinity, and concentrations of most cations. We also measured cation concentrations on samples of ambient groundwater from wells used to conduct in-situ groundwater incubations in 2002. We measured pH and alkalinity at the water quality laboratory at the University of Rhode Island and cation concentrations at the nutrient analysis laboratory in the Department of Crop and Soil Science at Cornell University using inductively coupled plasma spectrometry. Samples of ambient, amended, and incubated groundwater samples from 2002 were analyzed for $[Br^-]$, $[NO_3^-]$, and $[SO_4^{2-}]$ by ion chromatography at the analytical laboratory at the Institute of Ecosystem Studies, Millbrook, NY.

Data analysis

** DIC production*

We calculated DIC production using the following equations:

(1) $[DIC]_{prod} = [DIC]_{pull} - [DIC]_{push} - ([DIC]_{amb} * (1-F_{inc}))$

$[DIC]_{prod}$ is the amount of DIC produced from mineralization during the incubation.

$[DIC]_{pull}$ is [DIC] measured in the groundwater at the end of the incubation period,

$[DIC]_{push}$ is [DIC] in the groundwater reintroduced to the piezometer,

$[DIC]_{amb}$ is [DIC] in the ambient groundwater,

F_{inc} is the fraction of groundwater in the post-incubation sample derived from the degassed water calculated from the change in $[Br^-]$, and

$1\text{-}F_{inc}$ is the fraction of groundwater in the post-incubation sample derived from ambient groundwater mixed with the plume.

$$(2)\ \Delta DIC_{prod} = \frac{[DIC]_{prod}}{t_{inc}}$$

ΔDIC_{prod} is the rate of DIC mineralization over the course of the incubation.

t_{inc} is the duration of the incubation.

$[DIC]_{push}$ is pH dependent. We eliminated all aqueous CO_2 from the groundwater before reintroducing it into the well, but in cases of pH > 5, HCO_3^- remained in solution. We calculated $[DIC]_{push}$ based on measurements of pH and total DIC, and measurements of alkalinity from 2003 ambient groundwater samples.

** Contribution of old SOC to mineralization*

First, we calculated the contribution of old SOC to total C mineralization during the course of the incubation by combining a mass balance for DIC with ^{14}C-DIC signatures of ambient and incubated groundwater. Our DIC mass balance relies on our measurements of DIC and bromide concentrations in the ambient groundwater and the incubated plume. The ^{14}C signature of DIC produced during the course of the incubation, i.e. $^{14}C_{prod}$.is given by:

$$^{14}C_{prod} = \frac{^{14}C_{pull}*[DIC]_{pull} - (\ ^{14}C_{amb}*[DIC]_{amb}*(1-F_{inc})) - (^{14}C_{amb}*[DIC]_{push}*F_{inc})}{[DIC]_{prod}}$$

We applied this approach first considering only dilution via advection and second considering both advection and the presence of bicarbonate at the beginning of the incubation.

Second, we calculated a minimum potential contribution of old C depending upon the proportion of DIC in the incubated sample produced by mineralization over the course of the incubation, but independent of measured DIC concentrations. We constrained this estimate using only the ^{14}C signatures of DIC in the incubated plume and ambient groundwater and estimates of the age of mineralizeable ancient C at specific locations. We assigned an age of 1 ybp ($\Delta^{14}C$ value +90‰) to the contemporary pool and ages consistent with dates of buried horizons at different depths to the ancient pool. At site C, 260 cm, we initially assigned the ancient pool an age of 16,663 ybp ($\Delta^{14}C$ value -825‰) and then ran the same set of calculations assuming ages of 12,000, 8,000, and 4,000 ybp.

Our assumptions tend to minimize the contribution of ancient C mineralization. Initially, we assumed no dilution during the incubation and no bicarbonate present at the beginning of the incubation. There are additional sources of DIC to this groundwater, such as DIC in ambient groundwater that mixes into the incubation plume via advection, and these DIC sources have mean ages younger than that of DIC in the incubated plume. Therefore, including those terms would increase the importance of old C mineralization. Although multiple pools of C could be contributing to mineralization, using a maximum age for the old end member yields a minimum contribution for mineralization of old C. Using 1 ybp for the modern end member also yields a minimum estimate for the contribution of old C. Had we chosen SOM formed 20 ybp or 40 ybp for this end member, it would have had a considerably

more enriched ^{14}C signature owing to the spike of ^{14}C put into the atmosphere by atomic weapons testing in the 1950's, and hence would have required more depleted (old) C mineralization to balance it. Thus, this approach yields a conservative estimate of the importance of old C to DIC produced in-situ in the riparian subsurface.

** Potential for carbonate rock dissolution*

It was critical to address the possibility that some portion of the ^{14}C-DIC pool originated from carbonate rock dissolution rather than from SOC mineralization. Carbonate rocks typically have no ^{14}C remaining because all ^{14}C originally present has decayed. Therefore, a small contribution of rock-derived carbonate to the groundwater DIC pool can have large influence on the ^{14}C-DIC signature. We addressed this possibility by measuring calcium and magnesium concentrations in groundwater and by comparing cation concentration gradients within and among sites to gradients of ^{14}C-DIC.

** Statistical analysis*

Our in-situ groundwater incubations, which are time- and labor-intensive, and ^{14}C analysis, which is expensive, give us highly realistic measurements at the expense of large sample sizes. Most of our data interpretation relies on reasonable assignment of causation based on multiple lines of evidence rather than strong statistical power. In cases where statistical tests were possible, we used SAS (2002-2003).

Results

Ambient groundwater chemistry

We measured concentrations of magnesium and calcium to evaluate the potential contribution of carbonate rock dissolution to ^{14}C-DIC signatures. Neither calcium nor

magnesium concentration varied systematically between outwash sites and (A and B) and alluvial sites (C and D), nor did concentrations of calcium plus magnesium change regularly with depth at site C (Figure 3.1, Table 3.1). At all depths, both calcium and magnesium concentrations were lower at site C than at site A (Table 3.1).

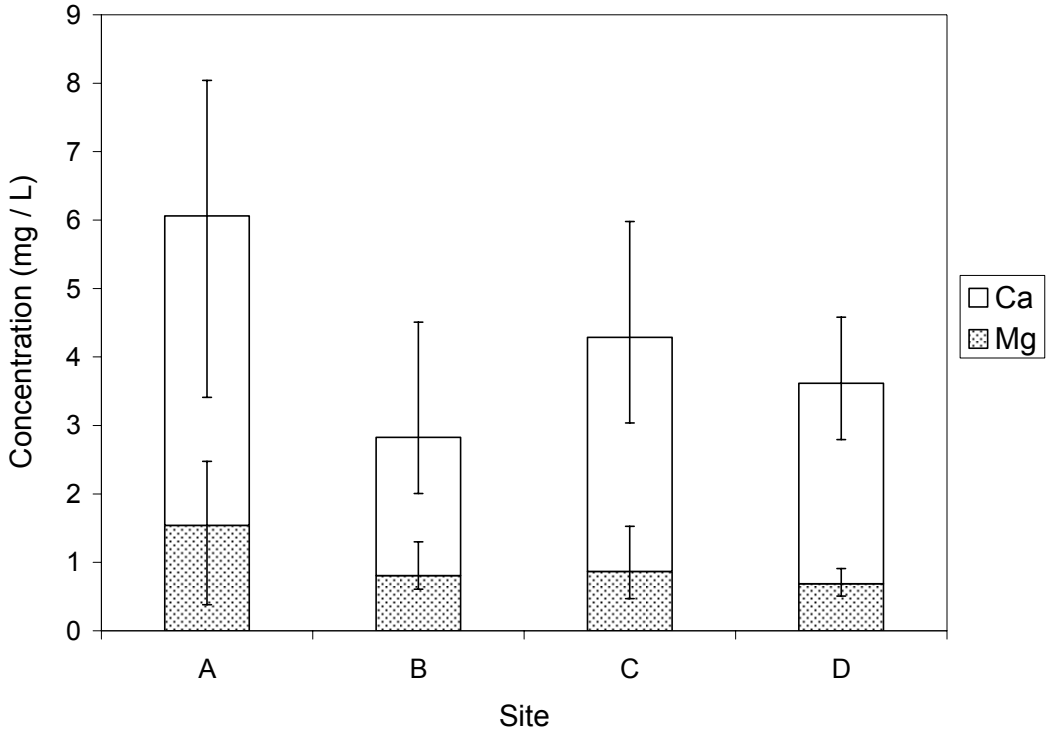


Figure 3.1. Calcium and magnesium concentrations in ambient groundwater from August-September 2003, by site. Error bars show maximum and minimum concentrations.

Table 3.1. Concentrations (mg L^{-1}) of elements in ambient groundwater sampled in August-Sept 2003, measured by inductively coupled plasma emission spectroscopy (ICP). Values are means from multiple wells at each site-depth.

	Site			
Element / depth	A	B	C	D
Al				
65 cm	0.23	0.27	0.39	0.03
150 cm	b.d.	b.d.	0.16	b.d.
300 cm	0.01	0.18	0.19	b.d.
site mean	0.12	0.22	0.30	0.03
Ca				
65 cm	3.63	2.46	3.54	2.24
150 cm	4.46	2.27	3.73	2.33
300 cm	5.34	1.38	2.97	3.31
site mean	4.52	2.02	3.42	2.93
Fe				
65 cm	b.d.	0.08	2.68	0.19
150 cm	0.01	b.d.	5.42	b.d.
300 cm	0.14	b.d.	4.25	0.13
site mean	0.06	0.08	4.04	0.15
K				
65 cm	0.83	1.67	0.94	0.44
150 cm	1.42	2.83	1.89	0.56
300 cm	1.70	1.65	3.09	1.21
site mean	1.35	1.99	1.92	0.94
Mg				
65 cm	1.18	0.97	1.06	0.53
150 cm	1.56	0.73	0.86	0.56
300 cm	1.83	0.70	0.65	0.77
site mean	1.54	0.81	0.87	0.69
Na				
65 cm	0.80	0.50	0.61	0.63
150 cm	1.06	0.53	0.67	0.56
300 cm	1.48	0.48	0.64	0.57
site mean	1.13	0.50	0.64	0.58
S				
65 cm	2.01	3.20	2.38	1.41
150 cm	2.36	3.16	2.48	1.08
300 cm	2.55	2.90	0.33	0.40
site mean	2.33	3.08	1.76	0.74
P				
65 cm	0.00	0.00	0.00	0.00
150 cm	0.00	0.00	0.04	0.00
300 cm	0.00	0.00	0.08	0.00
site mean	0.00	0.00	0.07	0.00
Mn				
65 cm	0.002	0.046	0.051	0.039
150 cm	0.002	0.041	0.136	0.029
300 cm	0.005	0.080	0.125	0.117
site mean	0.003	0.057	0.101	0.088

Background concentrations of anions relevant to our experiment were generally low (Table 3.2). Bromide concentrations were always < 0.1 mg L^{-1} except at 260 cm wells at site C, where they were < 0.35 mg L^{-1}. Nitrate concentrations were near or below detection limits at sites B, C, and D and ranged from 1.8 mg L^{-1} (65 cm) to 3.9 mg L^{-1} (300 cm) at site A (Table 3.2). Sulfate concentrations displayed contrasting patterns at sites A and C (Table 3.2), declining with depth at site C (15.4 to 0.5 mg L^{-1}) and, like NO_3^-, increasing with depth at site A (6.6 to 9.0 mg L^{-1}). Data on other measurements of ambient groundwater chemistry (alkalinity, pH, cation concentrations, specific conductance, temperature) are summarized in Table 3.1, 3.2, & 3.3.

Alluvial sites (C and D) had higher DIC concentrations than outwash sites (A and B) at shallow and intermediate depths (Figure 3.2). In 2003, sites C and D had higher DIC concentrations than sites A and B at 65 cm and 150 cm ($p<0.05$, 1-tailed t-test, 65 and 150 cm samples combined). In 2002, DIC concentrations in 65 and 150 cm samples combined were higher at site C than at sites A and B ($p<0.05$, 2-tailed t-test). In both years, DIC concentrations in the deepest piezometers did not differ between alluvial and outwash sites ($p>0.3$, 2-tailed t-test). The inter-site difference disappeared in the deepest piezometers because concentrations at alluvial sites decreased markedly with depth whereas concentrations in groundwater at outwash sites remained relatively constant (Figure 3.2).

Table 3.2. Anion and dissolved oxygen (D.O.) concentrations (mg L^{-1}) and groundwater temperature at sites and wells used for in-situ incubation experiments in 2002. Anion and D.O. measurements are means from both wells at each site-depth combination; temperature of ambient groundwater was measured immediately prior to beginning the incubation at each well.

	Site														
	A					B					C				
Depth (cm)[1]	Date	T (^{o}C)	NO_3^-	SO_4^{2-}	D.O.	Date	T (^{o}C)	NO_3^-	SO_4^{2-}	D.O.	Date	T (^{o}C)	NO_3^-	SO_4^{2-}	D.O.
65	11/26	9.4	1.8	6.6	4.5	12/15	7.1	0.1	10.8	2.2	11/30	7.9	0.0	15.4	0.7
	12/10	7.2				12/15	6.6				12/7	6.4			
150	11/21	11.4	2.5	7.0	6.1						11/30	9.3	0.1	12.8	1.0
	12/10	7.6									11/30	8.4			
300	11/21	10.7	3.9	9.0	5.9						11/30	10.2	0.1	0.4	0.7
	11/26	9.1									12/7	7.5			

[1] Actual depth at site C = 260 cm.

Table 3.3. Alkalinity, pH, specific conductance, and temperature of ambient groundwater in 2003, by site and depth.

	Site			
Variable / depth	A	B	C	D
pH				
65 cm	5.5	5.4	5.8	5.5
150 cm	5.8	5.4	6.7	5.9
300 cm	5.8	5.0	6.9	6.6
Alkalinity (mg $CaCO_3$ L^{-1})				
65 cm	3.1	2.8	11.6	4.6
150 cm	5.8	1.5	23.4	8.7
300 cm	7.6	0.2	31.7	23.7
Temperature (deg C)				
65 cm	16.4	17.6	17.4	17.5
150 cm	15.5	16.0	16.2	16.3
300 cm	14.4	14.6	15.0	14.4
Specific conductance (uS)				
65 cm	62	51	54	47
150 cm	84	49	69	43
300 cm	103	44	69	65

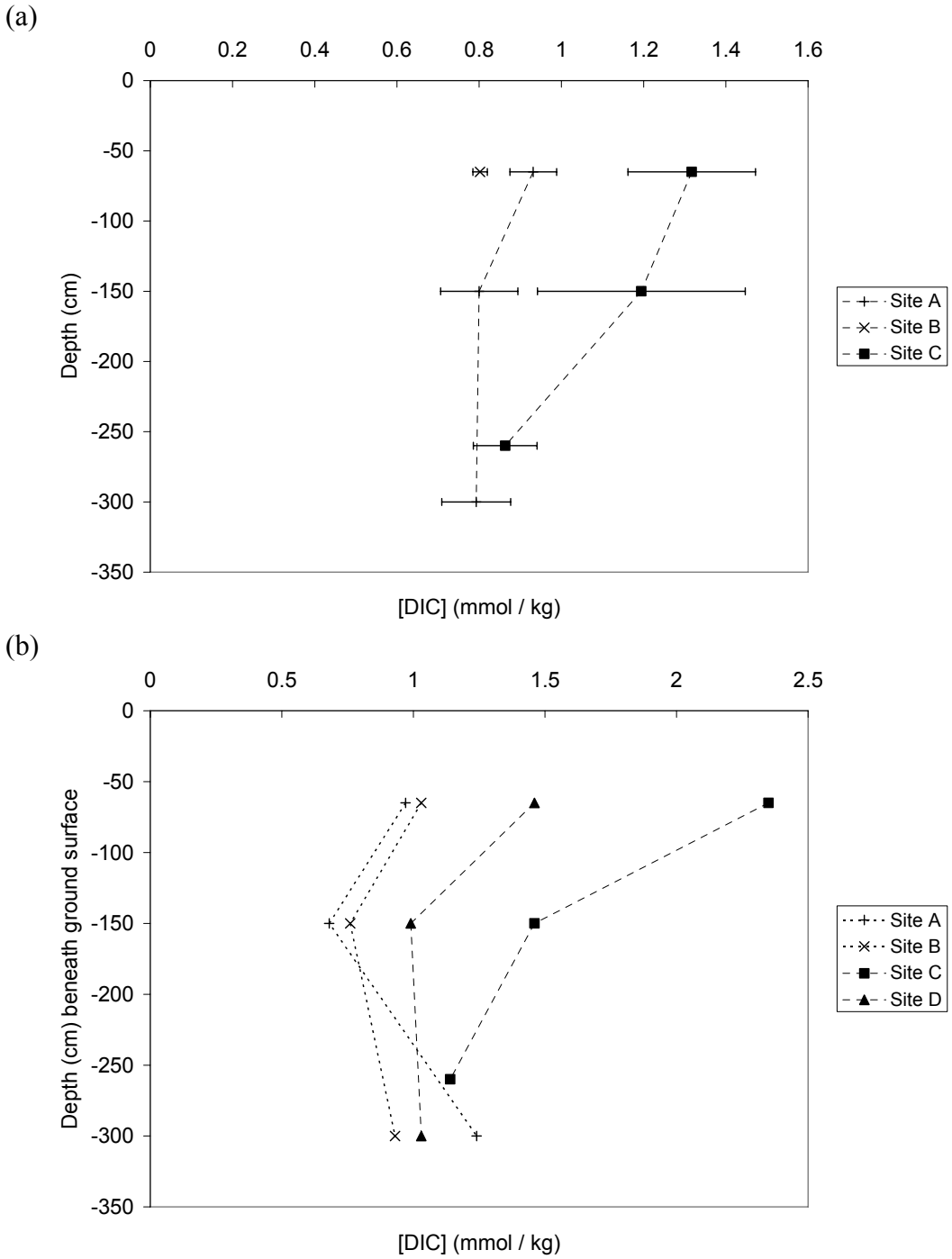


Figure 3.2. Concentration gradients of dissolved inorganic carbon (DIC) in ambient groundwater. (a) Sites and wells used in the 2002 incubation experiment. Each point is the mean of samples from two wells except site A 300 cm, which is the mean of 3 wells; bars show the concentration range. (b) Wells sampled in August-September 2003; we sampled one well at each site-depth combination.

Isotope signatures of ambient groundwater DIC

In 2002, the radiocarbon signature of ambient DIC decreased markedly with depth at site C (Figure 3.3a), and ^{14}C signatures from both 260-cm wells (-76 and -89‰) and the 150-cm well (-23‰) at this site were less than 0‰ (Figure 3.3a, Table 3.4). Radiocarbon signatures of DIC in 2002 ambient groundwater from site A (outwash) were more enriched than those from site C, ranging from 129.9‰ at 65 cm to 32.8‰ at 300 cm (Figure 3.3a, Table 3.5). In addition, the modest depletion in $\Delta^{14}C$ with depth at outwash site A presents a different pattern than the strong decline observed at alluvial site C (Figure 3.3a). Samples from the 65 cm piezometers at site B (outwash) were more depleted than shallow samples from the other sites (Figure 3.3a, Table 3.5).

In summer, 2003, we observed two patterns in ambient groundwater ^{14}C of DIC. First, as in 2002, ^{14}C signatures at site C (alluvial) declined monotonically with depth (from +71‰ at 65 cm to -41‰ at 260 cm, Figure 3.3b, Tables 3.4 & 3.5), but this pattern did not appear for samples from site D (alluvial) (range +15.9 to +36‰, Table 3.5). Second, ^{14}C-DIC values from 150 cm and deeper were always more depleted at sites C and D (alluvial) than at sites A and B (outwash) (Figure 3.3b), and ^{14}C signatures from the outwash sites fell within a narrow range (66-93‰) compared to those from the alluvial sites (Figure 3.3b). We observed no trend in ^{14}C signatures with depth at Sites A, B, or D (Figure 3.3b).

In three instances we observed ambient groundwater DIC with ^{14}C signatures <34‰ from the shallowest piezometers (65 cm). The ^{14}C-DIC values from 65 cm wells at site B (outwash) in winter 2002 were 10.7 and 33.4‰ respectively, and in summer 2003 a sample from 65 cm at site D (alluvial) had a ^{14}C-DIC signature of 15.9‰.

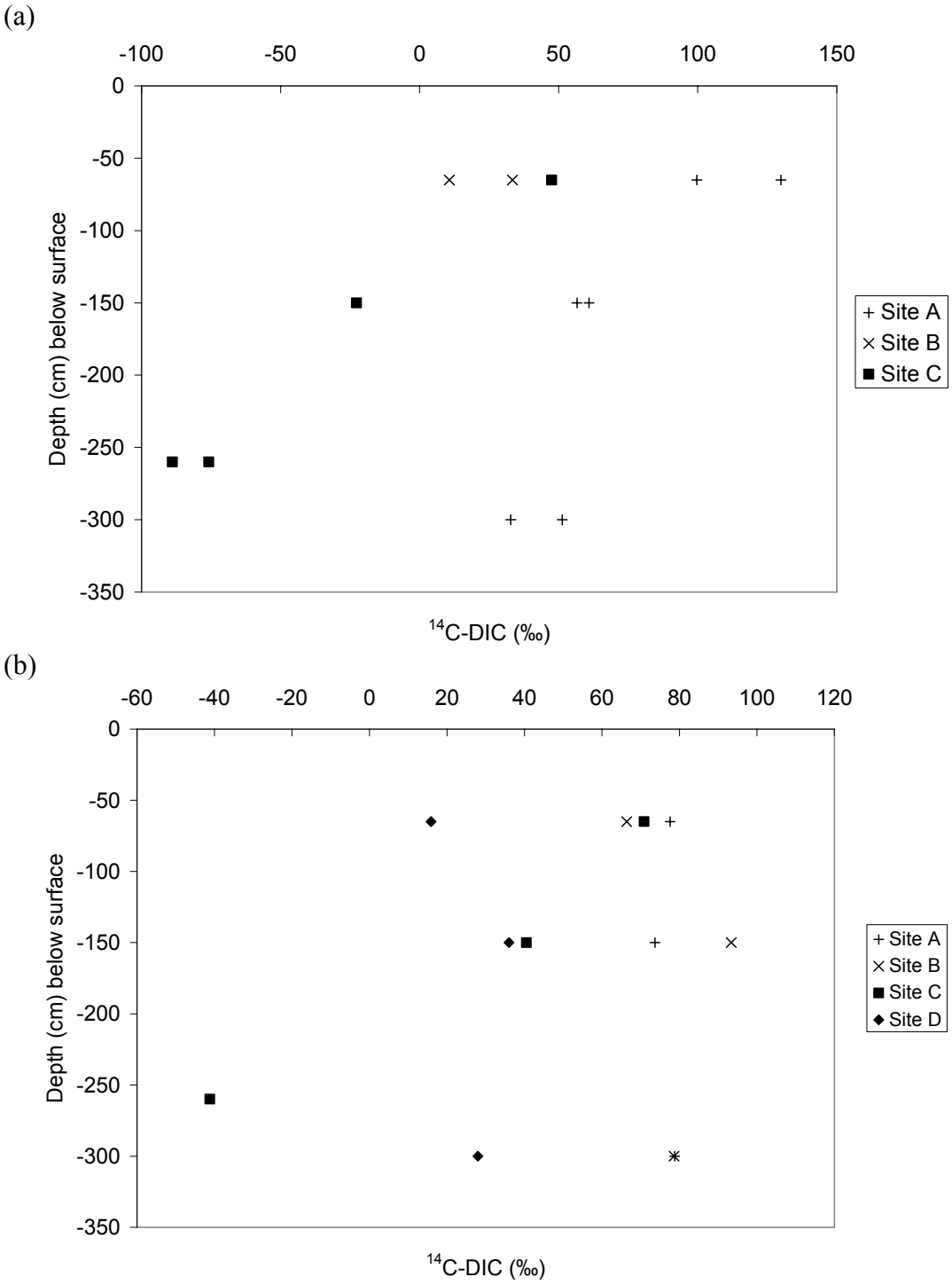


Figure 3.3. ^{14}C signatures of dissolved inorganic carbon (DIC) in ambient groundwater sampled in 2002 and 2003. (a) Sites and wells used in the 2002 incubation experiment; each point is value from a single well. (b) Wells sampled in August-September 2003; we sampled one well at each site-depth combination.

Table 3.4. Isotopic signatures for samples with premodern ^{14}C signatures.

Sample description				**^{13}C and ^{14}C signatures**			
Sample year	Site	Depth (cm)	Ambient or Incubated	$^{13}CO_2$ (‰)	$^{14}CO_2$ (‰)	F modern	Fm Error
2002	C	150	A	-24.7	-22.7	0.9835	0.0043
2002	C	260	A	-21.69	-88.9	0.917	0.0048
2002	C	260	A	-22.48	-75.8	0.9301	0.0034
2003	C	260	A	-21.38	-41.2	0.965004	0.0028
2002	A	150	I	-20.81	-33.2	0.973	0.0035
2002	B	65	I	-27.03	-63.3	0.9427	0.0035
2002	C	150	I	-23.48	-55.7	0.9504	0.0033
2002	C	260	I	-22.46	-193.5	0.8117	0.0033
2002	C	260	I	-28.24	-166.8	0.8385	0.0025

Table 3.5. Isotopic signatures for samples with modern ^{14}C signatures.

Sample description				**^{13}C and ^{14}C signatures**			
Sample year	Site	Depth (cm)	Ambient or Incubated	$^{13}CO_2$ (‰)	$^{14}CO_2$ (‰)	F modern	Fm Error
2002	A	65	A	-23.68	99.7	1.1067	0.0049
2002	A	65	A	-24.35	129.9	1.1372	0.0044
2002	A	150	A	-22	60.9	1.0678	0.0047
2002	A	150	A	-23.28	56.6	1.063375	0.0029
2002	A	300	A	-22.96	51.3	1.0581	0.0046
2002	A	300	A	-22.78	32.8	1.0394	0.0051
2002	B	65	A	-25.23	10.7	1.0172	0.0037
2002	B	65	A	-25.86	33.4	1.0400	0.0051
2002	C	65	A	-25.58	47.5	1.0542	0.0052
2003	A	65	A	-23.92	77.6	1.084539	0.0032
2003	A	150	A	-22.17	73.7	1.080597	0.0039
2003	A	300	A	-22.68	78.8	1.0857	0.0029
2003	B	65	A	-25.58	66.4	1.073284	0.0045
2003	B	150	A	-24.8	93.4	1.100451	0.0032
2003	B	300	A	-24.73	78.6	1.085501	0.0029
2003	C	65	A	-24.6	70.9	1.077811	0.0027
2003	C	150	A	-24.48	40.5	1.047176	0.0026
2003	D	65	A	-25.67	15.9	1.022433	0.0029
2003	D	150	A	-24.45	36	1.04262	0.0033
2003	D	300	A	-22.91	28	1.034571	0.0026
2002	A	65	I	-22.76	31.2	1.0378	0.0047
2002	A	65	I	-20.35	43.7	1.050525	0.0037
2002	A	300	I	-19.71	154.8	1.1623	0.0061
2002	A	300	I	-21.52	44.6	1.0513	0.0038
2002	B	65	I	-27.86	23.6	1.0301	0.005
2002	C	65	I	-26.92	68.6	1.0755	0.0038

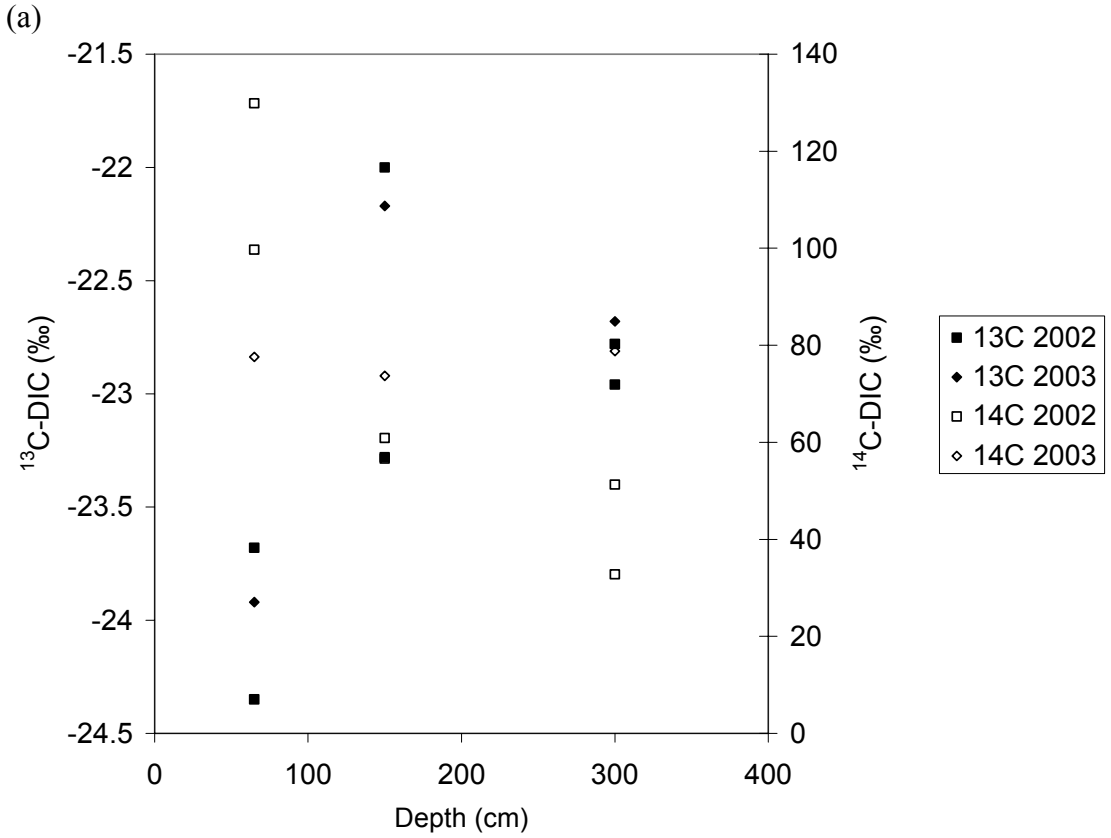
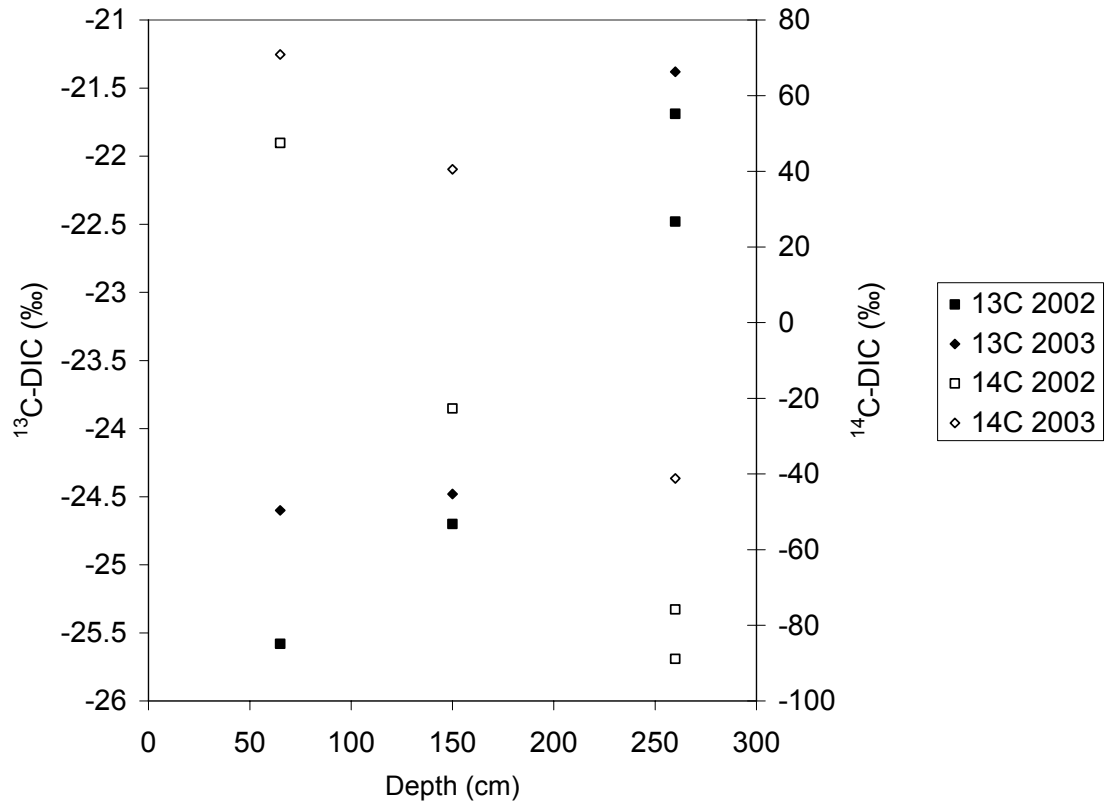


Figure 3.4. Relationships between depth, ^{13}C-DIC, and ^{14}C-DIC in ambient groundwater in 2002 and 2003 at (a) site A and (b) site C.

Figure 3.4. (Continued).

(b)



^{14}C-DIC and ^{13}C-DIC were inversely correlated at site C (r= -0.81, p<0.05 n=7) and site A (r= -0.63, p<0.07 n=9) (Figure 3.4). ^{14}C-DIC also correlated negatively with alkalinity at these sites in 2003 (r = -0.95, p<0.005 n=6), but this relationship emerged exclusively from patterns at site C (r = -0.98, p = 0.13) and not at all from site A (r = 0.12, p = 0.9) (Figure 3.5). ^{14}C-DIC signatures bore no relationship to DIC concentrations (Figure 3.6).

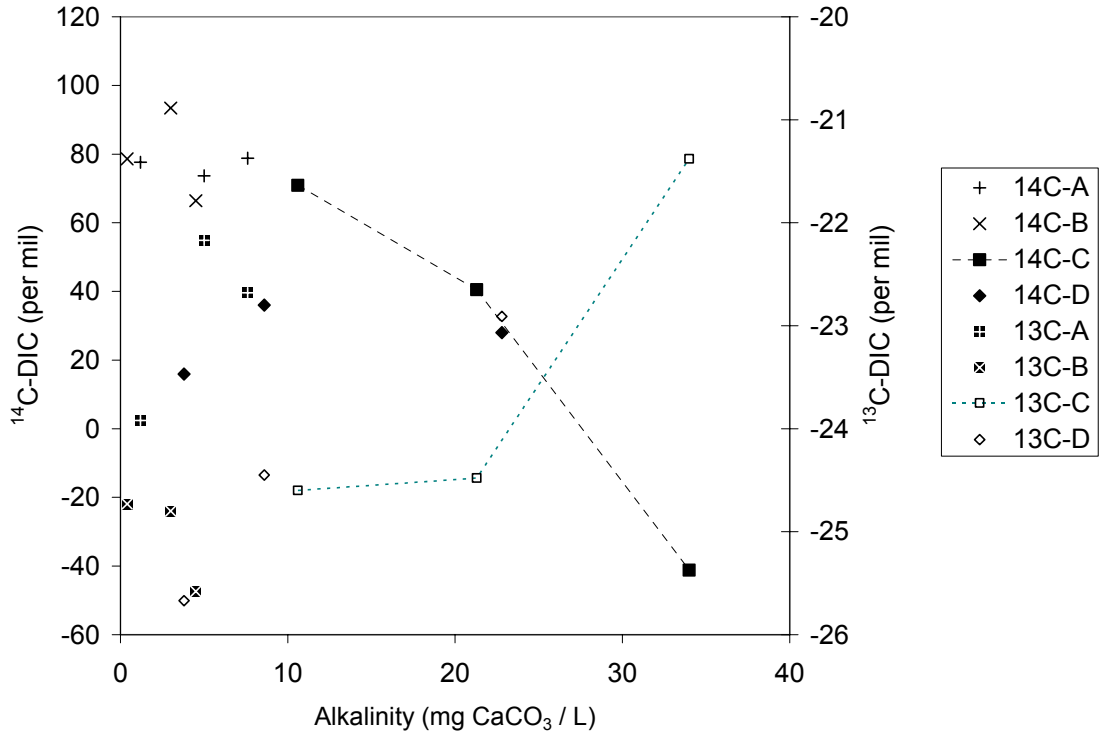


Figure 3.5. Relationships between alkalinity, ^{13}C-DIC, and ^{14}C-DIC in ambient groundwater in 2003, at each of four sites.

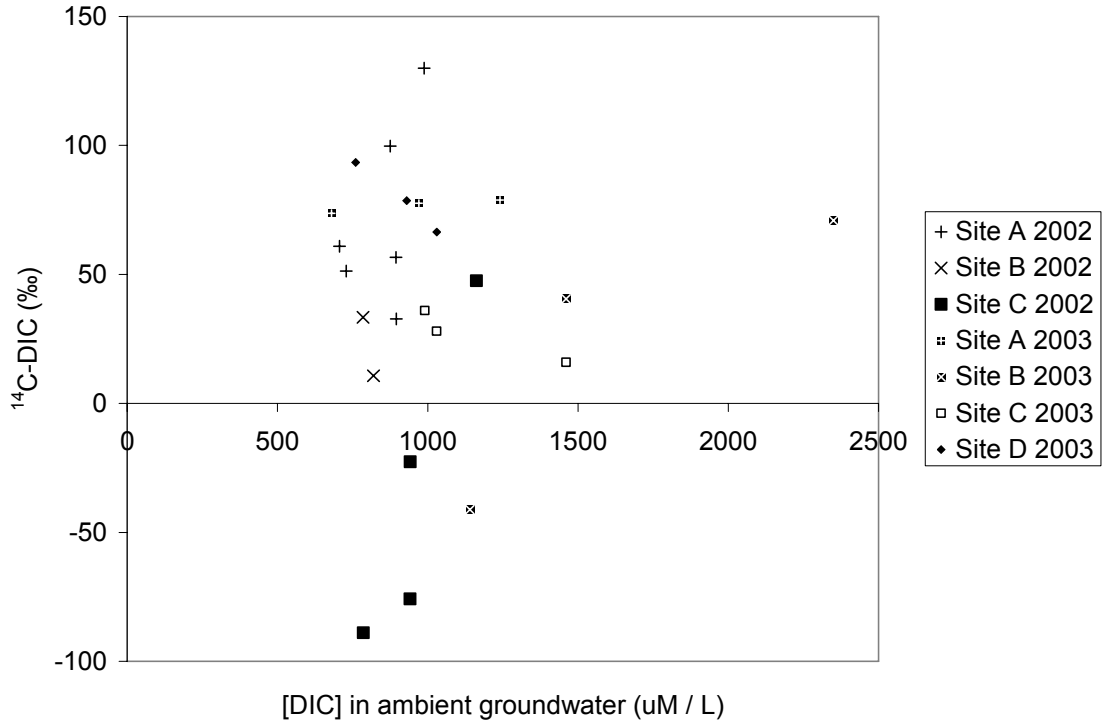


Figure 3.6. Correlation between [DIC] and ^{14}C-DIC in ambient groundwater, by site and year.

In-situ incubations ("push-pull" experiments)

In most cases, we recovered >75% of our tracer confirming that our incubation times were suitable given flow conditions in the aquifer. The 65 cm well at site B yielded 100% recovery, and only the 65 cm well at site C (18%) yielded recovery < 55%.

We observed two notable patterns in ^{14}C-DIC of incubated groundwater. First, ^{14}C-DIC of incubated groundwater was usually more depleted than ^{14}C-DIC of the corresponding ambient groundwater (Figure 3.7). In addition to the highly depleted signatures at 260 cm at site C (alluvial), we observed ^{14}C signatures <50‰ and sometimes <0‰ in incubated samples from 65 cm and 150 cm wells at sites A and B (outwash) (Figure 3.7, Tables 3.4 & 3.5). Differences between ambient and incubated samples were often >50‰ (Figure 3.7, Table 3.6). A notable exception to the pattern

of more depleted values in incubated samples was the sample from a 300 cm piezometer at site A (outwash), which had a ^{14}C signature of 154.8‰ compared to an ambient value of 51.3‰ (Figure 3.7, Tables 3.5 & 3.6). Second, the pattern of ^{14}C depletion with depth in samples of incubated groundwater at site C (alluvial) (Figure 3.8) mirrored data from the ambient groundwater (Figure 3.3a), and incubated samples from the 260 cm wells at this site exhibited ^{14}C-DIC signatures in the range of -166 to -194‰ (Figures 3.7 & 3.8, Table 3.4).

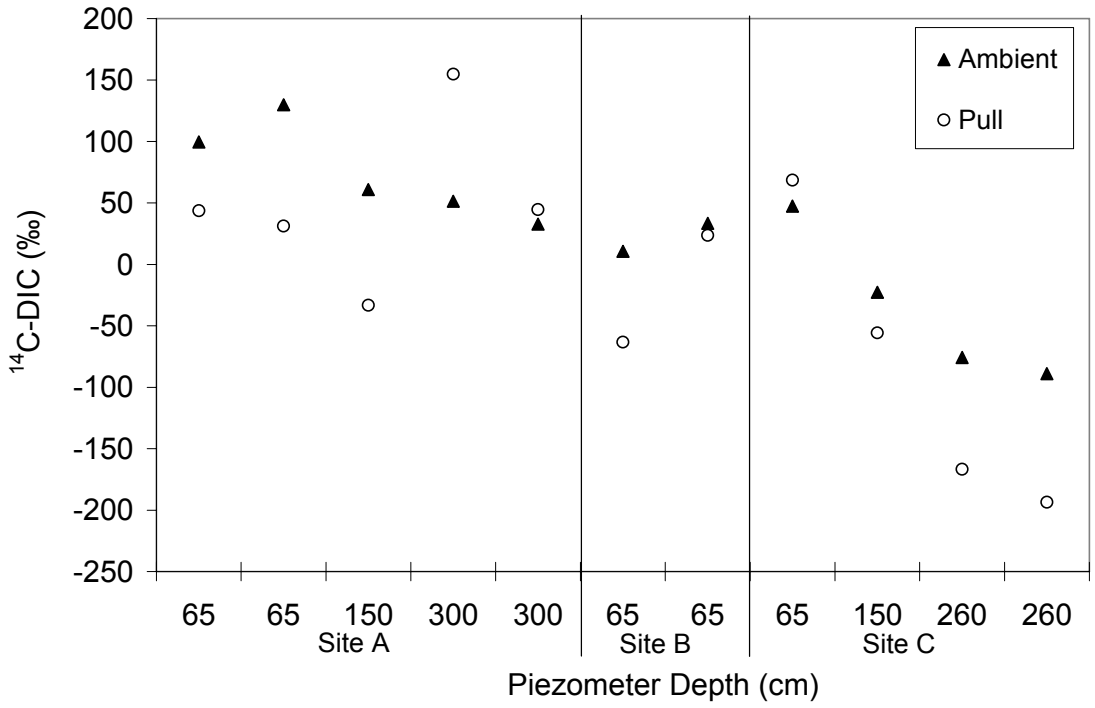


Figure 3.7. ^{14}C-DIC of groundwater in ambient samples and samples following in-situ incubation at alluvial (C) and outwash (A,B) sites.

Carbon mineralization rates at sites A and B (outwash) exceeded those at site C (alluvial) ($p=0.05$, 2-tailed t-test) and showed no strong pattern with depth (Figure 3.9a). Rates of DIC production adjusted for dilution and dispersion of the dosed plume were as high as 0.07 uM CO_2 kg soil^{-1} second^{-1} (Figure 3.9a). At site C, where pH and alkalinity were high by comparison with our other study sites, accounting for

bicarbonate in the initial groundwater yielded negative values for DIC production at one 150 cm and two 260 cm wells (Figure 3.9b). Accounting for bicarbonate also yielded an estimate of DIC production < 0 fro one 65 cm well at site A (Figure 3.9b). We know of no DIC consumption processes likely to be operating at these sites. In future calculations, we consider scenarios with and without accounting for bicarbonate initially present in the groundwater.

Despite these uncertainties in the DIC budget for our incubations, the very good recovery rates leave us confident that we executed our in-situ incubations effectively. Moreover, because ^{14}C signatures are reported with respect to a ^{13}C signature of -25‰, any processes that could have associated fractionation affects on isotopic signatures would not have influenced the radiocarbon data. Although the uncertainties in our DIC budgets add some limitations to our ability to quantify the contribution of old C to subsurface C mineralization, using multiple approaches (e.g., data from wells with low pH and alkalinity, modeling) enabled us to make sound estimates regarding the importance of ancient C in these ecosystems.

Using a mass balance approach, and assuming no bicarbonate in groundwater at the start of the incubations, we infer that C mineralized during the incubation must have had ^{14}C signatures ranging from -515‰ at site C, 260 cm, to +396‰ at site C, 65 cm (Figure 3.10, Table 3.7). Depleted C was not restricted to alluvial soils and great depth; C mineralized at shallow and intermediate depths at site A had ^{14}C signatures of < -100‰ and < -285‰ respectively (Figure 3.10, Table 3.7). Conversely, ^{14}C-enriched C was not restricted to outwash soils and shallow depths; C mineralized at 65 cm at site C and at 300 cm at site A had mean ^{14}C signatures >200‰ (Figure 3.10, Table 3.7).

Table 3.6. Minimum ages of mineralized SOM required to account for ^{14}C-DIC signatures of ambient and incubated groundwater samples. Calibrated ages determined using: (1) Calib 5.0.1 and the IntCal04 data set (Reimer et al. 2004) for premodern ^{14}C signatures; and (2) CaliBomb using the Northern Hemisphere Zone 1 data set for samples with modern ^{14}C signatures (http://calib.qub.ac.uk/CALIBomb/frameset.html). Values are means of the youngest and oldest ages with > 15% probability reported by the calibration software.

Sampling year	Site	Depth (cm)	^{14}C-DIC (‰)		Minimum required age of a contributing C source (ybp) based on the ^{14}C-DIC of the:	
			ambient sample	incubated sample	ambient sample	incubated sample
2002	A	65	99.7	43.7	5	47
2002	A	65	129.9	31.2	9.5	47
2002	A	150	60.9	-33.2	45	279
2002	A	300	51.3	154.8	45.5	12
2002	A	300	32.8	44.6	45	47
2002	C	65	47.5	68.6	45	4
2002	C	150	-22.7	-55.7	193.5	526
2002	C	300	-75.8	-166.8	644	1,378
2002	C	300	-88.9	-193.5	677	1,658
2002	B	65	10.7	-63.3	47.5	571
2002	B	65	33.4	23.6	45	47
2003	A	65	77.6	[redacted]	44.5	[redacted]
2003	A	150	73.7	[redacted]	44.5	[redacted]
2003	A	300	78.8	[redacted]	44.5	[redacted]
2003	B	65	66.4	[redacted]	45	[redacted]
2003	B	150	93.4	[redacted]	24.5	[redacted]
2003	B	300	78.6	[redacted]	44.5	[redacted]
2003	C	65	70.9	[redacted]	45	[redacted]
2003	C	150	40.5	[redacted]	45.5	[redacted]
2003	C	260	-41.2	[redacted]	414.5	[redacted]
2003	D	65	15.9	[redacted]	47.5	[redacted]
2003	D	150	36	[redacted]	45.5	[redacted]
2003	D	300	28	[redacted]	45.5	[redacted]

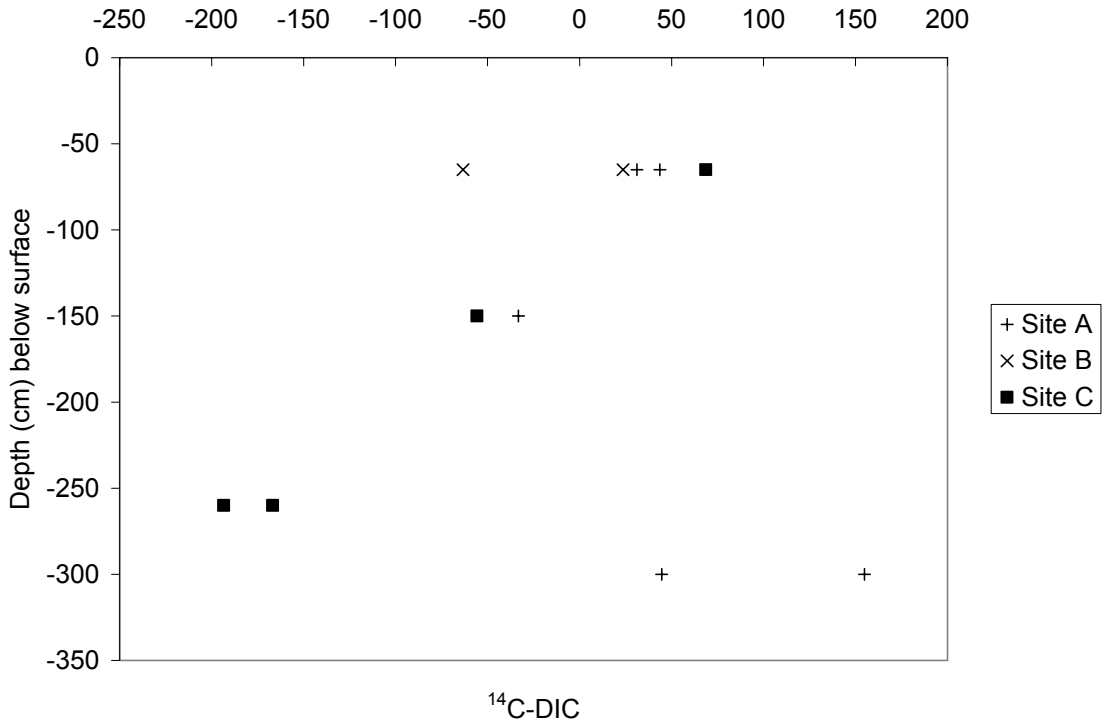
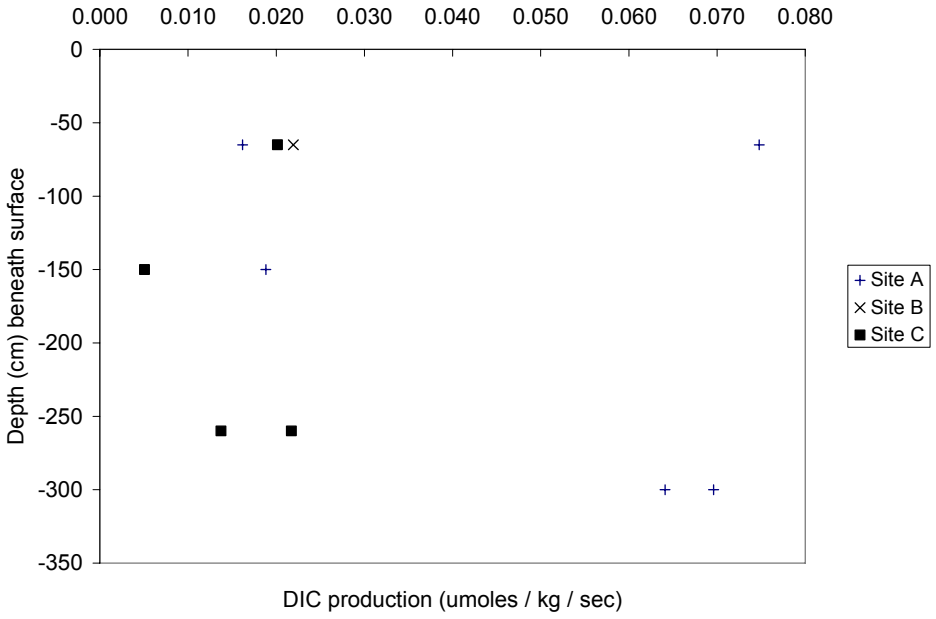


Figure 3.8. ^{14}C-DIC of groundwater following in-situ incubation vs depth at alluvial (C) and outwash (A,B) sites.

We repeated our mass balance calculation of the mean radiocarbon signatures of mineralized C incorporating estimates of bicarbonate present in the groundwater at the beginning of the incubations, and compared these estimates to those derived assuming no bicarbonate at time zero. This was not possible in cases where estimates of bicarbonate present at T zero resulted in DIC production estimates < 0. In most cases where we were able to make this comparison, the estimates differed little (Figure 3.10), reflecting the fact that >80% of total DIC in ambient groundwater at sites A and B occurs as CO_2, owing to the generally low alkalinity and pH of these groundwaters (Table 3.3).

(a)



(b)

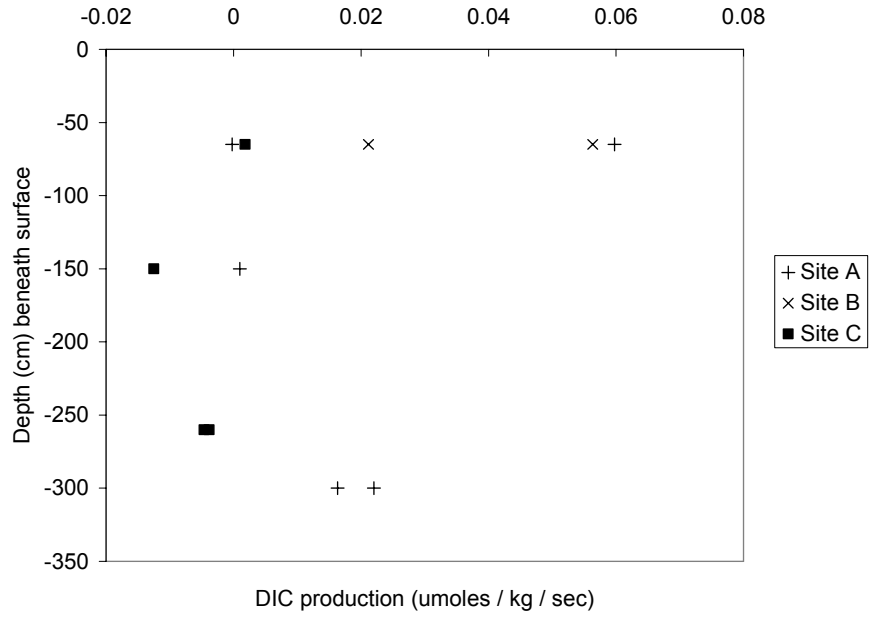


Figure 3.9. Production of dissolved inorganic carbon (DIC) during in-situ groundwater incubations. DIC production estimated from [DIC] in ambient and incubated groundwater: (a) accounting for dilution during the incubation but not for bicarbonate present at the beginning of the incubation; and (b) accounting for both dilution during the incubation and bicarbonate present at the beginning of the incubation.

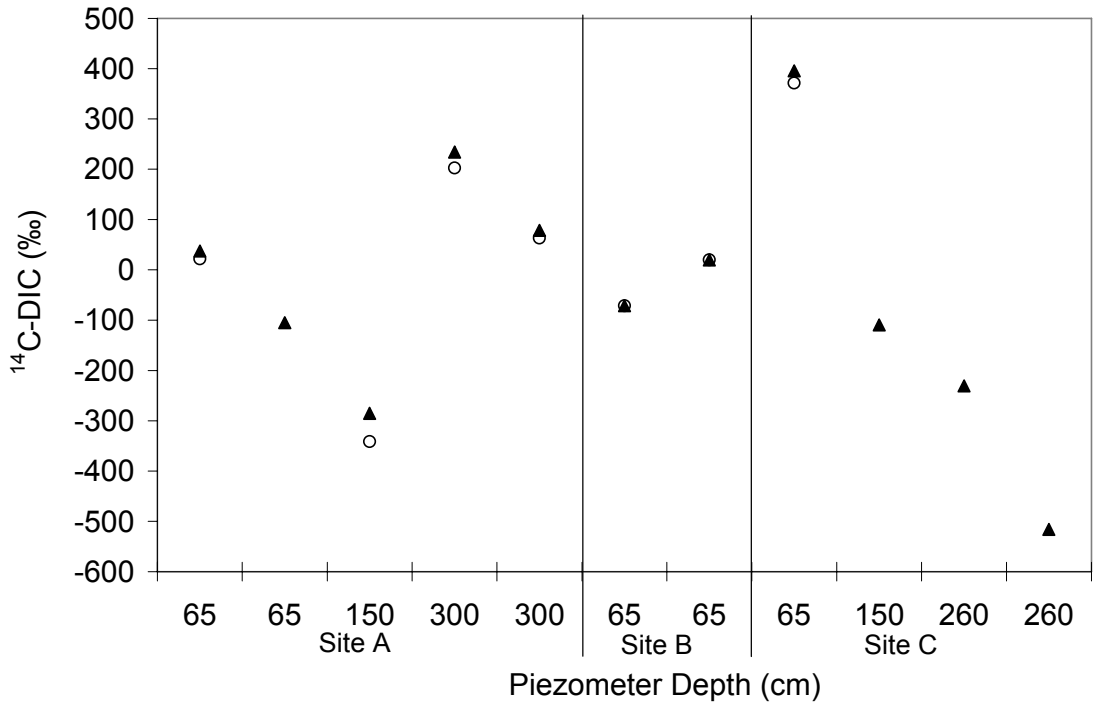


Figure 3.10. ^{14}C-DIC signatures (calculated) of mineralized C for each incubation site: (1) assuming no DIC present at the beginning of the incubation (filled triangles); and (2) where we calculated DIC production > 0, using estimated bicarbonate present at time zero (open circles).

Our analysis of the contribution of ancient C to total C mineralization, independent of [DIC] measurements, showed that the ^{14}C-depleted end member contributed a minimum of 31% of C mineralization at 260 cm at site C (alluvial). This value corresponds to the case in which the ancient end member source has a ^{14}C signature of -825‰, and 100% of the DIC in the incubated groundwater sample derived from C mineralization during the incubation (Figure 3.11). Processes that could realistically contribute to the DIC pool in the incubated sample include advection of ambient groundwater into the plume and the presence of bicarbonate in the sample at the start of the incubation. Notably, the contribution of depleted C increases according to a power function as the contribution of mineralized DIC decreases. Thus, if >60% of the DIC in the incubated sample was derived from advection or bicarbonate initially

present in the groundwater, then depleted C contributed >50% of C mineralized during the incubation.

Assuming that the actual source of ancient C formed 8,000 ybp, and that mineralization contributed between 40-100% of DIC in the sample, then ancient C accounts for 42-68% of C mineralized during the incubation at site C, 260 cm beneath the surface (Figure 3.11).

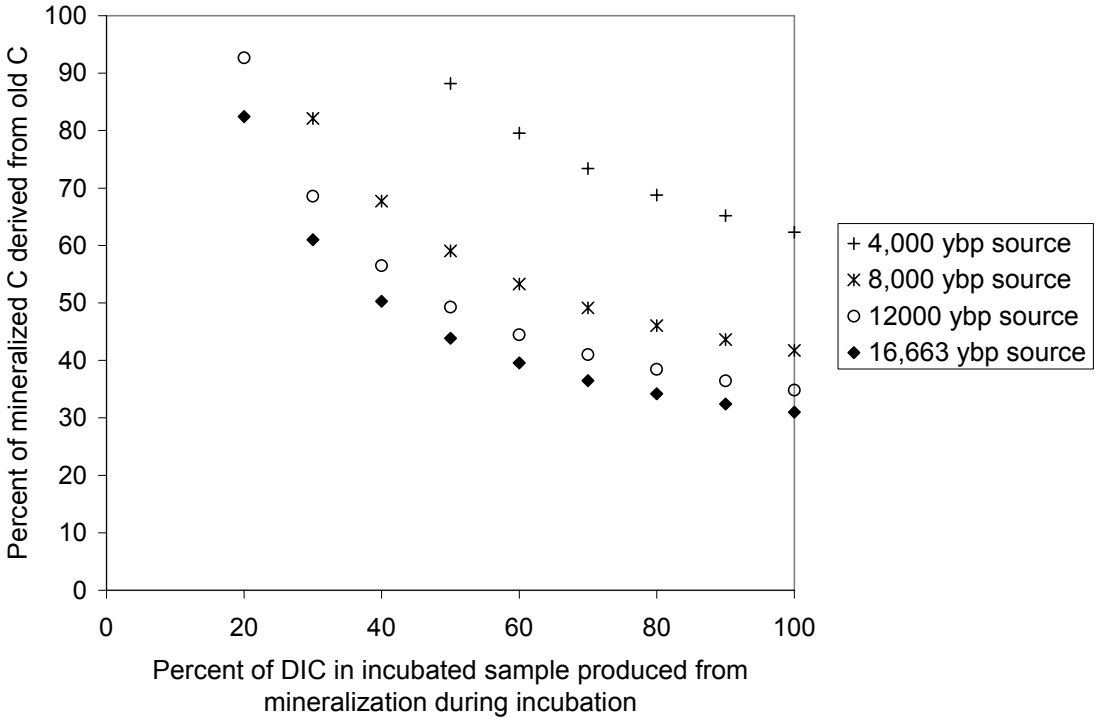


Figure 3.11. Relationship between the contribution of: (1) C mineralization during the incubation to total DIC in the incubated sample; and (2) ancient carbon to C mineralization. Calculated using ^{14}C signatures of ambient and incubated groundwater and realistic estimates of C source age at site C, 260 cm.

It is conceivable that C mineralization accounted for <40% of total DIC in the incubated sample. The relatively high pH (6.9) and alkalinity (31.7 mg $CaCO_3$ L^{-1}) of groundwater at this site-depth (Table 3.3) suggest bicarbonate may have been present in appreciable quantities at the start of the incubation. Further, the measured

concentrations of DIC were higher in ambient groundwater (786, 941 uM L^{-1}) than in the incubated samples (598, 635 uM L^{-1}), suggesting that dilution by advection could add significant quantities of DIC. The recovery rates at these wells (39 and 74%) also indicate that DIC addition occurred via advection. If 20% of total DIC in the sample resulted from C mineralization during the incubation, then microbial use of depleted (i.e., ancient) C would account for 80-100% of C mineralization at this site-depth (Figure 3.11).

Carbon mineralization rates were highest at those locations where ^{14}C-DIC signatures of mineralized DIC failed to show strong evidence of depleted C, i.e., 0‰ < ^{14}C-DIC < 300‰ (Figure 3.12).

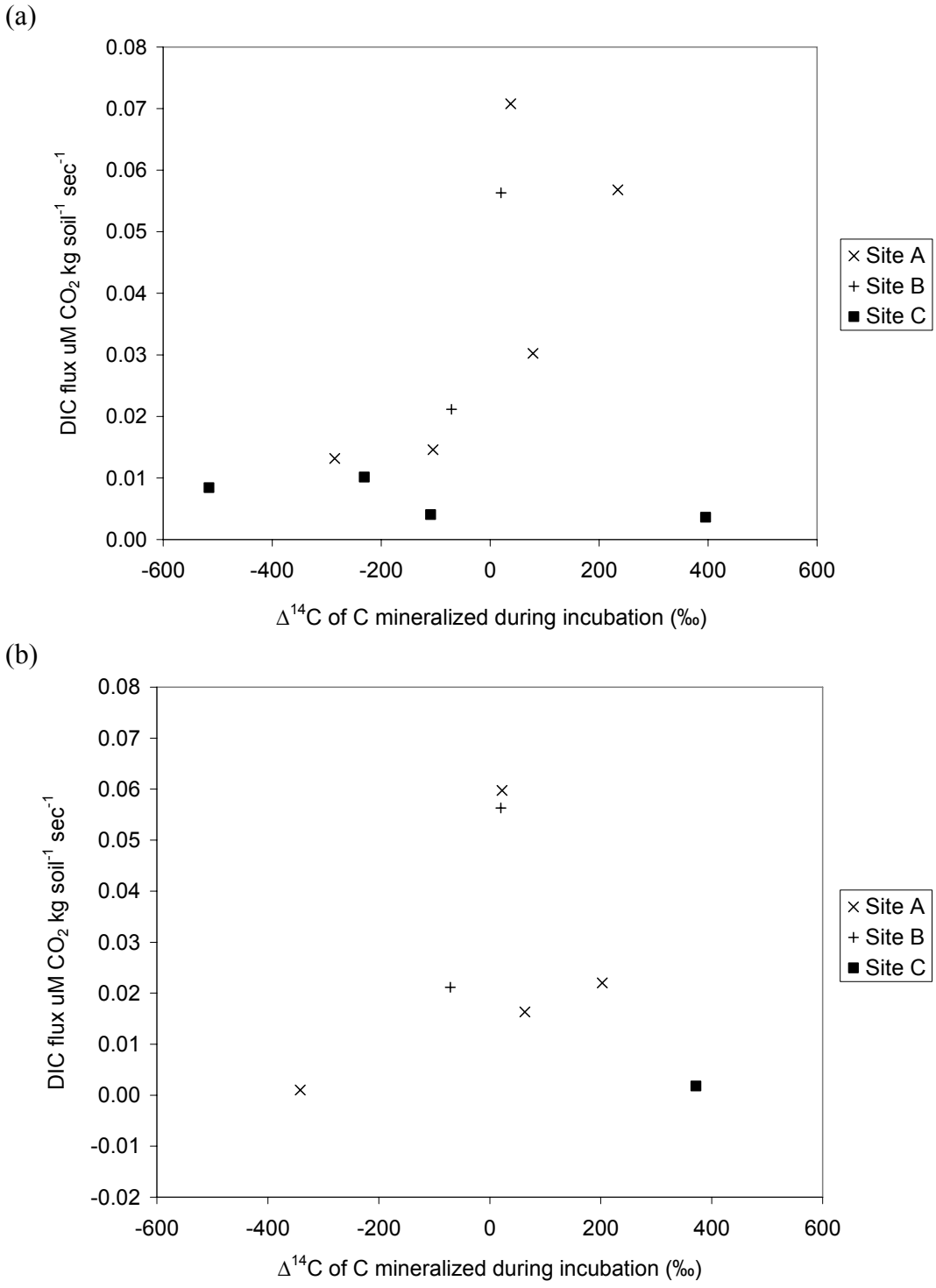


Figure 3.12. Relationship between calculated radiocarbon signatures of C mineralized during the in-situ incubations and C mineralization rate: (a) assuming dilution via advection; and (b) assuming dilution via advection and bicarbonate present at the start of the incubations, estimated from measurements of alkalinity, pH, and total DIC in ambient groundwater where estimated DIC production >0.

Discussion

Interpretation of ^{14}C-DIC signatures

We can use the ^{14}C signature of DIC in a groundwater sample to infer a minimum age of the oldest source contributing to that sample (Table 3.6). This differs from assigning an age to the DIC pool of a sample, a step we feel would not be justified because the DIC in any groundwater sample is almost certainly comprised of a mix of DIC pools of varying ^{14}C signatures.

Metabolism of old carbon

Data from ambient groundwater samples strongly support the hypothesis that C thousands of years old is being respired in the riparian subsurface. Ambient DIC in 260 cm-deep piezometers at site C had ^{14}C signatures of -75.8 and -88.9 in 2002 (Figure 3.3, Tables 3.4 & 3.6). Had all the DIC in these samples evolved from SOM of uniform age, the C sources would have had ages of 644-677 ybp and 414.5 ybp in winter 2002 and summer 2003, respectively (Table 3.6). Because these ^{14}C signatures very likely derived from a heterogeneous pool that included some recently-fixed C, the ^{14}C value of the oldest SOM contributing to the pool must have been considerably more depleted than the measured value, with correspondingly older age. For example, these signatures are consistent with contributions of: (1) 18-20% C fixed 16,663 ybp (Δ^{14}C -825‰) and 80-82% C fixed ~3 ybp (Δ^{14}C +90‰); (2) 25% C fixed 16,663 ybp, 50% C fixed ~3 ybp, and 25% C fixed ~23 ybp (+290‰); or (3) 55% C fixed ~3 ybp, 15% C fixed ~23 ybp, and 30% C fixed 8,300 ybp (-605‰). Although it is impossible to identify the particular components (i.e., SOM pools of varying age) contributing to this mix, nor the proportions of each, at least one of these sources must have been as old as the youngest age implied by the observed ^{14}C signatures.

In addition, the extent of ^{14}C depletion declined monotonically with depth at this site during both samplings (Figure 3.3), suggesting that the contribution of ancient C to the C cycle increased progressively with depth at this alluvial riparian zone. This scenario is consistent with the observation that the age of buried horizons, and hence the opportunity for mineralization of increasingly old SOM, increases with depth.

The ^{14}C-DIC values of samples from our in-situ groundwater incubations provide further evidence for microbial use of ancient C in the subsurface. Ambient groundwater DIC is a mixture of C produced locally as well as C that has been mineralized and transported from the surface or other points along the flowpath, whereas groundwater from our in-situ incubations contains a much higher proportion of DIC produced locally and immediately. Therefore, the signal of local C mineralization should be stronger in ^{14}C signatures of samples recovered at the end of each incubation than in ambient groundwater samples. The significant declines in ^{14}C-DIC signatures that we observed over the course of most of our in-situ incubations (Figure 3.7, Table 3.6) strongly imply metabolism of C at least as depleted (where we had 100% recovery) and — because advection adds ambient DIC to the recovered sample — usually considerably more depleted than the bulk DIC recovered from the incubated samples (Figures 3.7 & 3.10, Tables 3.6 & 3.7). Incubated samples had minimum ^{14}C-DIC signatures of -167‰ and -194‰, compared with -75.8‰ and -88.9‰ in ambient samples. Had DIC with these signatures been produced by mineralization of a homogeneous SOM pool, that SOM would have had an age of 1,378-1,658 ybp (Table 3.6). Combining our data from ambient and incubated samples, we conclude that the DIC produced during our incubations 260 cm deep at site C (alluvial) must have had ^{14}C signatures as depleted as -516‰ (Table 3.7); this requires mineralization of SOM formed at least 6,614 ybp.

Table 3.7. Minimum ages of mineralized SOM required to account for calculated ^{14}C-DIC signatures. Calibrated ages determined using: (1) Calib 5.0.1 and the IntCal04 data set (Reimer et al. 2004) for premodern ^{14}C signatures; and (2) CaliBomb (http://calib.qub.ac.uk/CALIBomb/frameset.html) using the Northern Hemisphere Zone 1 data set for samples with modern ^{14}C signatures. Values are means of the youngest and oldest ages with > 15% probability reported by the calibration software.

Site	Depth (cm)	^{14}C-DIC of C mineralized during the incubation (‰):		Minimum required age of a contributing C source (ybp):	
		calculated considering advection only.	calculated considering advection and initial bicarbonate.	based on Δ^{14}C of DIC mineralized during incubation, calculated using advection only.	based on Δ^{14}C of DIC mineralized during incubation, calculated using advection and initial bicarbonate.
A	65	37.5	22	47	47
A	65	-104.8	*	790	*
A	150	-285.3	-342	2,842	3,595
A	300	234.1	203	20	17
A	300	78.4	63	4	4
C	65	395.5	372	27	27
C	150	-109.3	*	859	*
C	300	-230.8	*	2,085	*
C	300	-515.9	*	6,614	*
B	65	-71.1	-71	591	591
B	65	20	20	47	47

* Accounting for initial bicarbonate present yielded estimates of DIC production < 0, which precludes calculation of ^{14}C signature of mineralized SOM.

Although the signatures from samples 260 cm deep at site C were more depleted than those from other site-depth combinations we studied, they were not unique in demonstrating metabolism of premodern C. Of the twelve in-situ incubations conducted, six demonstrated metabolism of SOM formed >500 ybp (Figure 3.13, Table 3.7). The high frequency with which our in-situ incubations provided evidence for metabolism of old C, combined with the limited number of sites included in this study and the high incidence of microbially-available C in buried horizons as indicated by laboratory studies (Gurwick et al. chapter 1), suggests that metabolism of ancient C in the riparian subsurface is common in the types of landscapes we studied.

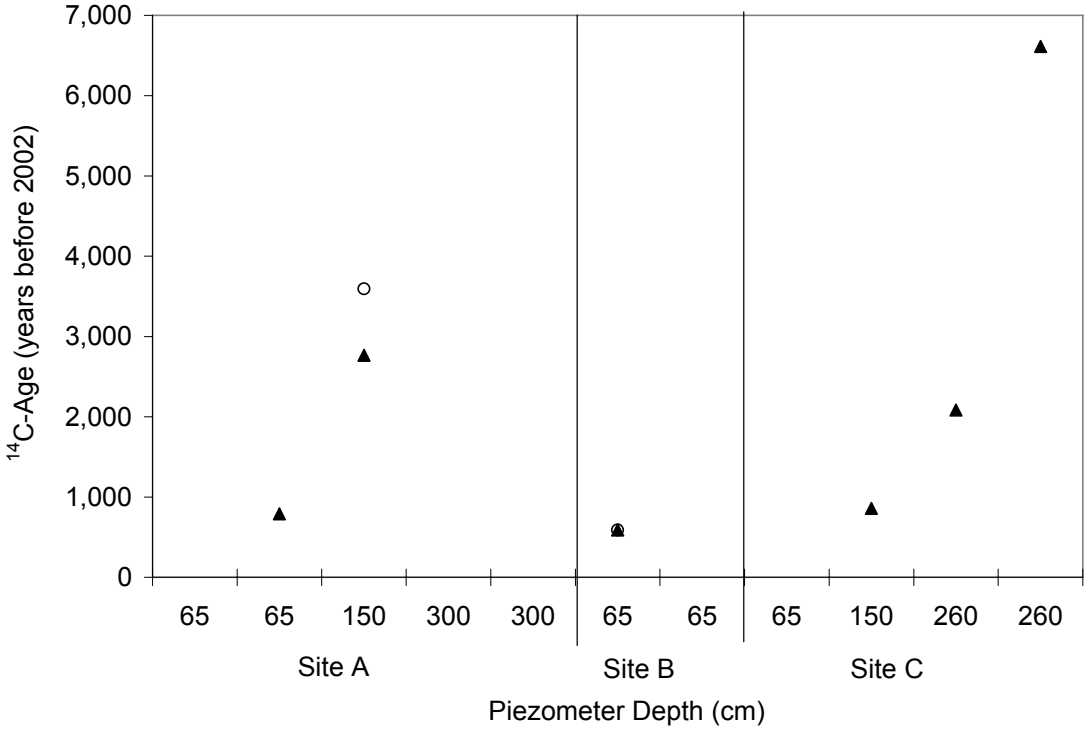


Figure 3.13. ^{14}C-calibrated ages of mineralized C for each incubation with pre-modern estimated ^{14}C signatures: (1) calculated assuming the groundwater introduced to the well had no DIC present at the beginning of the incubation (filled triangles) and (2) calculated using estimates of bicarbonate, calculated using measurements of [DIC] in ambient groundwater in 2002 and alkalinity and pH, where we estimated DIC production > 0 (open circles). Ages of mineralized SOM for cases with modern ^{14}C signatures are discussed in the text. Units are years before present, where present is defined as 2002.

Potential for carbonate mineral dissolution to explain ^{14}C patterns

The strong correlations between alkalinity and ^{14}C-DIC, and alkalinity and ^{13}C-DIC, at site C (Figure 3.5) force us to consider the possibility that carbonate minerals contribute to both increased alkalinity and depleted ^{14}C signatures at this site. Carbonate minerals tend to be enriched in ^{13}C, and because they are generally very old they also have highly depleted ^{14}C signatures. Small amounts of a highly-depleted end member can influence the mean ^{14}C signatures of the DIC pool. However, several lines of evidence suggest that carbonates are not driving the depleted ^{14}C-DIC signatures from 150 and 260 cm at site C. We would expect large spatial differences in carbonate dissolution to yield corresponding patterns of calcium and/or magnesium in groundwater. Therefore, if carbonate dissolution drove depleted ^{14}C signatures, we would expect to have seen higher concentrations of calcium and magnesium where we found more depleted ^{14}C-DIC. This was not the case at our sites; while ^{14}C signatures were lower at site C compared to other sites, calcium and magnesium concentrations were highest at site A (Figure 3.1). In addition, ^{14}C signatures at site C declined markedly with depth, but calcium and magnesium concentrations did not (Table 3.1). These data suggest that the declines we observed in DIC-^{14}C signatures with depth at site C (alluvial) did not result solely from dissolution of carbonate minerals.

To provide additional insight into the potential for dissolution of carbonate minerals to explain the observed patterns of ^{14}C signatures, we calculated the change in ^{13}C-DIC and ^{14}C-DIC signatures that would be expected to result from DIC production associated with a doubling of the concentration of calcium + magnesium. The molar ratio in carbonate minerals of Ca (or Mg) to carbon is 1:1, the concentration of (Ca+Mg) in our ambient groundwater samples ranged from 100-200 umoles L^{-1}, and the ambient DIC concentrations ranged from 700-1,500 umoles L^{-1}. Doubling the

(Ca+Mg) concentration would therefore result in a 9-25% increase in DIC (Table 3.8), which would require a DIC production rate of ~0.01 umole $kg^{-1}sec^{-1}$. For the two deep wells at site C, the resulting ^{14}C-DIC signatures would be -165 and -192‰ and ^{13}C-DIC signatures of -28.3 and -23.5‰. While these predicted ^{14}C signatures are consistent with those observed in our incubated samples, before accounting for advection, the observed ^{13}C-DIC signatures are much more depleted than both the predicted values and than the observed ambient ^{13}C-DIC values (Table 3.8). Further, because alkalinity is relatively high at site C, the total DIC in ambient groundwater was 60-70% bicarbonate, implying that the actual DIC increase there was considerably lower than 0.01 umole $kg^{-1}sec^{-1}$. Lesser — and more realistic — increases in Ca + Mg, with correspondingly lower DIC increases, fail to account for the observed ^{14}C depletion (Table 3.9).

We conclude that the correlation between alkalinity, ^{13}C, and ^{14}C in ambient groundwater at site C does not reflect increasing carbonate contributions to the DIC pool at depth at this site, and that depleted ^{14}C-DIC signatures there reflect mineralization of old organic matter in buried soil horizons rather than DIC contributions from carbonate minerals.

Table 3.8. Estimated changes in ^{13}C- and ^{14}C-DIC of riparian groundwater from dissolution of carbonate minerals associated with a doubling of [calcium + magnesium] in groundwater. ^{13}C and ^{14}C of C inputs from carbonate mineral dissolution assumed to be 0‰ and -1000‰, respectively. All isotopic values given in units of per mil.

Site	Depth (cm)	[Mg + Ca] (umole/L)	Alkalinity (um / L)	[DIC] (um/ L)	DIC / Alk	% C increase	^{14}C amb	^{14}C-DIC final (modeled)	^{14}C pull	^{13}C amb	^{13}C-DIC final (modeled)	^{13}C pull
A	65	160	32	875	27	18	99.7	-70	43.7	-23.68	-20.02	-20.35
A	65	160	32	988	31	16	129.9	-27	31.2	-24.35	-20.96	-22.76
A	150	176	58	706	12	25	60.9	-151	-33.2	-22.00	-17.61	-20.81
A	300	207	76	963	13	21	51.3	-134	154.8	-22.96	-18.91	-19.71
A	300	207	76	896	12	23	32.8	-161	44.6	-22.78	-18.51	-21.52
C	65	131	107	1472	14	9	47.5	-38	68.6	-25.58	-23.49	-26.9
C	150	128	230	942	4	14	-22.7	-140	-55.7	-24.70	-21.75	-23.48
C	260	101	317	941	3	11	-75.8	-165	-166.8	-22.48	-20.31	-28.24
C	260	101	317	786	2	13	-88.9	-192	-193.5	-21.69	-19.23	-22.46
B	65	102	45	820	18	12	10.7	-102	-63.3	-25.23	-22.43	-27.03
B	65	102	45	785	17	13	33.4	-86	23.6	-25.86	-22.88	-27.86

Table 3.9. Estimated changes in ^{13}C- and ^{14}C-DIC of riparian groundwater from dissolution of carbonate minerals associated with varying increases in the [calcium + magnesium] 260 cm deep at site C. ^{13}C and ^{14}C of C inputs from carbonate mineral dissolution assumed to be 0‰ and -1000‰, respectively. ^{13}C and ^{14}C of DIC in ambient groundwater were -21.69‰ and -88.9‰ respectively, [DIC] in ambient groundwater = 786 umole L^{-1}, and initial [Ca+Mg] = 101 umole L^{-1}.

[Ca+Mg] increase (%)	^{14}C-DIC final (modeled)	^{13}C-DIC final (modeled)
10	-100	-21.42
20	-112	-21.15
30	-122	-20.89
40	-133	-20.64
50	-143	-20.39
60	-154	-20.15
70	-163	-19.92
80	-173	-19.69
90	-183	-19.46
100	-192	-19.24

Utility of ambient ^{14}C measurements

Measurements of ^{14}C-DIC in ambient groundwater reflect DIC produced at multiple points along a flow path. Because C mineralization rates are much higher in surface soils than in the subsurface, and because all flow paths must begin at the surface, some DIC produced in surface soils will be carried along the flow path. Slower rates of C mineralization in the subsurface will also contribute to the DIC pool as groundwater proceeds along the flow path. It is likely that some of these C pools formed between 1965-1980, and mineralization of this SOM would contribute DIC far more enriched in ^{14}C than present-day atmospheric CO_2. The mix of these pools could easily mask any contribution of ancient organic C to the DIC pool.

Given the multiple DIC sources represented in a sample of ambient groundwater, the clear signature of premodern C at our study sites suggests a somewhat surprising utility for this technique. Although it affords only limited ability to quantify the

contribution of ancient C to DIC fluxes and cannot rule out contributions from an ancient C source, ambient samples with depleted ^{14}C-DIC signatures establish that premodern C contributes to the DIC pool. Because ambient groundwater samples can be collected using far less time and fewer resources than would be required to conduct in-situ incubations, they can be collected in greater numbers, allowing coverage across broad spatial scales. Future studies of riparian groundwater chemistry in landscapes where soil carbonates are rare or absent should consider including ambient ^{14}C sampling to provide initial clues about both spatial variation and seasonal patterns of the contribution of old C to DIC pools.

Spatial patterns of carbon sources and implications for riparian classification

Contrary to expectations, old C appears to play an important role in outwash as well as in alluvial riparian zones, and the spatial distribution of ancient C mineralization did not follow a regular, predictable pattern. Soil profiles at our alluvial sites contain buried soil horizons that are an obvious source of ancient, but labile C (Gurwick et al. chapter 1). We expected that: (1) mineralization of ancient C would be more common at alluvial than at outwash sites, and (2) DIC at alluvial sites would become progressively older with depth, corresponding to the increasing age of buried horizons. Consistent with these expectations, ^{14}C-DIC signatures at site C declined with depth, and ^{14}C-DIC signatures of ambient groundwater collected from alluvial sites in summer 2003 were generally more depleted than values from outwash sites (Figure 3.3b). However, ^{14}C-DIC signatures at site D (alluvial) failed to show a strong decline with depth, as would be expected if microbes were metabolizing SOM associated with buried horizons at this site. In addition, our incubations showed clear evidence of ancient C metabolism at outwash sites, where buried horizons are much less common (Figures 3.7, 3.8, 3.10, & 3.13). Even ambient groundwater from outwash sites

showed clear evidence that microbes were metabolizing SOM formed at least 50 ybp. More striking, our incubations led us to infer mean ages of > 750 ybp for mineralized C at shallow and intermediate depths at site A (outwash) and >590 ybp at a 65 cm well at site B (outwash) (Table 3.7, Figure 3.13). The spatial distribution of microbially-available ancient C in the riparian subsurface thus appears to be somewhat idiosyncratic and dependent upon site characteristics often not captured by our conceptual model of outwash and alluvial landscapes.

Descriptions of the soil profile at site A based on soil pits and augur transects failed to reveal ubiquitous buried soil horizons, but our data leave little doubt that old C is being mineralized in the subsurface. One possible explanation for this discrepancy is simply intra-site heterogeneity. Attempts to obtain soil cores from within the specific area where we located our piezometers failed due to an omnipresent horizon of cobbles approximately 75 cm beneath the surface (Gurwick, unpublished data). Site A may in fact harbor more alluvial characteristics beneath these cobbles. Apparently, even relatively intensive efforts to characterize SOM pools in riparian zones fail to predict effectively the C supply available to microbes. Combined with observations of relatively high denitrification rates at site A (Kellogg et al., 2005), our observations of old C metabolism at this outwash site suggest we should exercise considerable caution before assigning low denitrification potential to riparian zones where soil investigations have yielded minimal evidence of SOM in the subsurface.

Complementing our observation of old C metabolism at shallow and intermediate depths at outwash sites, we found that relatively young C (<50 years) played a significant role where our conceptual model of riparian landscape and C supply would not necessarily have predicted it. Two key inferences in this regard were mean ages of

mineralized C 65 cm beneath the surface at site C (alluvial), and at 300 cm at site A (Table 3.7), both of which reflect a "bomb carbon" signal from ~1962-1985. The bomb C signal 300 cm under the surface at site A is particularly curious because it appears beneath piezometers where we detected metabolism of considerably older C.

A possible source of decades-old C 300 cm deep at site A is DOC or POC moving from upland fields and into the riparian zone along deep flow paths, similar to the mechanism proposed by Fierer et al. (2005) to account for subsurface CO_2 production in a California annual grassland. Concentrations of calcium, potassium, sodium, nitrate, and sulfate increase with depth at site A, likely reflecting the influence of nearby agricultural fields and suggesting that some flow paths bypass the upland edge of the riparian forest, allowing transport to within the vicinity of our sampling wells. It is reasonable to suppose that the same land use practices and hydrolgeologic factors that create these gradients of inorganic compounds also facilitate disturbance and transport of previously-stabilized soil C.

Soil disturbance associated with agriculture has been proposed to explain sources of old C metabolized in large rivers (Howarth et al. 1991, McCallister, 2004). The same mechanism may operate at smaller scales and via the subsurface in riparian zones. If so, then subsurface C supply and associated N cycling in the riparian subsurface needs to be understood with respect to both: (1) highly localized controls (i.e., stream channel dynamics and consequent formation and persistence of buried horizons); and (2) the nature of the surrounding landscape, e.g., soil stability and hydrologic connections.

Our conclusion that C cycling does not appear to vary systematically between riparian zones in alluvial and outwash settings mirror previous findings that denitrification failed to vary systematically between these riparian zone types (Kellogg et al. 2005). In addition, whereas C source varied systematically with depth at site C (Figures 3.3, 3.6), denitrification did not (Kellogg et al. 2005). Similarly, denitrification rates were high, and comparable, in deep minipiezometers at sites A and C (Kellogg et al. 2005), but the age of mineralized C differed by an order of magnitude between these locations. Although our small number of sites limits our ability to generalize to the landscape, these observations raise questions about the utility of distinguishing between alluvial and outwash riparian zones as a basis for predicting subsurface microbial activity and associated N removal. Other hydrogeologic metrics such as proposed by Hill et al. (2004) and Vidon and Hill (2004) may prove more fruitful in an applied context. Despite the large number of studies on riparian zone nitrogen cycling (see Martin et al. 1999), systematic investigations of hydrogeologic setting and riparian subsurface biogeochemistry are rare and the negative results reported here should not be interpreted as reason to curtail efforts in this direction. As a practical matter, variation among riparian zone types is greater when considered over larger spatial scales (e.g., among physiographic provinces) and inter-province comparisons me meet with more rapid success in developing functional typologies of riparian zones.

Seasonal and spatial patterns of C supply and DIC age

Our data are consistent with the proposition that mineralized ancient C is more apparent at locations and times where the supply of young SOM is low. In summer in the northeastern U.S., we expect faster decomposition and relatively high rates of plant-mediated C supply to soils compared to the dormant season. We observed more

^{14}C-depleted ^{14}C-DIC signatures and lower DIC concentrations in the winter 2002 ambient sampling than in the summer 2003 sampling. These results suggest that younger sources of carbon are less plentiful in winter than in summer and that microbes do not fully compensate for variations in supply.

Inter-site differences in C mineralization and the age of mineralized C may also be explained by the proposition that ancient C supports low rates of microbial activity while SOM formed more recently can, when present, support higher rates of microbial activity. Carbon mineralization rates during our in-situ incubations, conducted in winter 2002, were low at sites where we found evidence of ancient C mineralization (Figure 3.12). At sites where mineralized C had a more contemporary source, we were more likely to observe high C mineralization rates.

C mineralization rates

Measured rates of C mineralization during our incubations were as high 0.071 uM CO_2 kg soil^{-1} sec^{-1}, and were generally higher at sites A and B (outwash) than site C (alluvial) (Figure 3.9). This difference may have resulted from the higher concentrations of dissolved oxygen at sites A and B (outwash) than at sites C and D (alluvium) (2-8 vs <2 mg L^{-1}, respectively, Kellogg et al. 2005).

Our C mineralization rates overlap rates measured in laboratory incubations of buried horizons from Rhode Island riparian zones, but with higher maximum rates at outwash sites believed to lack buried horizons (Gurwick et al., chapter 1). Our highest measured rate of C mineralization in-situ nearly equaled rates associated with surface O horizons from these sites in laboratory incubations (Gurwick et al, chapter 1). Our C mineralization estimates also resemble rates from riparian zones in Ontario (Hill and

Cardaci 2004) and are of the same order of magnitude as C mineralization rates associated with peat from 40 cm beneath the surface of sphagnum peatlands in North America (Yavitt et al. 2000).

Our measured in-situ rates are several orders of magnitude greater than those measured using 132-day incubations of large, intact soil cores obtained from 61 cm beneath the soil surface in poorly-drained soils at an outwash riparian zone in Rhode Island (4.6-7.3 * 10^{-5} uM CO_2 kg $soil^{-1}$ sec^{-1}) (Jacinthe et al. 2003). This discrepancy is particularly striking because we measured C mineralization rates of .056 and .021 uM CO_2 kg $soil^{-1}$ sec^{-1} using the two 65 cm deep wells at site B, the origin of aquifer material used in the large core experiment. Although Jacinthe et al. (1998) reported significantly higher C mineralization rates in response to DOC amendments, these increases do not significantly lessen the discrepancy between these two studies. The measurements might be reconciled if the bulk of C mineralization depends on continual inputs of C from surface. Our analysis of radiocarbon signatures of DIC at these wells suggests that at least in one case premodern C contributes significantly to C mineralization, implying that external inputs as a direct source of microbially-available C are unlikely to explain the discrepancy between these two studies. A second possibility is that external inputs of labile C, while a small component of the total C budget, are required to prime the continued mineralization of otherwise-recalcitrant C; our data do not allow us to evaluate that hypothesis.

After accounting for advection and estimating bicarbonate present at the start of the in-situ incubation, we calculate DIC consumption, or removal, at some of our sites (Figure 3.9b), particularly those at site C with relatively high pH and alkalinity (Table 3.3). These losses cannot be explained by realistic errors that might be associated with

measurement of key variables like pH, alkalinity, or total DIC. We must therefore consider possible explanations for DIC loss during the course of the incubation.

Two biotic process that could potentially remove CO_2 from groundwater are methanogenesis and nitrification. While methanogenesis could perhaps account for DIC consumption at site C, where groundwater dissolved oxygen levels are typically < 1 and often < 0.5 mg O_2 L^{-1}, it would require methanogens to compete effectively with denitrifiers where NO_3^- concentrations exceed 20 mg L^{-1}, which is unlikely. Nitrification is also unlikely as ammonium (the energy substrate for nitrifiers) is present at very low levels in the groundwater at our sites.

An alternative DIC removal mechanism is precipitation of carbonate during the course of the incubation. This mechanism may be plausible because removing CO_2 from the groundwater raised the pH. When this now-basic groundwater was reintroduced into the subsurface, it came into contact with a large surface area of sand particles, which facilitates precipitation from a supersaturated solution. Using the concentration of DIC in ambient groundwater and the alkalinity of this groundwater, we estimated the proportion of DIC from each well present as different DIC species. With this information, we then estimated the pH of the groundwater after removing the CO_2 and, finally, combined this with information about ion contents in the groundwater to determine whether this groundwater was likely to have been supersaturated with respect to carbonate. We cannot conclusively demonstrate the occurrence or absence of this process during our experiments, but our analyses to date, using aqueous geochemistry models, do not suggest this is a significant loss pathway.

We gain insight about C mineralization rates by considering rates of denitrification measured in the same mini-piezometers by Kellogg et al. (2005) using enriched ^{15}N, high NO_3^- concentrations, and SF_6, a highly sensitive gas tracer. Mean denitrification rates measured 260 cm deep at site C were 22 and 109 ug N kg^{-1} day^{-1} in Fall and Spring, respectively (Kellogg et al. 2005), which require C mineralization of 2 x 10^{-4} and 4.5 x 10^{-5} umole C kg^{-1} sec^{-1}. We conducted our experiments in late Fall, and the Fall rates reported by Kellogg et al. (2005) would yield DIC increases of 15-30 uM DIC L^{-1} over the course of our incubations. Changes of this magnitude could easily be lost given between-sample variation in DIC concentrations and errors associated with estimating advection. Because sites C and D (alluvial) typically had dissolved oxygen concentrations <2 mg L^{-1} (Kellogg et al., 2005, N. Gurwick unpublished data), chances are good that when NO_3^- is in excess, the bulk of DIC production is associated with denitrification and that DIC production rates during Kellogg et al.'s (2005) study were within an order of magnitude of those estimated based on their denitrification measurements. This analysis therefore supports the view that C mineralization rates at site C below 150 cm were very low (<0.0001 uM kg^{-1} sec^{-1}), within the margin of error around zero.

Ancient C and ecosystem processes

From an ecosystem perspective, our data demonstrate that metabolism of ancient C during winter can constitute a minimum of 31% of total C mineralization that occurs at depth in streamside subsurface soils, and the ancient C contribution to C mineralization in winter 2002 could have been 100% of C mineralized during our in-situ incubations (Figure 3.11). These data contrast the finding that ancient C (up to 8,000 ybp) does not contribute significantly to CO_2 production in 3-meter deep soil profiles in California (Fierer et al. 2005) and the absence of an ancient OM signal in C

fluxes from the Amazon River to the atmosphere (Mayorga et al. 2005). It is possible that ancient C is being metabolized in those systems as well, but that it has not been detected because rates of C mineralization associated with recently-fixed C mask the signal in bulk CO_2 pools measured in those studies. Our data are in accord with evidence that ancient C contributes >25% of microbial C assimilation in the Hudson River (McCallister et al. 2004). The picture of multiple C pools, varying widely in age, that contribute to C mineralization in the riparian subsurface, is also consistent with data showing that age of respired CO_2 in peatlands reflects both younger and older C sources (Chanton et al. 1995; Chasar et al. 2000, Dioumaeva, 2003).

Although rates of C mineralization in the deep subsurface can be very low, the contribution of ancient C in this ecosystem matters because riparian zones are considered to be hot spots of landscape-scale N removal, and microbial activity in these soils has been shown to be C limited (N Gurwick, unpublished data). Further, even very low rates of C mineralization can support denitrification rates that are a large fraction of N fluxes across the terrestrial-aquatic interface. Although total C mineralization is low in subsurface soils compared to surface ecosystems, our findings imply that ancient C plays a major role in the control of landscape-scale N fluxes.

Literature Cited

Addy, K., D. Q. Kellogg, A. J. Gold, P. M. Groffman, G. Ferendo and C. Sawyer (2002). "In Situ Push-Pull Method to Determine Ground Water Denitrification in Riparian Zones." Journal of Environmental Quality 31: 1017-1024.

Bilbrough, C. J. and M. M. Caldwell (1995). "The Effects of Shading and N-Status On Root Proliferation in Nutrient Patches By the Perennial Grass Agropyron-Desertorum in the Field." Oecologia 103(1): 10-16.

Blazejewski, G. A. (2002). Carbon in riparian zone subsoils: Morphology and spatial distribution. Dept of Natural Resources Science. Kingston, RI, University of Rhode Island.

Bradley, P. M., M. Fernandez and F. H. Chapelle (1992). "Carbon Limitation of Denitrification Rates in an Anaerobic Groundwater System." Environmental Science & Technology 26(12): 2377-2381.

Buckau, G., R. Artinger, S. Geyer, M. Wolf, P. Fritz and J. I. Kim (2000). "Groundwater in-situ generation of aquatic humic and fulvic acids and the mineralization of sedimentary organic carbon." Applied Geochemistry 15(6): 819-832.

Chanton, J. P., J. E. Bauer, P. A. Glaser, D. I. Siegel, C. A. Kelley, S. C. Tyler, E. H. Romanowicz and A. Lazrus (1995). "Radiocarbon Evidence For the Substrates Supporting Methane Formation Within Northern Minnesota Peatlands." Geochimica Et Cosmochimica Acta 59(17): 3663-3668.

Chasar, L. S., J. P. Chanton, P. H. Glaser, D. I. Siegel and J. S. Rivers (2000). "Radiocarbon and stable carbon isotopic evidence for transport and transformation of dissolved organic carbon, dissolved inorganic carbon, and CH4 in a northern Minnesota peatland." Global Biogeochemical Cycles 14(4): 1095-1108.

Davidson, E. A. and I. A. Janssens (2006). "Temperature sensitivity of soil carbon decomposition and feedbacks to climate change." Nature 440(7081): 165-173.

Devito, K. J., D. Fitzgerald, A. R. Hill and R. Aravena (2000). "Nitrate dynamics in relation to lithology and hydrologic flow path in a river riparian zone." Journal of Environmental Quality 29(4): 1075-1084.

D'Hondt, S., B. B. Jorgensen, D. J. Miller, A. Batzke, R. Blake, B. A. Cragg, H. Cypionka, G. R. Dickens, T. Ferdelman, K.-U. Hinrichs, N. G. Holm, R. Mitterer, A. Spivack, G. Wang, B. Bekins, B. Engelen, K. Ford, G. Gettemy, S. D. Rutherford, H. Sass, C. G. Skilbeck, I. W. Aiello, G. Guerin, C. H. House, F. Inagaki, P. Meister, T. Naehr, S. Niitsuma, R. J. Parkes, A. Schippers, D. C. Smith, A. Teske, J. Wiegel, C. N. Padilla and J. L. S. Acosta (2004). "Distributions of Microbial Activities in Deep Subseafloor Sediments." Science 306(5705): 2216-2221.

Dioumaeva, I., S. Trumbore, E. A. G. Schuur, M. L. Goulden, M. Litvak and A. I. Hirsch (2003). "Decomposition of peat from upland boreal forest: Temperature dependence and sources of respired carbon." Journal of Geophysical Research-Atmospheres 108(D3).

Dunnivant, F. M., P. M. Jardine, D. L. Taylor and J. F. McCarthy (1992). "Transport of naturally occurring dissolved organic carbon in laboratory columns containing aquifer material." Soil Science Society of America Journal 56: 437-444.

Eissenstat, D. M. and M. M. Caldwell (1988). "Seasonal Timing of Root-Growth in Favorable Microsites." Ecology 69(3): 870-873.

Fierer, N., O. A. Chadwick and S. E. Trumbore (2005). "Production of CO2 in soil profiles of a California annual grassland." Ecosystems 8(4): 412-429.

Fustec, E., E. Chauvet and G. Gas (1989). "Lignin Degradation and Humus Formation in Alluvial Soils and Sediments." Applied and Environmental Microbiology 55(4): 922-926.

Gold, A. J., P. A. Jacinthe, P. M. Groffman, W. R. Wright and R. H. Puffer (1998). "Patchiness in groundwater nitrate removal in a riparian forest." Journal of Environmental Quality 27(1): 146-155.

Groffman, P. M., A. J. Gold and R. C. Simmons (1992). "Nitrate Dynamics in Riparian Forests Microbial Studies." Journal of Environmental Quality 21(4): 666-671.

Gurwick, N. P., P. M. Groffman, A. J. Gold, G. Blazejewski and M. Stolt (in prep). "Microbially-available carbon in buried soils." To be submitted to Biogeochemistry.

Hackley, K. C., C. L. Liu and D. Trainor (1999). "Isotopic identification of the source of methane in subsurface sediments of an area surrounded by waste disposal facilities." Applied Geochemistry 14(1): 119-131.

Haycock, N. E. and G. Pinay (1993). “Groundwater Nitrate Dynamics in Grass and Poplar Vegetated Riparian Buffer Strips During the Winter.” Journal of Environmental Quality 22(2): 273-278.

Hill, A. R. and M. Cardaci (2004). “Denitrification and Organic Carbon Availability in Riparian Wetland Soils and Subsurface Sediments.” Soil Science Society Of America Journal 68(1): 320-325.

Hill, A. R., K. J. Devito, S. Campagnolo and K. Sanmugadas (2000). “Subsurface denitrification in a forest riparian zone: Interactions between hydrology and supplies of nitrate and organic carbon.” Biogeochemistry 51(2): 193-223.

Hill, A. R., P. G. F. Vidon and J. Langat (2004). “Denitrification Potential in Relation to Lithology in Five Headwater Riparian Zones.” Journal Of Environmental Quality 33(3): 911-919.

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(14 June): 789-791.

Howarth, R. W., J. R. Fruci and D. Sherman (1991). “Inputs of Sediment and Carbon to an Estuarine Ecosystem Influence of Land Use.” Ecological Applications 1(1): 27-39.

Istok, J. D., M. D. Humphrey, M. H. Schroth, M. R. Hyman and K. T. Oreilly (1997). “Single-well, ‘‘push-pull’’ test for in situ determination of microbial activities.” Ground Water 35(4): 619-631.

Jacinthe, P. A., P. M. Groffman and A. J. Gold (2003). “Dissolved Organic Carbon Dynamics in a Riparian Aquifer: Effects of Hydrology and Nitrate Enrichment.” Journal Of Environmental Quality 32(4): 1365-1374.

Kellogg, D. Q., A. J. Gold, P. M. Groffman, K. Addy, M. H. Stolt and G. Blazejewski (2005). “In Situ Ground Water Denitrification in Stratified, Permeable Soils Underlying Riparian Wetlands.” Journal of Environmental Quality 34(2): 524-533.

Krumholz, L. R., S. H. Harris and J. M. Suflita (2002). “Anaerobic microbial growth from components of cretaceous shales.” Geomicrobiology Journal 19(6): 593-602.

Levin, I. and B. Kromer (2004). “The Tropospheric 14CO2 level in Mid-Latitudes of the Northern Hemisphere (1959–2003).” Radiocarbon 46(3): 1261-1272.

Martin, T. L., N. K. Kaushik, J. T. Trevors and H. R. Whiteley (1999). "Review: Denitrification in temperate climate riparian zones." Water Air and Soil Pollution 111(1-4): 171-186.

Mayorga, E., A. K. Aufdenkampe, C. A. Masiello, A. V. Krusche, J. I. Hedges, P. D. Quay, J. E. Richey and T. A. Brown (2005). "Young organic matter as a source of carbon dioxide outgassing from Amazonian rivers." Nature 436(7050): 538-541.

McCallister, S. L., J. E. Bauer, J. E. Cherrier and H. W. Ducklow (2004). "Assessing sources and ages of organic matter supporting river and estuarine bacterial production: A multiple-isotope (Delta C-14, delta C-13, and delta N-15) approach." Limnology and Oceanography 49(5): 1687-1702.

McCarty, G. W. and J. M. Bremner (1992). "Availability of Organic-Carbon For Denitrification of Nitrate in Subsoils." Biology and Fertility of Soils 14(3): 219-222.

McMahon, P. B., J. K. Bohlke and B. W. Bruce (1999). "Denitrification in marine shales in northeastern Colorado." Water Resources Research 35(5): 1629-1642.

Minderman, G. (1968). "Addition, decomposition and accumulation of organic matter in forests." Journal of Ecology 56: 355–362.

Moore, T. R., J. A. Trofymow, B. Taylor, C. Prescott, C. Camire, L. Duschene, J. Fyles, L. Kozak, M. Kranabetter, I. Morrison, M. Siltanen, S. Smith, B. Titus, S. Visser, R. Wein and S. Zoltai (1999). "Litter decomposition rates in Canadian forests." Global Change Biology 5(1): 75-82.

Nelson, W. M., A. J. Gold and P. M. Groffman (1995). "Spatial and Temporal Variation in Groundwater Nitrate Removal in a Riparian Forest." Journal of Environmental Quality 24(4): 691-699.

Parkin, T. B. and J. J. Meisinger (1989). "Denitrification Below the Crop Rooting Zone as Influenced by Surface Tillage." Journal of Environmental Quality 18(1): 12-16.

Parkin, T. B. and W. W. Simpkins (1995). "Contemporary Groundwater Methane Production from Pleistocene Carbon." Journal of Environmental Quality 24(2): 367-372.

Parton, W. J., D. S. Schimel, C. C.V. and D. S. Ojima (1987). "Analysis of factors controlling soil organic matter levels in Great Plains grasslands." Soil Science Society Of America Journal 51: 1173-l 179.

Petsch, S. T., K. J. Edwards and T. I. Eglinton (2003). "Abundance, distribution and delta C-13 analysis of microbial phospholipid-derived fatty acids in a black shale weathering profile." Organic Geochemistry 34(6): 731-743.

Petsch, S. T., T. I. Eglinton and K. J. Edwards (2001). "C-14-dead living biomass: Evidence for microbial assimilation of ancient organic carbon during share weathering." Science 292(5519): 1127-1131.

Rethemeyer, J., C. Kramer, G. Gleixner, G. L. B. Wiesenberg, L. Schwark, N. Andersen, M. J. Nadeau and P. M. Grootes (2004). "Complexity of soil organic matter: AMS C-14 analysis of soil lipid fractions and individual compounds." Radiocarbon 46(1): 465-473.

Simmons, R. C., A. J. Gold and P. M. Groffman (1992). "Nitrate Dynamics in Riparian Forests - Groundwater Studies." Journal of Environmental Quality 21(4): 659-665.

SAS (2002-2003). Version 9.1 for Windows. SAS Institute Inc. Cary, NC, USA,

Six, J., R. T. Conant, E. A. Paul and K. Paustian (2002). "Stabilization mechanisms of soil organic matter: Implications for C-saturation of soils." Plant and Soil 241(2): 155-176.

Sollins, P., P. Homann and B. A. Caldwell (1996). "Stabilization and destabilization of soil organic matter: mechanisms and controls." Geoderma 74(1-2): 65-105.

Starr, R. C. and R. W. Gillham (1993). "Denitrification and Organic-Carbon Availability in 2 Aquifers." Ground Water 31(6): 934-947.

Stuiver, M., P. J. Reimer and R. Reimmer (2005). Calib 5.0.1. 2005: http://www.calib.qub.ac.uk/crev50/.

Vidon, P. G. F. and A. R. Hill (2004). "Landscape controls on nitrate removal in stream riparian zones." Water Resources Research 40: W03201.

Wakeham, S. G., A. P. McNichol, J. E. Kostka and T. K. Pease (2006). "Natural-abundance radiocarbon as a tracer of assimilation of petroleum carbon by bacteria in salt marsh sediments." Geochimica et Cosmochimica Acta 70(7): 1761-1771.

Well, R., H. Hoper, O. Mehranfar and K. Meyer (2005). "Denitrification in the saturated zone of hydromorphic soils—laboratory measurement, regulating factors and stochastic modeling." Soil Biology and Biochemistry 37(10): 1822-1836.

Yavitt, J. B., C. J. Williams and R. K. Wieder (2000). "Controls on microbial production of methane and carbon dioxide in three Sphagnum-dominated peatland ecosystems as revealed by a reciprocal field peat transplant experiment." Geomicrobiology Journal 17(1): 61-88.

Yeomans, J. C., J. M. Bremner and G. W. McCarty (1992). "Denitrification Capacity and Denitrification Potential of Subsurface Soils." Communications in Soil Science and Plant Analysis 23(9-10): 919-927.

Zimov, S. A., Y. V. Voropaev, I. P. Semiletov, S. P. Davidov, S. F. Prosiannikov, F. S. Chapin, III, M. C. Chapin, S. Trumbore and S. Tyler (1997). "North Siberian Lakes: A Methane Source Fueled by Pleistocene Carbon." Science 277(5327): 800-802.

CHAPTER FOUR: CARBON SUPPLY TO RIPARIAN SURFACE AND SUBSURFACE SOILS VIA ROOTS

Introduction

Numerous studies have established the importance of roots to C cycling in the upper 20-40 cm of the soil profile, below which root abundance declines dramatically (McClaugherty et al. 1982, Vogt, 1986, Bottner 1988, Bowden, 1993, Pregitzer, 1995, Jackson, 1996). Although they comprise a small proportion of total plant biomass in forests, fine roots grow and decompose rapidly and can account for a large fraction of NPP (Hendrick and Pregitzer 1992; Fahey and Hughes 1994; Eissenstat and Yanai 1997; Ruess et al. 2003). Fine roots mediate C delivery from plants to soil microbes via exudation (Qualls et al. 1991; Grayston et al. 1997), direct transfer to mycorrhizae, and sloughing, as well as through production and microbial decomposition of plant tissue. In all, root-associated carbon, much of which is derived from recent photosynthate, appears to account for the majority (52-80%) of total soil respiration in northern forests (Bowden et al. 1993; Hogberg et al. 2001; Fahey et al. 2005).

In addition to driving the C cycle in surface soils, roots influence many aspects of the soil nitrogen (N) cycle, including denitrification, a process of great interest in riparian ecosystems (Woldendorp 1962; Brar 1971; Bottner et al. 1988; Bottner et al. 1991). The links between roots, the C cycle, and the N cycle exist because soil microbial activity is often C limited and because microbes mediate key transformations in the nitrogen cycle. Because root tissues form patches of high concentration organic matter in the soil matrix, microbial respiration associated with the rhizosphere can outpace oxygen resupply via diffusion, creating anaerobic microsites. By supplying C directly, and creating anaerobic conditions indirectly, roots create conditions

conducive to denitrification, assuming the presence of nitrate and the ability of microbes to compete with plants for nitrate in soil pore water.

Does this well-established model of plant influences on C and N cycling in surface soils, which is primarily derived from study of upland ecosystems, apply to saturated subsurface soils in riparian zones? Both tree and grass roots can influence carbon and nitrogen cycling as deep as 30 meters in seasonally-dry soils (Stone and Kalisz 1991; Fisher et al. 1994; Nepstad et al. 1994), and wetland plants are well-known to have physiological adaptations that allow their roots to survive hypoxic environments (Armstrong 1964). Field studies have established that shallowly-rooted seepwillow roots increase denitrification rates in desert streams because they supply organic matter to microbes in the hyporheic zone (Schade et al. 2001). Finally, mesocosm experiments have established that plants positively influence nitrification and denitrification in wetland soils (Reddy et al. 1989), and that root-derived, microscale patches in the riparian subsurface act as hot spots of denitrification at depth (60 – 100 cm) in the soil profile (Jacinthe et al. 1998). Our knowledge of wetland plant physiology and the available empirical data therefore suggest that roots are key drivers of denitrification in riparian aquifers.

A few studies have directly addressed root biomass, distribution, or dynamics for saturated and C-limited subsoils beneath riparian forests (Hill 1996), and others have investigated similar ecosystems. At a riparian zone of a 3rd-order stream in northern New York, with a mean water table depth of 46-107 cm, Kiley and Schneider (2005) found 200-400 g m^{-3} of roots at 90-120 cm depth. In a study of 14 forested riparian zones in Appalachia with water tables >75 cm below the surface, Wynn et al. (2004) reported small (<1 cm cm^{-3}) but non-zero values for root length density at 90-100 cm

and did not report biomass. Roots growing at least 50 cm below the water table and 1-2 meters below the surface have been found in non-forested peatlands (Saarinen 1996; Moore et al. 2002). Moore et al. (2002) found that biomass of sedge and herb roots was greatest near the water table (~50 cm), rather than at the surface, and extended to 1 meter; shrub roots, while shallower, nevertheless grew beneath the depth of the water table. A number of teams have measured root distributions in forested floodplains in the southeastern U.S., but limited their sampling depth to <50 cm and usually <30 cm (Jones et al. 1996; Clawson et al. 2001; Burke and Chambers 2003; Giese et al. 2003). The tendency to limit sampling depths to shallow soils also characterizes well-known studies of root biology in the Great Dismal Swamp (Powell and Day 1991, Megonigal, 1992, Day, 1993) and others in forested peatlands (Conlin and Lieffers 1993). Collectively, these investigations of root distributions in riparian zones and other wetland ecosystems are consistent with the hypothesis that deep roots occur and function 1 meter below the surface and well below the water table, but most have not directly investigated the locations where most groundwater flows, and where denitrification has been shown to influence landscape-scale N fluxes.

The broad goal of this study was to further understanding of the role plant roots can play in subsurface riparian ecosystems and to ascertain whether it is analogous to their thoroughly-investigated role in surface soils. My specific objectives were to: (1) characterize root distributions in saturated, subsurface riparian soils; (2) quantify turnover times for roots in the subsurface; and (3) compare root biomass and turnover in surface and subsurface riparian soils. The work was associated with a broader study of C and denitrification dynamics in a series of riparian ecosystems in Rhode Island, USA (Blazejewski et al. 2005; Kellogg et al. 2005).

Methods

Site descriptions

I collected soil samples at three riparian zones with shallow (<3%) surface slopes on low (1^{st} - 3^{rd}) order streams in the Pawcatuck River watershed, Rhode Island. All sites had vegetation dominated by approximately 80 year old *Acer rubrum* L with a lower proportion of *Quercus alba* and an abundant shrub layer of *Vaccinium corymbosum* L, and *Clethra alnifolia* L. These sites occurred on two hydrogeologic settings, outwash and alluvial, distinguished primarily by the high frequency of buried soil horizons at alluvial sites (Kellogg et al. 2005). The outwash site (B) had soils classified as inceptisols (Walpole series, a sandy, mixed, mesic Aeric Endoaquept) and groundwater with pH 5.0 – 5.8 and dissolved oxygen ranging from 2 – 6 mg L^{-1}. Cobbles at 65 cm prevented coring at a second outwash site (A). The alluvial sites (C, D) were characterized by entisols, a coarse-loamy, mesic Fluvaquentic Endoaquept and a coarse-loamy, mesic Fluvaquentic Humaquept. Groundwater was less acidic than at site B (pH 5.3 – 6.5) and had dissolved oxygen concentrations <2 and often <1 mg L^{-1}. Site designations here follow nomenclature used by Gurwick et al. (in prep, chapter 3) and (Kellogg et al. 2005). Sites are described in more detail in Kellogg et al. (2005).

Root biomass

Because root biomass tends to be patchily-distributed, and because infrequent high concentrations of organic matter can have large impacts on landscape-scale nutrient fluxes, I chose to sample intensively at one site (B) and to augment this with modest sampling at two additional sites (C, D). In addition, because most groundwater flow occurs in the subsurface and because much less is known about root distributions in the subsurface than in surface soils, I concentrated efforts on measuring root

distributions below 50 cm in most samples. I chose a subset for measuring root biomass in the upper 50 cm in order to characterize the surface-subsurface gradient.

** Root and soil sampling techniques*

I collected most samples using a 120-cm long hardened steel tube with a chemically-inert plastic liner 6 cm i.d. (Giddings Machine Co., Windsor CO). To prevent soil loss from the bottom of the core, I installed a "basket catcher," a series of flexible steel fins mounted on a ring secured near the bottom of the tube just behind the cutting bit. During insertion, the fins pressed against the side of the tube; upon extraction, they collapsed. I pounded the tube into the ground using a 70-pound slide hammer and extracted it with a jack mounted on a 30 cm x 40 cm steel plate. Upon extraction, the liner tubes were capped at both ends and stored at 4 deg C until processed.

At site B, in October 1999 I sampled to 1 meter depth along two 60-meter transects in poorly-drained (PD) soils oriented parallel to the stream and 8 meters apart from each other. Cores were collected at 3-meter intervals along each transect, regardless of distance from trees, hummocks, or hollows. In August 2002, at sites C and D, I collected 8 cores at each site along a single transect located in poorly-drained (PD) soils adjacent to piezometers previously established for a companion study. At the same time, I collected an additional 8 cores from the transects at site B. In 2002, I increased my sampling depth from 100 to 120 cm.

Using soil cores had several key advantages for estimating root biomass in the context of C supply and N fluxes in subsoils near the terrestrial-aquatic boundary. First, it allowed me to sample over a broad spatial area and therefore to estimate root biomass of the site. Second, it minimized the chance that soils from shallower depths would

mix with deeper samples. This can occur when the sides of an auger hole collapse while collecting deep samples and is common when working in saturated, sandy soils. Third, because I generally observed minimal compaction it allowed us to have strong confidence in the depth from which any particular sample came. Fourth, the cores largely preserved soil structure, and this enabled me to section cores and incubate intact soils from different depths. Fifth, the cross-sectional area of the sampled location was well-constrained, yielding sound estimates of root biomass on a per-area basis. Finally, cores could be sectioned lengthwise to reveal horizon breaks and lenses, and samples could then be divided accordingly.

In the laboratory, I measured from the bottom of each core and divided it into five 10-cm long sections, assuming that most compaction occurred in the upper, organic-rich soil layers. For most samples, I separated roots from soil by immersing the sample in water, agitating it, and pouring the water through 3 nested sieves (1 mm, 0.5 mm, 53 um). I repeated this procedure three times per sample and sorted roots caught on the 1 mm sieve into 3 or 4 size classes by diameter (>2 mm, 1-2 mm, and either < 1 mm or 0.5-1 mm and < 0.5 mm). In this paper, I report only total biomass of roots captured on the 1 mm sieve.

To compare root biomass in surface and subsurface horizons, I measured root biomass soil horizons from the top 50 cm of a subset of cores. These sections also included highly organic soils, and in those cases I separated roots by hand, with forceps.

I did not distinguish between live and dead roots because standard criteria used for this purpose could not be applied effectively to the roots growing in the saturated, sandy soils I studied. Roots were generally not white and often lacked the tensile strength

associated with live roots in surface soils of terrestrial ecosystems. I also observed numerous different root morphologies in the subsurface, all of which were distinct from fine roots I observed in surface soils. To develop a live/dead typology in which I had confidence for these roots went beyond the scope of this study.

To complement the coring, I dug 8 soil pits, also at site B, approximately 1 m^2 and ~60-80 cm deep. Pit depth was a function of what I could reasonably accomplish given water tables (usually < 60 cm) and continual recharge. I collected samples from the sides of soil pits by clipping them and storing them in ziplock bags at 4 deg C until processing.

Collecting roots from the soil pits afforded two advantages. First, I was able to observe root systems and their orientation with respect to surrounding soils. Second, based on these observations I was able to sample root systems with specific characteristics, and from particular environments, for radiocarbon analysis.

I used a bucket augur to collect samples from the bottoms of pits and thereby extend the maximum sampling depth. In these ecosystems, sampling depth was often limited by the length of the coring tube and by the tendency of wet, sandy soils to collapse quickly. However, in a few cases I was able to collect samples from as deep as 150 cm.

Root production and turnover

** Terminology*

Because methodological limitations of measurements of root dynamics often preclude measuring precisely the parameter of interest, a brief reminder on terminology is

appropriate. Root production is the mass of roots produced per area or volume in a given time interval. The actual measurement may reflect either gross production or net production. Root turnover is the proportion of a root population that is replaced per year, and root turnover time is the time period over which a root grows, dies, and is replaced in the population.

** Ingrowth cores*

To measure root production, in October 1999 I installed ingrowth cores in holes from which I had extracted soil cores along the transects at site B (Neill 1992, Fahey, 1999). I constructed "plugs" of root-free mortar sand encased in fiberglass window screen (20 cm long, 8 cm diameter). Using a bucket auger, I enlarged holes previously occupied by soil cores, and simultaneously removed sand that had accumulated in them. I immediately pushed an ingrowth plug as deep as possible (63-76 cm below the surface) and then filled surrounding gaps with mortar sand. I measured the distance from the top of the hole to the top of the plug, inserted a second plug on top of the first, and repeated this procedure until the top of the final plug sat within a few cm, or above, the soil surface. Because I was primarily interested in root production at depth, some holes were left without plugs extending all the way to the soil surface. No ingrowth cores were established at other sites, which were added in later years of the study.

I extracted ingrowth cores by first excavating an area approximately 0.5 m diameter around the near-surface plug and using the resulting space to access deeper plugs. I used clippers to sever roots around surface plugs and long knives to cut around deeper plugs, which were always surrounded by saturated sand. Plugs were stored at $4^{o}C$ until processing.

To measure root biomass in the ingrowth cores, I first clipped all roots penetrating the plug at the screen surface and included in my measurements only roots that were clearly within the core. I then immersed the plug in a large beaker of water and slit the core to allow sand to escape. I retrieved roots adhering to the mesh with scissors and forceps and roots associated with the sand matrix by wet-sieving (as above). Roots were oven-dried for 24 hours at 60^{o}C before weighing.

I excavated ingrowth cores in Fall 2000 (8), Spring 2001 (7), Fall 2001 (7), and Spring 2002 (7). Because the installation procedure precluded having ingrowth cores near the surface occupy the soil profile to equal depths, I express accumulated biomass as grams of oven-dry root per m^2 per vertical cm.

^{14}C-AMS dating

Root production can also be inferred from information about the age distribution of a population of roots, and root age can be estimated from the $^{14}C/^{12}C$ ratio of either individual roots or composite samples (Gaudinski et al. 2001, Tierney, 2002). On short time scales (several years to decades), ^{14}C can be used to date organic matter by taking advantage of a spike in the $^{14}C/^{12}C$ ratio of atmospheric CO_2 created by atmospheric testing of nuclear weapons in the mid-20th century. On longer time scales, age is estimated from the decay rate of ^{14}C, calibrated to account for changes in atmospheric $^{14}CO_2$.

The main advantages of the radiocarbon dating approach to age roots are that: (1) it relies on samples that have grown in an undisturbed environment; and (2) it enables a flux-based estimate from a snapshot collected at one point in time. The former is particularly important with regard to root biology because nearly all other methods of

measuring root turnover suffer from potentially strong influences of measurement devices on growth rates (Hendricks et al. 2006). The advantages become more pronounced in deep, saturated soils where more traditional methods are difficult to apply.

A primary limitation associated with estimating root production or turnover by radiocarbon dating is inability to characterize the age distribution of a root population. Although this could be achieved in theory, the per-sample analysis cost is high (hundreds of dollars) and the number of samples required is relatively large (>30 per population). Therefore, I aimed to capture the range of variation in root ages rather than to achieve a precise estimate of root turnover in these subsurface ecosystems.

I chose roots for ^{14}C dating based on site, depth, diameter, morphology and microenvironment (e.g., sand, buried wood, or decomposing roots colonized by more recently-grown roots). To maximize my ability to interpret the ^{14}C data, wherever possible I submitted an individual root rather than a composite sample. When individual roots did not yield sufficient mass for analysis, I combined roots of similar morphology from the same soil sample. Finally, I photographed each sample I submitted to document morphology and appearance (Appendix A). I focused my efforts on small-diameter roots from intermediate and deep samples at site B (Table 4.1). The decision to concentrate sampling effort at site B reflected the presence of ingrowth cores and the extensive root biomass data I collected there. The decision to focus on small-diameter roots rested on preliminary evidence that turnover rates were unusually slow and a consequent interest in estimating maximum likely turnover rates, and although rates of fine root turnover have been subject to debate, root longevity is believed to increase with diameter. At sites C and D, small diameter roots were very

rare in the subsurface, and I therefore analyzed larger roots more representative of the root populations in those soils (Table 4.1).

Table 4.1. Number of root samples analyzed for ^{14}C signatures by AMS by diameter class and site.

Diameter Class	Site B	Site C	Site D
<0.5 mm	9*	1	
.5-1 mm	2**		
1 – 2 mm		2	1
> 2 mm	3		2

* Includes two roots that were colonizing roots > 5 mm diameter, both of which are included in the > 2 mm class in this table.

** includes a sample from an intact root system with trunk ~2 mm diameter. The submitted sample included roots 0.5 – 1 and < 0.5 mm diameter.

For analysis by accelerator mass spectrometry (AMS), I submitted samples to the National Ocean Sciences AMS facility (NOSAMS) in Woods Hole, MA and the W.M. Keck Carbon Cycle AMS laboratory at the University of California, Irvine. Prior to submitting samples, I cleaned each one carefully with water, a scalpel, and a dissecting needle under a dissecting microscope to remove all mineral and organic particles that adhered to the sample. Any root tissue that appeared to have soil organic matter ingrained in its surface was excised. Prior to AMS analysis, samples were subjected to acid and base hydrolysis to remove protein and other organic matter not associated with the roots' structural matrix.

Data analysis

Statistical analyses were conducted using SAS (2002-2003). I used histograms to describe the distribution of roots in the riparian subsurface and ANOVA to test for differences in root biomass among sites and depths.

^{14}C ages were calculated according to methods of Stuiver and Pollach (1977) and calibrated using the IntCal04 data set embedded in Calib Rev 5.0.1 (Stuiver et al. 2005) for premodern ^{14}C values, and using the Northern Hemisphere Zone 1 data set in Cali-Bomb (http://calib.qub.ac.uk/CALIBomb) for samples with modern (post-1950) ^{14}C signatures. I report all ^{14}C ages as years before present (ybp) where present is defined as 2002, the later of the two sampling dates. Where radiocarbon age calibration yielded multiple solutions, I report both, along with the associated probabilities. ^{14}C signatures are reported as both fraction modern and $\Delta^{14}C$ (Stuiver and Polach 1977 and http://www.nosams.whoi.edu/clients/data.html).

I calculated root turnover, the proportion of the population replaced each year, by dividing production by standing biomass. This approach requires the simplifying assumption that the population can be treated as a single pool (Tierney and Fahey 2002). In fact, root populations may have a skewed age distribution with a relatively small number of long-lived roots, making a multiple-pool model more appropriate (Tierney and Fahey 2002, Joslin, in press 2006). Because the single-pool assumption is often violated, the calculated value might be better termed a root turnover index (Joslin et al. in press 2006). I chose to use conventional terminology while recognizing the inherent uncertainties and limitations of the method.

I estimated root turnover for surface and subsurface soils using production data collected with ingrowth cores and radiocarbon analysis respectively. To capture a range for surface soils, I used the 95% confidence interval around the mean production calculated from ingrowth cores and the median and mean values for root biomass. For the subsurface, to estimate realistic boundaries for root turnover given available data, I used the upper and lower quartile values for root biomass between 50-100 cm and the

range of fine root ages indicated by ^{14}C AMS analysis for roots between 40-75 cm depth.

Results

Root biomass

At site B (outwash), root biomass declined with depth from the surface but did not decline systematically below 50 cm (Figure 4.1, ANOVA $p>0.5$, $n=167$). Median root biomass at site B in shallow (<30 cm) soils was 57 g m^{-2} cm^{-1}, or approximately 1,710 g m^{-2},and mean biomass was 168 g m^{-2} cm^{-1}, or 5,040 g m^{-2}. Despite a dramatic decline in mean biomass below 25 cm, the maximum biomass observed in a single core increment in the more intensively-sampled subsurface soils (50-100 cm) was nearly 50% as large as most values in the more sparsely-sampled surface horizons (Figure 4.1).

Across site B, root biomass in the subsurface was unevenly distributed, with a relatively small number of samples yielding high values (Figure 4.2). Mean subsurface root biomass was 192 +/- 81 g m^{-2} (95% CI, $n=35$), with a maximum value of 834 g m^{-2} and an upper quartile of 286 g m^{-2}. Roots frequently occurred 90-100 cm beneath the surface, and the water table at this site rarely drops below 50 cm.

Root biomass profiles at sites C and D (alluvial) showed the majority of roots concentrated in surface horizons, but in contrast to the pattern at site B root biomass tended to increase regularly between 60-100 cm from a minimum between 30-60 cm at these sites (Figure 4.3). Although total root biomass did not differ among the three sites, mean root biomass below 50 cm was higher at sites C and D than at site B (outwash) (ANOVA, $p<0.005$, $n=48$, Figure 4.4).

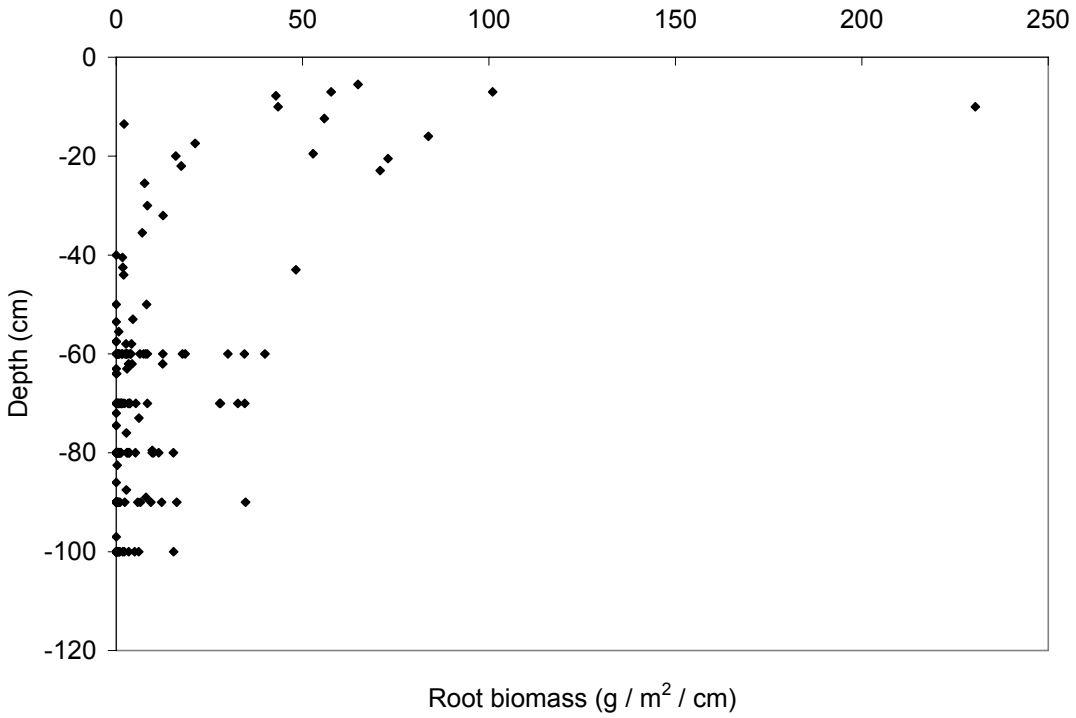


Figure 4.1. Root biomass from soil cores at site B (outwash). Sampling density is higher below 50 cm than in the 0-50 cm interval.

I observed wide variation in root abundance in the subsurface among the eight soil pits at site B. In two pits I observed no subsurface roots. In three pits, roots occurred frequently but these three pits differed markedly in their soil environments. In one pit, I found abundant roots in a layer of buried wood as deep as 80 cm, which I interpret as a tree blown over in a storm and subsequently buried. In a second, with relatively stable, gravelly soils, roots protruded from the pit walls. In a third, with fine sandy soils, roots appeared to grow vertically through the subsoil and were visible as the pit walls collapsed leaving cavities beneath 60 cm. Here I also observed coarse roots (~10 mm diameter) growing into the bottom of the pit, below depths where I could sample.

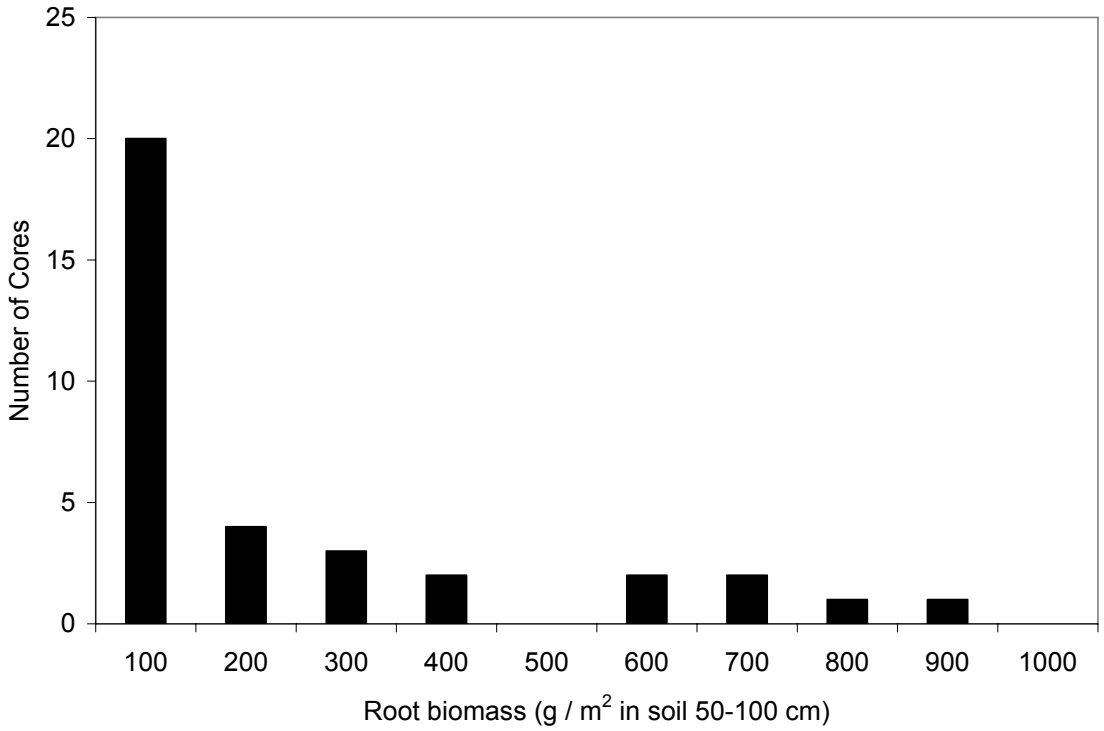


Figure 4.2. Frequency distribution of root biomass 50-100 cm at site B.

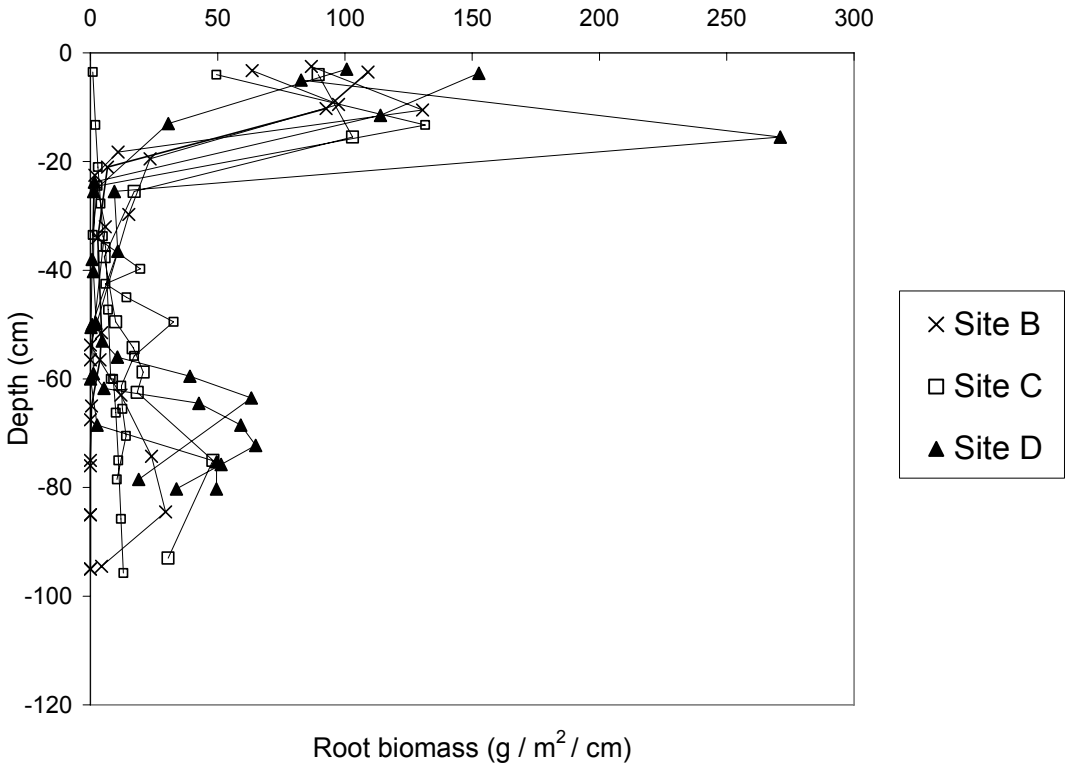


Figure 4.3. Root biomass with depth in three cores at each of three sites. Site B is outwash; Sites C and D are alluvial.

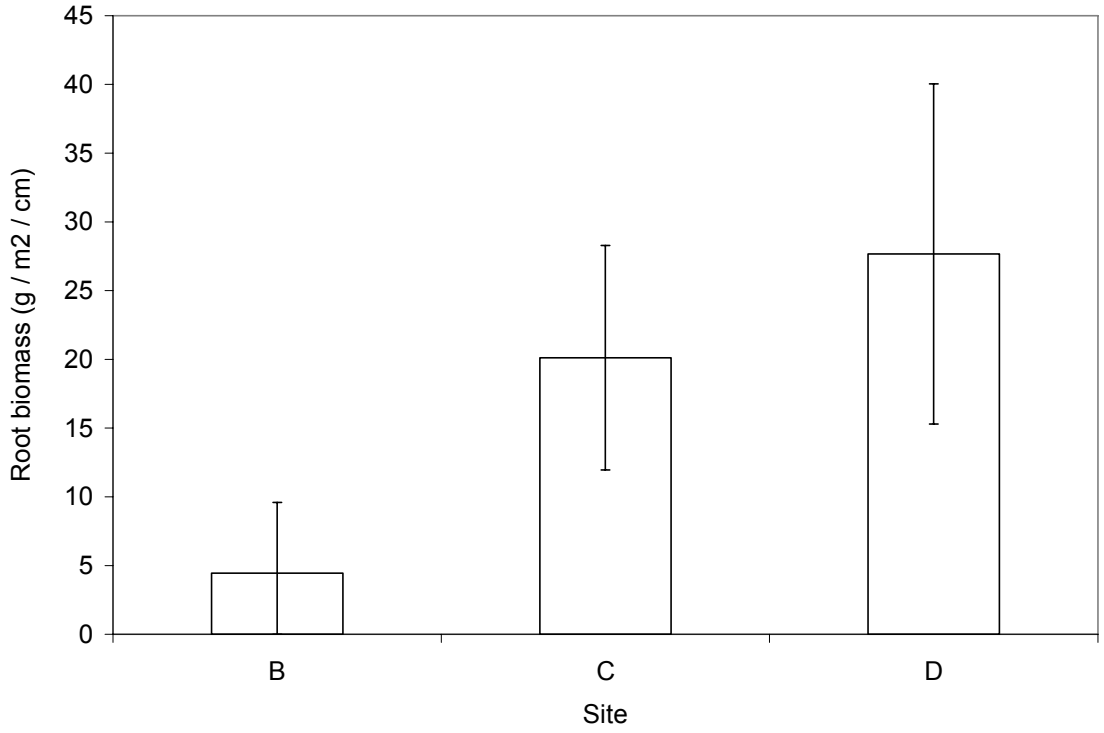


Figure 4.4. Mean root biomass below 50 cm at three sites (3 cores per site). Site B is outwash; Sites C and D are alluvial. Error bars show 95% confidence intervals.

Root colonization and morphology

Dissecting roots in the laboratory frequently revealed unequivocal evidence of roots in the subsurface colonizing other roots (Appendix A, Figures A.8 & A.9). Invasion occurred at a variety of scales, with 0.5 mm diameter roots invading 2 mm diameter roots and 2 mm roots (as well as 0.5 mm roots) invading 5 mm roots. Where invasion occurred, it was common to see extremely long (>5 cm) and narrow (<0.2 mm) lateral roots extending from fine roots that had colonized host roots. Host roots were sometimes obviously in a state of decay and at other times appeared intact and possibly alive based on initial visual inspection, until dissection revealed the extent of colonization.

Root morphology changed dramatically between surface and subsurface horizons. In particular, subsurface roots frequently exhibited a bladder-like morphology with prominent air spaces and short lateral branches that increased in diameter away from the parent root.

Root production from ingrowth cores

Biomass in ingrowth cores varied with depth and collection date (ANOVA, $p < 0.001$, Figure 4.5). After 30 months, very few roots had penetrated ingrowth cores below 40 cm despite substantial standing stocks of roots at those depths. I did observe at least one instance of a fine root growing along the edge of a saturated ingrowth core, with short (< 1 cm) laterals beginning to grow through the mesh. In contrast, all ingrowth cores from the top 20-30 cm of the soil profile contained roots after 12 months, a feature I never observed in the deeper cores.

The ingrowth cores yield annual belowground production estimates of 331-680 g m^{-2} yr^{-1}. Ingrowth within the top 30 cm of the soil profile was substantially greater in cores harvested in Fall 2001 (24 months) and Spring 2002 (31 months) than in cores harvested in Fall 2000 (12 months) and Spring 2001 (20 months) (Figure 4.6). I interpret the greater ingrowth biomass during the second year to reflect time required for roots to reach the core during the first growing season following installation and therefore calculated production as the difference between Autumn 2000 and Autumn 2001.

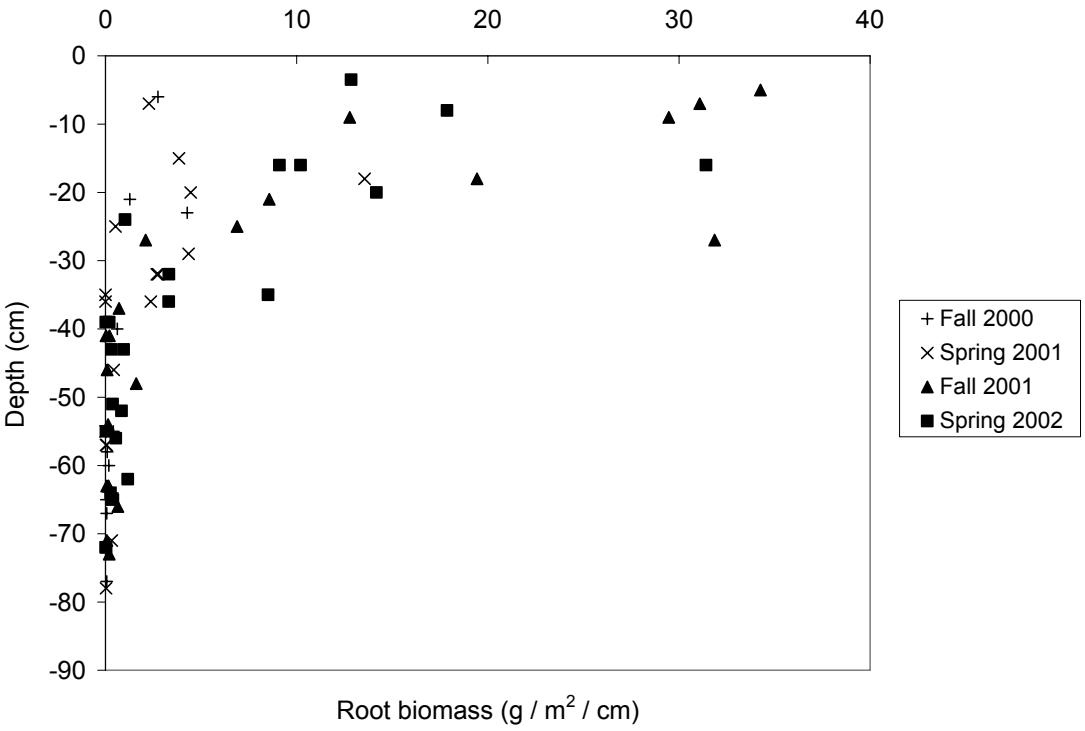


Figure 4.5. Root biomass accumulated in ingrowth cores over 12, 20, 24, and 31 months. Depth refers to the bottom of each ingrowth core. Cores were installed in Fall, 1999; legend shows harvest dates for different cores.

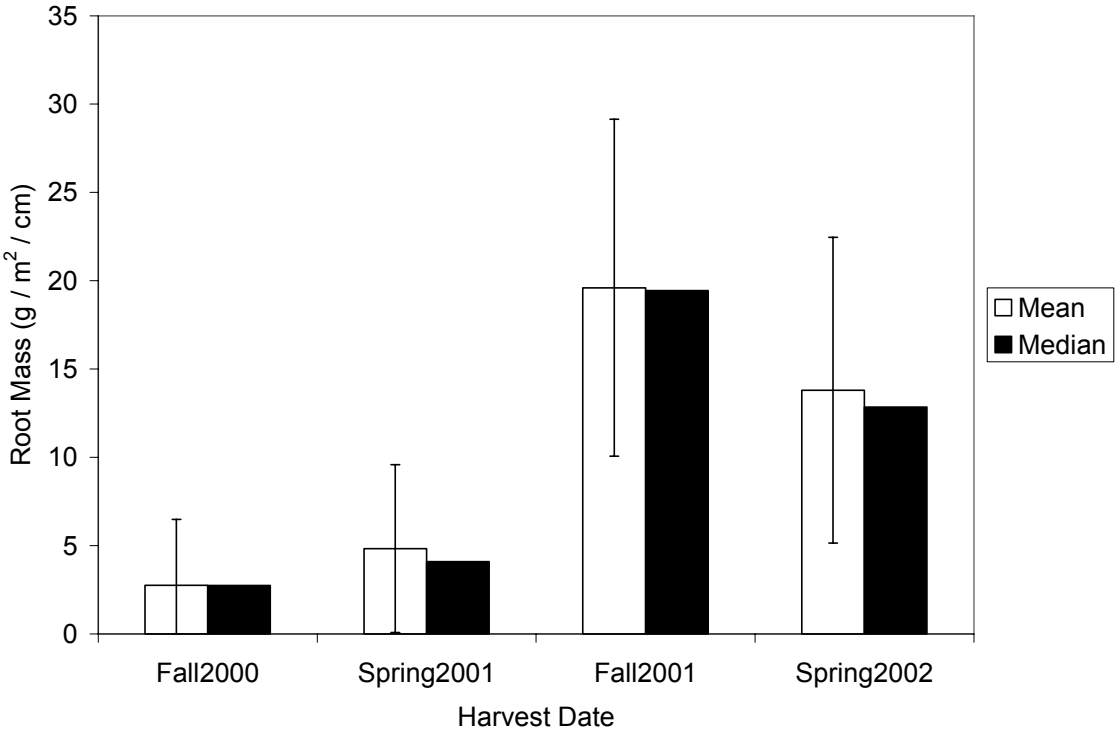


Figure 4.6. Mass in ingrowth cores within the top 30 cm of the soil column. Error bars indicate 95% confidence intervals.

Root ages and production estimated from ^{14}C dating

Radiocarbon analysis of roots showed a striking pattern of ^{14}C depletion with depth (Figure 4.7, Table 4.2). Ages ranged from ~10 ybp to ~8,000 ybp indicating currently-growing vegetation and a wide range of relic roots.

Between 40 and 75 cm, $\Delta^{14}C$ values ranged from -49 to 388‰, and all but two had ^{14}C signatures > 0, evidence of C derived from atmospheric nuclear weapons testing in the 1950s. The two samples with depleted values were coarse roots and had calibrated ^{14}C ages of 200-550 ybp. The fine roots colonizing them varied in diameter (Appendix A, Figures A.8 & A.9), but the ones submitted for analysis were <0.5 mm and had ^{14}C signatures of 388.4 and 230.4‰. The eight remaining samples from this depth interval included (from site B) four <0.5 mm, one 0.5-1 mm, and one 1-1.5 mm as

well as two 2-3 mm roots, one from site C and one from site D. Although the small sample size precludes statistical comparisons, these samples yield no apparent trends in age with respect to site or diameter class. All showed evidence of atmospheric ^{14}C enrichment with estimated ages between 14 and 47 ybp (Table 4.2).

Assuming this ecosystem is at steady state and that root age is a realistic estimate of root longevity, I combined these estimates of fine root age with estimates of subsurface biomass to calculate fine root production in the subsurface. The boundaries on my estimate of root biomass (upper and lower quartile) were 41 and 286 g m^{-2} yr^{-1}, respectively, yielding fine root production estimates of 0.9 – 20 g m^{-2} yr^{-1}.

With the exception of a sample of fine roots from 150 cm that had a ^{14}C signature of 52.3‰, roots collected from below 80 cm had ^{14}C signatures less than 0 ‰, and usually less than -100 ‰, corresponding to ages of 100-8,400 ybp. Although I analyzed only 3 samples from sites C and D, respectively (Figure 4.7, Table 4.2), the 3 oldest roots came from these two sites. The most ^{14}C-depleted roots came from site C and included a < 0.5 mm sample as well as a 2 mm diameter root. Three different diameter classes from a single soil sample 90-100 cm depth at site B had values of -37 (<0.5 mm), -31.7 (0.5-1 mm), and -24‰ (1-2 mm).

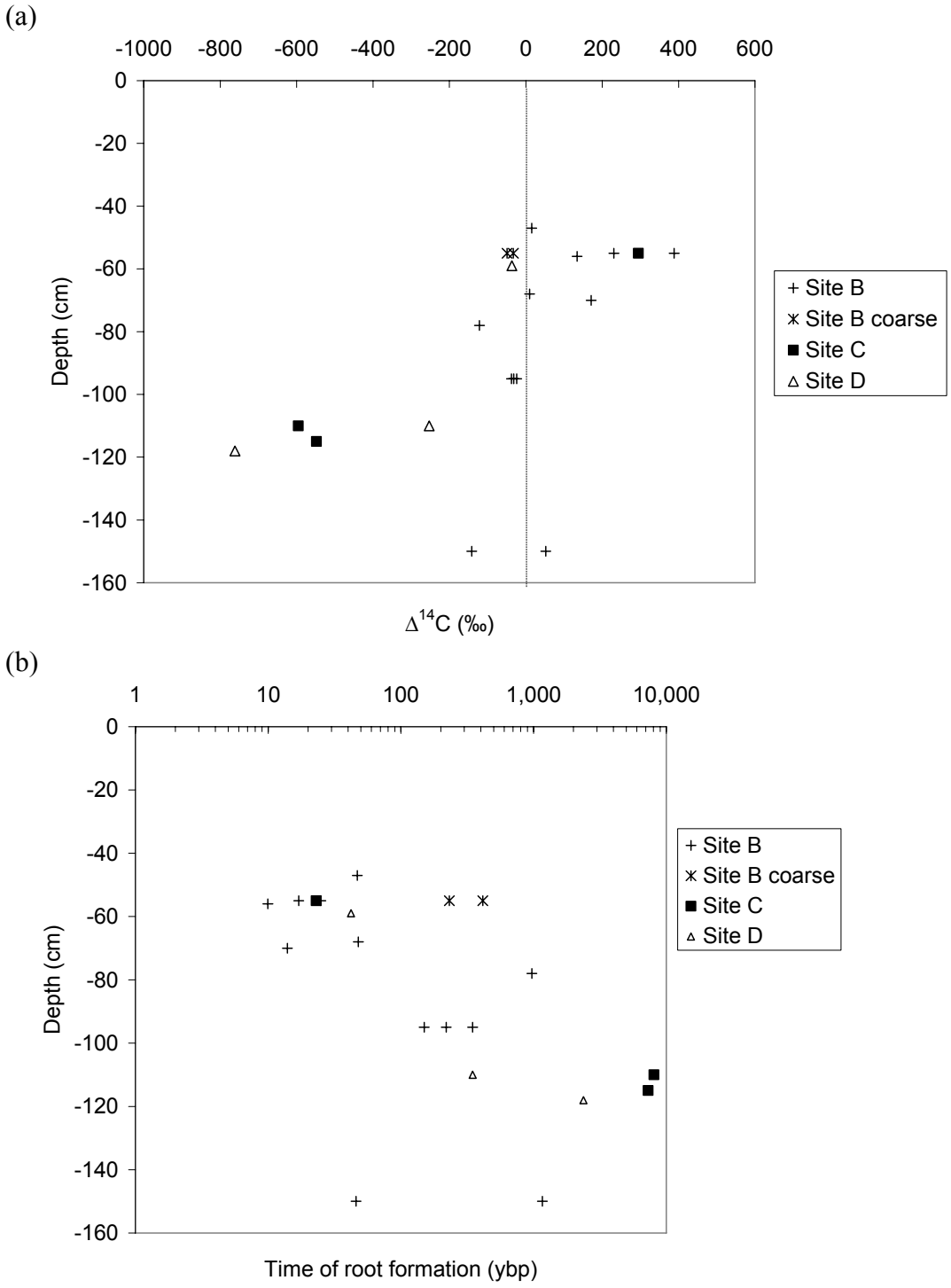


Figure 4.7. Radiocarbon signatures of individual roots (a); and inferred ages (b) with depth. The two coarse roots at site B were 5-10 mm in diameter and were both colonized by fine roots, which were also analyzed for delta ^{14}C. Note log scale in (b).

Table 4.2. Radiocarbon signatures of roots. Where a ^{14}C signature was consistent with multiple ages, only the two most probable ranges are reported here. Age calibration using methods of Stuiver and Pollach (Calib 5.0.1) for ages > 200 ybp and Cali-Bomb for ages < 80 ybp. Ages reported as years before present (ybp) where present is defined as 2002.

Collection year	Site	Depth (cm)	Diameter (mm)	F modern	FM error	$\Delta^{14}C$ (‰)	^{14}C age (ybp)	Age error (yrs)	Calibrated age 1 (ybp)	Calibrated age 1 probability	Calibrated age 2 (ybp)	Calibrated age 2 probability
2002	B	40-55	<0,5	1.0223	0.0017	15.4	> Mod		47	1.00		
2002	B	50-60	<0.5	1.2382	0.0158	230.4	> Mod		17	0.68	30	0.32
2002	B	50-60	1-1.5	1.3972	0.0143	388.4	> Mod		25	0.75	39	0.25
2002	B	50-60	10	0.9734	0.0038	-32.7	215	30	197-267	0.51	319-358	0.35
2002	B	50-60	10	0.9567	0.0035	-49.3	355	30	359-462	0.53	473-548	0.47
1999	B	50-62	<0.5	1.1421	0.0017	134.4	> Mod		10	0.98	44	0.02
2002	B	60-75	<0.5	1.0167	0.0017	9.8	> Mod		48	1.00		
2002	B	65-75	<1	1.1790	0.0018	171.0	> Mod		14	0.99	44	0.003
2002	B	75-80	<0.5	0.8848	0.0016	-121.1	985	15	956-986	0.80	881-910	0.17
1999	B	90-100	0.5-1	0.9749	0.0017	-31.7	205	15	201-238	0.54	322-349	0.30
1999	B	90-100	<0.5	0.9693	0.0014	-37.2	250	15	337-359	0.91	209-217	0.09
1999	B	90-100	1-2	0.9826	0.0019	-24.0	140	20	224-283	0.28	116-170	0.25
2002	B	150	<0.5	0.8643	0.0019	-141.5	1,170	20	1,105-1,224	0.92	1060-1080	0.08
2002	B	150	<0.5	1.059	0.0148	52.3	> Mod		46	0.98	6	0.02
2002	C	100-120	<0.5	0.4072	0.0053	-595.5	7,220	110	7,889-8,276	0.95	8284-8366	0.05
2002	C	110-120	2	0.455	0.0028	-547.9	6,330	50	7,217-7,385	0.90	7435-7468	0.06
2002	C	50-60	2-3	1.3038	0.0029	295.0	> Mod		23	0.73	40	0.27
2000	D	106-114	1-2	0.9700	0.0019	-36.6	245	20	334-361	0.76	205-220	0.23
2002	D	115-120	2-3	0.7518	0.003	-252.9	2,290	30	2355-2405	0.69	2232-2295	0.30
1999	D	57-62	2-3	1.2174	0.0061	209.2	> Mod		42	0.55	17	0.45

Root turnover

Applying the low and high-end estimates for both root biomass (median value 1,700-5,000 m^{-2}) and production (331-680 g m^{-2} yr^{-1}) in the surface soil, and assuming this system is at steady state, I calculated root turnover in the surface as 6 - 40% per year, corresponding to a residence time of 2.5-15 years. In the subsurface, I estimated the boundaries on root production based on root ages of 14-47 ybp, which correspond to turnover rates of 2% and 7% respectively.

Discussion

Root biomass, production, and turnover in near-surface soils

The decline in root biomass with depth in the upper 50 cm (Figures 4.1, 4.3) resembles the pattern found in many studies of root distributions (Jackson et al. 1996). It contrasts data of Moore et al. (2002) who found peak biomass near the water table in a peatland; possibly reflecting differences in vegetation (forest vs herbaceous) or a relatively stable water table in the peatland compared to the flashy hydrologic regime at my study sites. The sharp decline in biomass observed at my study sites is also consistent with the mechanistic understanding that fine roots function largely to acquire nutrients and hence are most abundant in surface soils where organic matter and nutrient concentrations are highest. It also supports the view that flooded soils diminish root biomass and production (e.g., Burke and Chambers 2003). Despite the paucity of root growth into deeper parts of the soil profile relative to the surface, this growth may be a critical component of C supply to microbial populations and associated nutrient transformations (e.g., denitrification) in these C-poor environments.

Changes in root biomass in ingrowth cores over time (Figure 4.6) strongly reflected seasonal patterns expected to occur in northeastern deciduous forests. Root biomass accumulated in ingrowth cores over the growing season (spring 2001– fall 2001) but not over either period that fell mostly outside of the growing season (Figure 4.6). The biomass increase from fall 2000 to Fall 2001 exceeded the increase between installation in fall 1999 (zero) and fall 2000; this difference may have resulted from time required for roots to move laterally through the sand between the surrounding soil and the ingrowth core, or to move towards the center of the ingrowth core during the first year. The apparent trend towards lower biomass in spring 2002 compared to fall 2001, while not statistically significant, occurred in the direction expected from decomposition over the dormant season, particularly during late fall and early spring.

My ingrowth core measurements (Figure 4.5) suggested annual belowground production 0-30 cm of 331-680 g m^{-2} yr^{-1}, consistent with other estimates of fine root production in hydric soils using ingrowth cores. At a 3rd-order stream in northern New York, Kiley and Schneider (2005) found a similar rate of mean net root production; they measured accumulation of ~375 g m^{-2} in the top 30 cm of the soil profile between June and November, 2000, with higher net production over shorter time periods. Belowground production in wetland forest ecosystems of the Great Dismal Swamp was estimated at 59-366 g m^{-2} yr^{-1} (Day and Megonigal 1993). Day et al. (1993) also found less production, and higher decomposition, in continuously-flooded compared to periodically-flooded mesocosms. Forested riparian zones in the northeastern U.S., including my study sites, have seasonally flashy water tables, suggesting that the high end of Day et al.'s (1993) estimates, which overlap my measured values, can reasonably be compared to mine. (Hendricks et al. 2006) reported root production of 70-107 g m^{-2} yr^{-1} for a longleaf pine-wiregrass forest ecosystem in Georgia, somewhat

lower than my estimates. However, Hendricks et al. (2006) defined fine roots as <= 0.5 mm and excluded necromass from their fine root biomass estimates, whereas a substantial fraction of roots in my ingrowth cores had diameters 0.5 – 2 mm, and I did not distinguish between live and dead roots. Thus, it seems likely that the rates estimated by these studies are actually very similar.

Measurements of root production using ingrowth cores can reflect a number of potential artifacts (Fahey et al. 1999, Lauenroth, 2000) and have been found to underestimate fine root production in forest ecosystems by 50% or more (Steele et al. 1997; Hendricks et al. 2006). One confounding factor associated with ingrowth cores is that belowground competition is greatly reduced in root-free soil and this may lead to artificially high rates of root growth. A second is that differences in nutrient concentrations may differ between the ingrowth core and the surrounding soil. In my experiment, I was mainly concerned with production in the subsurface and chose mortar sand as a medium that closely resembled the subsurface soils at my site. As a result, the ingrowth cores in the organic-rich surface horizons were nutrient-poor by comparison with the surrounding soils in the upper part of the soil profile, and roots proliferate less in nutrient-poor soil compared to nutrient-rich patches (Hodge 2004). I expect these sand-filled ingrowth cores retarded root production more than those filled with sieved soil or peat. Together, data from method comparison studies and the details of my implementation strongly suggests that this study yielded a conservative estimate of root production and underestimated turnover; actual root production in surface soils of these *Acer rubrum*-dominated riparian forests could easily be >1,000 g m^{-2} yr^{-1}.

I estimated root turnover to be 6-40% yr^{-1}, and the lower boundary in particular is low by comparison with other studies that have used similar methods. For example, using data from (Hendricks et al. 2006), I calculated a turnover rate of 98-110% yr^{-1} for a longleaf pine-wiregrass forest on hydric soils in Georgia. Data from a swamp tupelo wetland forest in South Carolina (Burke and Chambers 2003) yielded a rate of 21-42% yr^{-1}, comparable to my findings on the high end but exceeding my findings on the low end by a wide margin. Finally, Moore et al. (2002) calculated fine root turnover for a northern peatland as 20%-100% yr^{-1}, again suggesting a rapid replacement of the root system compared with my estimates.

There are several reasons to believe that turnover is actually faster than my initial estimates suggest. First, if I have underestimated production, as seems likely, then I have also underestimated turnover. Second, my estimate of root biomass includes both live and dead roots; hence I have almost certainly overestimated live root biomass, and decreasing that estimate would increase turnover. Third, I have used a single-pool model to calculate turnover. This assumes a population structure that is normally-distributed around the mean — an assumption that has been violated in thoroughly-characterized populations (Tierney and Fahey 2002). Assuming an age structure with a long tail (i.e., median age<mean age) would also lead to more rapid turnover.

Subsurface biomass distribution and production

The mean root biomass between 50-100 cm (192 +/- 81 g m^{-2}) greatly exceeds the value of 19.5 g m^{-2} arrived at by sampling from pit faces from a different area at site B (Rotkin-Ellman et al. 2004), but is in remarkably close agreement with the 200-400 g m^{-3} found by Kiley and Schneider (2005) for a forested riparian zone on a 3rd-order

stream in northern New York using soil cores. This similarity is particularly striking because the Adirondack site appeared to have less consistently saturated soils and lower minimum water tables than my study sites. At site B, water tables were usually within 10 cm of the surface during the dormant season; they dropped during the growing season but still usually remained within 50 cm of the surface. At sites C and D, water tables were generally higher than that. Studies comparing root distributions among wetland soils differing in water table depth have repeatedly shown decreased rooting depth with increasing extent of saturation (Lieffers and Rothwell 1987, Megonigal, 1992, Day, 1993, Clawson, 2001, Baker III, 2001), but see Rodgers et al. (2003) for an exception). Sampling method and intensity may be as important as inter-site differences in explaining discrepancies between studies.

Data from ingrowth cores and radiocarbon analyses suggest that fine root turnover occurs on decadal time scales at depths from ~45-75 cm, much more slowly than turnover in surface soils of upland forests (Hendrick and Pregitzer 1992; Pregitzer et al. 1995; Tierney et al. 2001; Ruess et al. 2003; Tierney et al. 2003; Hendricks et al. 2006). Previous research using stable and radioisotope signatures of C to estimate root turnover in surface soils (Gaudinski et al. 2001, Matamala, 2003) typically have reported slower turnover (longer residence times) than those determined by sequential coring or minirhizotrons. However, the explanation for these discrepancies appears to reside in measuring mean values without regard to the age structure of a root population, particularly the relatively small number of long-lived fine roots (Tierney and Fahey 2002). In this study, I frequently avoided composite samples and, notably, found no signatures corresponding to the mean northern hemisphere ^{14}C signature. Methodological differences alone are therefore unlikely to account for the slow turnover rates that I measured.

Radiocarbon ages of roots deeper in the soil profile (>80 cm) suggest that these are relic plant tissues. The associated ages (hundreds to thousands of years) preclude the possibility that these roots are simply long-lived and suggest more than one process is responsible for their presence. The 8,000-year-old roots at site C likely grew when the horizons in which they now sit were near to the surface. However, the samples with ages hundreds of years old raise the possibility that episodic events such as extreme droughts lead to periods of deeper root growth and subsequent death. This scenario suggests that, during droughts, the pool of subsurface labile carbon increases and is then available to fuel anaerobic processes under subsequent hypoxic conditions.

A possible objection to this scenario is that many roots yielding ^{14}C-ages hundreds or thousands of years old appeared remarkably intact, with very fine lateral branches (Appendix A, e.g., Figures A.7, A.13, A.17), and that, given their appearance, an alternative explanation for the ^{14}C signatures must be sought. However, given the care with which I cleaned roots prior to AMS analysis and removed possible contaminants (apparent from the condition of the roots as shown in Appendix A), the only alternative explanation is that roots in the subsurface took up old C from their environment and incorporated it into structural tissues. At site C, roots had ^{14}C-calibrated ages thousands of years old; had these roots actually grown in recent decades, nearly all the recently-derived C in these tissues would have had to have been replaced with old C. Where researchers have harvested roots growing through screens and measured the ^{14}C activity, they have found good correspondence between known root age and the current ^{14}C signature of atmospheric CO_2 (Gaudinski et al. 2001). However, recent data suggests that over longer time scales roots can incorporate C from surrounding organic matter into their tissues (E. Hobbie, personal communication). Although I cannot entirely rule out the contribution of old C to plant

tissue in some cases, it seems unlikely that this process could contribute enough C to root tissue to yield the ^{14}C signatures reported here, and I conclude that the measured ^{14}C ages accurately report the time at which the roots formed.

Controls on fine root turnover

A number of studies have attempted to explain variation in fine root production and turnover (Tierney et al. 2003). Likely candidates for explanatory variables have included root diameter and branching order, soil temperature, soil moisture, and nutrient availability. The absence of roots with ^{14}C ages <5 ybp suggests that fine roots live for many years in riparian subsurface soils, where nutrient availability is extremely low. It is possible that all roots in this study were dead, but I have no evidence that root production in the subsurface ceased 5 ybp, and I observed a large fraction of sampled roots that formed 5-40 ybp. This observation is consistent with theory about resource allocation and root longevity and with observations that roots live longer in nutrient-poor environments (Eissenstat and Yanai 1997; Eissenstat et al. 2000). My observations of varying root ages and root morphologies with depth in riparian forest soils raise the possibility that soil depth or factors that covary with depth, such as degree of saturation, control fine root turnover at a much wider temporal scale (years to decades) than is typically observed in surface soils (months to years).

The often-used character of root diameter appears to be of marginal utility for understanding root longevity and turnover in the riparian subsurface. In most cases, I observed no clear relationship between diameter and ^{14}C signature. In the case where I analyzed roots of three separate diameter classes (<0.5 mm, 0.5-1 mm, 1-2 mm) from the same soil sample (site B, 90-100 cm), the smallest diameter root yielded the most

depleted, and therefore oldest, ^{14}C signature, and the widest-diameter root yielded the least depleted signature.

As a practical matter, root morphology in the riparian subsurface 40-75 cm deep precludes the possibility of using diameter as a diagnostic tool. Whereas diameter is often related to root branching in surface soils, with low-order roots having narrow diameters, lateral roots in the saturated riparian subsurface frequently increase in diameter after branching from a parent root. In addition, a single root often increases and decreases along its length, so assigning roots to diameter classes is somewhat arbitrary. The frequent colonization of roots by other roots, and the extent to which roots of varying size and colonization are tangled together further complicates the task of assigning fine roots to specific classes based on size or morphology. What appears to be a 2 mm root may, upon dissection, turn out to be the shell of a 2 mm root that has since been colonized my tens of 0.2-0.5 mm roots.

Surface-subsurface connections

The powerful control that roots exert over biogeochemical cycles in surface soils follows from the rapid, direct conduit formed between C-fixation aboveground and microbial processes in the soil, and it appeared likely that roots played a similar connective role in the riparian subsurface. This expectation followed from several lines of evidence including understanding of wetland root physiology (Armstrong 1964, 1979; Colmer 2003) and data showing significant root biomass at depths of 1 meter in riparian zones (Wynn et al. 2004; Kiley and Schneider 2005) and other wetlands (Saarinen 1996; Moore et al. 2002). The finding that root-derived microsites have high concentrations of C relative to the soil matrix and that they supported denitrification 60-100 cm beneath the soil surface in riparian zones (Gold et al. 1998;

Jacinthe et al. 1998) bolstered the case that plant roots drive C supply and denitrification at the terrestrial-aquatic interface.

Data on root production and age strongly suggests that coupling between the surface and subsurface in riparian zones is strong in surface soils, moderately weak at depths of 40-75 cm and very weak below 80 cm depth. Fine roots from 40-75 cm depth ranged in age from 14-47 ybp (Figure 4.7, Table 4.2). Because roots that could easily have been identified as live or recently-live by visual inspection in fact were hundreds of years old (Appendix A), there is no way to be sure whether these roots were alive at the time of collection. Nevertheless, it is clear that roots have grown into this depth range within the past decade at multiple sites, that none of the roots sampled from this part of the subsurface were relics, and that current surface vegetation was delivering C to these soil horizons, though at a relatively slow rate.

This view of a moderately weak surface-subsurface connection at 40-75 cm is consistent with data from my ingrowth core measurements, which showed little ingrowth below 30 cm, but rarely revealed a fine root growing along the side of a core between 30-75 cm depth. Applying my biomass estimates from 50-100 cm and turnover times of 10 and 40 years, one would expect on average 0.1 – 0.8 grams of fine root to have accumulated in an ingrowth core plug at 40-75 cm during that time. However, because root biomass in the subsurface is patchily distributed (Figure 4.2), actual accumulation of roots in deep ingrowth plugs would occur infrequently. Therefore it would not be surprising to miss these hot spots of subsurface root production given my ingrowth core sampling strategy, which was sparse by comparison with root biomass sampling.

In contrast to the 40-75 cm section of the soil profile, the soil horizons below 80 cm appear largely disconnected from surface biotic influence. Only one root from deeper than 80 cm had a ^{14}C signature (52.3‰) indicating formation after 1950 (Figure 4.7, Table 4.2); all other roots from >80 cm depth formed at least 100 ybp. Given the age of the forest at these sites, none of these older roots could have been alive, nor could they recently have been contributing C to the subsurface via exudation or direct transport to mycorrhizae.

In soil pits, I observed coarse roots descending through the sediments in the pit bottom and speculated these might give rise to fine roots at greater depths. Radiocarbon dating revealed these coarse roots to be hundreds of years old, refuting that hypothesis, although fine roots colonizing these coarse roots were considerably younger (Figure 4.7, Table 4.2, Appendix A). It is conceivable that this process of root colonization occurs more deeply than I was able to sample in this study and constitutes an infrequent but concrete surface-subsurface carbon conduit.

Given the observation of ecosystem-relevant rates of denitrification below 1 meter in riparian zones (including sites B, C, and D in this study) (Kellogg et al. 2005), some C source besides contemporary root production must be supporting microbial activity in the subsurface. Available evidence points to mineralization of old C in buried horizons (Gurwick, chapters 1, 2). Buried soil horizons with C contents much higher than the surrounding soil matrix fuel microbial activity in the laboratory, including denitrification (Gurwick and Hayn, unpublished data), and ^{14}C signatures of dissolved inorganic carbon at ambient conditions and during in-situ groundwater incubations demonstrate that old C from many riparian zones in Rhode Island, U.S.A. is

potentially mineralizeable, and that it is mineralized in-situ at my intensively studied sites.

Consequences of potential changes in riparian landscapes for rooting depth

Given the rapid pace of landscape change, it is likely that the conditions currently governing rooting depth and production in riparian zones will shift. For example, climate change and species invasions can lead to vegetation change. Vegetation at my study sites was dominated by *Acer rubrum* L with a lower proportion of *Quercus alba* and an abundant shrub layer of *Vaccinium corymbosum* L, and *Clethra alnifolia* L. *Acer rubrum* is classified as facultative, meaning that it is equally likely to occur in wetlands or uplands, though it is known to have wet and dry ecotypes. It is not unreasonable to suppose that replacement by facultative-wet or obligate wetland plants like *Salix* (http://plants.usda.gov/wetland.html) would result in a deeper rooting zone in saturated soils and sediments.

Alternatively, a decrease in water table could result from changes in either precipitation patterns or incoming solar radiation, both of which are components of climate change (IPCC 2001), or from incision of the nearby stream. Stream incision, a characteristic of urban stream syndrome, leads to a depression of water tables near the stream and a diminished connection between the stream and the riparian zone (Groffman et al. 2002). A decrease in water table level from any of these causes would likely lead to increased rooting depth, but with little benefit for landscape-scale N removal.

A related but distinct shift in hydrology is the periodicity of water table fluctuations at many time scales from seasons to centuries. It appears likely that the root-derived

patches identified as hot spots of denitrification at 60-100 cm depth at site B (Gold et al. 1998; Jacinthe et al. 1998) form from roots that descend into the subsurface and then die. If mean water table depth remains the same but the duration of periodic droughts — and hence the range of water table depth — increases, it is conceivable that such microsites could form over a greater depth range than they have so far been observed.

Literature Cited

Armstrong, W. (1964). "Oxygen Diffusion from the Roots of some British Bog Plants." Nature **204**: 801-802.

Armstrong, W. (1979). "Aeration in Higher Plants." Advances in Botanical Research **7**: 226-332.

Blazejewski, G. A., M. H. Stolt, A. J. Gold and P. M. Groffman (2005). "Macro- and Micromorphology of Subsurface Carbon in Riparian Zone Soils." Soil Science Society Of America Journal **69**(July-August): 1320-1329.

Bottner, P., J. Cortez and Z. Sallih (1991). Effect of living roots on carbon and nitrogen of the soil microbial biomass. Plant Root Growth: An Ecological Perspective (special publication #10 of the British Ecological Society). D. Atkinson. London, Blackwell Scientific**:** 201-210.

Bottner, P., Z. Sallih and B. G. (1988). "Root activity and carbon metabolism in soils." Biology and Fertility of Soils **7**(71): 71-78.

Bowden, R. D., K. J. Nadelhoffer, R. D. Boone, J. M. Melillo and J. B. Garrison (1993). "Contributions of Aboveground Litter, Belowground Litter, and Root Respiration to Total Soil Respiration in a Temperature Mixed Hardwood Forest." Canadian Journal of Forest Research **23**(7): 1402-1407.

Brar, S. S. (1971). "Influence of roots on denitrification." Plant and Soil **36**(1-3): 713-715.

Burke, M. K. and J. Chambers (2003). "Root dynamics in bottomland hardwood forests of the Southeastern United States Coastal Plain." Plant and Soil **250**(1): 141-153.

Clawson, R. G., B. G. Lockaby and B. Rummer (2001). "Changes in Production and Nutrient Cycling across a Wetness Gradient within a Floodplain Forest." Ecosystems **4**: 126-138.

Colmer, T. D. (2003). "Long-distance transport of gases in plants: a perspective on internal aeration and radial oxygen loss from roots." Plant Cell and Environment **26**(1): 17-36.

Conlin, T. S. S. and V. J. Lieffers (1993). "Seasonal Growth of Black Spruce and Tamarack Roots in an Alberta Peatland." Canadian Journal of Botany **71**(2): 359-360.

Day, F. P. and J. P. Megonigal (1993). "The Relationship Between Variable Hydroperiod, Production Allocation, and Belowground Organic Turnover in Forested Wetlands." Wetlands **13**(2): 115-121.

Eissenstat, D. M., C. E. Wells, R. D. Yanai and J. L. Whitbeck (2000). "Research View: Building Roots in a Changing Environment: Implications for Root Longevity." New Phytologist **147**(1).

Eissenstat, D. M. and R. D. Yanai (1997). The ecology of root lifespan. Advances in Ecological Research, Vol 27. **27:** 1-60.

Fahey, T. J., C. S. Bledsoe, F. P. Day, R. Ruess and A. J. M. Smucker (1999). Fine root production and demography. Standard soil methods for long term ecological research. G. P. Robertson, D. C. Coleman, C. S. Bledsoe and P. Sollis. New York, Oxford University Press**:** 437-455.

Fahey, T. J. and J. W. Hughes (1994). "Fine-Root Dynamics in a Northern Hardwood Forest Ecosystem, Hubbard Brook Experimental Forest, NH." Journal of Ecology **82**(3): 533-548.

Fahey, T. J., G. L. Tierney, R. D. Fitzhugh, G. F. Wilson and T. G. Siccama (2005). "Soil respiration and soil carbon balance in a northern hardwood forest ecosystem." Canadian Journal of Forest Research **35**(2): 244-253.

Fisher, M. J., I. M. Rao, M. A. Ayarza, C. E. Lescano, J. I. Sanz, R. J. Rhomas and R. R. Vera (1994). "Carbon Storage by Introduced Deep-Rooted Grasses in the South American Savannas." Nature **371**: 236-237.

Gaudinski, J. B., S. E. Trumbore, E. A. Davidson, A. C. Cook, D. Markewitz and D. D. Richtter (2001). "The age of fine-root carbon in three forests of the eastern United States measured by radiocarbon." Oecologia **129**(3): 420-429.

Giese, L. A. B., W. M. Aust, R. K. Kolka and C. C. Trettin (2003). "Biomass and carbon pools of disturbed riparian forests." Forest Ecology and Management **493–508**: 493–508.

Gold, A. J., P. A. Jacinthe, P. M. Groffman, W. R. Wright and R. H. Puffer (1998). "Patchiness in groundwater nitrate removal in a riparian forest." Journal of Environmental Quality **27**(1): 146-155.

Grayston, S. J., D. Vaughan and D. Jones (1997). "Rhizosphere carbon flow in trees, in comparison with annual plants: The importance of root exudation and its impact on microbial activity and nutrient availability." Applied Soil Ecology **5**(1): 29-56.

Groffman, P. M., N. J. Boulware, W. C. Zipperer, R. V. Pouyat, L. E. Band and M. F. Colosimo (2002). "Soil Nitrogen Cycle Processes in Urban Riparian Zones." Environmental Science & Technology **36**(21): 4547-4552.

Hendrick, R. L. and K. S. Pregitzer (1992). "The Demography of Fine Roots in a Northern Hardwood Forest." Ecology **73**(3): 1094-1104.

Hendricks, J. J., R. L. Hendrick, C. A. Wilson, R. J. Mitchell, S. D. Pecot and D. L. Guo (2006). "Assessing the patterns and controls of fine root dynamics: an empirical test and methodological review." Journal of Ecology **94**(1): 40-57.

Hill, A. R. (1996). "Nitrate removal in stream riparian zones." Journal of Environmental Quality **25**(4): 743-754.

Hodge, A. (2004). "The plastic plant: root responses to heterogeneous supplies of nutrients (Tansley review)." New Phytologist **162**(1): 9-24.

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**(14 June): 789-791.

IPCC (Intergovernmental Panel on Climate Change) (2001). Climate Change 2001. The Third Assessment Report, Working Group I. WMO/UNEP, Cambridge University Press, Cambridge, England.

Jacinthe, P.-A., P. M. Groffman, A. J. Gold and A. Mosier (1998). "Patchiness in microbial nitrogen transformations in groundwater in a riparian forest." Journal of Environmental Quality **27**(1): 156-164.

Jackson, R. B., J. Canadell, J. R. Ehleringer, H. A. Mooney, O. E. Sala and E. D. Schulze (1996). "A global analysis of root distributions for terrestrial biomes." Oecologia **108**(3): 389-411.

Jones, R. H., B. B. Lockaby and G. L. Somers (1996). "Effects of microtopography and disturbance on fine-root dynamics in wetland forests of low-order stream floodplains." American Midland Naturalist **136**: 57-71.

Joslin, J. D., J. B. Gaudinski, M. S. Torn, W. J. Riley and P. J. Hanson (in press 2006). "Fine-root turnover patterns and their relationship to root diameter and soil depth in a 14C-labeled hardwood forest." New Phytologist **0**(0).

Kellogg, D. Q., A. J. Gold, P. M. Groffman, K. Addy, M. H. Stolt and G. Blazejewski (2005). "In Situ Ground Water Denitrification in Stratified, Permeable Soils Underlying Riparian Wetlands." Journal of Environmental Quality **34**(2): 524-533.

Kiley, D. K. and R. Schneider (2005). "Riparian roots through time, space and disturbance." Plant and Soil **269**(1-2): 259-272.

Lieffers, V. J. and R. L. Rothwell (1987). "Rooting of Peatland Black Spruce and Tamarack in Relation to Depth of Water-Table." Canadian Journal of Botany **65**(5): 817-821.

McClaugherty, C. A., J. D. Aber and J. M. Melillo (1982). "The Role of Fine Roots in the Organic-Matter and Nitrogen Budgets of 2 Forested Ecosystems." Ecology **63**(5): 1481-1490.

Moore, T. R., J. L. Bubier, S. E. Frolking, P. M. Lafleur and N. T. Roulet (2002). "Plant biomass and production and CO2 exchange in an ombrotrophic bog." Journal of Ecology **90**(1): 25-36.

Neill, C. (1992). "Comparison of soil coring and in-growth core methods for measuring belowground production." Ecology **73**: 1919-1921.

Nepstad, D. C., C. R. De Carvalho, E. A. Davidson, H. Jipp-Peter, P. A. Lefebvre, G. H. Negreiros, E. D. Da Silva, T. A. Stone, S. E. Trumbore and S. Vieira (1994). "The role of deep roots in the hydrological and carbon cycles of amazonian forests and pastures." Nature **372**(6507): 666-669.

Powell, S. W. and F. P. Day (1991). "Root Production in Four Communities in the Great Dismal Swamp." American Journal of Botany **78**(2): 288-297.

Pregitzer, K. S., D. R. Zak, P. S. Curtis, M. E. Kubiske, J. A. Teeri and C. S. Vogel (1995). "Atmospheric CO2, Soil-Nitrogen and Turnover of Fine Roots." New Phytologist **129**(4): 579-585.

Qualls, R. G., B. L. Haines and W. T. Swank (1991). "Fluxes of dissolved organic nutrients and humic substances in a deciduous forest." Ecology **72**(1): 254-266.

Reddy, K. R., W. H. Patrick, Jr. and C. W. Lindau (1989). "Nitrification-Denitrification at the Plant Root-Sediment Interface in Wetlands." Limnology and Oceanography **34**(6): 1004-1013.

Rodgers, H. L., F. P. Day and R. B. Atkinson (2003). "Fine root dynamics in two Atlantic white-cedar wetlands with contrasting hydroperiods." Wetlands **23**(4): 941-949.

Rotkin-Ellman, M., K. Addy, A. J. Gold and P. Groffman (2004). "Tree species, root decomposition and subsurface denitrification potential in riparian wetlands." Plant and Soil **263**(1-2): 335-344.

Ruess, R. W., R. L. Hendrick, A. J. Burton, K. S. Pregitzer, B. Sveinbjornsson, M. E. Allen and G. E. Maurer (2003). "Coupling fine root dynamics with ecosystem carbon cycling in black spruce forests of interior Alaska." Ecological Monographs **73**(4): 643-662.

Saarinen, T. (1996). "Biomass and production of two vascular plants in a boreal mesotrophic fen." Canadian Journal of Botany **74**: 934-938.

SAS Institute (2002-2003). SAS. Cary, NC, USA, SAS Institute Inc.

Schade, J. D., S. G. Fisher, N. B. Grimm and J. A. Seddon (2001). "The influence of a riparian shrub on nitrogen cycling in a Sonoran Desert stream." Ecology **82**(12): 3363-3376.

Steele, S. J., S. T. Gower, J. G. Vogel and J. M. Norman (1997). "Root mass, net primary production and turnover in aspen, jack pine and black spruce forests in Saskatchewan and Manitoba, Canada." Tree Physiology **17**(8-9): 577-587.

Stone, E. L. and P. J. Kalisz (1991). "On the maximum extent of roots." Forest Ecology and Management **46**: 59-102.

Stuiver, M. and H. A. Polach. (1977). "Discussion: Reporting of 14C Data." Radiocarbon **19**(3): 355–363.

Stuiver, M., P. J. Reimer and R. Reimmer (2005). Calib 5.0.1. **2005:** http://www.calib.qub.ac.uk/crev50/.

Tierney, G. L. and T. J. Fahey (2002). "Fine root turnover in a northern hardwood forest: a direct comparison of the radiocarbon and minirhizotron methods." Canadian Journal of Forest Research **32**(9): 1692-1697.

Tierney, G. L., T. J. Fahey, P. M. Groffman, J. P. Hardy, R. D. Fitzhugh and C. T. Driscoll (2001). "Soil freezing alters fine root dynamics in a northern hardwood forest." Biogeochemistry **56**(2): 175-190.

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**(5): 670-679.

Woldendorp, J. W. (1962). "The quantitative influence of the rhizosphere on denitrification." Plant and Soil **17**: 267–270.

Wynn, T. M., S. Mostaghimi, J. A. Burger, A. A. Harpold, M. B. Henderson and L.-A. Henry (2004). "Variation in Root Density along Stream Banks." Journal of Environmental Quality **33**(6): 2030-2039.

CHAPTER FIVE: SYNTHESIS: RIPARIAN RIFTS IN THE SPACE-TIME CONTINUUM

Introduction

Intensive nitrogen (N) inputs to terrestrial ecosystems cause diverse problems for human health and environmental conservation in upland forests and down-gradient ecosystems (Vitousek et al. 1997; Carpenter et al. 1998). The strong link between N enrichment of terrestrial ecosystems and eutrophication points to two needs: (1) the ability to predict nitrogen fluxes to coastal waters as N-additions to terrestrial ecosystems change; and (2) the development of effective strategies to manage landscape-scale N fluxes. An improved understanding of how N sinks in the landscape operate would address both of these knowledge gaps.

Nitrate removal from shallow groundwater is thought to occur primarily in specific landscape elements, and riparian zones sit atop the list of N removal hot spots in the landscape (Hill 1996). Looking across studies of riparian zone N retention reveals considerable variability in N removal rates both within and among sites (Hanson et al. 1994; Gold et al. 2001; Johnston et al. 2001), and this variability is sufficiently large to impede effective evaluation of riparian zones as N sinks in watersheds. There is a clear need to explain this variability, especially in terms of mappable features or ones that can be measured or estimated reasonably at large spatial scales.

One line of research that begins to link studies of riparian zone N cycling with investigations of landscape N flux considers how and why riparian zone N removal capacity varies with hydrogeologic setting (e.g., Pinay et al. 1995, 2002; Hill 1996; Jordan et al. 1997; Devito et al. 2000; Puckett 2004, Vidon and Hill 2004, Kellogg et al. 2005; Merrill 2006). This approach is potentially powerful because it seeks to

explain variation in N sinks at the scale of recognizable functional landscape units. Organizing riparian zone ecosystem processes according to a typology of sites has theoretical appeal because features which arise from hydrogeologic processes operating over large spatial and long temporal scales (e.g., soil texture, land surface slope, surficial geology, landscape position), in turn influence variables that control denitrification rates on small spatial and short temporal scales (e.g., organic matter distribution, groundwater residence time, water table depth).

At small scales, denitrification potential depends primarily on the supply of microbially-available C where nitrate-laden groundwater flows (Trudell et al. 1986, Slater and Capone 1987, Smith and Duff 1988, Francis et al. 1989, Obenhuber and Lowrance 1991, Yeomans et al. 1992, Starr and Gillham 1993, Hill 1996, Clement et al. 2002, Puckett 2004). To provide information that is both useful for management and relevant to landscape scale processes, we must identify site characteristics that: (1) create, or are associated with high concentrations of microbially-available C; and (2) lead to long contact times between nitrate-bearing groundwater and zones of high C supply.

First principles suggest three possible C sources for subsurface microbes: (1) dissolved organic carbon (DOC) leached from surface soils; (2) plant roots growing through subsoils; and (3) buried, C-rich soil horizons formed by erosion and deposition in alluvial landscapes (Figure 1.1, chapter 1). The objective of this research was to evaluate the relative importance of these three C sources in the riparian subsurface and to assess whether it varied between riparian zones in different hydrogeologic settings.

Here we use the main findings of this research to build a conceptual model of C supply in the riparian subsurface. Key features of this framework include: (1) geomorphic processes operating over long time scales create legacies that influence contemporary biogeochemical cycles in the riparian subsurface; (2) these legacy effects can be viewed as episodic strong connections between surface and subsurface ecosystems; (3) the short-term influence of surface ecosystem processes on belowground ecosystems is strong in surface soils, moderate in the shallow subsurface, and very weak below about 80 cm; and (4) the spatial distribution of microbially-available C suggests "hot spots" of C mineralization at multiple spatial scales from centimeters to kilometers.

Legacy effects in riparian zones and functional classifications

A critical feature of riparian soil profiles formed by alluvial processes is the ubiquitous presence of buried C-rich horizons. In Rhode Island, nearly all riparian soil profiles mapped as either outwash or alluvium (Rector 1981) included buried horizons within 10 meters of the stream, although they occur very infrequently below 2 meters (Blazejewski 2002).

Several data sets from this research underscore the need to include buried horizons in functional classifications of riparian zones. First, laboratory measurements of C mineralization and microbial biomass demonstrated that buried horizons harbor microbially-available C at levels considerably greater than occur in the surrounding matrix of B and C horizons (Figures 2.2 & 2.3, chapter 2). Contrary to expectations, C availability did not diminish with depth within the subsurface, although buried horizons occurred infrequently below 2 meters (Blazejewski 2002). Soil horizon type explained 50% of the variation in C mineralization per mass of soil, suggesting that

soil characteristics at the time of burial are more important than time since burial for determining the potential for buried soil horizons to support microbial activity in the subsurface. Hence, the potential for N removal from groundwater in the subsurface does not diminish with depth or age of buried horizons, underscoring the likely role of these soils in preventing hydrologic bypass.

Second, the radiocarbon signatures of dissolved inorganic carbon (DIC) in ambient groundwater (Figure 3.3, chapter 3) and groundwater incubated in-situ (Figure 3.6, chapter 3) demonstrated conclusively that microbes metabolize ancient C in the riparian subsurface. While buried horizons may trap DOC leached from surface soils, metabolism of ancient C accounts for a substantial fraction of C mineralization at depth in the subsurface, particularly at alluvial sites. At one alluvial site, mineralization of ancient C in winter accounted for a minimum of 31% of total C mineralized (Gurwick et al. in prep, chapter 3).

Third, although we found considerable root biomass in the deep subsurface, particularly in alluvial soils (Figures 4.3 & 4.4, chapter 4), nearly all roots collected from below 80 cm formed more than 100 years before present (Figure 4.7, chapter 4), also supporting the argument that relic C dominates microbial activity in the deep subsurface and that these landscape features need to be incorporated into functional classifications of riparian zones.

In the glaciated northeast, we are unlikely to derive great benefits from distinguishing between alluvial and outwash riparian zones, or among alluvial sites on 1^{st}-3^{rd} order streams. Soils mapped as outwash nearly always include alluvial features within 10 meters — and in many cases beyond 10 meters — from the stream (Blazejewski

2002). The high frequency of buried horizons near streams, combined with our findings that old C is microbially-available are consistent with the rapid decline in nitrate concentrations over short distances at the soil-stream interface that has been observed in previous studies. Companion studies (Blazejewski 2002) have failed to identify strong predictive relationships between mappable landscape attributes and the frequency of buried horizons at particular sites.

Differentiating between 1st-3rd and 4th-order streams may be useful for modeling landscape-scale N flows because higher-order streams exhibited a greater abundance of buried horizons, particularly below 2 meters (Blazejewski 2002). Two questions need to be resolved in order to evaluate the potential importance of these deep buried horizons for N removal. First, we found relatively low C-availability associated with the deep buried horizons but had only two samples from > 2 meters (Figure 2.2, chapter 2). Additional measurements would be required to determine whether low C mineralization rates (and associated low N removal potentials) are a general feature of these deep soils. Second, because most movement of groundwater across the terrestrial-aquatic interface occurs on low-order streams, the potential for interaction between nitrate-bearing water and buried horizons on higher-order streams depends largely upon the extent to which these soils intersect hyporheic flow. If these horizons occur within the hyporheic zone, then the potential for interaction with water previously delivered to the channel via lower-order streams remains very high. The combined roles of C availability and denitrification in the riparian zone and hyporheic zone have been explored very little and remain a promising area for future research at the terrestrial-aquatic interface.

The dominant role that buried horizons appear to play in the glaciated northeast is less apparent in some areas of the Chesapeake Bay Watershed (Lowrance et al. 1997) but probably play a larger role than previously recognized in some of those regions. Shallow confining layers that are common in the Inner Coastal Plain physiographic region force interaction between groundwater and plant roots, and increase the potential for overland hydrologic bypass of the biologically active zone. In their assessment of riparian forest buffers, Lowrance et al. (1997) emphasized that denitrification potential would likely be minimal in areas such as tidal creeks that lacked a confining layer and where the depth to water table was below the root zone. This aspect of the riparian forest buffer typology presented in Lowrance et al. (1997) needs to be reconsidered in light of the data on buried horizons presented here. Tidal creeks are subject to floods and the potential for buried C-rich soil horizons seems high. In parts of the Piedmont, most discharge to streams occurs through fractured bedrock and the potential for buried horizons in this geomorphic setting seems low. The conclusion that denitrification in parts of the Piedmont depends on deeply-rooted vegetation in the regolith points to the potential for roots to play a larger role in some subsurface ecosystems than in the deep subsurface at our study sites, though our findings about root turnover underscore the need to augment observations of intact roots with measurements of root age or production.

Surface-subsurface connections

The old age of mineralized C (Figures 3.7 & 3.8, Table 3.4, chapter 3) and the very low contribution of modern plant roots to C cycling at depth (Figure 4.7, chapter 4) resolve long-standing questions about the contribution of plant roots to N removal in the riparian subsurface (Hill 1996) and, contrary to expectations, emphasize the decoupling of surface and subsurface ecosystem on time scales of months to years.

We expected roots in the subsurface to function more like roots in surface soils, where they exert powerful control over biogeochemical cycles by directly connecting C-fixation aboveground and microbial processes in the soil. This expectation followed from several lines of evidence including understanding of wetland root physiology (Armstrong 1964, 1979; Colmer 2003) and data showing significant root biomass at depths of 1 meter in riparian zones (Wynn et al. 2004; Kiley and Schneider 2005) and other wetlands (Saarinen 1996; Moore et al. 2002). However, despite appearing intact, nearly all roots collected from below 80 cm formed more than 100 years before present, and roots from between 40-75 cm formed at least 10 ybp (Figure 4.7 and Table 4.2, chapter 4). Data from ingrowth cores (Figures 4.5 & 4.6, chapter 4) supported the interpretation that root turnover is rapid in the top 20-30 cm of soil, slow between 40-75 cm, and very slow below 80 cm. In addition, C mineralization associated with relic roots appears low relative to that associated with SOM of buried horizons (N. Gurwick, unpublished data). These data imply that N removal below 75 cm at our study sites is disconnected from surface ecosystem processes such as plant uptake or C supply by live plant roots, and are consistent with previous observations of denitrification in shallow riparian groundwater during winter (Nelson et al. 1995).

On long time scales, the interaction of climate and terrestrial ecosystem processes control the rapid incorporation of C into the subsurface. Periodic droughts may control the penetration of roots into the shallow subsurface (40-75 cm) and the associated formation of root-derived patches that have high N removal capacity (Gold et al. 1998; Jacinthe et al. 1998). On longer time scales, hurricanes can uproot trees, sometimes forcing large branches deep below the surface where they can be metabolized and colonized by fine roots (see chapter 4, Appendix A). At the longest time scale, floods deposit sediment on top of surface soil horizons, creating buried C-

rich soil horizons that then become part of the subsurface and that continue to support microbial activity for millennia.

Hot spots at multiple scales

The concept of hot spots in soil biogeochemical cycles has received considerable attention since it was first introduced nearly twenty years ago (Parkin 1987; Christensen 1998). It has now been applied in a number of contexts from microbiology to conservation biology, but usually at a single spatial or temporal scale. Data from this study and others suggest that N removal in the landscape can be understood in a framework of hot spots at multiple spatial scales. These include: roots across transects in the shallow subsurface (Figure 4.2, chapter 4), buried horizons within soil profiles (Figure 2.2, chapter 2), soil profiles within riparian zones (Blazejewski 2002, Gurwick unpublished data), riparian zones within landscapes, and alluvial landscapes within glacial regions. These landscape and soil features vary temporally, with the smallest features shifting most rapidly.

Future research directions

Several findings from these investigations point towards worthwhile future research. Perhaps the most pressing need is for additional information on ^{14}C signatures associated with roots and DIC from multiple riparian zones across multiple depths, hydrogeologic settings, distances from streams, and seasons. The data sets presented here, while a valuable first step, are unusual and need to be expanded to resolve questions associated with outliers in our relatively limited data sets. Radiocarbon measurements of DIC in ambient groundwater, while costly to analyze, could easily be incorporated into existing studies of riparian groundwater from a logistical point of view.

Additional study of chemistry associated with different buried horizons and their associated lability could yield unique insights about the stabilization of soil organic matter (SOM). Our understanding of SOM stabilization is undergoing a rapid shift (Sutton and Sposito 2005), and these old soils provide an unusual window into the relationship between SOM chemistry and microbial activity.

Of the three potential C sources to the riparian zone identified in chapter 1, DOC received minimal attention in this study, in part because previous research had suggested that labile DOC is rapidly metabolized, leaving recalcitrant material to be carried along the flow path (McCarty and Bremner 1992). However, much remains to be understood about DOC production, lability, and fate (Kalbitz et al. 2000), and ^{14}C-DIC signatures at 300 cm depth at site A suggest DOC may be important in subsurface C metabolism, particularly where upgradient surface soils have been disturbed. Our studies of ancient C metabolism underscore the point that rates of C metabolism considered unimportant in surface soils can constitute dominant fluxes in the subsurface, and this perspective may apply to DOC as well as to SOM.

Riparian and hyporheic processing have yet to be combined in studies of biogeochemical cycles along the land-water continuum. This remains a promising area for future research. One specific need is to assess whether buried horizons beneath stream channels and in stream-side soils influence hyporheic zone biogeochemistry to the extent that they appear to influence groundwater moving across riparian zones.

The clear importance of buried soil horizons at the terrestrial-aquatic interface also underscores the need to revisit existing riparian zone classification schemes (e.g.,

Lowrance et al. 1997), evaluate their treatment of vegetation and hydrogeologic features, and include buried horizons as we develop new classification systems. This effort could involve both new syntheses of existing data and additional data collection efforts. One implication of data presented here is that the depth of the active rooting zone and the general importance of vegetation may have been overestimated while the presence and importance of buried horizons, which are easily identified, may have been ignored.

Rapid shifts in land use and climate could alter the factors that control C supply in the riparian subsurface, and the consequences of such shifts need to be anticipated and investigated. Hypotheses about the relationship between soil saturation, vegetation composition, and root dynamics could be tested to good effect at sites where changes in land use or regulation of stream flow have led to incised banks and/or lower water tables. Questions about the consequences of vegetation shifts and the relative dominance of different species in the subsurface could perhaps be explored using cross-site comparisons.

Finally, the emerging understanding of how buried horizons influence C and N cycling in the riparian subsurface needs to be incorporated into strategies of riparian restoration. Field experiments with buried wood have yielded important insights in this regard (Schipper and Vojvodic-Vukovic 2001; Schipper et al. 2004, 2005) but they have been conducted away from the stream and are limited in number. Given the ubiquity of buried horizons in northeastern riparian landscapes, the dominant role they appear to play in landscape-scale N removal, and the extent to which we have altered native stream channel morphologies, there is a clear need for research on: (1) the extent to which stream alteration has eliminated or reduced the frequency of buried

horizons near the stream channel; and (2) the feasibility of creating subsurface features that mimic the functions of buried horizons, particularly at depth, in riparian landscapes.

Literature Cited

Armstrong, W. (1964). "Oxygen Diffusion from the Roots of some British Bog Plants." Nature 204: 801-802.

Armstrong, W. (1979). "Aeration in Higher Plants." Advances in Botanical Research 7: 226-332.

Blazejewski, G. A. (2002). Carbon in riparian zone subsoils: Morphology and spatial distribution. Dept of Natural Resources Science. Kingston, RI, University of Rhode Island.

Carpenter, S. R., N. F. Caraco, D. L. Correll, R. W. Howarth, A. N. Sharpley and V. H. Smith (1998). "Nonpoint pollution of surface waters with phosphorous and nitrogen." Ecological Applications 8(3): 559-568.

Christensen, S., S. Simkins and J. M. Tiedje (1990). "Spatial variation in denitrification." Soil Science Society of America Journal 54: 1614-1618.

Clement, J. C., G. Pinay and P. Marmonier (2002). "Seasonal dynamics of denitrification along topohydrosequences in three different riparian wetlands." Journal of Environmental Quality 31(3): 1025-1037.

Colmer, T. D. (2003). "Long-distance transport of gases in plants: a perspective on internal aeration and radial oxygen loss from roots." Plant Cell and Environment 26(1): 17-36.

Devito, K. J., D. Fitzgerald, A. R. Hill and R. Aravena (2000). "Nitrate dynamics in relation to lithology and hydrologic flow path in a river riparian zone." Journal of Environmental Quality 29(4): 1075-1084.

Francis, A. J., J. M. Slater and C. J. Dodge (1989). "Denitrification in Deep Subsurface Sediments." Geomicrobiology Journal 7(1-2): 103-116.

Gold, A. J., P. M. Groffman, K. Addy, D. Q. Kellogg, M. Stolt and A. E. Rosenblatt (2001). "Landscape attributes as controls on ground water nitrate removal capacity of riparian zones." Journal of the American Water Resources Association **37**(6): 1457-1464.

Gold, A. J., P. A. Jacinthe, P. M. Groffman, W. R. Wright and R. H. Puffer (1998). "Patchiness in groundwater nitrate removal in a riparian forest." Journal of Environmental Quality 27(1): 146-155.

Hanson, G. C., P. M. Groffman and A. J. Gold (1994). "Symptoms of Nitrogen Saturation in a Riparian Wetland." Ecological Applications 4(4): 750-756.

Hill, A. R. (1996). "Nitrate removal in stream riparian zones." Journal of Environmental Quality 25(4): 743-754.

Jacinthe, P.-A., P. M. Groffman, A. J. Gold and A. Mosier (1998). "Patchiness in microbial nitrogen transformations in groundwater in a riparian forest." Journal of Environmental Quality 27(1): 156-164.

Johnston, C. A., S. D. Bridgham and J. P. Schubauer-Berigan (2001). "Nutrient Dynamics in Relation to Geomorphology of Riverine Wetlands." Soil Science Society Of America Journal 65: 557-577.

Jordan, T. E., D. L. Correll and D. E. Weller (1997). "Relating nutrient discharges from watersheds to land use and streamflow variability." Water Resources Research 33(11): 2579-2590.

Kalbitz, K., S. Solinger, J. H. Park, B. Michalzik and E. Matzner (2000). "Controls on the dynamics of dissolved organic matter in soils: A review." Soil Science **165**(4): 277-304.

Kellogg, D. Q., A. J. Gold, P. M. Groffman, K. Addy, M. H. Stolt and G. Blazejewski (2005). "In Situ Ground Water Denitrification in Stratified, Permeable Soils Underlying Riparian Wetlands." Journal of Environmental Quality 34(2): 524-533.

Kiley, D. K. and R. Schneider (2005). "Riparian roots through time, space and disturbance." Plant and Soil 269(1-2): 259-272.

Lowrance, R., L. S. Altier, J. D. Newbold, R. R. Schnabel, P. M. Groffman, J. M. Denver, D. L. Correll, J. W. Gilliam, J. L. Robinson, R. B. Brinsfield, K. W. Staver, W. Lucas and A. H. Todd (1997). "Water Quality Functions of Riparian Forest Buffers in Chesapeake Bay Watersheds." Environmental Management 21(5): 687-712.

McCarty, G. W. and J. M. Bremner (1992). "Availability of Organic-Carbon For Denitrification of Nitrate in Subsoils." Biology and Fertility of Soils **14**(3): 219-222.

Merrill, A. G. and T. L. Benning (2006). "Ecosystem type differences in nitrogen process rates and controls in the riparian zone of a montane landscape." Forest Ecology and Management 222(1-3): 145-161.

Moore, T. R., J. L. Bubier, S. E. Frolking, P. M. Lafleur and N. T. Roulet (2002). "Plant biomass and production and CO2 exchange in an ombrotrophic bog." Journal of Ecology 90(1): 25-36.

Nelson, W. M., A. J. Gold and P. M. Groffman (1995). "Spatial and Temporal Variation in Groundwater Nitrate Removal in a Riparian Forest." Journal of Environmental Quality 24(4): 691-699.

Obenhuber, D. C. and R. Lowrance (1991). "Reduction of Nitrate in Aquifer Microcosms by Carbon Additions." Journal of Environmental Quality 20(1): 255-258.

Parkin, T. B. (1987). "Soil microsites as a source of denitrification variability." Soil Science Society of America Journal 51: 1194-1199.

Pinay, G., C. Ruffinoni and A. Fabre (1995). "Nitrogen Cycling in Two Riparian Forest Soils Under Different Geomorphic Conditions." Biogeochemistry 30: 9-29.

Pinay, G., J. C. Clement and R. J. Naiman (2002). "Basic principles and ecological consequences of changing water regimes on nitrogen cycling in fluvial systems." Environmental Management 30(4): 481-491.

Puckett, L. J. (2004). "Hydrogeologic controls on the transport and fate of nitrate in ground water beneath riparian buffer zones: results from thirteen studies across the United States." Water Science and Technology 49(3): 47-53.

Saarinen, T. (1996). "Biomass and production of two vascular plants in a boreal mesotrophic fen." Canadian Journal of Botany 74: 934-938.

Schipper, L. A., G. F. Barkle, J. C. Hadfield, M. Vojvodic-Vukovic and C. P. Burgess (2004). "Hydraulic constraints on the performance of a groundwater denitrification wall for nitrate removal from shallow groundwater." Journal of Contaminant Hydrology 69(3-4): 263-279.

Schipper, L. A., G. F. Barkle and M. Vojvodic-Vukovic (2005). "Maximum Rates of Nitrate Removal in a Denitrification Wall." Journal of Environmental Quality 34(4): 1270-1276.

Schipper, L. A. and M. Vojvodic-Vukovic (2001). "Five years of nitrate removal, denitrification and carbon dynamics in a denitrification wall." Water Research 35(14): 3473-3477.

Slater, J. M. and D. G. Capone (1987). "Denitrification in Aquifer Soil and Nearshore Marine-Sediments Influenced by Groundwater Nitrate." Applied and Environmental Microbiology 53(6): 1292-1297.

Smith, R. L. and J. H. Duff (1988). "Denitrification in a Sand and Gravel Aquifer." Applied and Environmental Microbiology 54(5): 1071-1078.

Starr, R. C. and R. W. Gillham (1993). “Denitrification and Organic-Carbon Availability in 2 Aquifers.” Ground Water 31(6): 934-947.

Sutton, R. and G. Sposito (2005). “Molecular structure in soil humic substances: The new view.” Environmental Science & Technology 39(23): 9009-9015.

Trudell, M. R., R. W. Gillham and J. A. Cherry (1986). “An in-situ study of the occurrence and rate of denitrification in a shallow unconfined sand aquifer.” Journal of Hydrology 83: 251-268.

Vidon, P. G. F. and A. R. Hill (2004). “Landscape controls on nitrate removal in stream riparian zones.” Water Resources Research 40: W03201.

Vitousek, P. M., J. D. Aber, R. W. Howarth, G. E. Likens, P. A. Matson, D. D. Schindler, W. H. Schlesinger and D. G. Tilman (1997). “Human alteration of the global nitrogen cycle: sources and consequences.” Ecological Applications 7(3): 737-750.

Wynn, T. M., S. Mostaghimi, J. A. Burger, A. A. Harpold, M. B. Henderson and L.-A. Henry (2004). “Variation in Root Density along Stream Banks.” Journal of Environmental Quality 33(6): 2030-2039.

Yeomans, J. C., J. M. Bremner and G. W. McCarty (1992). “Denitrification Capacity and Denitrification Potential of Subsurface Soils.” Communications in Soil Science and Plant Analysis 23(9-10): 919-927.

APPENDIX A. CHARACTERISTICS AND ^{14}C-AGES OF ROOTS FROM THE RIPARIAN SUBSURFACE

Photographs of roots analyzed for ^{14}C activity, with associated information about sampling depth, ^{14}C signatures, and inferred time of formation (ybp relative to 2002). Multiple ranges reported where no single range had a probability of >90%. More detailed information reported in Chapter 4, Table 4.2.

Figure A.1. Root sample 1.
Site: B Depth: 40-55 cm Diameter: <0.5 mm
$\Delta^{14}C = 15.4$ Age = 47 ybp

Figure A.2. Root sample 2.
Site: C Depth: 50-60 cm Diameter: 1-2 mm
$\Delta^{14}C = 295$ Age = 23 or 40 ybp

Figure A.3. Root sample 3.
Site: B Depth: 50-62 cm Diameter: <0.5 mm
$\Delta^{14}C = 134.3$ Age = 10 ybp

Figure A.4. Root sample 4.
Site: D Depth: 57-62 cm Diameter: >2 mm
$\Delta^{14}C = 209.2$ Age = 17 or 42 ybp

Figure A.5. Root sample 5.
Site: B Depth: 60-75 cm Diameter: <0.5 mm
$\Delta^{14}C = 9.8$ Age = 48 ybp

Figure A.6. Root sample 6.
Site: B Depth: 65-75 cm Diameter: <2 mm
$\Delta^{14}C = 171$ Age = 14 ybp

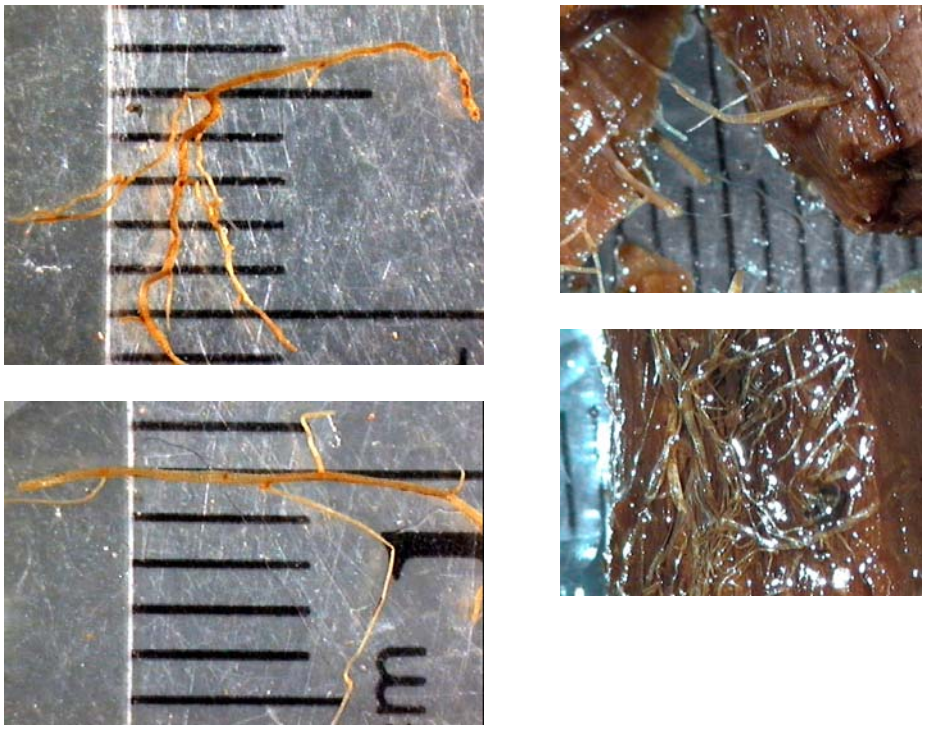


Figure A.7. Root sample 7.
Site: B Depth: 75-80 cm Diameter: <0.5 mm
$\Delta^{14}C$ = -121.1 Age = 881-986 ybp

Figure A.8. Root sample 8.
Site: B Depth: 65-85 cm
Diameter (host): > 5 mm Diameter (colonizer): <0.5 mm
$\Delta^{14}C$ (host) = -32.7 $\Delta^{14}C$ (colonizer) = 388.4
Age (host) = 197-267 ybp Age (colonizer) 25 or 39 ybp

Figure A.9. Root sample 9.

Site: B	Depth: 65-85 cm
Diameter (host): > 5 mm	Diameter (colonizer): <0.5 mm
$\Delta^{14}C$ (host) = -49.3	$\Delta^{14}C$ (colonizer) = 230.4
Age (host) = 359-548 ybp	Age (colonizer) 17 or 30 ybp

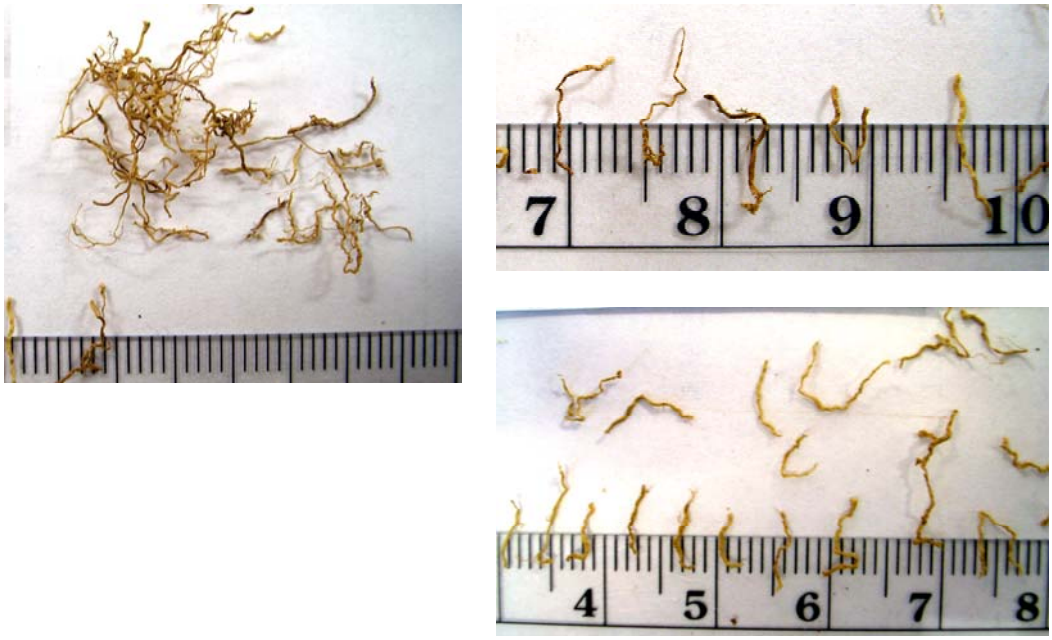


Figure A.10. Root sample 10.

Site: B	Depth: 90-100 cm	Diameter: <0.5 mm
$\Delta^{14}C$ = -37.2	Age = 337-359 ybp	

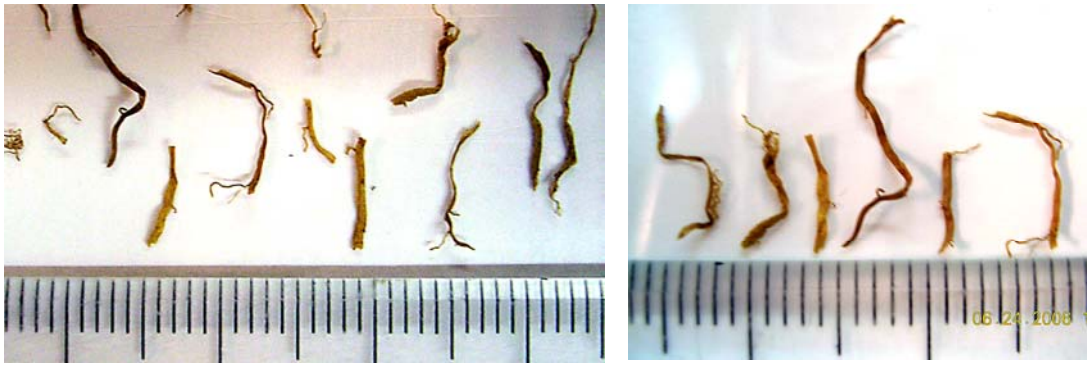


Figure A.11. Root sample 11.
Site: B Depth: 90-100 cm Diameter: 0.5-1 mm
$\Delta^{14}C$ = -31.7 Age = 201-238 or 322-349 ybp

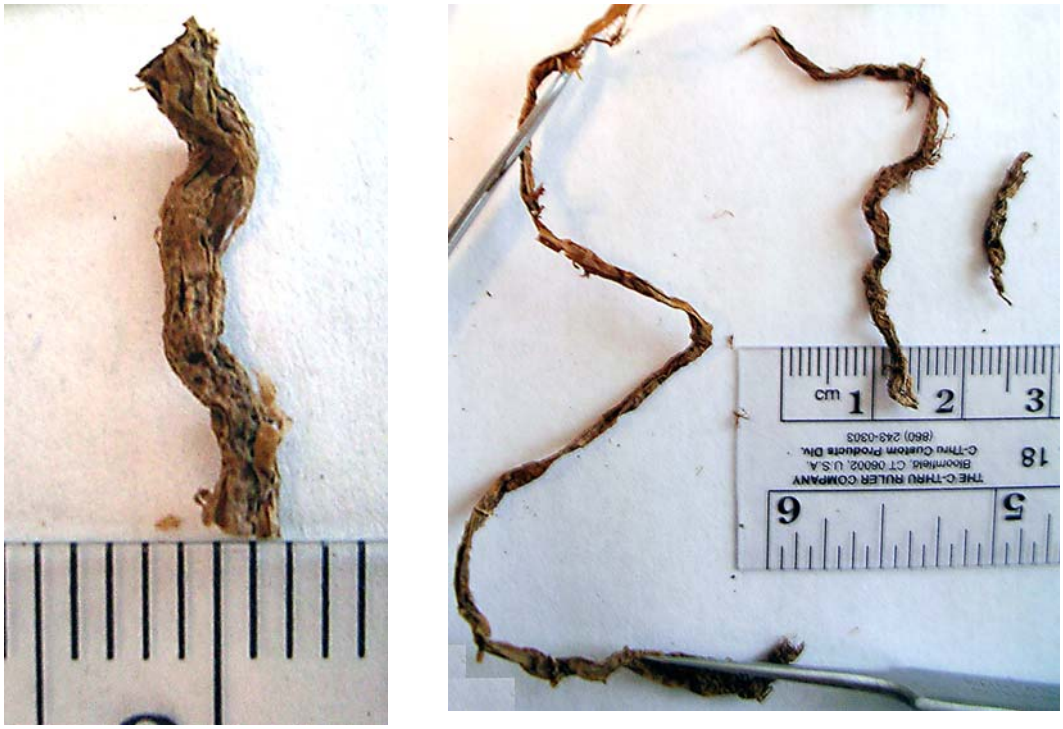


Figure A.12. Root sample 12.
Site: B Depth: 90-100 cm Diameter: > 2 mm
$\Delta^{14}C$ = -24 Age = 56 - 334 ybp

Figure A.13. Root sample 13.
Site: C Depth: 100-120 cm Diameter: <0.5mm
$\Delta^{14}C$ = -595.5 Age = 7,889 – 8,276 ybp


Figure A.14. Root sample 14.
Site: D Depth: 106-114 cm Diameter: 1-2 mm
$\Delta^{14}C$ = -36.6 Age = 205-220 or 334-361 ybp

Figure A.15. Root sample 15.
Site: C Depth: 110-120 cm Diameter: 2 mm
$\Delta^{14}C$ = -547.9 Age = 7,217-7,385 ybp

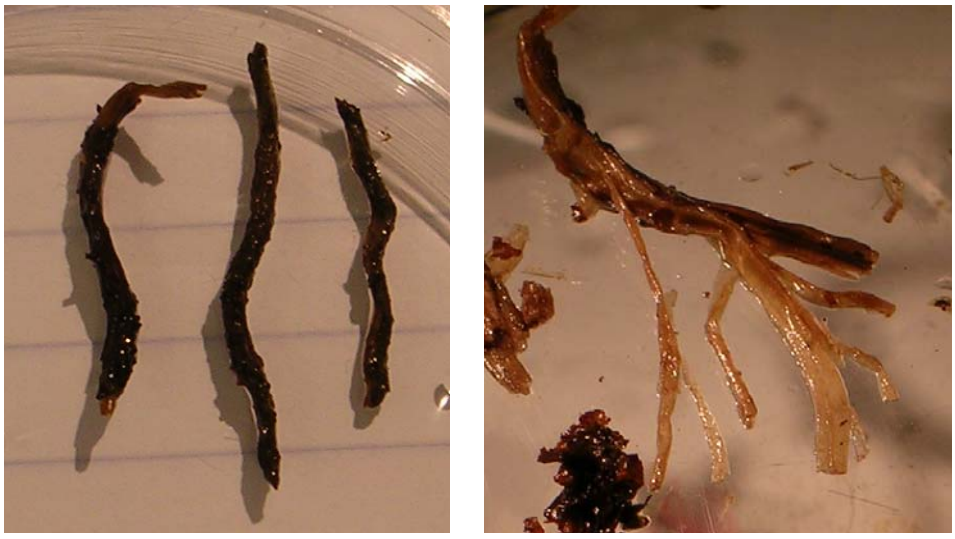


Figure A.16. Root sample 16.
Site: D Depth: 115-120 cm Diameter: 2-3 mm
$\Delta^{14}C$ = -252.9 Age = 2,232-2,405 ybp

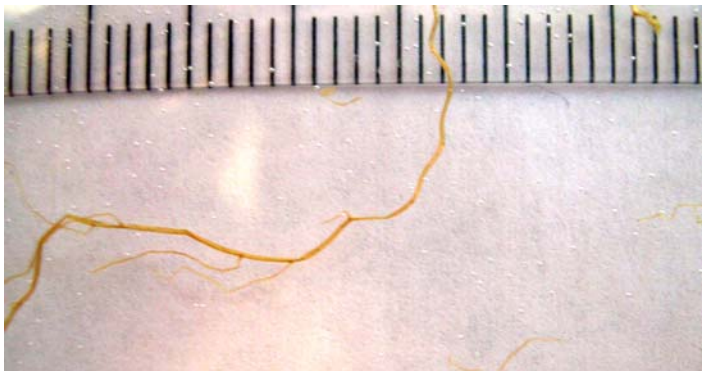


Figure A.17. Root sample 17.
Site: B Depth: 150 cm Diameter: <0.5 mm
$\Delta^{14}C$ = -141.5 Age = 1,105 – 1,224 ybp

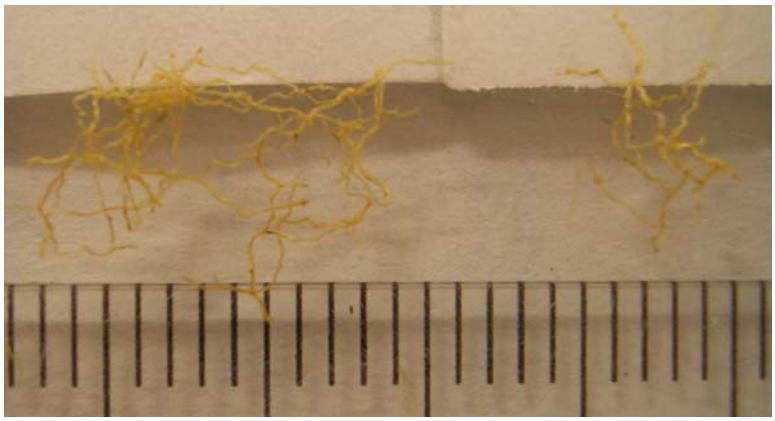


Figure A.18. Root sample 18.
Site: B Depth: 150 cm Diameter: <0.5 mm
$\Delta^{14}C$ = 52.3 Age = 46 ybp