Impacts of Agricultural Management and Landscape Factors on Soil Carbon and Nitrogen Cycling

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Dedication

This dissertation is dedicated to A.R; I could not have done this without you.

ABSTRACT

Agricultural management has altered soil carbon (C) and nitrogen (N) inputs, losses, and turnover rates. Understanding how management interacts with landscape factors to regulate soil C and N losses is essential to addressing climate change. Through research conducted in agricultural systems in Minnesota I investigated: (1) how the loss of corn root-derived C as carbon dioxide (CO_2), and N as nitrous oxide (N_2O) differed among five management systems, and (2) how hillslope position and soil moisture affected the size and turnover of soil C pools.

In a field study using stable isotope techniques, I found that the fraction of root-derived C and N emitted as CO_2 and N_2O, the C and N emission factors, were 35% and less than 1% respectively. Individually, each emission factor was lower in systems with increased rotation diversity. Conversely, the relationship between C and N emission factors differed with tillage and fertilization intensity, not with rotation diversity. The magnitude of root-derived C and N emission factors has agricultural policy implications. Currently an emission factor of 1% is used for all N inputs to agricultural systems. My research suggests that a lower emission factor would better reflect N_2O emissions from belowground N sources.

In a laboratory study, both position and soil moisture significantly impacted the size and mean residence time of soil C pools along a low slope hillslope. Intact core sections of the upper four horizons from three hillslope positions were incubated at 50, 75, 90 and 100% water-filled pore space (WFPS) for 355 days. Total soil C (TC), N, and the resistant fraction of TC (64%) increased downslope. Under saturated conditions, 100% WFPS treatment, the size and mean residence time of the labile C fraction (<1%

of TC) increased. Increased moisture, between 50% and 90% WFPS, also lengthened the mean residence time of slow C. In this low slope landscape I found effects of both position and moisture on C pool dynamics; soil moisture had the most significant impacts on labile C pool size and the slow C pool mean residence time.

TABLE OF CONTENTS

List of Tables viii

List of Figures ix

Chapter 1 Introduction 1

Agricultural Management Effects on Carbon and Nitrogen Cycling in Soils .. 2
Spatial Patterns of Carbon and Nitrogen Cycling in Soils 5
Research Objectives 8
Summary 10

Chapter 2 Root-Derived Contributions to Soil Respiration as Influenced by Agricultural Management Systems 13

Introduction 17

Materials and Methods 22

Research Site 22
Treatments and Experimental Design 22
Isotope Labeling 24
Gas Flux Sampling and Analysis 26
Determination of Residue-Derived Respiration 27
Data Analysis 29

Results 31

Climate and Site Environmental Data 31
Root-Derived Carbon Inputs and Root-Derived Soil Organic Carbon 32
Daily Soil and Root-Derived Respiration Rates 33
Management System Effects on Cumulative Respiration 34
Root-Derived Carbon Turnover 35

Discussion 36

Seasonal Patterns of Root-Derived Carbon Cycling 36
Management Effects on Root-Derived Carbon Cycling 38
Contrasting Environmental and Management Control of Root-Derived Carbon Cycling 39
Factors Influencing the Magnitude of Root-Derived Carbon Turnover 40
Conclusions 42

Chapter 3 Root-Derived Nitrous Oxide Emissions and Carbon-Nitrogen Coupling in an Agricultural Ecosystem 50

Introduction 53

Materials and Methods 58

Research Site 58
Treatments and Experimental Design 59
Isotope Labeling 60
Nitrous Oxide Emissions 61
Root-Derived Nitrous Oxide Emissions 63
Data Analysis 65

Results 67

Climate and Site Environmental Data 67
Root-Derived Nitrogen and Soil Nitrogen 68
Daily Nitrous Oxide Emission Rates 69
Cumulative Total and Root-Derived Nitrous Oxide Emissions 71
Coupling of Root-Derived Carbon and Nitrogen Cycling 73

Discussion 74

Magnitude of Nitrous Oxide Emissions 75
Environmental and Management Effects on N_2O Emissions 76
Root-Derived Carbon and Nitrogen Emission Factors 77
Management Effects on Root-Derived Emission Factors 79
Directions for Future Research 81
Conclusions 82

Chapter 4 Landscape Position and Soil Moisture Effects on Carbon Pool Dynamics of Agricultural Soils 92

Introduction 96

Materials and Methods 103

Research Site Description and Sample Collection 103
Experimental Design 104
Moisture Treatments 105
Nitrogen Mineralization 106
Carbon Mineralization Incubations 107
Determination of Carbon Pool Size and Turnover 109
Data Analysis 112

Results 113

Soil Respiration Data and Treatment Effectiveness 113
Landscape Position Effects on Soil Characteristics, Carbon Pool Sizes and Turnover Rates 114
Moisture Treatment Effects on Carbon Pool Size and Turnover Rates 116
Nitrogen Mineralization 118

Discussion 119

Landscape Position Effects on Carbon Pools and Turnover Rates 119
Moisture Treatment Effects on Carbon Pools and Turnover Rates..... 121
Nitrogen Mineralization and Carbon-Nitrogen Coupling 123
Conclusions 125

Chapter 5 Conclusions 135

Research Summary..... 136
Directions for Future Research..... 140
Conclusions 142

Bibliography.....143

LIST OF TABLES

Table 2.1 Management System Characteristics. 44

Table 2.2 Monthly Precipitation and Soil Temperature. 45

Table 2.3 Root-Derived Carbon and Soil Organic Carbon Content. 46

Table 2.4 Management Effects on Cumulative Carbon Emissions. 47

Table 3.1 Management System Characteristics. 84

Table 3.2 Soil Environmental Characteristics. 85

Table 3.3 Root-Derived Nitrogen and Total Soil Nitrogen. 86

Table 3.4 Models of Cumulative Total and Root-Derived N_2O Emissions. 87

Table 4.1 Cumulative Incubation Emissions of CO_2, N_2O, and CH_4. 127

Table 4.2 Soil Characteristics.. 128

Table 4.3 Hillslope Position and Moisture Treatment Effects on C Pool Size and MRT 129

Table 4.4 Net N Mineralization Rates. 130

LIST OF FIGURES

2.1 Daily Carbon Emission Rates. 48

2.2 Cumulative Carbon Emissions. 49

3.1 Daily Nitrogen Emission Rates. 88

3.2 Cumulative Nitrogen Emissions. 89

3.3 Root-Derived Carbon and Nitrogen Contributions to Total Emissions. 90

3.4 Management Effects on Root-Derived Carbon and Nitrogen Coupling. 91

4.1 Hillslope Diagram. 131

4.2 Hillslope Position and Moisture Treatment Effects on CO_2 Emissions. 132

4.3 Soil Carbon Pools by Hillslope Position and Horizon. 133

4.4 Cumulative Carbon and Nitrogen Mineralization. 134

CHAPTER 1

Introduction

Decisions about land use management have direct impacts on the total quantity of soil carbon (C) and nitrogen (N) stored in soils, and the vulnerability of that soil C and N to loss from the soil system. Consequences of our management decisions regarding soil C and N can have strong climate feedbacks effects (Pacala and Socolow, 2004). Due to the positive relationship between soil C content and crop productivity (Kravchenko and Robertson, 2007), historically, cropland in the USA has been preferentially located on high C soils. Losses of soil C upon conversion from forest or pasture to cropland have been on the order of 50% (Guo and Gifford, 2002). In spite of these losses, agricultural ecosystems in the United States rank second among ecosystems contributing to the national soil organic C (SOC) stock, 24% in the upper two meters of soil (Guo et al., 2006). Soil C loss with conversion to agriculture is thought to be a result of both decreased C inputs due to the loss of perennial vegetation, and increased C losses due to the destructive effect of tillage on physical and chemical C protection mechanisms in soils (Six et al., 2000). The relative importance of changes in C inputs versus losses is unclear, and agricultural ecosystems on average remain C sinks (Van Oost et al., 2007). Soils contain the largest terrestrial reservoir of carbon (C), estimated to be 2344 Pg C in the upper three meters of soil (Jobbagy and Jackson, 2000). Consequently, small changes in the rate of any soil C cycle process, resulting from management decisions, have the potential to significantly impact the global C cycle.

Agricultural ecosystems, specifically row crop agriculture, are an important source of greenhouse gas emissions. Globally, agriculture represents 11% of the land area and contributed 13% of the global carbon dioxide (CO_2), methane (CH_4), and nitrous oxide (N_2O) emissions in 2004 measured as CO_2 equivalents (IPCC, 2007). The increase in fertilized acres has made agricultural ecosystems the primary source of N_2O emissions globally (IPCC, 2007). As the global population increases, decisions about intensification or extensification (land clearing) of agricultural production could greatly alter the climate impacts of this population growth (Tilman et al., 2011). The prospect of either agricultural intensification or extensification underscores the importance of understanding how management decisions influence rates of C and N cycling in agricultural soils and examining the relative importance of management versus state factor control over C and N cycling rates in soils.

Agricultural Management Effects on Carbon and Nitrogen Cycling in Soils

Substantial efforts have been made toward identification and quantification of agricultural management practice (tillage, fertilization, and crop rotation) effects on C and nitrogen (N) cycling in soils. Current models of soil C such as CENTURY (Parton et al., 1987) have been extensively tested and can often accurately predict management effects on C stocks (e.g., Bricklemyer et al., 2007). Improvements in our understanding of both the physical, biological, and chemical mechanisms by which C is protected in soils (Plante et al., 2006a; Six et al., 2006; Marschner et al., 2008; von Lützow et al., 2008), as well as management effects on those mechanisms (Six et al., 2000; Poirier et al., 2009) have been rapid. In spite of these advances, changes in management practices

such as tillage reduction or elimination of tillage have not consistently resulted in the expected increase in soil C storage (Angers et al., 1997; Dolan et al., 2006; Baker et al., 2007). Further, neither the direction nor magnitude of management practice effects on greenhouse gas emissions is consistent, though recent work suggests regional differences due to climate and soil texture (Almaraz et al., 2009; Johnson et al., 2010).

In light of both the greater contribution of belowground C inputs to SOC (Balesdent and Balabane, 1996; Rasse et al., 2005) and the increasing likelihood of aboveground C residue removal in agricultural ecosystems (Wilhelm et al., 2004; Wilhelm et al., 2010), interest in the fate of belowground C inputs to SOC is increasing. The greater recalcitrance and proximity to soil particles of root C than aboveground C are thought to be the primary reasons for greater root than shoot C contributions to SOC (Wander and Yang, 2000; Puget and Drinkwater, 2001). Management effects are more often detectable on a single process, such as soil respiration, than on the total soil C pool (Wander and Bidart, 2000). Nevertheless, studies of agricultural management effects on the contribution of root-derived C to total SOC (Allmaras et al., 2004; Wilts et al., 2004) are more common than studies of the root-derived C contribution to a single process such as soil respiration (Broadbent and Nakashima, 1974; Gale and Cambardella, 2000; Puget and Drinkwater, 2001). Understanding how management systems influence C retention or loss from individual sources, such as roots, can improve our ability to model soil C dynamics, which could be useful for improvement of land use management practices with respect to soil fertility, for C credit allocation, and more broadly for improvement of biogeochemical models.

Effects of agricultural management on root-derived N inputs are similarly not well studied. Recent research has focused on contributions of fertilizer-derived N to N_2O emissions; fertilizer-derived losses are thought to equal roughly 1% of fertilizer N added to the soil (IPCC, 2006). Emission factors (EF), the fraction of any given N source emitted, are useful for estimation of N_2O emissions in situations where sufficient field testing of N_2O rates is not feasible, but data on N application rates are available. As N_2O emissions are highly spatially and temporally variable, the data requirements for N_2O flux quantification are significant even over a small area of land (Parkin, 2008). In many locations, collecting the requisite number of samples needed for an accurate measurement of N_2O flux is not feasible. For this reason, determination of accurate EFs is needed for a broad range of ecosystems and management systems. Currently, a single IPCC EF is applied for fertilizer, manure, and aboveground residue N sources. Recent studies have suggested that the use of a single EF overestimates N emissions as N_2O from organic N sources including manure and aboveground residue (Gardner and Drinkwater, 2009; Delgado et al., 2010). Given that belowground sources of N, as well as C, contribute more to stable C and N pools than aboveground residue (Broadbent and Nakashima, 1974), it is likely that the EF for organic belowground sources of N is even lower than estimated for organic aboveground N sources.

Studies of agricultural management effects on total CO_2 and N_2O emissions from soils, in combination with studies of root-derived C and N retention in the soil form the basis for my research and hypotheses. However, it is unclear if management effects on root-derived CO_2 or N_2O emissions mimic effects on total CO_2 or N_2O emissions, or if effects on root-derived C emission as CO_2 mimic effects on root-derived C retention in

the soil. No prior field studies have quantified the effects of management practices on root-derived C or N emission as CO_2 or N_2O. Positive effects of rotation diversity (the number of crops grown in sequence, each for one growing season) on root-derived C inputs (Varvel, 2006) and retention in the soil (Bolinder et al., 1999) have been observed, while effects of tillage on root-derived C retention in the soil are inconsistent (Clapp et al., 2000; Allmaras et al., 2004). A laboratory study of hairy vetch roots indicated root C contributions to total CO_2 emissions were 30%, early in the growing season, with the contribution decreasing to 5% over a growing season (Puget and Drinkwater, 2001), but this measure does not include root exudate C, and includes no measure of management effects on this contribution. Similarly, as yet, laboratory incubations of root-amended soils exclude root exudate N (Lou et al., 2007), which can be a significant N source (Soon and Arshad, 2002). Therefore, losses of 24 and 43% of oat and rye root N respectively from the soil N pool (excluding exudate), over 112 days, may overestimate root N loss rates (Malpassi et al., 2000). To our knowledge a root-derived EF that accounts for root exudate N has not been quantified. Doing so, would further our ability to accurately model N_2O emissions given knowledge of N inputs by accounting for belowground sources of N and their contribution to total N loss as N_2O. Determining the effects of agricultural management practices on root-derived EFs for a common crop such as corn would further improve our ability to model N_2O emissions.

Spatial Patterns of Carbon and Nitrogen Cycling in Soils

State factors such as climate and topography also exert considerable control over global patterns of soil C storage and greenhouse gas emissions (Jenny, 1941; Yoo et al.,

2006). Historically, most studies of hillslope C dynamics have occurred in high relief landscapes where erosion is the primary driver of C transport downslope. In these landscapes, greater C storage downslope occurs due to C burial or organic matter binding to the fine textured particles also transported downslope by erosion (e.g., Stallard, 1998; Rosenbloom et al., 2006). However, most agricultural ecosystems are located in regions with low relief where C sinks generated by erosion are small; the disproportionate number of hillslope studies conducted in high relief locations has led to the overestimation of erosion induced C sinks in agricultural ecosystems (Van Oost et al., 2007). In low relief temperate landscapes such as the Prairie Pothole region of the Upper Midwest, USA, hydrology is a relatively more important control over C cycling rates than in locations where hillslope studies are more frequently conducted. However, most studies of hydrologic impacts of C storage in this landscape have been conducted in wetlands (e.g., Gorham, 1991). This gap exists in spite of clear recognition that mean annual precipitation can significantly influence soil C storage and C cycling rates (Guo et al., 2006).

The general patterns of how soil moisture influences soil C stocks as well as greenhouse gas emissions have been extensively studied (e.g., Post et al., 1982; Linn and Doran, 1984). As with most biological processes, an optimal moisture level exists: for aerobic soil processes such as soil respiration and nitrification this level is often between 50–70% water-filled pore space (WFPS) , for anaerobic processes the optimal level may increase up to 100% WFPS (Davidson et al., 2000). With changes in precipitation patterns anticipated due to climate change it becomes imperative to determine how soil C and N cycling will respond to changing soil moisture levels. Amounts of winter

precipitation in the Upper Midwest are expected to increase, but, offset by warmer temperatures and lower summer precipitation, average soil moisture in the region may decrease (Kling et al., 2003). These changes in climate will have consequences for both soil moisture and water table levels, and ultimately for soil C pools. Manipulations of soil moisture levels and water tables in other landscapes have shown that moisture can result in significant changes in rates of biologically-driven soil processes (Freeman et al., 1993; Mikha et al., 2005; Yurova et al., 2007).

The potential to detect management effects on a single C pool or process is much greater than the potential to detect changes on the entire soil C pool (Wander and Bidart, 2000). Past studies along hillslopes have indicated that the total C pool size and mean residence time (MRT) increase downslope (Sherrod et al., 2005) and with depth (Baisden et al., 2002; Harden et al., 2002). Wetland studies demonstrate the importance of saturated organic soils as a long term C sink and the potential of these soils to be a very large source of C under lowered water table conditions (Gorham, 1991; Freeman et al., 1993; Oechel et al., 1998; Blodau et al., 2004). High C storage in wetlands results primarily from slowed decomposition rates due to anaerobic conditions during saturation (Gorham, 1991). The same mechanism should apply in seasonally saturated mineral soils. However, due to the paucity of research in seasonally saturated mineral soil landscapes, it is unclear whether saturation conditions are an important driver of SOC pool dynamics along these hillslopes or with depth in the soil profile. Further work is needed to determine the applicability of the high slope soil C pool size and turnover patterns to low slope, seasonally saturated mineral soils, and to determine how the sensitivity of soil C pools to changing moisture varies along hillslopes.

Research Objectives

The goal of my research is to improve our understanding of both management and environmental effects on soil C and N cycling. My work has focused on agricultural soils of the Upper Midwest, USA due to their intense management and the potential consequences that management has for causing soil C and N loss with consequences for climate change and water quality in particular. My research addresses management effects on the loss rates of belowground inputs of C and N as the greenhouse gases CO_2 and N_2O. A second component of my research examines the effects of topography and hydrology on the size and turnover rate of soil C pools. In both components I use rates of soil respiration and denitrification to quantify losses from a specific C or N substrate, or from specific soil C or N pools. Additionally, my work contributes to our understanding of the relative importance of management versus select state factor effects on soil C and N pools and fluxes, and the effects of each on the coupling of C–N cycles in soil.

Specifically, through a field based experiment I determine agricultural management effects on the loss of root-derived C and N as CO_2 and N_2O. These are the first field-based estimates of root-derived C and N contributions to total CO_2 and N_2O emissions, and the first estimates of management effects on these loss rates. The results of this work are presented in Chapters 2 and 3. Through a laboratory based study in Chapter 4, I examine effects of hillslope position and soil moisture on the size and turnover rates of soil C pools. This research represents a first step toward understanding

the relative importance of topography and moisture as C mineralization controls in low slope, seasonally saturated mineral soils.

In Chapter 2, I examine the effects of a five agricultural management systems on corn root-derived C emissions as CO_2 in an Upper Midwest agricultural ecosystem. I employed stable isotope techniques to quantify root-derived C inputs (root biomass plus root exudates) and trace root-derived C emissions. This research tests three hypotheses: 1) that seasonal patterns, environmental drivers and management effects on root-derived C emissions would not differ from effects on total CO_2 emissions, 2) that root-derived C emissions, contributions to total C emissions, and turnover (defined as the fraction of initial root-derived C respired over the growing season) are greater from systems with high tillage and fertilization intensity and decreased crop rotation diversity, and 3) that organically fertilized systems exhibit higher root-derived C losses, but lower root-derived contributions to C emissions and turnover than inorganically fertilized systems.

In Chapter 3, I determine the EF for root-derived N across a range of agricultural management systems and examine the effects of management on root-derived C and N coupling. As part of the same experiment described in Chapter 2, I test the hypotheses that: 1) tillage and fertilization intensity would increase root-derived N emissions through effects of fertilizer quantity on corn root quality (C:N ratio), 2) increased tillage and decreased diversity would positively affect root-derived N emissions during non-fertilized rotation phases, 3) the EF would be smaller in organically fertilized systems, and 4) losses of root-derived C would be positively related to losses of root-derived N.

In Chapter 4, I build on the results of Chapters 2 and 3 to determine effects of hillslope position (topography) and soil moisture on soil C pool size and turnover rates.

The first objective of this research was to estimate both the patterns of C pool size and MRT along a low slope, seasonally saturated hillslope. The second objective was to quantify the effects of changing moisture conditions, ranging from near the wilting point to saturation, on these C pool sizes and MRTs throughout soil profiles along a hillslope. I used long-term laboratory-based soil incubations to test: 1) if patterns of soil C distribution and MRT along a low slope hillslope mimic patterns of higher C storage and longer C MRTs observed downslope in higher relief, more arid ecosystems, and 2) if the MRT of the labile C pool converges when exposed to a uniform moisture treatment, indicating dominance of moisture as a control on labile C turnover in this landscape.

Summary

Through my dissertation research I aim to further our understanding of agricultural management and environmental effects on the loss of C and N from specific soil C pools. I quantify the contribution of root-derived C and N to emissions of CO_2 and N_2O, and the coupling of root-derived C and N cycling across management systems. By measuring rates of root-derived C and N emission I addresses a gap in our understanding of both the importance of recent belowground C and N inputs as sources of CO_2 and N_2O, and contributes to the development of accurate N emission factors. Through the research in Chapter 4 I assess whether traditional erosion-based patterns of C distribution along hillslopes are upheld in low slope, seasonally saturated, landscapes. Further, I quantify the response of soil C pool sizes and turnover rates to changes in soil moisture. This research contributes to our understanding of how hillslope soil C

dynamics may change with increased precipitation resulting from climate change or land use.

October 17, 2011

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CHAPTER 2

Root-Derived Contributions to Soil Respiration as Influenced by Agricultural Management Systems

With Jennifer Y. King

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Agricultural management practices have the potential to significantly alter soil C storage through changes in C inputs and losses. Plant roots represent a significant, but poorly understood source of C inputs. Corn (*Zea mays* L.) root C was 13C-labeled in a field experiment to examine the effects of tillage and fertilization, crop rotation diversity, and fertilizer type on root-derived C cycling in Mollisols in the Upper Midwest. Daily and cumulative root-derived C emissions, their contribution to total soil respiration, and the turnover of root-derived C were quantified during the soybean [*Glycine max* (L.) Merr.] phase of crop rotations. Over two growing seasons, the average corn root-derived contribution to cumulative soil C emissions was 8.5%, and on average 35% of the initial root-derived C was respired. Environmental conditions were the primary control over daily root-derived C emissions, but management system effects on cumulative growing season emissions were evident. In both seasons organic fertilization led to lower cumulative root-derived emissions and root-derived contributions to cumulative C emissions than synthetic fertilization. In 2005, more intense tillage and synthetic fertilization positively affected cumulative root-derived C emissions and the root-derived fraction of cumulative soil C emissions (moldboard > chisel plow). Root-derived C turnover was negatively affected by rotation diversity in 2006 (2-yr > 4-yr rotation). We suggest that rotation diversity has an important influence on soil C storage and root-derived C turnover through effects on the quantity of root-derived C inputs.

ABBREVIATIONS

AICc	corrected Akaike's information criteria
C	carbon
CO_2	carbon dioxide
HPI	plots with broadcast high purchased inputs and moldboard plow
LPI	plots with banded low purchased inputs and chisel plow
LR	labeled root microplot
MLRA	major land use resource area
N	nitrogen
NH_4^+	ammonium
NO_3^-	nitrate
ORG	plots with organic inputs and moldboard plow
SOC	soil organic carbon
UR	unlabeled root microplot
VICMS	variable input crop and management system

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INTRODUCTION

The potential to increase carbon (C) storage in agricultural soils through selection of management practices that reduce C loss rates has garnered significant scientific and political attention. Soils contain the largest terrestrial reservoir of C (Jobbagy and Jackson, 2000); this can be enhanced either through increased C inputs or decreased C losses. As a mitigation strategy, more emphasis is frequently placed on reducing C losses than increasing inputs, due in part to the increasing number of uses for aboveground plant material (e.g., cellulosic ethanol production).

Interest in the C storage potential of agricultural soils, and the potential for increased harvesting of aboveground C sources, necessitates a better understanding of the dynamics of belowground C inputs. In annual cropping systems, due to slower decomposition, root-derived C (detrital root biomass and more labile root exudates) contributes nearly twice as much C to stable soil organic carbon (SOC) pools as does aboveground residue (Rasse et al., 2005). Slower decomposition of root-derived C may reflect: 1) greater chemical recalcitrance, and 2) greater chemical and physical protection through increased mineral association and occlusion in particulate organic matter fractions (Wander and Yang, 2000; Puget and Drinkwater, 2001; Rasse et al., 2005).

Due to the longer mean residence time of root-derived than shoot-derived C inputs to the soil, others have emphasized the need to determine the controls on detrital root decomposition (Johnson et al., 2007). Studies of management practice effects on the storage and turnover of root-derived C typically measure or model contributions to soil C pools (Allmaras et al., 2004; Wilts et al., 2004). Yet management effects are more

likely to be detected on fractions of soil C (Wander and Bidart, 2000), or on a single process such as soil respiration, than on the total soil C pool. Very few studies of root-derived C contributions to soil respiration exist, and these have not compared management practice effects (Broadbent and Nakashima, 1974; Gale and Cambardella, 2000; Puget and Drinkwater, 2001). Past studies of specific management practice effects on soil respiration, SOC content, and root C contributions to SOC may provide an indication of effects on root-derived C emissions. However, there is insufficient evidence to determine whether management practice (tillage, fertilization, crop rotation, and form of nutrient addition) effects on root-derived C emissions parallel effects on total soil C emissions.

Tillage affects soil respiration primarily through changes to C availability and the decomposition environment. Positive effects of tillage on soil C emissions (e.g., Ussiri and Lal, 2009) are attributed to: tillage induced increases in SOC availability and aeration (Balesdent et al., 2000), decreased fungal hyphae stabilization of aggregates (Beare et al., 1997), and short-term tillage induced pulses of carbon dioxide (CO_2) emission (Reicosky et al., 2002). Neutral or negative effects of tillage on soil respiration may result from differences in the decomposition conditions for residue within the soil profile; with chisel plowing, residue remains primarily in the upper 10 cm of soil, whereas the majority of residue is buried between 10 and 20 cm depth with moldboard plowing (Allmaras et al., 1996). Additionally, regional differences in tillage effects are suggested by the number of studies conducted in the central and northeastern U.S. or southeastern Canada which have found neutral or negative effects of tillage on

cumulative growing season soil CO_2 emissions (Almaraz et al., 2009; Johnson et al., 2010), or whole profile SOC content (Angers et al., 1997; Dolan et al., 2006).

Both tillage and fertilization can influence root-derived C input quantity, quality, and retention. Isolated studies suggest positive effects of tillage (Clapp et al., 2000; Allmaras et al., 2004) and nitrogen (N) fertilization on root-derived C input quantity (Poirier et al., 2009), and quality (lower C:N) (Russell et al., 2009). Effects of tillage on root-derived contributions to SOC differed by root input estimation method; using literature based root:shoot ratios, Clapp et al. (2000) found positive effects of tillage on root-derived contributions to SOC, while isotope based calculations for the same study led Allmaras et al. (2004) to reach the opposite conclusion. In a labeling study, where equal masses and $\delta^{13}C$ signatures of root and shoot inputs were assumed, no long-term tillage effect was found on root-derived C retention (Wander and Yang, 2000).

The relative strength of tillage effects on overall soil C cycling appears greater than fertilization effects, and there is no strong evidence of tillage and fertilization interactions (Moran et al., 2005; Poirier et al., 2009). However, tillage effects on root-derived C cycling are unclear, and fertilization effects are not known; effects of both are likely to differ for soil versus root-derived respiration. The absence of N fertilization effects on SOC content suggests that added N both increases input quantity and the decomposition rate of recent residue (Dalal et al., 2007; Poirier et al., 2009). Short-term increases in recent residue decomposition rates are frequently offset by negative effects of fertilization on cumulative residue decomposition (Berg and Matzner, 1997; Moran et al., 2005), an idea supported by the lack of N fertilization effects on cumulative growing season soil respiration (Reicosky et al., 2002). These results highlight the differing

temporal patterns in fertilization effects on soil versus root-derived respiration rates and suggest that soils with greater N availability could have greater long-term retention of root-derived C (Moran et al., 2005).

The strongest tillage effects on soil respiration are evident at hourly or daily scales; in contrast crop rotation effects are most evident over longer time periods, such as a growing season (Omonode et al., 2007). While shifts from continuous corn to a corn–soybean rotation result in lower soil respiration (Omonode et al., 2007), effects of more diverse rotation systems on soil respiration are not well studied. Increased SOC content, as a result of enhanced rotation diversity (greater than two crops) (Kuratomi, 2003; Franzluebbers and Follett, 2005), suggests enhanced rotation diversity should similarly decrease soil respiration. The suggested mechanisms driving a positive effect of crop rotation diversity on SOC are reduced pest abundance (Dick, 1992), greater microbial biomass and fungal:bacterial ratios (Six et al., 2006), increased diversity or complementarity of water and nutrient demands, and increased diversity of rooting depths and residue decomposition rates (Grant et al., 2002). Limited evidence suggests that effects of rotation diversity on corn root-derived C retention (based on doubled root:shoot ratios) appear to mimic effects on SOC, with greater retention in 4-yr than 2-yr rotations or corn monoculture systems (Bolinder et al., 1999; Gregorich et al., 2001). The relative importance of rotation, tillage, and fertilization on both daily and cumulative root-derived respiration are unknown.

Our research addresses several knowledge gaps regarding management system effects on root-derived respiration and turnover. To address these gaps we used stable isotope techniques to calculate corn root-derived C inputs (detrital root biomass and root

exudates) and to examine the effects of management systems, varying in tillage and fertilization intensity, crop rotation diversity, and form of nutrient input, on cycling of root-derived C in an Upper Midwest agricultural ecosystem. Specifically, we measured daily and cumulative root-derived and total C emissions as CO_2, the contribution of root-derived C to daily and cumulative soil C emissions, and the turnover of root-derived C over the growing season (the fraction of initial root-derived C respired). Though no fertilizer was added during the period of respiration measurements, residual effects of fertilizer type (organic versus synthetic) on soil N availability were expected. Organic N loss rates are less than those of synthetic N (Gregorich et al., 2005), and soil respiration rates with organic fertilization can be significantly higher than with synthetic fertilization (Alluvione et al., 2010; Heller et al., 2010).

This research tests the hypotheses that: (1) seasonal patterns, environmental drivers, and management effects on root-derived C emissions mimic patterns and effects on total C emissions, (2) root-derived C emissions, contributions to total C emissions, and turnover are greater from systems with high tillage and fertilization intensity and decreased crop rotation diversity, and (3) organically fertilized systems, with high inputs of non-root C, have higher root-derived C emissions, but lower root-derived contributions to C emissions and turnover, than inorganically fertilized systems.

MATERIALS AND METHODS

Research Site

Research plots at the University of Minnesota's Southwest Research and Outreach Center (44°14' N, 95°19' W) have been under continuous management since 1989 as part of the variable input crop management system plots (VICMS) (Porter et al., 2006). This long-term history makes it likely that rates of soil C cycling reflect steady state process rates for these management systems. Variation in elevation and soil series at our study site are typical of the major land resource area 103 (MLRA 103), which spans south-central Minnesota and north-central Iowa. As of 2006, 80% of MLRA 103 was cropland (USDA, 2006), and in 2008, farmland in these two states represented 26% and 23% of total planted corn and soybean land area nationally (Economic Research Service, 2010). Three soil series extend across the 3.6 m elevation gradient. Ves loam (mesic Calcic Hapludolls) and Normania loam soils (mesic Aquic Hapludolls) are found at higher elevations, and Webster silty clay loam soils (mesic Typic Endoaquolls) are found at lower elevations. Soils in all plots were Ves–Normania intergrades with the exception of three plots measured in 2006 dominated by Webster soils.

Treatments and Experimental Design

The five management systems included in this study ranged in tillage type and frequency, crop rotation diversity, and fertilizer type (Table 2.1). Tillage co-varied with fertilization amount only during the corn phase of crop rotations. *HPI* refers to high tillage–high fertilization practices (moldboard plow), and *LPI* refers to low tillage–low fertilization practices (chisel plow). During the soybean phase of crop rotations, when all

respiration measurements were collected, there was no fertilizer application (as recommended by the University of Minnesota Soil Testing Service). We expected minimal residual differences in soil N availability based on the previous season's fertilization amount (HPI versus LPI); a test of this assumption is presented with the results. Two year crop rotations included corn and soybean; four year rotations included corn (*Zea mays* L.), soybean (*Glycine max* (L.) Merr.), oats (*Avena sativa* L.) interseeded with alfalfa (*Medicago sativa* L.), and alfalfa. Fertilizer inputs to the corn rotation were either synthetic (HPI and LPI) or organic (beef manure) additions (*ORG*); systems with organic fertilizer also received moldboard tillage. Both 2- and 4-yr rotations of HPI and LPI were included in the study, but only 4-yr organic rotations were included as 2-yr rotations do not meet organic certification criteria.

Root-derived C cycling was measured in two seasons (2004 and 2005) on two separate sets of 15 plots (30 x 55 m) within a single field (386 x 375 m). All plots selected for the study were planted with corn during isotope labeling and planted with soybeans the following growing season when root-derived C emissions were measured. In each season, one plot representing each management system was selected per block; three blocks were arranged perpendicular to the slope to capture topographic and soil variation at the site. Paired microplots within each plot contained either ^{13}C labeled (*LR*, 1.0 m^2), or unlabeled corn root-derived material (*UR*, 1.2 m^2). Due to the microplot size, we simulated fall primary tillage practices to incorporate unlabeled corn shoot residue after the corn harvest; detrital roots remained *in situ*. In systems that normally received moldboard plowing (HPI and ORG), shoot residue was incorporated with shovels by distributing it on the surface and inverting the upper 20 cm of soil. In systems normally

chisel plowed (LPI), shoot residue was incorporated throughout the upper 20 cm of soil. Throughout the soybean growing season, cultivation was completed with a small tiller.

Isotope Labeling

Live corn plants were pulse-labeled between five and six times with $^{13}C–CO_2$ during the vegetative phase of development following Wander and Yang (2000). Plants were covered with a transparent tent into which ^{13}C-enriched CO_2 (99-atom %) was introduced. Labeling was done on clear, sunny days, and timed to coincide with periods of high photosynthetic activity in order to maximize $^{13}C–CO_2$ uptake. Tent CO_2 concentrations and temperature were monitored to track CO_2 uptake and ensure that temperature did not greatly limit CO_2 uptake.

Root material was heavily labeled with ^{13}C in both growing seasons. Root samples were analyzed on an elemental analyzer (Fisons NA 1500, Thermo Scientific, Waltham, MA) coupled with a continuous flow isotope ratio mass spectrometer (Fisons Optima, Manchester, UK). The average labeled root $\delta^{13}C$ value was 144.1 ± 11.8‰ while the average unlabeled root $\delta^{13}C$ value was -11.6 ± 0.2‰ in 2005. In 2006, the average labeled root $\delta^{13}C$ value was 237.8 ± 26.9‰ while the average unlabeled root $\delta^{13}C$ value was -11.9 ± 0.3‰ (D. Allan, *personal communication*, 2010).

A major challenge in any study of root-derived C dynamics is quantification of the inputs (both detrital root C and C from exudates). In this study, roots were left in place after labeling to mimic the normal decomposition conditions for each management system (location within the soil profile, root density, etc.). Soil core samples were collected just prior to fall tillage and shoot residue incorporation in 2004 and 2005. Root

C estimates based on root picking from core samples (root C per soil volume) were 58% of root-derived C calculated from $\delta^{13}C$ values of the root biomass and the soil in each microplot. The isotope-based estimate is likely larger and more accurate due to inclusion of fine-root biomass and exudate C. Both fine roots and exudates are missed by root-picking techniques or root:shoot ratios, although estimates based on the latter are often doubled to account for root exudate C (Rasse et al., 2005). The fraction of root-derived C in the soil of each plot in each spring was estimated by combining two mixing models (e.g., Puget and Drinkwater, 2001), one developed for each microplot (LR and UR). The amount of root-derived C in the soil (0–30 cm) was based on a weighted average of the root-derived C calculated for 0–10 and 10–30 cm:

$$F_{root-C} = (\delta^{13}C_{soil-LR} - \delta^{13}C_{soil-UR}) / (\delta^{13}C_{root-LR} - \delta^{13}C_{root-UR}),$$
$$C_{root} = (F_{root-C} * C_{soil}), \qquad \text{(Equation 2.1)}$$

where F_{root-C} represents the fraction of root-derived C in the total SOC pool, and $\delta^{13}C$ is the isotope value of the bulk soil ($\delta^{13}C_{soil}$), or of the root biomass ($\delta^{13}C_{root}$), in the LR or UR microplots. Labeled root-derived C is assumed to be the only source of differences in $\delta^{13}C$ between the LR and UR microplots. The F_{root-C} numerator is necessarily smaller than the denominator as the impact of root C signatures on the signature of the total SOC pool is small relative to the difference between labeled and unlabeled root-derived C. Hereafter, the subscript "root" is used to identify root-derived C. The root-derived C in each LR microplot, C_{root} (g C/cm^3), is the product of F_{root-C} and the total SOC content, C_{soil} (g C/cm^3).

Gas Flux Sampling and Analysis

Soil C emissions were measured at two to four week intervals throughout the growing seasons of 2005 (n=7) and 2006 (n=6) using vented static stainless steel chambers. Soil respiration rate was measured in four chambers within each of 15 plots (5 management systems, 3 blocks): two chambers in the LR microplots and two in the UR microplots on each sampling date. Chamber bases were located side-by-side covering the area between two rows of soybeans, but not covering any aboveground plant material. Bases measured 29 x 49.5 x 9 cm (l x w x h), and chamber tops measured 29 x 49.5 x 10 cm. The average height of each chamber base side (soil surface to upper edge) was determined on each date in order to accurately calculate chamber headspace volume. To ensure a tight seal, weather stripping lined the chamber edges and metal clips held the top and base together. Tops were covered with reflective insulation to minimize heating and were vented to maintain atmospheric pressure. To increase the chamber volume sampled, the sampling port was connected to four, 13 cm long, 0.32 cm OD pieces of tubing arrayed across the inside of chamber tops.

Five headspace samples were collected at 10 minute intervals over a 40 minute period. Fifteen mL samples were injected into 9 mL glass vials capped with butyl rubber septa. Over-pressurizing reduced the possibility of leakage into sample vials before analysis. Samples were analyzed for CO_2 concentration on a gas chromatograph fitted with a thermal conductivity detector (HP 8580, Foster City, CA) and equipped with a headspace autosampler (Model 7000/7050, Teledyne Tekmar, Mason, OH). Three gas standards were used for calibration (Scott Specialty Gas, Plumsteadville, PA). The air temperature at each plot and the volume of each chamber were used to calculate the

headspace gas concentration at the time each vial was collected. Flux rates were determined from the regression slope of the headspace gas concentrations over time. On occasion, high CO_2 concentrations at the final time points created a non-linear regression (the result of altered diffusion rates). In these cases, the final time points were not included in the flux calculation. Cumulative respiration was calculated using linear interpolation between sampling dates.

On each sampling date, air temperature, soil temperature, moisture, and nitrate (NO_3^-) and ammonium (NH_4^+) concentrations were measured with respiration rates. Three air and soil temperature measurements were averaged for each plot (thermocouple probes, 10 cm above and 10 cm below the soil surface). Similarly, three soil samples (0–10 cm) from each plot were homogenized and sub-sampled for gravimetric soil moisture (105°C, 48 hours) and inorganic N analyses. Inorganic N concentrations were determined by extraction in a 2M KCl solution and flow injection colorimetric analysis (Quickchem 8000, Lachat, Loveland, CO). Soil data measured by collaborators includes bulk density (J. Strock, *personal communication,* 2006), SOC, total soil nitrogen (TN), and both root biomass and soil ^{13}C data (D. Allan, *personal communication,* 2010).

Determination of Residue-Derived Respiration

Headspace samples for isotopic analysis were collected from one chamber in each LR and UR microplot using custom-made 170 mL glass flasks (Chemglass, Vineland, NJ), at least three samples per chamber. Samples were injected through an inlet system (Mass Spec Solutions, Manchester, UK) to separate and preconcentrate the samples before introduction to the continuous flow isotope ratio mass spectrometer.

Three NOAA/CMDL–calibrated standards analyzed by the Stable Isotope Laboratory at the University of Colorado's Institute of Arctic and Alpine Research (Boulder, CO) were used to determine the $\delta^{13}C$ values for samples relative to the international standard, VPDB (Trolier et al., 1996). The standard tank CO_2 concentrations were 350, 500, and 1000 μL/L with $\delta^{13}C$ values of -8.5, -14.2, and -25.2‰.

Calculation of the daily root-derived contribution to soil respiration followed the methods of Puget and Drinkwater (2001). The CO_2 concentration in each flask was determined based on its collection time and chamber flux rate, and the estimated $\delta^{13}C$ of substrates respired were determined using Keeling plots (Phillips and Gregg, 2001). As with Equation 2.1, two mixing models, one per microplot, were combined to calculate the root-derived fraction of C emitted and the root-derived C emission rate from each plot:

$$F_{root} = (\delta^{13}C_{CO_2-LR} - \delta^{13}C_{CO_2-UR}) \ / \ (\delta^{13}C_{root-LR} - \delta^{13}C_{root-UR}),$$
$$R_{root} = (F * R_{total}), \qquad \text{(Equation 2.2)}$$

where $F_{root\text{-}R}$ is the contribution of root-derived C to total C respired, and $\delta^{13}C$ is the isotopic value of the substrate respired ($\delta^{13}C_{CO2}$), or the root biomass ($\delta^{13}C_{root}$), in the LR and UR microplots. R_{total} is the soil respiration rate from each LR microplot (g $C/m^2/d$), and R_{root} is the root-derived respiration rate from each plot (g $C_{root}/m^2/d$). The root-derived fraction of cumulative growing season C emissions is the quotient of each plot's cumulative root-derived emissions and cumulative total C emissions.

Data Analysis

Two statistical approaches are presented to facilitate comparison of our results with studies that do not include multivariate analysis and to determine effects of both management and environmental controls. Results based on linear mixed effects models with management system as the only main effect, are hereafter referred to as "main effect models." Results of a second approach including covariates are hereafter referred to as "multivariate models." In the latter, management system was included as a main fixed effect, and soil temperature, moisture, SOC content, and inorganic N concentration were included as covariates. Plot soil temperature and moisture data, not the site meteorological data (Table 2.2), were used in all models. Soil NO_3^- data were log transformed for both seasons, as were the 2006 soil NH_4^+ data. All respiration rate data were square root transformed. Figures and tables show non-transformed data, while statistical results are based on transformed data. The best fit model was identified through stepwise simplification using Akaike information criterion values corrected for small sample sizes (AICc) (Johnson and Omland, 2004). Covariates were not included in models if significant covariate correlation was found (variance inflation factor>3).

In all models, specific one-sided a priori contrasts were defined to compare management system characteristics: 1) tillage and fertilization intensity (HPI versus LPI), 2) crop rotation diversity (2- versus 4-yr rotations, 4ORG excluded), and 3) fertilizer type (4HPI and 4LPI versus 4ORG). Contrast results were Bonferroni corrected. Block was included as a random effect in all models to account for spatial variation in soil characteristics. To address temporal pseudoreplication (repeated sampling of plots), daily models included a random effect term of plot nested within

block on each date. To avoid inaccuracies of spatial pseudoreplication, respiration rates were calculated for each plot (average of chambers with flux rate slope R^2>0.85) (Hurlbert, 1984).

In 2005, soil respiration was measured in all plots on all dates, but root-derived respiration was only measured in all plots beginning mid-season. To estimate cumulative full-season root-derived respiration for 2005, it was necessary to extrapolate based on late season measurements. A strong linear relationship between the 2006 cumulative full and cumulative late season root-derived respiration for each plot (R^2=0.85) indicated that application of the 2006 late:full season ratio to data from the same late season date range in 2005 should result in reasonable full season estimates for each plot, as long as seasonal patterns were similar. A strong correlation (R^2=0.99) between the running cumulative amounts of residue-derived C on five dates in each season confirmed the similarity of seasonal root-derived respiration rate patterns. The late:full season relationship from 2006 was then used to scale the late 2005 season cumulative root-derived C emissions for an estimate of cumulative full season root-derived emissions.

Statistical analyses were completed using R (version 2.10.1, R Development Core Team, 2009) with the lme4 (Bates and Maechler, 2009), languageR (Baayen, 2009), and HH packages (Heiberger, 2009) for analysis of mixed effects models. AICc values and variance inflation factors were determined with the MuMIn (Barton, 2009) and car packages (Fox, 2009). Differences were considered significant when $p \leq 0.05$, and marginally significant when p-values were between 0.1 and 0.05. Agronomically interesting, but not statistically significant, trends reflect values of $p \leq 0.2$.

RESULTS

Climate and Site Environmental Data

Over both seasons, the average soil temperature (at 10 cm) was 25.1 ± 0.4°C, and the average soil moisture (upper 10 cm) was 20.0 ± 0.3%. Based on these field measurements, 2005 was a wetter and marginally warmer season than 2006. Compared to the 44 year historical site averages, maximum and minimum soil temperatures at 10 cm were comparable, but annual precipitation was higher than average in both seasons (Table 2, Southwest Research and Outreach Center, 2009). Soil NH_4^+ concentrations were higher in 2005 (0.96 ± 0.05 μg N-NH_4^+/g soil) than in 2006 (0.57 ± 0.04 μg N-NH_4^+/g soil). Soil NO_3^- concentrations did not differ between seasons (mean = 5.16 ± 0.28 μg N-NO_3^-/g soil).

As all soils in the 2005 plots were an intergrade between Ves and Normania series, comparisons among soil series were made only in 2006. Webster soils had higher soil moisture, higher SOC content (0–30 cm), and lower bulk density than Ves-Normania soils. However, due to a greater range in SOC among plots in 2006 (6.2–13.7 kg C/m^2) than 2005 (8.3–10.5 kg C/m^2), SOC content did not differ between seasons.

Management had only minimal effect on non-C soil properties. No effect of management was found on seasonal patterns or season average soil temperature or moisture in either season. Bulk density was marginally higher in 2-yr than 4-yr rotations in 2005, but a similar effect was not observed in 2006. There was no effect of tillage and fertilization or rotation diversity on soil NH_4^+ in either season and no effect on soil NO_3^-

in 2005. In 2006, soil NO_3^- was actually marginally higher in low- than high fertilization systems (LPI>HPI). Total soil N was also higher in LPI than HPI systems in both seasons; these soil N data support our assumption that the amount of N added during corn development had no residual effect on soil N availability during the period of respiration measurements. Thus, for both daily and cumulative root-derived respiration, the contrast between HPI and LPI systems is effectively a test of tillage effects. For effects on SOC, root-derived C inputs, and root-derived C turnover, we cannot distinguish between fertilizer and tillage effects; in these cases, management intensity is used to refer to the joint tillage–fertilization practices. Total soil N was also higher in more diverse rotation systems in 2005 and showed a similar trend in 2006. The fertilizer type applied to developing corn affected soil N availability in both seasons; NO_3^- levels were higher in 4ORG than 4HPI or 4LPI systems.

Root-Derived Carbon Inputs and Root-Derived Soil Organic Carbon

Management system effects on SOC content were stronger than effects on root-derived C inputs (Table 2.3). Root-derived C inputs were marginally greater in high diversity rotations in 2006 (4-yr>2-yr), and a similar trend was evident in 2005. More intense practices reduced SOC content in 2005 and marginally reduced it in 2006. High diversity crop rotations had marginally greater SOC content than low diversity rotations in 2005. Surprisingly, SOC content did not differ between systems receiving organic (4ORG) and synthetic inputs (4HPI, 4LPI). In 2006, likely due to management intensity effects on SOC, the root-derived contribution to SOC was marginally greater in more intense systems and in systems with synthetic rather than organic inputs.

Daily Soil and Root-Derived Respiration Rates

Daily soil respiration rates showed stronger environmental than management effects in both seasons. Both the main effect and multivariate models indicated no effect of management system on soil respiration rates (Fig. 2.1a, 2.1b). The best fit models for 2005 and 2006 indicated a positive relationship with soil temperature. Additionally, the 2005 model included a negative relationship with NH_4^+ (model R^2=0.56), while the 2006 model included a negative relationship with soil temperature squared (model R^2=0.75).

Daily rates of root-derived respiration again indicated an important effect of soil temperature and generally weak management system effects (Fig. 2.1c, 2.1d). Root-derived respiration showed a trend of higher rates in high tillage systems in 2005 and in low diversity rotations in 2006. Also in 2006, synthetic fertilization resulted in higher rates of root-derived respiration than organic fertilization (4HPI, 4LPI>4ORG). Multivariate analysis of the 2005 root-derived respiration rate data was not done due to the short sampling period. In 2006, the best fit model indicated soil temperature was positively related to root-derived respiration rates (R^2=0.56); rates were high through late June, then decreased consistently through the season.

The root-derived contribution to daily rates of soil C emissions in 2005 was marginally higher in high-tillage than low-tillage systems; there were no management effects in 2006. Though not significant, this contribution was consistently low in the 4ORG and 2LPI systems in both seasons. Based on the best fit model for 2006, soil NO_3^- was negatively related to the root-derived contribution to soil respiration (R^2=0.38).

Between growing seasons, seasonal patterns of both soil and root-derived respiration rates were very similar (Fig. 2.1a, 2.1b). Early in 2005, CO_2 samples from only a subset of the 15 plots (n=3) were analyzed isotopically, including one replicate of each 4-yr management system. These data are shown (Fig. 2.1a) as an indication of early season patterns, but were not included in subsequent figures or statistical analyses. In each season, the timing of peak root-derived respiration rates was much earlier than peak rates of total soil respiration. Compared to the 2005 season, the longer 2006 sampling period made it possible to discern seasonal patterns of root-derived respiration rates by management system and the root-derived contribution to respiration (Fig. 2.1c–2.1f). In late May (pre-planting), the root-derived contribution to soil C emissions was 13.4 ± 3.4% (n=3, data not shown) in 2005 and 12.8 ± 1.3% (n=15) in 2006. Through August, the root-derived contribution to C emissions decreased; as soybeans senesced (August–September) root-derived contributions increased briefly in both seasons (Fig. 2.1e, 2.1f).

Management System Effects on Cumulative Respiration

Cumulative soil respiration showed very little effect of management system (Fig. 2.2a), but showed clear effects of environmental conditions. The main effect model indicated no significant management system effect on the cumulative amount of soil C respired in 2005, and only a marginal negative effect of rotation diversity in 2006 (Table 2.4). Cumulative soil C emissions were positively related to soil temperature in 2005 (R^2=0.21), and were negatively related to soil moisture in 2006 (R^2=0.69). The difference between seasons could reflect greater overlap between major precipitation events and periods of high soil respiration in 2006 than 2005 (Table 2.2).

Both cumulative root-derived C emissions and the root-derived fraction of cumulative C emissions showed strong management system effects (Fig. 2.2b, 2.2c). Cumulative root-derived C emissions were 27 and 25 g C_{root}/m^2 in the 2005 and 2006 growing seasons. These cumulative emissions represent root-derived contributions to cumulative C emissions of 9.0 and 7.9%. With the exception of the 4HPI system, both measurements showed similar management system patterns between the two seasons: each was consistently high in the 2HPI system, intermediate in both LPI systems, and low in the 4ORG system. The main effect model was the best model for each measurement in both seasons. Tillage intensity was positively related to both cumulative root-derived C emissions and the root-derived fraction of cumulative respiration in 2005 (Table 2.4). Rotation diversity had a marginal negative effect on cumulative root-derived C emissions in 2006, but had no effect on the root-derived contribution, likely due to the parallel marginal negative rotation effect on total C emissions. In both seasons, the strongest management effect was due to fertilizer type, with greater root-derived emissions and root-derived contributions from synthetically rather than organically fertilized systems.

Root-Derived Carbon Turnover

The fraction of initial root-derived C emitted as CO_2 was similar in the two growing seasons, though the effects of management differed. Over 120 days, 22–43% of root-derived C inputs were emitted in 2005. Over 148 days, 27–62% of root-derived C inputs were emitted in 2006 (Fig. 2.2d). Though management system contrasts were not significant in 2005, management could not be removed from a model of the root-derived

fraction of C emitted without a significant reduction in the model fit. The main effect model was the best model in 2005 (R^2=0.26) and 2006 (R^2=0.62). In 2006, root-derived C turnover was lower in high diversity than low diversity rotation systems (Table 2.4).

DISCUSSION

Building on the importance of root-derived C as a source of SOC, the objective of this study was to further our understanding of management and environmental effects on both daily and cumulative corn root-derived C emissions. Seasonal patterns suggest important differences between the temporal dynamics of corn root-derived and total soil respiration. Management effects, while weak on daily rates, influenced multiple indicators of root-derived C cycling, though the effects of specific management practices differed among indicators and seasons. Interestingly, our results indicate stronger effects of management systems on root-derived than total soil respiration, and stronger environmental than management system effects on cumulative soil respiration rates. Potential sources of variability in the magnitude of root-derived C turnover are discussed.

Seasonal Patterns of Root-Derived Carbon Cycling

Seasonal patterns of root-derived respiration do not mimic patterns of total soil respiration. There are two likely factors contributing to the earlier peak in root-derived, rather than total, respiration rates. First, though temperature likely limited root-derived respiration rates early in the season (regression slopes were highest early in the season,

data not shown), through most of the season rates were likely limited by the availability of root-derived C. The C content of the free-light fraction (pool of partially decomposed, non-occluded C) is strongly correlated with soil respiration (Gregorich et al., 2006). Averaged over both growing seasons, the root-derived contribution to free-light fraction C decreased from 21% in the spring to 8.3% in the fall, indicating decreased root C availability (D. Allan, *personal communication*, 2010). Second, soil respiration rates (reflecting all heterotrophic and autotrophic respiration) were 2–8 times greater than rates of root-derived (heterotrophic) respiration. Rhizosphere respiration (live root plus associated heterotrophic respiration) can contribute substantially to total soil respiration; soybean rhizosphere respiration contributed 40–95% of the C respired in a greenhouse study (Sey et al., 2010). Rates of rhizosphere respiration have been shown to increase through the flowering stage of plant development (Fu et al., 2002). Thus, the later peak in total soil respiration than root-derived respiration likely reflects both increased C availability and the mid-season peak in rhizosphere respiration rates.

The timing differences in peak root-derived and total soil respiration rates contributed to two seasonal patterns that appear characteristic of the root-derived contributions to soil respiration. First, the contribution decreases over the growing season, due to both decreasing root-derived respiration rates and a later peak in total soil respiration rates. In this study, root-derived C represented a small and decreasing fraction of respired C, approximately 8.5% over each season, decreasing from 12–14% in the spring to 5–10% by senescence. In a comparable study, the hairy vetch root-derived contribution to soil respiration decreased roughly from 30 to 5% over a growing season (Puget and Drinkwater, 2001). Second, there is a spike in the root-derived

fraction of soil respiration late in each growing season, likely related to plant senescence and rapidly declining rates of live root respiration; a similarly timed increase was observed by Puget and Drinkwater (2001). The spike was not a function of tillage as measurements were made before fall tillage; the spike also appears short-lived based on 2006 data.

Management Effects on Root-Derived Carbon Cycling

The joint effects of management on C inputs and decay rates may explain the consistent rotation and inconsistent tillage effects on root-derived C cycling observed across root-derived C cycling studies. Similar to the results of this study, others have shown positive rotation diversity effects on root C input quantity (Varvel, 2006) and root-derived C retention (Bolinder et al., 1999; Gregorich et al., 2001). Studies of tillage have shown inconsistent effects on root-derived C inputs (e.g., Qin et al., 2005) and retention in the soil (Clapp et al., 2000; Wander and Yang, 2000; Allmaras et al., 2004). In this study, though both management intensity (in 2005) and synthetic fertilization (in both seasons) showed positive effects on cumulative root-derived respiration, neither affected C input quantity, and ultimately neither influenced root C turnover. In contrast, a marginal negative rotation diversity effect on root-derived respiration in 2006 was augmented by a marginal positive effect of rotation diversity on root-derived C inputs, resulting in a strong negative rotation diversity effect on root C turnover. These results indicate that management systems only affected root C turnover when the effects on C input quantity and decay rate were complementary.

Though there was no effect of fertilizer type on root-derived C turnover, there was a negative effect of organic fertilizer on root-derived respiration. Similarly, Kong and Six (2010) found greater root-derived contributions to SOC in organically fertilized systems than synthetically fertilized systems. We observed that root-derived C represented a smaller fraction of the total SOC pool in organic systems in 2006; this could explain the lower root-derived C emissions and lower root-derived contribution to total C emissions than was measured in synthetically fertilized systems. Over the long-term, lower losses of root-derived C could result in greater root-derived contributions to SOC pools in organically fertilized systems.

Contrasting Environmental and Management Control of Root-Derived Carbon Cycling

Comparison of the main effect and multivariate models helped determine the relative strength and interactions between environmental and management controls on C cycling. Our results indicate, surprisingly, that environmental controls over cumulative total soil respiration exceeded management system effects. Soil temperature and, to a lesser degree, soil moisture were related to daily rates of root-derived and total soil respiration as well as to cumulative total soil respiration. In each of these models, comparison of AICc values indicated that there were no significant reductions in the model fits when management system was removed. Importantly, for the interpretation of this result, management system did not significantly influence seasonal patterns, or the average season soil temperature or moisture at this site.

Unlike cumulative soil respiration, cumulative root-derived respiration was better explained by management system effects than by environmental conditions. Studies

partitioning autotrophic and heterotrophic respiration indicate a greater dependence of autotrophic respiration on plant biomass and gross primary productivity, whereas heterotrophic respiration is more dependent on the quantity and C:N ratio of soil organic matter; temperature influences both pathways (Bond-Lamberty et al., 2004). In our study, cumulative soil respiration, a large fraction of which was likely autotrophic respiration, was best fit by models including environmental conditions, and management had weak effects on root-derived C inputs, indicative of effects on plant biomass. In contrast, cumulative root-derived respiration (one source of heterotrophic respiration) was best fit by models including management system, and management did influence SOC and TN content, as well as root C:N ratios (lower in more intensely managed systems, data not shown). Thus, differences between management effects on root-derived and total soil respiration appear consistent with the presence or absence of management effects on the underlying controls of heterotrophic and autotrophic respiration.

Factors Influencing the Magnitude of Root-Derived Carbon Turnover

Root-derived C cycling is likely to be influenced by both the quantity and quality of root C inputs; it is therefore likely to differ among crops. For example, early season contributions of hairy vetch root C to soil respiration (Puget and Drinkwater 2001) were nearly double the corn root contributions measured in this study. This difference could be a function of higher hairy vetch root quality, or greater vetch root contributions to SOC (6.5% versus 1% in this study), though the latter could also reflect higher SOC content at our study site. Over one growing season (5 months), we found that

approximately 35% of corn root-derived C present in the soil in early spring was respired. In laboratory incubations, 47% of labeled oat root-derived C was respired as $^{14}CO_2$ after 6 months (Gale and Cambardella, 2000), and 65% of barley root C was respired after 5 months (Broadbent and Nakashima, 1974); the latter study did not account for root-exudate C. To expand the comparison of root-derived C turnover to additional crops, the retention of corn root-derived C in the soil was estimated to be 38–80%, with an average of 65% over the two growing seasons. Other studies, using a variety of root C estimation methods, indicate a range of 49 to 80% root C retention for an assortment of crops (hairy vetch [*Vicia villosa* Roth], wheat [*Triticum aestivum* L.], pea [*Pisum sativum* L.], canola [*Brassica napus* L.], and corn) over a period of 5–6 months (Puget and Drinkwater, 2001; Soon and Arshad, 2002; Kong and Six, 2010). Though it appears that corn root-derived C turnover may be lower than that of higher quality crops (lower C:N), the large range in root C retention, or respiration, makes further investigation necessary.

Regional climate and soil texture differences could greatly influence the magnitude of root-derived C cycling. Given the differences in controls over root-derived and total soil respiration and the expected changes in climate, more studies such as this that help illustrate how specific C sources within the soil respond to both changes in management and changes in climate are needed. The strong temperature dependence of total soil respiration suggests that, particularly in the face of climate change, mitigating total soil respiration through management changes in this region may be hard to achieve, at least with the range of management practices evaluated here. As with tillage effects on SOC content, it is likely that regional differences in soil texture as well as climate will

significantly influence management effects on root-derived C cycling. While rotation diversity decreased root-derived C turnover in this study, high clay soils tend to be more retentive of residue C than sandy soils. This suggests that management effects on root-derived C inputs and cycling may be reduced in regions with less clay-rich soils (van Veen et al., 1989; Liang et al., 1998). Accordingly, greater regional understanding of management and environmental effects on both root-derived C inputs and emissions are needed to improve allocation systems for C credits.

Conclusions

By tracking the fate of ^{13}C-labeled root C, we found that approximately 8.5% of the cumulative soil C emissions over each growing season were root-derived and that on average 35% of root-derived C was respired over the growing season. Isotopic labeling enabled our identification of management system effects on multiple indicators of root-derived C cycling. Without specifically tracing the turnover of recent root C, we would have concluded that tillage, fertilization, rotation diversity, and fertilizer type effects were minimal, and that C cycling rates and cumulative C emissions at this site were primarily a function of soil temperature and moisture. Neither seasonal patterns nor management effects on root-derived respiration mimicked patterns of total soil respiration. Cumulative root-derived respiration, the root-derived fraction of cumulative respiration, and the turnover of root-derived C each showed some significant influence of management. Management intensity, likely due to tillage, enhanced root-derived C emissions and the root-derived fraction of total C emissions in 2005, but not 2006. While enhanced rotation diversity greatly reduced root-derived C turnover in 2006, the

same pattern was not evident in 2005. The rotation diversity effect appears to be largely a function of management effects on root C input quantity. The effects of management on input quantity and quality, as well as on C emissions, should be further examined and included in development of C credit systems. Future research on root-derived C cycling must incorporate management and environmental effects on root exudate C as well as root biomass C and in particular should focus on developing a greater mechanistic understanding of rotation diversity effects on root-derived C cycling.

Table 2.1 Management system components: rotation length, crop rotation order, fertilizer amount and type, and tillage type and frequency. Isotope labeling was completed during the corn rotation while gas sampling was completed during the following soybean rotation.

Management System	Rotation length	Rotation order	Fertilizer	Tillage Type and Frequency
	(yr)		(amount, type)[†]	
High Purchased Input (4HPI)	4	corn soybean oat/alfalfa alfalfa	high N,P,K	spring field cultivator (10 cm), fall moldboard plow (20 cm)[§]
High Purchased Input (2HPI)	2	corn soybean	high N,P,K	spring field cultivator (10 cm), fall moldboard plow (20 cm)
Low Purchased Input (4LPI)	4	corn soybean oat/alfalfa alfalfa	low N,P,K	spring field cultivator (10 cm), mid-season cultivator, 2 times (8 cm), fall chisel plow (20 cm)[‡§]
Low Purchased Input (2LPI)	2	corn soybean	low N,P,K	spring field cultivator (10 cm), mid-season cultivator, 2 times (8 cm), fall chisel plow (20 cm)[‡]
Organic (4ORG)	4	corn soybean oat/alfalfa alfalfa	manure	spring disk tillage & field cultivator (10 cm), early season rotary hoe (2.5 cm), mid-season cultivator, 2 times (8 cm), fall moldboard plow (20 cm)[§]

[†] No fertilization during the soybean phase of the rotations. Fertilization of corn was based on 10% higher yield goals for HPI than LPI systems; fertilization in LPI systems was based on more realistic yield goals. Fertilizer was broadcast in the HPI system and banded in the LPI system.

[‡] No fall tillage in 2LPI or 4LPI following the soybean crop.

[§] No fall tillage in 4-yr rotations following the oat/alfalfa growing season.

Table 2.2 Monthly precipitation totals and maximum and minimum soil temperatures for 2005, 2006, and a 44-yr average†. The growing season was considered May–October, similar to the sampling period.

	Total Precipitation			Maximum Soil Temperature			Minimum Soil Temperature		
Month	2005	2006	44-yr average	2005	2006	44-yr average	2005	2006	44-yr average
	----- cm -----			----- °C, 10 cm -----					
January	1.6	0.5	1.6	-4	1	-2	-6	1	-2
February	1.8	1.1	1.4	-1	-1	-1	-2	-2	-2
March	2.2	5.7	3.8	1	1	2	-1	0	1
April	9.4	9.3	7.1	11	12	9	6	7	6
May	15.3	6.2	8.5	15	17	18	10	12	13
June	9.9	23.9	9.6	23	23	24	18	18	18
July	7.8	4.4	9.9	27	29	28	21	22	22
August	7.1	10.4	7.8	26	27	27	19	20	21
September	25.3	7.2	7.7	21	19	21	16	13	16
October	3.9	1.1	5.0	13	13	13	9	7	9
November	5.2	1.1	3.4	6	6	5	3	1	3
December	3.0	4.9	1.6	1	-1	0	1	-3	-1
Growing Season	69.2	53.2	48.5						
Annual	92.3	75.8	67.5						

† Source: Southwest Research and Outreach Center (2009).

Table 2.3 Root-derived carbon (C_{root}), soil organic carbon (SOC), and the root-derived fraction of SOC ($F_{root\text{-}C}$) by management system. Mean and standard error (SE) shown. Following each *a priori* contrast *p*-value ≤ 0.2 (one-sided and Bonferroni corrected) is the management practice (or system) that had the greater value.

Management System	Root-Derived C‡		SOC		Root-Derived SOC	
	2005	2006	2005	2006	2005	2006
	— g C_{root}/m^2, 0–30 cm —		— kg C/m^2, 0–30 cm —		——— % ———	
2HPI	85.3 (36)	73.2 (26)	8.6 (0.2)	7.0 (0.6)	0.9 (0.4)	1.0 (0.3)
2LPI	91.7 (22)	41.8 (7)	9.8 (0.4)	9.1 (0.4)	0.9 (0.3)	0.5 (0.1)
4HPI	118.4 (14)	100.0 (15)	9.4 (0.6)	8.7 (1.6)	1.3 (0.1)	1.2 (0.1)
4LPI	106.9 (21)	99.2 (16)	10.0 (0.3)	10.3 (1.6)	1.1 (0.2)	1.0 (0.2)
4ORG	100.7 (11)	68.7 (28)	9.6 (0.4)	10.9 (1.4)	1.1 (0.1)	0.6 (0.2)
			Contrast p-values			
HPI vs. LPI	--	--	< 0.001*** (LPI)	0.054† (LPI)	--	0.054† (HPI)
2-yr vs. 4-yr	--	0.073† (4-yr)	0.082† (4-yr)	0.142 (4-yr)	--	0.113‡ (4-yr)
4HPI, 4LPI vs. 4ORG	--	--	--	--	--	0.029* (4HPI, 4LPI)

* Significant at the $p \leq 0.05$ level.
*** Significant at the $p \leq 0.001$ level.
† Marginally significant at the $p \leq 0.1$ level.
‡ Two-sided *p*-values, *a priori* hypotheses were two-sided (root-derived C), or results contradicted one-sided hypothesis.

Table 2.4 Management system effects on cumulative soil and root-derived C respired, the root-derived fraction of cumulative respiration, and the fraction of initial root-derived C respired based on main effect models. Following each *a priori* contrast *p*-value ≤ 0.2 (one-sided and Bonferroni corrected) is the management practice (or system) that had the greater value. Mean management system values shown in Fig. 2.2.

Management Contrast	Cumulative Soil Respiration		Cumulative Root-Derived Respiration		Root-Derived Fraction of Cumulative Respiration		Root-Derived C Respired	
	2005	2006	2005	2006	2005	2006	2005	2006
HPI vs. LPI	--	--	< 0.001*** (HPI)	--	0.008** (HPI)	--	--	--
2-yr vs. 4-yr	--	0.094† (2-yr)	0.130‡ (4-yr)	0.078† (2-yr)	--	--	--	< 0.001*** (2-yr)
4HPI, 4LPI vs. 4ORG	--	--	< 0.001*** (4HPI, 4LPI)	0.011* (4HPI, 4LPI)	< 0.001*** (4HPI, 4LPI)	0.061† (4HPI, 4LPI)	--	--

* Significant at the $p \leq 0.05$ level.
** Significant at the $p \leq 0.01$ level.
*** Significant at the $p \leq 0.001$ level.
† Marginally significant at the $p \leq 0.1$ level.
‡ Two-sided *p*-value reported; result contradicted one-sided *a priori* hypothesis.

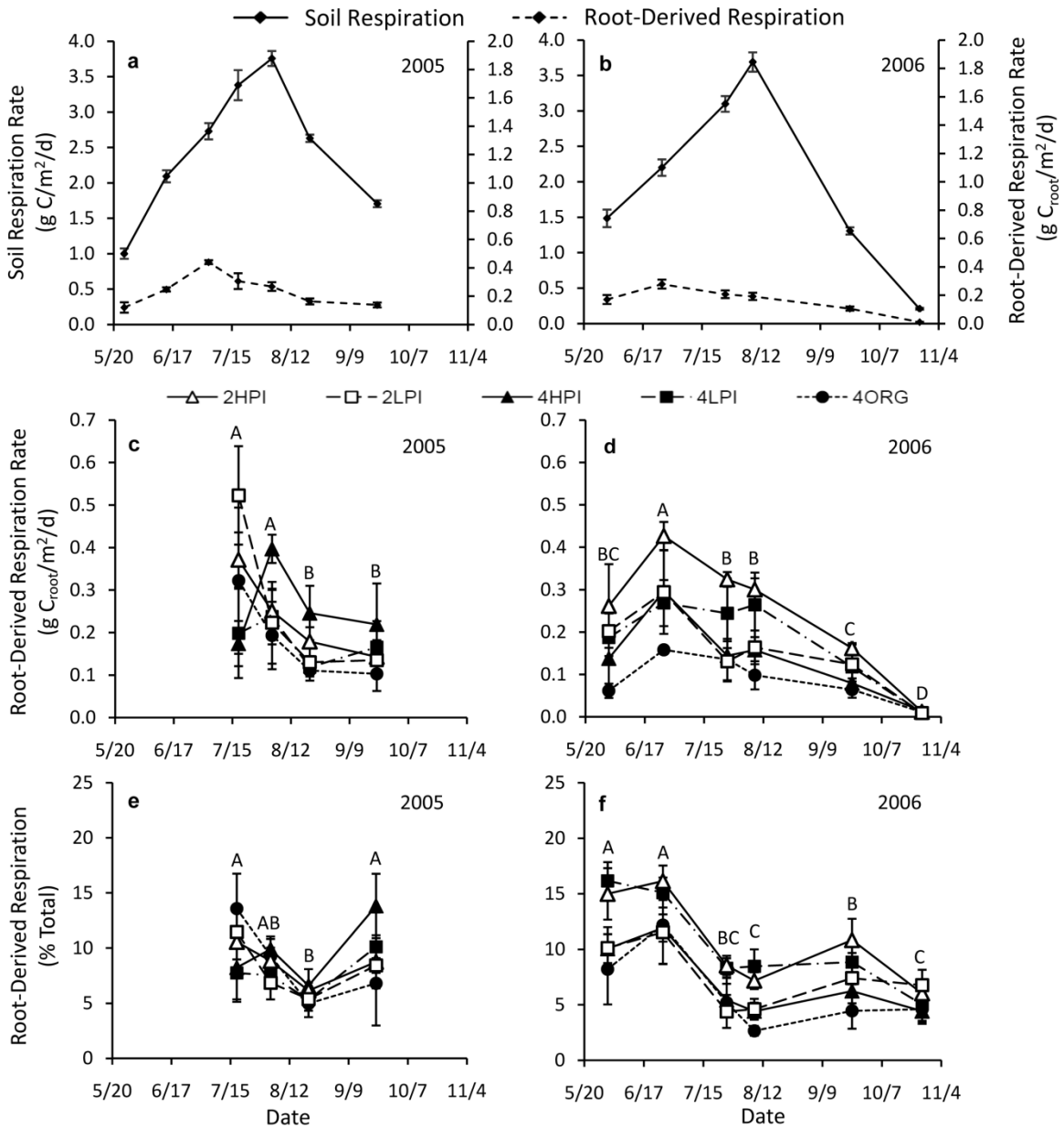

Figure 2.1 Seasonal patterns of soil and root-derived respiration rates, and the root-derived contribution to soil respiration. Different letters above the data points indicate significant differences between dates (averaged across management system). Soil and root-derived respiration rates, averaged across all management systems in (a) 2005 (*n*=3 on first three dates, *n*=15 remaining four dates), and (b) 2006 (*n*=15); mean and SE shown. Root-derived respiration rate by management system in (c) 2005 and (d) 2006, *n*=3. The root-derived contribution to soil respiration (%) by management system in (e) 2005 and (f) 2006, *n*=3.

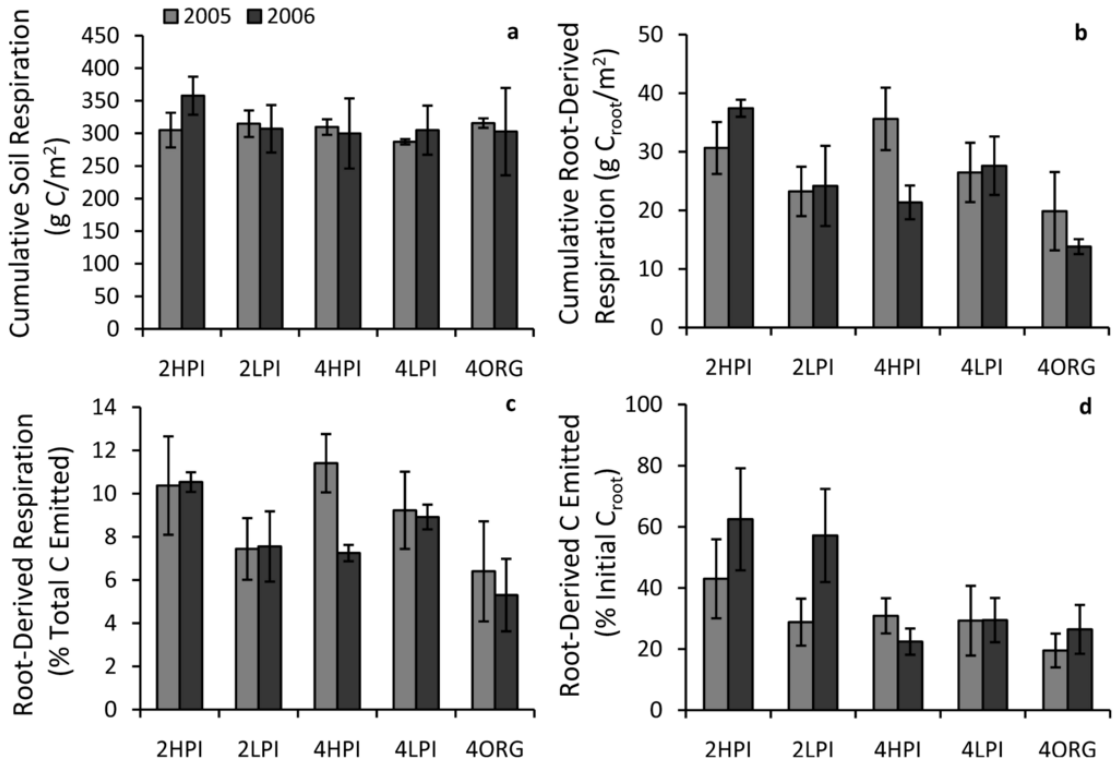

Figure 2.2 Cumulative C dynamics by management system for the 2005 and 2006 growing seasons, *n*=3, mean and SE shown. (a) Cumulative soil respiration. (b) Cumulative root-derived respiration. (c) Root-derived fraction of cumulative soil respiration. (d) Fraction of initial root-derived C respired.

CHAPTER 3

Root-Derived Nitrous Oxide Emissions and Carbon-Nitrogen Coupling in an Agricultural Ecosystem

Agricultural ecosystems are an important source of nitrous oxide emissions (N_2O) globally. As the primary source of N_2O emitted from agricultural soils, fertilizer-derived emissions have been well studied. In contrast, little attention has been given to contributions of nitrogen (N) from organic sources to N_2O emissions, particularly belowground organic N sources. *In situ* corn roots (*Zea mays* L.) were labeled with stable carbon (C) and N isotopes to examine effects of tillage and fertilization, the number of crops within rotations (either two or four), and fertilizer type (inorganic versus organic) on losses of root-derived N as N_2O. Additionally, root-derived C and N losses as carbon dioxide and N_2O were compared. All measurements were made over two growing seasons in the Upper Midwest, USA.

The average root-derived (root biomass and root exudate) contribution to N_2O emissions was 7% across management systems. Across both seasons, the root-derived contribution to N_2O emissions was higher in management systems with 2-yr (11%) than 4-yr crop rotations (6%). Similarly, the loss of initial root-derived N as N_2O (the emission factor) was higher in systems with 2-yr (0.24%) than 4-yr crop rotations (0.10%) in 2006. Across all management systems, the emission factor ranged from 0.04-0.52% in 2006. Root-derived N losses as N_2O and root-derived C losses as carbon dioxide were positively and linearly related. Our results suggest that an emission factor of 1%, currently used by the International Panel on Climate Change, results in an overestimate of N loss as N_2O from organic N sources.

ABBREVIATIONS

AICc	corrected Akaike's information criteria
C	carbon
CO_2	carbon dioxide
EF	emission factor
HPI	plots with broadcast high purchased inputs and moldboard plowing
LPI	plots with banded low purchased inputs and chisel plowing
LR	labeled root microplot
MLRA	major land use resource area
N	nitrogen
N_2	nitrogen gas
N_2O	nitrous oxide
NH_4^+	ammonium
NO_3^-	nitrate
ORG	plots with organic inputs and moldboard plowing
SOC	soil organic carbon
TN	total soil nitrogen
UR	unlabeled root microplot
VICMS	variable input crop and management system

INTRODUCTION

Agricultural soils are one of the primary sources of nitrous oxide (N_2O) contributing approximately 42% of anthropogenic N_2O emissions (Bouwman et al., 2002b; IPCC, 2007). N_2O contributes to destruction of stratospheric ozone (Ravishankara et al., 2009) and is a potent greenhouse gas with 298 times the global warming potential of carbon dioxide over a 100 year period (IPCC, 2007). Research aimed at reducing anthropogenic emissions of N_2O in agricultural systems has focused on nitrogen (N) applied as fertilizer and manure. Emission factor estimates (the fraction of applied N emitted as N_2O) range from near the IPCC value of 1% (Mosier et al., 1986; Mosier, 1994; IPCC, 2006), up to 2.5% if nitrogen gas (N_2) emissions are included (Davidson, 2009), and as low as 0.3% for N_2O only (Li et al., 2002).

Emission factors (EFs) are commonly used to estimate N_2O emissions when data on N application rates are available but accurate field-based measurements are not. Quantification of N_2O emissions via models and field-based measurements remains a challenge due in part to the high spatial and temporal variability in factors contributing to N_2O production (via nitrification and denitrification) and consumption (Firestone and Davidson, 1989; Davidson and Seitzinger, 2006; David et al., 2009; Groffman et al., 2009a). The difficulty of measuring N_2O emissions is largely the appeal of a simple EF calculation. However, the current IPCC EF overestimated N_2O emissions from systems with varying crop rotation and with both fertilizer and residue N sources (Jantalia et al., 2008). Inaccuracy of the IPCC EF may be due in part to the use of a single EF for fertilizer, manure, and residue-derived N losses as N_2O. In particular, N_2O emissions from organic N sources may be overestimated by the current IPCC EF. Application of

organic N sources, primarily crop residue, results in both higher N retention in plant and soil N pools (Gardner and Drinkwater, 2009) and lower N losses as N_2O than observed with fertilizer N sources (Delgado et al., 2010). To generate more accurate estimates of N_2O emissions based on applied N data, Delgado et al. (2010) suggested that the IPCC utilize a distinct EF for organic residue versus inorganic N sources.

The EF associated with root-derived N inputs may be even lower than that associated with other organic N sources such as manure and aboveground residue. Belowground sources of carbon (C) (Balesdent and Balabane, 1996; Bolinder et al., 1999) and N contribute more to stable soil C and N pools than aboveground sources (Broadbent and Nakashima, 1974). Due to the high degree of N recycling in soils (Sorenson, 1981), studies of belowground biomass N have more often focused on mineralization and immobilization of root-derived N (Broadbent and Nakashima, 1974; Trinsoutrot et al., 2000b; Chaves et al., 2004) than on gaseous loss pathways. Studies of agricultural management effects on residue-derived gaseous N losses have focused on aboveground material. This is largely a results of the challenges involved in quantifying belowground plant contributions to soil pools, a problem well described for root-derived C inputs (Rasse et al., 2005). Specifically, most studies have examined how residue quantity and quality (C:N ratio) interact with fertilizer N quantity and type to impact aboveground residue-derived N_2O emissions (Huang et al., 2004; Gentile et al., 2008; Jantalia et al., 2008; Delgado et al., 2010; Frimpong and Baggs, 2010). Studies of root-derived N_2O emissions have been primarily laboratory incubations of root-amended soil (Lou et al., 2007) which, for EF estimation, are less accurate than field studies due to the exclusion of root exudates. Exudates can represent a significant fraction of belowground

plant-derived N, between roughly 20–45% for some crops (Soon and Arshad, 2002). To our knowledge, there have been no field-based estimates of root-derived N losses as N_2O and no examination of agricultural management effects on those losses.

Whether agricultural management system effects on root-derived N_2O emissions mimic management effects on total soil N_2O emissions is an open question, and one that is difficult to answer given the lack of clear consensus on tillage, crop rotation, and fertilizer type effects on total soil N_2O emissions (e.g., Omonode et al., 2011). Studies of tillage effects on soil N_2O emissions suggest that the direction or presence of tillage effects may depend on whether tillage influences soil moisture and aeration or N availability. Reduced tillage can result in higher N_2O emissions when tillage reductions lead to greater water-filled pore space (WFPS) and reduced O_2 levels, thereby improving conditions for denitrification (Aulakh et al., 1984; Gregorich et al., 2005; Rochette et al., 2008). Most often, this negative effect of tillage on N_2O emissions has been observed in wet, high clay, soils. The effect of tillage also varies over time. Higher N_2O emissions with reduced or no-tillage may be short-lived resulting in no long-term effect of tillage on N_2O emissions (Six et al., 2004). Tillage can result in higher N_2O emissions when tillage results in the co-location of residue C and fertilizer N (Venterea et al., 2005; Drury et al., 2006; Del Grosso et al., 2008; Omonode et al., 2011).

In general, management systems with a greater diversity of crops grown in rotation (e.g., corn-soybean-alfalfa versus corn-corn) are expected to decrease N_2O emissions over the full rotation (Grant et al., 2002). Crop rotation diversity can affect N_2O emissions by changing soil organic C (SOC) content and soil N availability, or through effects on residue quality. Over the long-term, increased rotation diversity

positively affects SOC content (Van Vleck and King, 2011) and N availability (Kuratomi, 2003), which can each increase N_2O emissions (Bouwman et al., 2002a). Effects of crop rotation diversity on N availability can be due to individual crop effects on rates of soil N cycling or to crop-specific fertilization rates. Positive effects of fertilizer N on N_2O emission are well documented (Gregorich et al., 2005; Almaraz et al., 2009).

Comparisons of N_2O emissions from among individual crops may lead to different conclusions than comparisons over all phases of crop rotations. In two year corn-soybean rotations, N_2O emissions are not consistently higher, or lower, during the corn phase of the rotation (Rochette et al., 2004; Gregorich et al., 2005; Parkin and Kaspar, 2006). Over the full rotation period, higher N_2O emissions have been measured in continuous corn than corn-soybean rotations (Adviento-Borbe et al., 2006; Omonode et al., 2011). In some cases, comparisons between corn-soybean rotations and more diverse rotations (three or more crops) have found no significant effect of rotation diversity on total N_2O emissions (Johnson et al., 2010). In other studies, high N_2O emissions measured in high diversity rotation systems may reflect the inclusion of high residue N crops like alfalfa (Del Grosso et al., 2008). Based on studies of aboveground residue, higher inorganic N availability can result in higher residue N content (Malhi and Lemke, 2007) and lower C:N which can increase residue N mineralization rates (Trinsoutrot et al., 2000a) and residue-derived N_2O emissions (Gentile et al., 2008). However, it is unknown whether these relationships hold true for belowground residue.

Strong coupling of C and N cycles in agricultural ecosystems is generally not expected largely due to surplus fertilizer N additions (Woodmansee, 1984; Gardner and

Drinkwater, 2009). However, strong C and N coupling has been observed during the decomposition of plant residue and manure inputs (Huang et al., 2004). Highlighting the increased nutrient retention associated with coupling of C and N, Gardner and Drinkwater (2009) proposed a set of management practices that should increase C and N coupling, including more diverse rotations and greater inputs of organic matter. Few studies have tested the effects of management practices on soil C-N coupling and the subsequent effects on emissions of greenhouse gas emissions (Gärdenäs et al., 2011).

To advance understanding of the N sources which contribute to N_2O emissions from agricultural ecosystems, we measured emission of corn root-derived N as N_2O during two growing seasons and under a range of management systems varying in management intensity (tillage and fertilizer amount), crop rotation diversity, and fertilizer type (organic versus inorganic). Estimates of root-derived N inputs and their loss as N2O account for both detrital root N and root-exudate N. Additionally, to examine the relationship between root-derived C and N cycling, we compared emissions of root-derived C as carbon dioxide (CO_2) (Van Vleck and King, 2011) with root-derived N emissions as N_2O. Our main objectives were to assess the effects of agricultural management on: (i) the contribution of root-derived N to N_2O emissions, (ii) the fraction of initial root-derived N emitted as N_2O, analogous to the IPCC's EF, and (iii) root-derived C and N coupling. We use these results to quantify management effects on detrital root-derived N_2O emissions and determine the effects of agricultural management on belowground residue C and N coupling.

We hypothesized that: 1) management intensity (tillage and fertilization) would increase root-derived N emissions through positive effects of fertilizer quantity on corn

root quality (C:N ratio), 2) as N_2O measurements were made during non-fertilized phases of rotations, tillage would positively affect root-derived N emissions, 3) rotation diversity would decrease losses of root-derived N, 4) the root-derived emission factor (the fraction of initial root-derived N emitted as N_2O over the season), $EF_{N,}$ would be smaller with organic than inorganic fertilization, and 5) losses of root-derived C would be positively related to losses of root-derived N and that, when characterized relative to initial root-derived C and N, losses of root-derived N would be less than losses of root-derived C (EF_N versus EF_C).

MATERIALS AND METHODS

Research Site

Research plots at the University of Minnesota's Southwest Research and Outreach Center (44°14' N, 95°19' W) were part of the variable input crop management system (VICMS) study (Porter et al., 2006) and have been under continuous management since 1989. The site is characterized by mid-continental climate patterns with cold winters and warm summers; the average annual temperature is 7°C (Porter et al., 2006) and the 44-yr average annual precipitation (1961-2003) was 68 cm (Southwest Research and Outreach Center, 2009). Both soil series and topography at our study site are characteristic of the major land resource area 103 (MLRA 103) which spans south-central Minnesota and north-central Iowa. Land cover within MLRA 103 is roughly 80% cropland (USDA, 2006). Three soil series extend across the 3.6 m elevation gradient. Ves loam (mesic Calcic Hapludolls) and Normania loam soils (mesic Aquic Hapludolls)

are found at higher elevations, and Webster silty clay loam soils (mesic Typic Endoaquolls) are found at lower elevations. Soils in all plots were Ves-Normania intergrades with the exception of three plots measured in 2006 that were dominated by Webster soils.

Treatments and Experimental Design

The five management systems examined range in tillage type and frequency, crop rotation, and fertilizer type (Table 3.1). Tillage co-varied with fertilizer amount only during the corn phase of crop rotations. High tillage–high fertilization practices (moldboard plow) are hereafter referred to as *HPI*, and low tillage–low fertilization practices (chisel plow) are hereafter referred to as *LPI*. All N_2O emission measurements were made during the soybean phase of rotations when there was no fertilization. Fertilizer inputs to the corn phase of rotations were either inorganic (HPI and LPI) or organic (ORG). *ORG* systems were fertilized with beef manure, and the primary tillage method was moldboard plowing. Both 2- and 4-yr rotations of HPI and LPI were included in the study, but only 4-yr organic systems were included because 2-yr rotations do not meet organic certification criteria. Two year crop rotations included corn and soybean; four year rotations included corn (*Zea mays* L.), soybean (*Glycine max* (L.) Merr.), oats (*Avena sativa* L.) interseeded with alfalfa (*Medicago sativa* L.), and alfalfa.

Root-derived N cycling was measured in two seasons on two separate sets of 15 plots (30 x 55 m) within a single field. All plots were planted with corn during isotope labeling and planted with soybeans in the following growing season when root-derived N emissions were measured (2005 and 2006). Three blocks were arranged perpendicular to

the slope to capture topographic and soil variation at the site. In each season, one plot representing each management system was selected per block. Paired microplots within each plot contained either ^{15}N-labeled (*LR*, 1.0 m^2) or unlabeled corn root-derived material, detrital root biomass plus root exudates (*UR,* 1.2 m^2). Due to the microplot size we simulated fall primary tillage practices to incorporate unlabeled corn shoot residue after the corn harvest; detrital roots remained *in situ*. In systems that normally received moldboard plowing (HPI and ORG), shoot residue was incorporated with shovels by distributing it on the surface and inverting the upper 20 cm of soil. In systems normally chisel plowed (LPI), shoot residue was incorporated throughout the upper 20 cm of soil. Throughout the soybean growing season, cultivation was completed with a small tiller.

Isotope Labeling

Isotope labeling of corn was done during the 2004 and 2005 growing seasons. Live corn plants were pulse-labeled four times with ^{15}N labeled urea (98 atom%) during the vegetative phase of development by foliar spray application (Glassner et al., 1998). At harvest, corn roots were left in place to mimic the decomposition conditions typical within each management system. The fate of the labeled corn root-derived N was tracked during the following soybean growing season. Roots were collected at two depth intervals (0-10 and 10-30 cm) from each plot for analysis on an elemental analyzer (Fisons NA 1500, Thermo Scientific, Waltham, MA) coupled with a continuous flow isotope ratio mass spectrometer (CF-IRMS, Fisons Optima, Manchester, UK). Roots were heavily labeled: 1.08 ± 0.04 atom% ^{15}N for labeled roots compared to 0.37 ± 0.004 atom% ^{15}N for unlabeled roots in 2005 and 1.15 ± 0.03 atom% ^{15}N for labeled roots

compared to 0.38 ± 0.01 atom% ^{15}N for unlabeled roots in 2006 (D. Allan, *personal communication*, 2010). To estimate the root-derived N present in the soil in the spring of 2005 and 2006, we multiplied the root N content (g N_{root}/g_{root}) by the quantity of root-derived material estimated to be in the soil between 0–10 and 10–30 cm (g_{root}/m^2). The quantity of root-derived material in the soil was determined based on the C content of the root material and differences in the C isotope signatures of the soil and root-derived material between the labeled and unlabeled microplots (Van Vleck and King, 2011). The subscript "root" is used to differentiate total soil N pools or fluxes from recent corn root-derived N pools or fluxes (e.g., g N_{root}/m^2).

Nitrous Oxide Emissions

Emission rates of N_2O were measured during the soybean phase of rotations at two to four week intervals during the growing seasons of 2005 (*n*=5 over 88 days) and 2006 (*n*=6 over 148 days). Root-derived N_2O emissions were measured on a subset of the sampling dates in 2005 (*n*=3 over 34 days mid-season) and in 2006 (*n*=4 over 115 days). N_2O emission rates were measured using a static chamber approach in four chambers within each of fifteen plots (five management systems, three replicates): two chambers in the LR microplots and two in the UR microplots. Chamber bases were located side-by-side covering the area between two soybean rows, but not covering any aboveground plant material. Bases measured 29 x 49.5 x 9 cm (l x w x h), and chamber tops measured 29 x 49.5 x 10 cm. The average height of each chamber base side above the soil was determined on each date in order to accurately calculate chamber headspace volume and N_2O emission rate. Though our sampling frequency was below optimal

levels (Mosier et al., 1986; Parkin, 2008), underestimation of total and root-derived N_2O emissions should be minimized due to the absence of fertilization (and therefore fertilizer-induced pulses of N_2O emissions), the use of large chambers for relatively short deployment times (Venterea et al., 2009; Venterea, 2010), and our interest in determining relative differences among management systems.

From each chamber, four headspace samples (15 mL each) were collected at ten minute intervals over a forty minute period and injected into 9 mL glass vials capped with butyl rubber septa. Over-pressurizing reduced the possibility of leakage into sample vials before analysis. Samples were analyzed for N_2O on a gas chromatograph equipped with an electron capture detector (HP 8580, Foster City, CA) and a headspace autosampler (Model 7000/7050, Teledyne Tekmar, Mason, OH). Two gas standards (Scott Specialty Gas, Plumsteadville, PA) were used for calibration. N_2O data presented reflect the net exchange of N_2O between soils and the atmosphere with positive values indicating net movement of N_2O from soils into the atmosphere. Net negative N_2O exchange was occasionally measured; in these instances we could not calculate the exchange of root-derived N so while the negative total N_2O data are presented, the corresponding root-derived N_2O data could not be determined and are therefore not presented in the figures or analyses. Root-derived N_2O emissions were determined by comparing emissions from the LR and UR microplots as described below (Eq. 3.1).

On each sampling date soil characteristics likely to influence N_2O emissions were measured: air temperature, soil temperature, soil moisture, and nitrate (NO_3^-) and ammonium (NH_4^+) concentrations. Three air and soil temperature readings were averaged for each plot on each date (thermocouple probes, 10 cm above and below the

soil surface). The air temperature at each plot and the volume of each chamber were used to calculate the headspace gas concentrations over time, and emission rates were determined from the linear regression slope of the headspace concentrations over time (R^2>0.85). To calculate cumulative emissions for each season linear interpolation between sampling dates was used. To calculate the cumulative root-derived contribution to N_2O emissions, cumulative N_2O emissions were calculated for the subset of dates on which root-derived emission rates were measured (n=3 for 2005, n=4 for 2006). Three soil samples (0–10 cm) from each plot were homogenized and sub-sampled for gravimetric soil moisture (105°C, 48 hours) and inorganic N analyses on each date. Inorganic N concentrations were determined by extraction in a 2M KCl solution and flow injection colorimetric analysis (Quickchem 8000, Lachat, Loveland, CO). Soil characteristics measured by collaborators included bulk density (J. Strock, *personal communication,* 2006), SOC content, total soil nitrogen (TN), and both soil and root ^{13}C and ^{15}N data (D. Allan, *personal communication,* 2010).

Root-Derived Nitrous Oxide Emissions

Atmospheric and headspace samples for isotopic analysis were collected using custom-made 170 mL glass flasks (Chemglass, Vineland, NJ). Headspace samples were collected from one chamber in each LR and UR microplot at two or three of the following intervals after the chamber was sealed: 20, 30, and 40 minutes. Samples were injected through an inlet system (Mass Spec Solutions, Manchester, UK) to separate and preconcentrate the samples before introduction to the CF-IRMS. Three NOAA/CMDL–calibrated standards analyzed by the Stable Isotope Laboratory at the University of

Colorado's Institute of Arctic and Alpine Research (Boulder, CO) were used as reference standards to determine the $\delta^{15}N$ values for our N_2O samples relative to atmospheric N_2. The $\delta^{15}N$–N_2O of our standard tanks, filled at Niwot Ridge, was assumed to be equal to the $\delta^{15}N$ value measured for tropospheric N_2O at Niwot Ridge in 1996: 8.5 ± 0.4‰ (Sowers, 2001). Using the reference $\delta^{15}N$ value and the known isotope ratio ($^{15}N/^{14}N$) for atmospheric N_2, 0.0036765 (Junk and Svec, 1958), we calculated the isotope ratio for the standard tanks relative to atmospheric N_2. We found no significant difference between the 2/1 isotope ratio of N_2O in the standard tanks and in tropospheric flask samples collected at the University of Minnesota–Twin Cities campus (Saint Paul, MN). Thus standard tank and troposphere flask samples were used interchangeably as standards for our field N_2O samples.

Calculation of the daily root-derived contribution to total N_2O emissions followed the methods of Puget and Drinkwater (2001). We calculated the N_2O concentration in each flask based on its collection time, the chamber N_2O flux rate, and the estimated $\delta^{15}N$ values of N substrates determined using Keeling plots (Phillips and Gregg, 2001). The atmospheric $\delta^{15}N$-N_2O signature for each date (average of multiple samples collected at the field site) and the atmospheric N_2O concentration at each plot on each date (n=4) were used as an additional data point in each Keeling plot. This point reflects the N_2O concentration and $\delta^{15}N$-N_2O signature of the gas in the chamber headspace at the start of each sampling period. Two mixing model equations, one per microplot, were combined to calculate the root-derived fraction of N emitted and the amount of root-derived N emitted from each plot:

$$F_{root-N_2O} = (\delta^{15}N_{N_2O-LR} - \delta^{15}N_{N_2O-UR}) / (\delta^{15}N_{root-LR} - \delta^{15}N_{root-UR}),$$
$$N_2O_{root} = (F_{root-N_2O} * N_2O_{total})$$
(Equation 3.1)

where $F_{root-N2O}$ is the fractional contribution of root-derived N to the total N_2O emitted, and $\delta^{15}N$ is the isotopic value of the N source being emitted ($\delta^{15}N_{N2O}$), or the root-derived N ($\delta^{15}N_{root}$) in the LR and UR microplots. N_2O_{total} is the N_2O emission rate from all N sources within the LR microplot (g N/m^2/d), and N_2O_{root} represents the emission rate of root-derived N as N_2O from each plot (g N_{root}/m^2/d). The root-derived fraction of cumulative growing season N_2O was calculated as the quotient of each plot's cumulative root-derived emissions and cumulative total N_2O emissions.

Data Analysis

Two statistical approaches are presented to determine effects of both management and environmental factors on root-derived N cycling and to facilitate comparison of our results with studies that do not include mixed effects analysis with covariates. Linear mixed effects models with management system as the only main effect are hereafter referred to as "main effect models" to distinguish them from mixed effects models run with covariates. Statistical analyses were completed using R (version 2.10.1) (R Development Core Team, 2009) with lme4 (Bates and Maechler, 2009), languageR (Baayen, 2009), and HH packages (Heiberger, 2009) for analysis of mixed effects models. Specifically, the lmer function within the lme4 package was used for analysis of mixed effect models with and without covariates. Model comparison was completed using the maximum likelihood method, but final model results presented used the restricted maximum likelihood method (Crawley, 2007). AICc values and variance

inflation factors were determined with the MuMIn (Barton, 2010) and car packages (Fox, 2009). The best fit model was identified through stepwise simplification and model comparison using Akaike information criterion values corrected for small sample sizes (AICc). Models with AICc values differing by less than three from the best fit model AICc value were considered to fit the data equally well (Johnson and Omland, 2004).

Possible covariates for the daily mixed effects models included all measured soil characteristics: soil temperature, WFPS, soil NO_3^- and NH_4^+ concentrations. In cumulative mixed effects models with covariates soil TN and SOC content, and root-derived C:N ratios were additional covariate options. Square root and logarithm transformations were made as needed to improve normality; all figures and tables show non-transformed data, while all statistical results are based on transformed data. Unless otherwise noted, interactions between management and covariates could be excluded without significant reductions in model fit of mixed effect models with covariates. When correlation among covariates was found (variance inflation factor>3), the less significant covariate was excluded from the model.

In all models, specific *a priori* contrasts were defined to compare practices within the management systems: 1) high versus low intensity tillage and fertilization (HPI versus LPI), 2) crop rotation diversity (2 versus 4-yr rotations), and 3) fertilization type (4HPI and 4LPI versus 4ORG). Hypotheses tested by the contrasts were primarily one-sided, and all are Bonferroni corrected. To address temporal pseudoreplication (repeated sampling of plots), daily models included a random effect term nesting plot within block on each date (Crowley, 2007). To avoid inaccuracies of spatial pseudoreplication, daily rates of soil N_2O emission were calculated for each plot

(Hurlbert, 1984). Averaging flux rates at the plot level (for chambers with a flux rate slope R^2>0.70) was only completed for total N_2O emissions as root-derived N_2O data were calculated at the plot level (Eq. 3.1). Differences were considered significant when $p \leq 0.05$, and significant differences are described simply as higher or lower. Differences were described as marginally significant when p-values are between 0.1 and 0.05. Agronomically interesting trends are discussed when $p \leq 0.2$. Contrast p-values are indicated by the "c" as in the following notation, p_c.

RESULTS

Climate and Site Environmental Data

Based on field measurements, WFPS was higher in 2005 than in 2006, with an average of 64 and 57% respectively. Soil temperatures at 10 cm were marginally higher in 2005 than 2006, with an average of 25.1±0.4°C. Records from the climate station at our research site (Southwest Research and Outreach Center, 2009) indicated that annual precipitation in both 2005 (92.3 cm) and 2006 (75.8 cm) was higher than the 44-year historical site average (67.5 cm), while soil maximum and minimum temperatures at 10 cm were comparable to the historic monthly averages (Van Vleck and King, 2011). Major 2005 precipitation events occurred in May (data not shown) and September, before planting and after soybean senescence; major 2006 precipitation events occurred in June, shortly after planting (Fig. 3.1a, 3.1b).

Based on plot measurements from each date, there were no significant effects of management on soil temperature (range 9–36°C), WFPS (range 23–109%), or NH_4^+

concentrations in either season (Table 3.2, range <1–3 μg N-NH_4^+/g soil). Soil NO_3^- concentrations were marginally higher in low input management systems in 2006 (LPI>HPI, range <1–19 μg N-NO_3^-/g soil), and were higher in systems with organic inputs in both seasons (4ORG>4HPI,4LPI). SOC content ranged from 6.2–13.7 kg C/m^2 and was lower in high intensity systems (LPI>HPI) in 2005. SOC was also marginally lower in low diversity systems (4-yr>2-yr) in 2006, with similar SOC content trends in 2005 (for statistical analysis see Van Vleck and King, 2011)

Root-Derived Nitrogen and Soil Nitrogen

Management system effects on root-derived N inputs and their contribution to total soil N were not as pronounced in 2005 as in 2006 (Table 3.3). On average, labeled corn root-derived N represented 0.44±0.05% of TN in the spring of 2005 and 0.29±0.04% in the spring of 2006. The average C:N ratio of the root-derived material was 28.6±1.1 in 2005 and 29.4±1.2 in 2006. This C:N ratio was derived from the isotope based estimates of root-derived C and N present in the soil each spring and therefore reflects not only root biomass but also root exudate chemistry. Higher TN (Table 3.3) and equal or higher NO_3^- concentrations (Table 3.2) in LPI than HPI systems during the soybean phase of rotations support our assumption that greater N additions in HPI systems during corn development did not result in elevated N availability during the soybean phase of rotations. Thus, for both daily and cumulative root-derived N_2O emissions, the contrast between HPI and LPI systems was effectively a test of tillage effects. For TN, root-derived N inputs, C:N ratios, and EF_N, we cannot distinguish

between fertilizer and tillage effects; in these cases we use the broader term, management intensity, to refer to the joint tillage and fertilization effects.

Management significantly affected the quantity of root-derived N inputs, TN, the root-derived contribution to TN, and the C:N ratio of root-derived material. With lower intensity management, TN and the C:N ratio of root-derived inputs increased in both seasons. TN was also higher in more diverse crop rotation systems in both seasons and was marginally higher in ORG than other 4-yr systems in 2006 (Table 3.3; LPI>HPI and 4-yr>2-yr). In 2006, both root-derived N inputs and the contribution of those inputs to TN were greater in more diverse systems (4-yr>2-yr). In both seasons, the root-derived contribution to TN showed at least a trend of being greater in more intense management systems (HPI>LPI). Although in 2006 root quality also appeared higher in organically fertilized systems, this was an artifact of the contrast structure. Specifically, the significant difference in root quality was due to the high $C:N_{root}$ ratio in the 4LPI system; the ratios for 4HPI and 4ORG were not significantly different based on a Tukey HSD comparison.

Daily Nitrous Oxide Emission Rates

The average daily N_2O emission rate was 206.9±16.5 μg $N/m^2/d$ in 2005 and 281.3±19.1 μg $N/m^2/d$ in 2006 (Fig. 3.1a, 3.1b). Management system had no detectable effects on daily N_2O emission rates in 2005; the best fit model included only a positive trend with WFPS (p=0.169, R^2=0.19). In 2006, tillage had a marginal positive effect on daily N_2O emission rates (HPI>LPI, p_c=0.077) and there was a trend of higher emissions from organic than inorganic systems (4ORG vs. 4HPI, 4LPI, p_c=0.180). Even so, daily

N_2O emission rate data in 2006 were best fit by positive relationships with either soil temperature (p=0.003) or WFPS (p=0.006). Ultimately, none of these three models for 2006 emissions were strong predictors of the data ($R^2 \leq 0.11$).

Emission rates of root-derived N_2O followed total N_2O emission rate patterns in 2006. With the shorter measurement period in 2005, it was difficult to determine similarities between the seasonal patterns of total and root-derived N_2O emissions. However, it is clear that in both seasons the magnitude of total N_2O emission rates is substantially greater than that of N_2O_{root} emission rates (Fig. 3.1c, 3.1d). In both seasons, N_2O_{root} emissions were highest in late June–early July. Additional late-season sampling would have allowed us to determine if the high September 2006 emission rates represented a short pulse following soybean senescence.

The average daily N_2O_{root} emission rate was 13.7±1.1 μg $N_{root}/m^2/d$ in 2005 and 26.7±1.9 μg $N_{root}/m^2/d$ in 2006. N_2O_{root} emission rates increased marginally with tillage intensity in 2005 (HPI>LPI, p_c=0.059, R^2=0.30), but were not significantly influenced by management system in 2006 (Fig. 3.1e, 3.1f). In 2005, soil NO_3^- concentrations (p=0.026, R^2=0.25) were positively related to N_2O_{root} emission rates; this model fit the data better than the main effect model based on comparison of AICc values. In 2006, no measured environmental variables were related to daily N_2O_{root} emission rates.

The daily root-derived contribution to N_2O emissions, F_{root}, was 7.0±0.6% in 2005 and 9.5±0.6% in 2006. This contribution decreased slightly over time and was not affected by management system in 2005 or 2006 (Fig. 3.1g, 3.1h). In 2005, F_{root} was best described by a model including a positive relationship with soil temperature (R^2=0.37;

$p<0.001$). In 2006, in spite of no significant management system contrasts, management could not be removed, and the main effect model was the best fit model ($R^2=0.17$).

Cumulative Total and Root-Derived Nitrous Oxide Emissions

In 2005, cumulative N_2O emissions were positively related to soil NO_3^- concentrations (Table 3.4); none of the management contrasts were significant (Fig. 3.2a). In 2006, cumulative N_2O emissions showed a positive trend with respect to the average soil temperature and were marginally higher in systems with both more intense tillage practices (HPI>LPI) and more diverse crop rotations (4-yr>2-yr) (Table 3.4). In both seasons, the mixed effect model with covariates fit the data significantly better than the main effect model. Total N_2O emissions were measured on 5 dates over a period of 120 days in 2005 and on 6 dates over a period of 148 days in 2006, at least in part explaining the lower emissions in 2005. N_2O_{root} emissions were measured on 3 dates over 34 days in 2005 (mid-season) and on 4 dates over 115 days in 2006 (full growing season, planting to senescence). To calculate the residue-derived fraction of cumulative emissions cumulative N_2O emissions were also calculated for the shorter time period coinciding with root-derived emission measurements (data not shown).

Effects of management on cumulative root-derived emissions were only evident in 2005 (Fig. 3.2b). Cumulative N_2O_{root} emissions in 2005 were best fit by the main effect model with greater emissions from systems with more intense tillage practices and from less diverse crop rotations (Table 3.4). In 2006, cumulative N_2O_{root} emissions were best explained by a positive relationship with average plot soil temperature (Table 3.4).

Though the 2005 sampling period was shorter, the magnitude of root-derived N contributions to cumulative N_2O emissions, cumulative F_{root} values, were similar between seasons, with season averages of 7 and 9% (Fig. 3.2c). The similarity in magnitude was likely because the 2005 sampling period excluded dates with both the highest (early season) and the lowest (late season) daily F_{root} values based on 2006 seasonal patterns. The main effect model was the best fit for the 2005 and 2006 data (Table 3.4). In both seasons, the cumulative F_{root} was greater in systems with less diverse crop rotations than in those with more diverse rotations (2-yr>4-yr, Table 3.4). In 2005, the cumulative F_{root} was also marginally greater in systems with more intense tillage (HPI>LPI), whereas in 2006, this tillage effect was reversed. Additionally, the 2006 cumulative F_{root} was greater in inorganically fertilized systems (4HPI, 4LPI>4ORG).

The fraction of initial root-derived N emitted as N_2O, EF_N, was very low in both seasons, with season averages of 0.01 and 0.14%; it was especially low in 2005 largely due to the shorter sampling period than in 2006 (Fig. 3.2d). In both seasons, models combining management system and soil characteristics indicated that EF_N was significantly, or marginally significantly, greater in low diversity rotation systems (2>4-yr, Table 3.4). The best fit model in 2005 included management system and decreasing EF_N with increasing average WFPS data. As in 2005, a mixed effects model including a positive relationship with soil temperature fit the 2006 data significantly better than the main effect model (Table 3.4).

Coupling of Root-Derived Carbon and Nitrogen Cycling

We found strong relationships between root-derived C and N cycling. Along with the ^{15}N labeling and measurement of root-derived N emissions described here, ^{13}C labeling of corn roots and measurement of root-derived C emission as CO_2 were measured (Van Vleck and King, 2011). Over both growing seasons, there was a positive relationship between the contribution of root-derived C to daily CO_2 emission rates and the contribution of root-derived N to daily N_2O emission rates (Fig. 3.3a). In spite of differences in meteorological conditions and sampling period length, neither the slope nor intercept of this relationship differed significantly between seasons (data not shown). Contributions of both root-derived C and N to CO_2 and N_2O decreased over the season (shown for 2006, Fig. 3.3b). The similarity in seasonal patterns and relative contributions of root-derived C and N to total emissions exists in spite of large differences in the magnitude of cumulative root-derived C and N losses. Average cumulative root-derived C losses as CO_2 were 27.2 and 24.9 g C_{root}/m^2 in 2005 and 2006 respectively. Over the same time period, average cumulative root-derived N losses were 0.4 and 3.1 mg N_{root}/m^2 in 2005 and 2006.

Whether standardized by total C and N emissions as CO_2 and N_2O emissions (Fig. 3.3) or by total root-derived C and N inputs (2006 data, Fig. 3.4), the relationship between root-derived C and N emissions is linear. Calculated for the shorter date range over which we had root-derived N data in 2005, the fraction of root-derived C emitted, $EF_{C,}$ ranged from 2–89% with a mean of 23%. The comparable range of initial root-derived N loss was 0–0.51% with a mean of 0.09%. The fractions of initial root-derived C and N inputs emitted as CO_2 and N_2O over each season were again linearly related

with no significant difference between seasons in the slope or intercept of the EF_C–EF_N relationship (slope = 0.003, R^2 = 0.77).

In 2006, with root-derived C and N measurements spanning the full growing season, the slope describing the relationship between the fraction of initial root-derived C lost as CO_2 and N lost as N_2O differed with management intensity, but not rotation diversity. Relative to EF_C, EF_N was greatest in organic systems (4-yr rotation only), followed by low intensity, and then high intensity systems (each with both 2-yr and 4-yr rotations) (ORG>LPI>HPI, Fig. 3.4). Though decreased rotation diversity increased EF_C emissions, EF_N emissions showed a similar increase (see Van Vleck and King, 2011, and Table 3.4). As a result, the greater slope of the ORG system compared to LPI and HPI systems (Fig. 3.4) is not a result of differences in rotation diversity.

DISCUSSION

Recent studies of aboveground crop residue suggest that the N_2O emission factor for organic N sources should be less than the IPCC recommended 1%. We built on those previous studies to measure the management and soil environment effects on corn root-derived N_2O emissions and the coupling of root-derived C and N emissions. The use of two statistical approaches allowed us to readily compare the relative strength of environmental controls and management systems. Environmental controls proved more important for daily rates of total and root-derived N_2O emissions, while both management and environmental controls were key to understanding cumulative measures of N_2O_{root} emissions. Analysis of the root-derived C and N emissions factors

indicate some effects of management intensity on the coupling of root-derived C and N emissions as CO_2 and N_2O.

Magnitude of Nitrous Oxide Emissions

While total N_2O emissions measured in this study were low, they were similar to emissions measured under soybeans in southern Minnesota (Venterea et al., 2010). The occurrence of net N_2O uptake contributed to low rates of N_2O emissions; N_2O emissions reflect the balance of N_2O production and consumption processes. Net uptake has been measured under a range of soil moisture, temperature, and N availability conditions and in a number of different land-use categories (Flechard et al., 2005; Chapuis-Lardy et al., 2007; Billings, 2008; Philippot et al., 2011). Examination of the N_2O uptake data yielded some non-significant trends related to soil environmental conditions and management practices. Net N_2O uptake was higher in soils with high bulk density, but there was no clear relationship with WFPS. The frequency of net uptake measurements was also higher in low rotation diversity systems in both seasons (4>2 yr.).

The magnitude of N_2O_{root} emissions was small in comparison to total N_2O emissions, averaging 21 ± 2 μg $N_{root}/m^2/d$. Our values were also lower (2–5 μg N_{root}/kg soil over 34 days in 2005 and from 15–28 μg N_{root}/kg soil over 115 days in 2006) than a laboratory study of N_2O emissions from soil amended with rice root residue (49 μg N/kg soil over 55 days, Lou et al., 2007), likely because that study measured the effect of root-residue on total soil N_2O emissions thereby including residue priming effects.

Environmental and Management Effects on N_2O Emissions

Characteristics of the soil environment better predicted daily rates of total and root-derived N_2O emissions than management system. However, positive relationships with WFPS, soil temperature, and soil NO_3^- concentrations were often weak, explaining only 11–25% of the variability in the data. Soil NO_3^-, temperature, WFPS, available C, oxygen, and pH are frequently positively related to N_2O production in both field and incubation experiments (Skiba and Smith, 2000), though others have reported similarly weak relationships between total N_2O emissions and soil characteristics (e.g., Omonode et al., 2011). Due to the necessity of anaerobic conditions for denitrification, WFPS is often found to be an important control over N_2O emission rates. Peak N_2O production from denitrification has been estimated to occur between 60–80% WFPS (Davidson, 1991) with more recent studies suggesting peak emissions may occur under conditions closer to 80–85% WFPS (Skiba and Smith, 2000). The average WFPS over both seasons in this study was approximately 60%; being on the low end of the optimal WFPS range for N_2O production via denitrification, this may have contributed to the generally low N_2O emission rates. The difficulty in clearly identifying drivers of N_2O production reflects the high spatial and temporal variability of N_2O production, where hotspots of production can exist within otherwise unfavorable denitrification conditions (Groffman et al., 2009b).

The lack of management intensity effects on total N_2O emissions is not entirely surprising. Indeed, tillage effects on N_2O emissions have been inconsistent in past studies (Malhi and Lemke, 2007; Almaraz et al., 2009). Fertilizer, while it typically increases N_2O emissions (e.g., Gregorich et al., 2005) given sufficient soil C levels

(Matson et al., 1996), was not applied during the soybean phase of rotations in our study. The lack of rotation diversity effects is consistent with a recent study which found no effect of rotation on N_2O emissions (Johnson et al., 2010), and is also consistent with other studies indicating both positive and negative effects of crop rotation diversity on N_2O emissions (Rochette et al., 2004; Drury et al., 2006). Moreover, we found no clear evidence that fertilizer type applied (organic versus inorganic) during the corn phase of rotations influenced the magnitude of total or root-derived N_2O emissions.

In contrast to lack of management effects on total N_2O emissions, high intensity management and low diversity crop rotations tended to increase cumulative N_2O_{root} emissions, the cumulative F_{root}, and EF_N. The strength of these effects varied between the two growing seasons. The observed management system effects may reflect negative relationships between total soil N or root-derived N input quantity, and root-derived N emissions. Total soil N was higher in low intensity and high diversity systems in both seasons, while N_2O_{root} or F_{root} were lowest in these systems. In spite of higher TN in LPI systems in 2006, F_{root} was higher in LPI>HPI systems; this unexpected result may be attributable to higher total N_2O emissions in HPI systems. With constant root-derived emissions, greater root-derived N inputs would inevitably decrease the fraction of root-derived inputs emitted, EF_N. Though we cannot determine causality, greater root-derived N inputs were negatively related to EF_N in 4-yr systems.

Root-Derived Carbon and Nitrogen Emission Factors

Our results are in agreement with recent findings for aboveground residue that the EF for organic residues is, on average, less than the IPCC's default 1% (Delgado et

al., 2010). Lower losses of belowground residue N than aboveground residue N are expected (Lou et al., 2007), as with residue C (Rasse et al., 2005). The highest EF_N we measured from any plot was 0.5%, with an average of less than 0.2% (over 115 days in 2006). For the shorter 2005 measurement period, the EF_N was on average 0.03% (over 34 days). In a laboratory study of rice residue decomposition, rice straw amended soils had higher cumulative N_2O emissions and a higher straw emission factor over 55 days, 0.26%, than was measured for rice root residue amended soils, 0.15% (Lou et al., 2007). To our knowledge, this rice root residue study, though it excludes root residue N, provides the most comparable root-derived N_2O EF value to our estimates of a field-based corn root-derived EF_N.

The magnitude of root-derived N_2O-N losses was significantly lower than root-derived CO_2-C losses. Greater recycling of N than C is expected in soils, a fact evidenced by the decreasing C:N ratio of organic matter with increased microbial processing (Sorenson, 1981), and by studies showing lower losses of aboveground residue N than C from soils over time (Aita et al., 1997; Malpassi et al., 2000). While the absolute loss of root-derived C greatly exceeded the loss of root-derived N, contributions of root-derived C and N to CO_2 and N_2O emissions were very similar (0–30%) over each season.

Emissions of CO_2 and N_2O often are positively related (Rice et al., 1988; Garcia-Montiel et al., 2004; Miller et al., 2008; Omonode et al., 2011), but to our knowledge this is the first field-based test of the relationship between direct measures of root-derived C and N emissions as CO_2 and N_2O, and between the root-derived contribution to total CO_2 and N_2O emissions in an agricultural ecosystem. Following a method used

by Qian et al. (1997) we determined that approximately 0.1% of the root-derived C lost in 2006 was associated with the production of root-derived N emissions (N_2O and N_2), and that only 0.01% was associated with production of the root-derived N_2O emissions. For comparison, in laboratory incubations of soil planted with corn, Qian et al. (1997) determined that the CO_2 produced during denitrification (N_2O and N_2) reflected between 0.1–1.4% of total C lost via respiration. The estimates of total denitrification N and associated C losses were based on a N_2:N_2O ratio of 7:1 (CAST, 1976; Rolston et al., 1978; Mosier et al., 1986; Heinemeyer et al., 1998; Li et al., 2002) and the C requirements for N_2 and N_2O production (Qian et al., 1997). This calculation resulted in an estimate of 0.2–0.8% loss of root-derived N via denitrification (as N_2O and N_2).

Management Effects on Root-Derived Emission Factors

Our 2006 results highlight the greater potential of management to influence root-derived C rather than N emissions; the range of EF_N among management systems is very small in comparison to the range in EF_C. For example, if ORG, LPI and HPI systems each lost 0.15% of root-derived N as N_2O, based on 2006 results, the corresponding losses of root-derived C as CO_2 would have been roughly 25% from ORG, nearly 40% from LPI, and roughly 50% from HPI over the growing season.

Greater root-derived N losses per gram of root-derived C lost in ORG and LPI systems compared with HPI systems could be interpreted as increased N "leakiness", at least relative to root-derived C losses, although not in absolute terms. Total N_{root} emissions tended to be lower in ORG and LPI systems than HPI systems. Increased N availability in ORG and LPI systems (TN and NO_3^-) may have driven higher rates of

nitrification and denitrification, and thus greater N_2O production from ORG and LPI systems. With sufficient N, plant residue addition appears to stimulate N_2O production (Sarkodie-Addo et al., 2003). Specifically, root exudate C can increase rates of N cycling and N_2O production in the rhizosphere due to direct stimulatory effects of exudate C on denitrifiers, or indirectly through O_2 consumption during decomposition resulting in more favorable denitrification conditions (Smith and Tiedje, 1979; Henry et al., 2008; Philippot et al., 2009).

Alternatively, greater root-derived N loss per gram root-derived C lost in ORG and LPI systems than HPI systems may suggest less leaky C cycling, or tighter C–N coupling, in LPI and ORG systems than HPI systems. Occlusion of residue C and N in stable microaggregates likely contributes to residue C–N coupling (Aita et al., 1997; Angers et al., 1997) Greater stability of microaggregates in lower tillage systems (LPI) (Six et al., 2000) may partially explain the differences in the C-N coupling observed. Further exploration of management system effects on the relationship between C and N cycling is needed to determine if decreased management intensity and the addition of organic matter can increase the strength of C and N coupling (Gärdenäs et al., 2011). Largely in support of hypothesized management effects on total C and N proposed by Gardner and Drinkwater (2009), we observed lower losses of both root-derived C and N in systems with more diverse rotations. We also observed lower root-derived C losses from systems with organic inputs, though these low C losses were accompanied by increased root-derived N losses.

Directions for Future Research

Measurement of root-derived N_2O emissions allowed us to estimate the contribution of this particular N source to total N_2O emissions and management effects on root-derived N_2O emissions. In the current study, though important due to the strength of N_2O as a greenhouse gas, cumulative root-derived N_2O losses were less than 0.2% of the initial amount of root-derived N, while total losses of root-derived N from the soil pool ranged from 0–65% (D. Allan, *personal communication*, 2010). For comparison, over a period of 112 days, 24% of oat root N and 43% of rye root N (not including exudate N), were lost from the soil organic matter pool (Malpassi et al. 2000); this loss included N_2O losses, but also losses due to complete reduction to N_2 and leaching losses. Clearly, the high loss of root-derived N via other pathways serves as a reminder that N_2O is only one form in which root-derived N can leave soils, and the results of this study must be considered in combination with studies of management effects on other N loss pathways. For example, agricultural management has also been shown to influence residue-derived N leaching losses as NO_3^- (Delgado et al., 2010), and the ratio of N_2:N_2O emissions, with greater N_2 emissions from higher C, reduced tillage soils (Venterea and Stanenas, 2008). Further work integrating N loss via multiple pathways is needed to fully understand agricultural management system impacts on the fate of root-derived N.

Based on this study we cannot determine losses of root-derived N that occurred between corn harvest in the fall and the following spring prior to soybean planting. All losses of root-derived N were calculated relative to the amount of detrital corn root-derived N present in the soil in the spring prior to gas sampling. Losses during this

period have the potential to be significant, as N_2O emission rates can be high relative to growing season emission rates following harvest and during early spring freeze/thaw events (Rochette et al., 2004; Johnson et al., 2010). Year-long studies using this approach but also quantifying root-derived N_2 emissions would allow for a more complete understanding of management system effects on root-derived N cycling.

Parallel studies are needed on other soil types and with root-derived material from other crops. Fine textured soils are known to increase N_2O emissions (Bouwman et al., 2002a), as well as influence residue C-N coupling (Sorenson, 1981). N_2O emissions and EFs have been shown to vary with residue C:N ratios (Huang et al., 2004), though effects of residue C:N on decomposition may be influenced by soil N availability (Sall et al., 2007). Losses of root-derived C and N discussed here and in Van Vleck and King (2011) may underestimate the potential losses of root-derived C and N from crops other than corn. Corn roots have been shown to have a particularly large contribution to soil organic carbon compared to other common crops (Allmaras et al., 2004; Wilts et al., 2004; Johnson et al., 2007). If corn roots contribute more to stable soil N, as well as C, pools than other crops, and denitrification losses are lower under soybean than corn (Parkin and Kaspar, 2006; David et al., 2009), then we expect losses of root-derived N as N_2O to be higher from other crops than measured for corn root-derived N in this study.

Conclusions

There is great interest in determining how to accurately estimate the sources of greenhouse gas emissions (Delgado et al., 2010) and how to increase C–N coupling

(Gärdenäs et al., 2011) in agricultural ecosystems. In this study we investigated environmental and management effects on both root-derived N_2O emissions and the coupling of root-derived C and N. Crop rotation diversity exerted the greatest influence over the root-derived fraction of cumulative N_2O emissions and the fraction of initial root-derived N emitted as N_2O over the growing season. Lower crop rotation diversity was associated with greater root-derived N losses in both seasons, possibly due to differences in root quality. To our knowledge, this represents the first field-based estimate of a root-derived N emission factor and the first examination of management effects on root-derived C-N coupling. Growing season losses of corn root-derived N as N_2O were very low, representing less than 0.2% of the initial root-derived N. While the absolute loss of root-derived C greatly exceeded the loss of root-derived N, contributions of root-derived C and N to CO_2 and N_2O emissions were very similar (0–30%). Further research on total denitrification (N_2 plus N_2O) and leaching losses of root-derived N are needed in order to fully understand the effects of management on root-derived N cycling. Further studies are also needed to determine the causes and consequences of management effects on residue-derived C-N coupling.

Table 3.1 Management system components: system name, rotation length, fertilization, and tillage. Soybean followed corn in 2-yr crop rotations, while in 4-yr rotations corn was followed by soybean, oats interseeded with alfalfa, and alfalfa. Labeled corn root N emissions were measured in the soybean growing season.

System Name	Rotation Length	Fertilization[†]	Tillage Timing, Type, and Depth
	— yr —		——— cm ———
High Purchased Inputs (HPI)	2 & 4	high N, P, K (broadcast)	spring field cultivator (10) fall moldboard plow (20)[§]
Low Purchased Inputs (LPI)	2 & 4	low N, P, K (banded)	spring field cultivator (10) mid-season cultivator, 2 times (8) fall chisel plow (20)[‡§]
Organic (ORG)	4	manure	spring disk tillage & field cultivator (10) early season rotary hoe (2.5) mid-season cultivator, 2 times (8) fall moldboard plow (20)[§]

[†]No fertilization during the soybean phase of crop rotations. Fertilization of corn was based on 10% higher yield goals for HPI than LPI systems; fertilization in LPI systems was based on more realistic yield goals
[‡]No fall tillage in 2LPI or 4LPI following the soybean crop.
[§]No fall tillage in 4-year rotations following the oat/alfalfa season.

Table 3.2 Soil characteristics: average soil temperature, water-filled pore space (WFPS), ammonium and nitrate concentrations.

Management System	Soil Temperature		WFPS		Ammonium		Nitrate	
	2005	2006	2005	2006	2005	2006	2005	2006
	—— °C ——		—— % ——		—— µg N/g soil ——			
2HPI	26.2 (1.0)	24.8 (1.9)	59.5 (2.8)	55.3 (3.3)	0.82 (0.12)	0.67 (0.09)	4.19 (0.65)	3.76 (0.48)
2LPI	25.7 (0.9)	25.0 (1.8)	71.2 (3.3)	57.9 (3.5)	0.71 (0.09)	0.55 (0.08)	5.62 (0.90)	5.84 (0.65)
4HPI	25.6 (0.9)	23.6 (1.7)	64.5 (2.4)	57.9 (3.9)	1.00 (0.13)	0.57 (0.09)	4.73 (0.74)	4.50 (0.86)
4LPI	24.9 (0.8)	24.2 (1.5)	62.9 (3.4)	57.4 (4.0)	1.14 (0.13)	0.55 (0.10)	4.78 (0.83)	4.98 (0.90)
4ORG	26.2 (1.0)	24.5 (1.6)	62.9 (3.0)	54.5 (4.1)	0.83 (0.11)	0.74 (0.14)	8.10 (1.39)	7.62 (1.13)

Table 3.3 Management effects on total soil nitrogen (TN), root-derived nitrogen (N_{root}), the root-derived fraction of TN (TN_{root}), and the C:N ratio of root-derived material each spring as determined by main effect models. Mean and standard error (SE) shown. Significance shown for *a priori* management system contrasts (one-sided unless noted), dashes indicate *p*-values>0.2. Following *p*-values≤0.2 (Bonferroni corrected) the management practice (or system) with the greater value is indicated.

Management System	TN		N_{root}[‡]		TN_{root}		$C:N_{root}$[‡]	
	2005	2006	2005	2006	2005	2006	2005	2006
	– kg N/m^2, 0–30 cm –		– g N_{root}/m^2, 0–30 cm –		— % —		— —	
2HPI	0.79 (0.02)	0.72 (0.04)	4.70 (1.78)	2.27 (0.77)	0.59 (0.22)	0.31 (0.09)	26.6 (1.7)	31.7 (2.2)
2LPI	0.88 (0.03)	0.88 (0.04)	3.00 (0.49)	1.20 (0.26)	0.35 (0.07)	0.14 (0.03)	29.6 (2.9)	31.5 (0.3)
4HPI	0.87 (0.04)	0.82 (0.09)	4.37 (0.33)	3.97 (0.49)	0.50 (0.02)	0.48 (0.02)	27.1 (1.6)	25.0 (0.9)
4LPI	0.92 (0.03)	0.99 (0.14)	3.23 (0.72)	2.87 (0.48)	0.36 (0.08)	0.29 (0.05)	33.3 (0.8)	34.7 (1.6)
4ORG	0.92 (0.04)	1.06 (0.10)	3.83 (0.33)	2.87 (1.22)	0.42 (0.03)	0.26 (0.09)	26.4 (2.7)	24.6 (0.7)
Contrasts								
HPI vs. LPI	<0.001*** LPI	0.022* LPI	--	--	0.130[§] HPI	0.006** HPI	0.0815[†] LPI	0.004** LPI
2 vs. 4-yr.	0.005** 4-yr	0.184[§] 4-yr	--	0.044* 4-yr	--	0.022*[‡] 4-yr	--	--
4HPI, 4LPI vs. 4ORG	--	0.096[†] 4ORG	--	--	--	0.126[§] 4HPI, 4LPI	--	0.007** 4HPI, 4LPI

[†] Marginally significant at the $p \leq 0.1$ level.
*Significant at the $p \leq 0.05$ level.
**Significant at the $p \leq 0.01$ level.
***Significant at the $p \leq 0.001$ level.
[‡] Two-sided *p*-value, two-sided hypothesis or results contrary to *a priori* one-sided hypothesis.
[§] Non-significant trend, $0.1 \leq p \leq 0.2$.

Table 3.4 Main effect and best fit models for cumulative total and root-derived N_2O emissions (N_2O, N_2O_{root}), the root-derived fraction of cumulative N_2O emissions (F_{root}), and the fraction of initial root-derived N emitted (EF_N) over each season. Model components, direction of covariate-response relationship, covariate and contrast significance, and R^2 values. M = management system; NO_3^- = soil nitrate (µg N/g soil); temp = soil temperature at 10 cm (°C); WFPS = water filled pore space (%) in the upper 30 cm; TN = total soil nitrogen (kg N/m^2).

	N_2O		N_2O_{root}		F_{root}		EF_N	
Main Effect	**2005**	**2006**	**2005**	**2006**	**2005**	**2006**	**2005**	**2006**
	– mg N/m^2 –		– mg N_{root}/m^2 –		——— % ———			
Model:	M	M	M	M	M	M	M	M
Contrasts:								
HPI vs. LPI	—	HPI (0.153[§])	HPI (<0.001***)	—	HPI (0.080[†])	LPI (0.003**[‡])	—	—
2 vs. 4-yr	—	—	2 (0.058[†])	—	2 (0.033*)	2 (0.039*)	—	2 (0.044*)
4HPI, 4LPI vs. 4ORG	—	—	—	—	—	4HPI, 4LPI (0.047*)	—	—
main effect R^2:	—	0.40	0.72	—	0.50	0.74	—	0.42
Best Fit								
Model:	NO_3^-	M + temp	M	temp	M	M	M + WFPS	temp
relationship	(+)	(+)		(+)			(-)	(+)
p-value	0.024*	0.121[§]		0.048*			<0.001***	0.002**
Contrasts:								
HPI vs. LPI	—	HPI (0.058[†])		—			—	—
2 vs. 4-yr	—	4 (0.091[†‡])		—			2 (0.0190*)	—
4HPI, 4LPI vs. 4ORG	—	—		—			—	—
best fit R^2:	0.28	0.58		0.23			0.75	0.46

[†]Marginally significant at the $p \leq 0.1$ level.
*Significant at the $p \leq 0.05$ level.
**Significant at the $p \leq 0.01$ level.
***Significant at the $p \leq 0.001$ level.
[‡] Two-sided *p*-value indicated where result is contrary to *a priori* one-sided hypothesis.
[§] Non-significant trend, $0.1 \leq p \leq 0.2$.

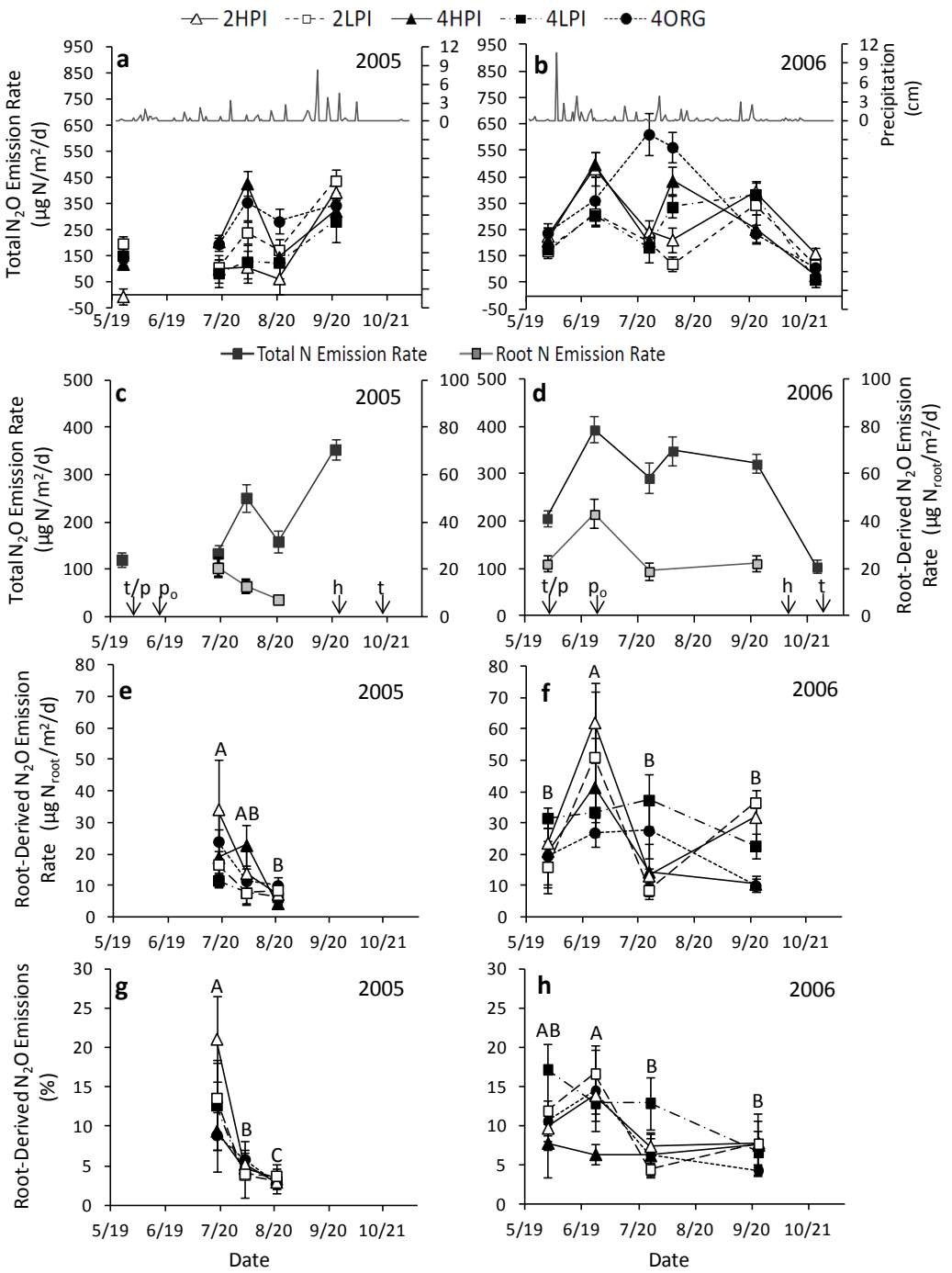

Figure 3.1 Precipitation and seasonal patterns of N_2O emission rates by management system in 2005 (a) and 2006 (b). Total and root-derived N_2O emission rates, averaged across management systems, in 2005 (c) and 2006 (d). Dates of spring tillage and planting for all but 4ORG (t/p), planting for 4ORG (p_o), harvest (h), and fall tillage (t) are indicated. Root-derived N_2O emission rates in 2005 (e) and 2006 (f). Root-derived contribution to N_2O emissions in 2005 (g) and 2006 (h). Mean and SE shown, *n*=3. Different capital letters indicate significant differences between dates.

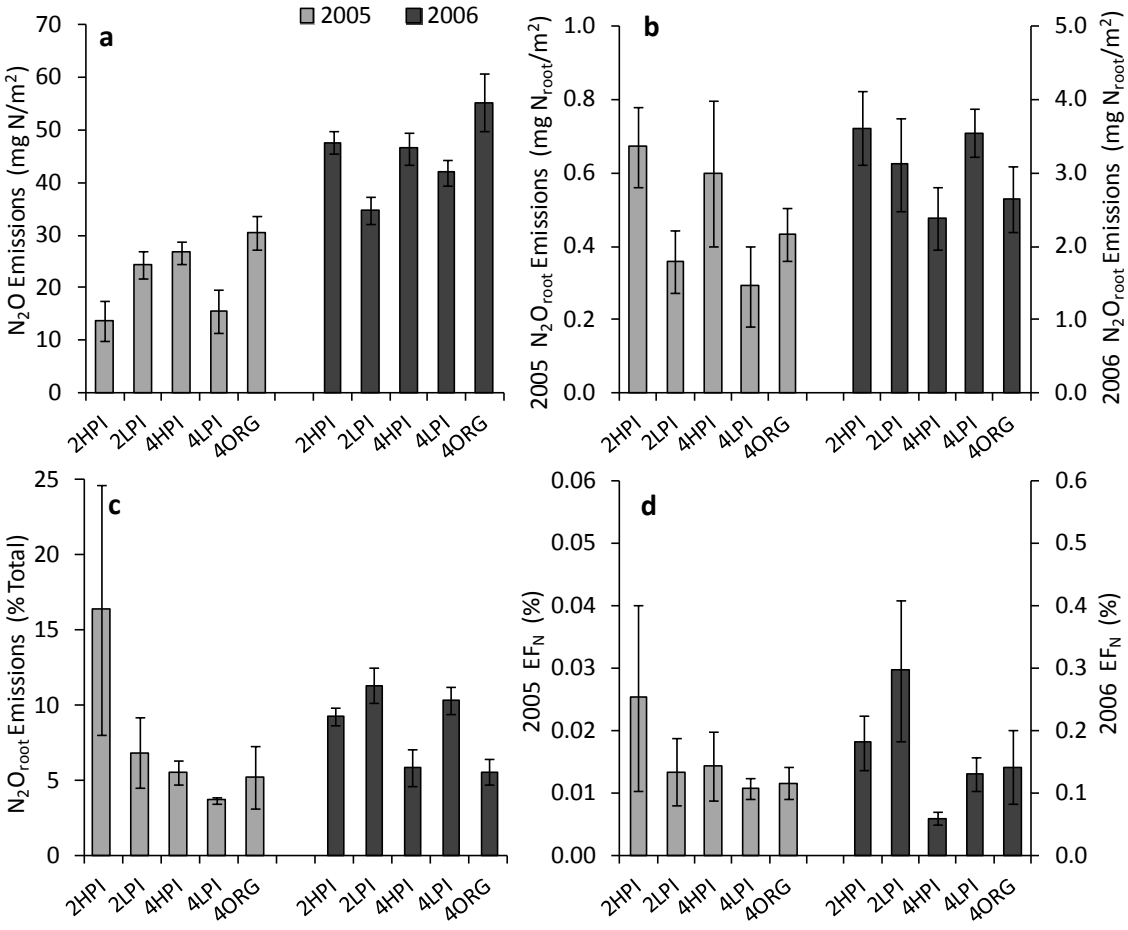

Figure 3.2 Cumulative N dynamics by management system for each growing season, *n*=3, mean and SE shown. Cumulative N_2O emissions: (a) total and (b) root-derived. (c) Root-derived fraction of cumulative N_2O emissions. (d) Fraction of initial root-derived N emitted as N_2O (EF_N). Differences in the sampling period length required two y-axes in panels (b) and (d).

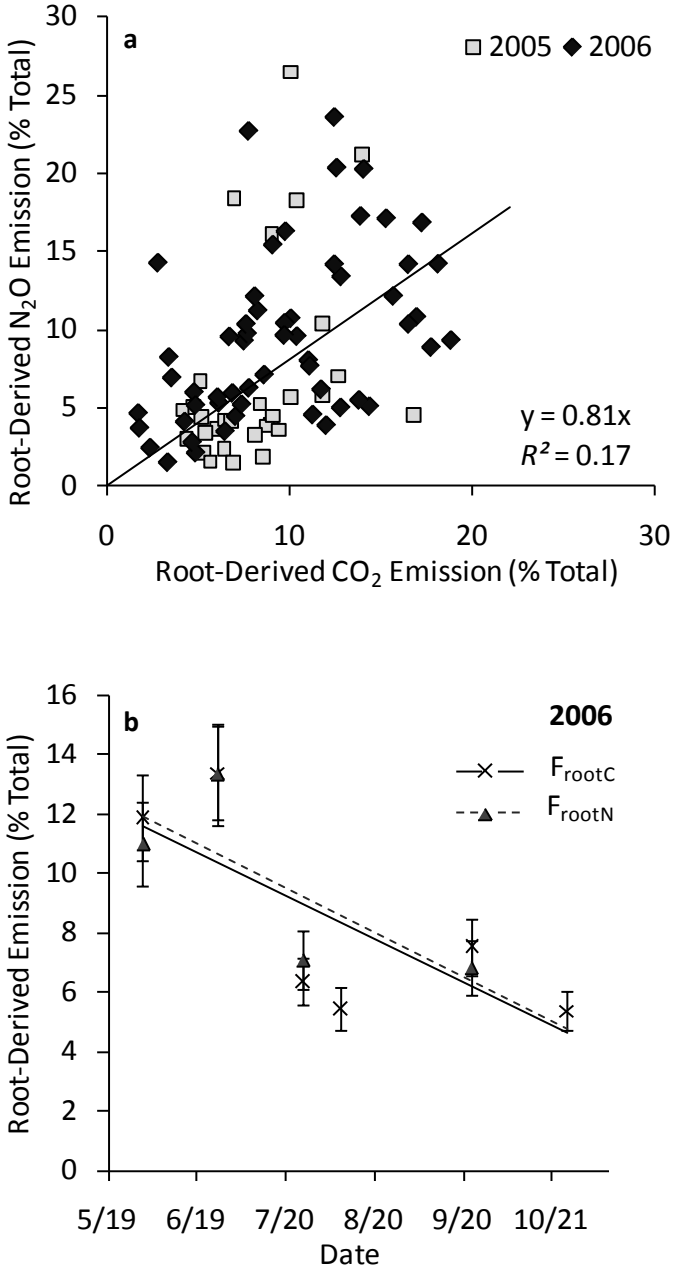

Figure 3.3 Relationship between the contribution of root-derived C and N to daily CO_2 and N_2O emissions. Each point represents a single plot on a single date. Root-derived C and N contributions to total CO_2 (F_{rootC}) and N_2O (F_{rootN}) emissions: (a) between seasons, single trendline and regression equation (n_{2005}=30, n_{2006}=57), and (b) over the 2006 season (n=15).

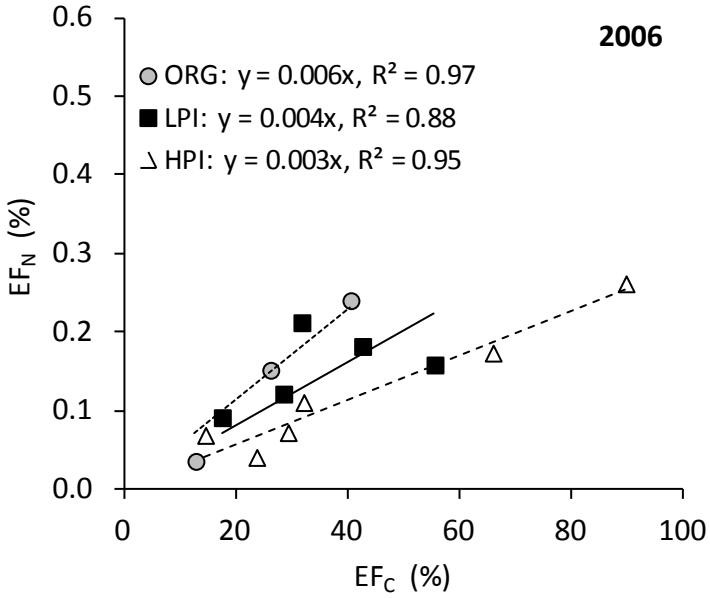

Figure 3.4 Fraction of initial root-derived C and N emitted as CO_2 and N_2O. In 2006, the slope of the EF_C-EF_N relationship differed between HPI, LPI, and ORG systems.

CHAPTER 4

Landscape Position and Soil Moisture Effects on Carbon Pool Dynamics of Agricultural Soils

Erosion-induced burial and protection of carbon transported down hillslopes is considered the primary mechanism driving the existence of soil carbon (C) sinks in agricultural ecosystems. We considered the potential for high soil moisture conditions to stabilize C along a low-slope hillslope in south-central Minnesota. Specifically, we investigated: (1) if patterns of C accumulation typical of erosion-dominated higher slope landscapes were evident on a low slope agricultural hillslope, and (2) how C pool sizes and their mean residence time (MRT) along the hillslope responded to changes in soil moisture.

Intact core sections from four horizons at three hillslope positions were incubated in the laboratory at 50, 75, 90 and 100% WFPS for 355 days. Total C (TC) and the nonhydrolyzable fraction of TC both (64% of TC, 2.4–27.0 mg C/cm^3) increased downslope. The labile C fraction (less than 1% of TC) was more responsive to moisture treatments than the slow C fraction (35% of TC, 0.5–14.5 mg C/cm^3). Across treatments and positions the labile C pool size of the uppermost horizon differed by 72% (0.31 mg C/cm^3) from the maximum pool size. The labile C pool size ranged from 0.08–0.56 mg C/cm^3. In the lower three horizons, the size of the labile C pool differed from the maximum by 70–79% (0.08–0.09 mg C/cm^3) with a range in the pool size of 0.01–0.16 mg C/cm^3 across treatments. The size of the labile C fraction was largest and the labile C MRT was longest in the 100% WFPS treatment in most horizons. In contrast, intermediate moisture treatment levels (90 and 75%) resulted in the longest MRT estimates for the slow C fraction. Treatments resulted in a range of slow C MRT estimates among horizons, from 11–126 years, representing a range of 86–89% change from the maximum MRT estimated for each horizon. Based on the significant changes

in the size of the labile C pool and the MRT of the slow C pool in response to changes in soil moisture observed in this study, further research into the effects of soil moisture on soil C stabilization and dynamics of C storage along hillslopes in similar low slope-high moisture landscapes is needed.

ABBREVIATIONS

BD	bulk density
C	carbon
CH_4	methane
CO_2	carbon dioxide
CRP	Conservation Reserve Program
F	footslope
LB	lower backslope
MRT	mean residence time
N	nitrogen
NHC	nonhydrolyzable C
N_2	nitrogen gas
N_2O	nitrous oxide
NH_4^+	ammonium
NO_3^-	nitrate
NPP	net primary productivity
SOC	soil organic carbon
US	upper shoulder
WFPS	water filled pore space

INTRODUCTION

Modeling and field studies show an increase in soil carbon (C) pools at low topographic positions across a wide range of ecosystems in both surface soils (Schimel et al., 1985; Burke et al., 1999; Hook and Burke, 2000; Garten and Ashwood, 2002) and at depth (Rosenbloom et al., 2006). Similarly, measures of soil C age indicate older C, or a greater abundance of C with a long mean residence time (MRT), in low topographic positions as compared with upslope soils (Harden et al., 1999; Sherrod et al., 2005). Mechanisms previously used to explain patterns of soil organic carbon (SOC) distribution and MRT along hillslopes include: 1) erosion induced transport and burial of SOC slowing decomposition in low topographic positions due to lower microbial activity at depth, 2) transport of fine textured soils to low slope positions leading to lower decomposition through binding of SOC to silt and clay particles, and 3) transport of nutrients downslope creating gradients in net primary productivity (NPP) along hillslopes and ultimately greater C inputs to soils in low slope positions. A fourth mechanism, frequently mentioned but less frequently examined, is slower decomposition downslope due to increased soil moisture and greater oxygen limitation of microbial activity in regions with near-surface water tables. Due to the co-variation of these mechanisms, identifying the principal cause of SOC accumulation downslope across a wide range of landscapes has proven a challenge.

The majority of hillslope C studies have focused on erosion and deposition of SOC as drivers of the correlation between SOC and topography (Stallard, 1998; Liu et al., 2003; Rosenbloom et al., 2006; Yoo et al., 2006). The hypothesis that erosion can result in the development of a C sink in low hillslope positions (Stallard, 1998) has been

supported in both agricultural ecosystems and in lower erosion, more pristine systems (Harden et al., 1999; Berhe et al., 2007). Rapid burial, followed by physical or chemical protection of the transported labile C, slows decomposition rates and is considered the primary mechanism creating this C sink (Van Oost et al., 2007). Results showing higher C sink strength in eroding rather than in depositional sites have been explained by either: a) high C input rates from plant residue combined with low CO_2 emissions at eroded sites (Liu et al., 2003), or b) high decomposition rates of transported labile C prior to deposition in a low slope position preventing the accumulation of C downslope (Jacinthe et al., 2001b). The size of the potential erosion-induced C sink in agricultural ecosystems and mechanisms controlling the sink size are still unclear (Van Oost et al., 2007). However, quantifying C sinks in agricultural landscapes is important for developing better landscape-level estimates of the net C balance of agricultural ecosystems, particularly given the vast land area used for agricultural production globally, 11% (IPCC, 2007).

In addition to direct C transport effects, erosion also indirectly influences hillslope C distribution through effects on soil texture. Erosion frequently creates gradients of soil texture along hillslopes such that low slope positions have a higher percentage of fine textured soils (Schimel et al., 1985; Kravchenko et al., 2006). Fine textured soils (high percent silt + clay) are frequently correlated with greater SOC pools (Schimel et al., 1985; Burke et al., 1999) due to increased chemical protection of SOC as mineral-associated SOC (Torn et al., 1997; Mikutta et al., 2006). In addition to chemical protection of SOC, fine soil texture promotes aggregate formation and stability (Liu et al., 2003; Six et al., 2004) and increases soil moisture retention (Chapin et al., 2002),

each of which could lead to increased C pool sizes through decreased rates of decomposition. While this relationship between fine textured soils and larger SOC pools is included in soil C models like CENTURY (Parton et al., 1987), the relationship between C mineralization rates and clay content is not consistently strong (Giardina et al., 2001), suggesting that the importance of the relationship between C pool size and soil texture may vary among ecosystems and landscapes.

Increased concentrations of nitrogen (N) in low slope positions have also been attributed to erosion and deposition (Schimel et al., 1985; Burke et al., 1999). Consequences of increased N concentrations downslope include positive effects on C input (i.e. NPP) rates (Berhe et al., 2007), while effects on C loss rates from litter and SOC can be negative (Craine et al., 2007; Hobbie, 2008). Overall, studies examining the effects of nutrient availability on SOC dynamics have shown mixed results (Dijkstra et al., 2004; Torn et al., 2005). Due to the typical high fertility of agricultural soils N availability is unlikely to be a key driver of C dynamics along hillslopes in agricultural ecosystems.

Insight into how changes in hydrology might influence C distribution and MRT along hillslopes comes primarily from wetland studies. Wetland soils store more C than any other individual life zone, 220 Pg C (Post et al., 1982). The abundance of studies examining the C response of wetland soils to altered moisture and water table levels is due to concern over the vulnerability of this large C stock to decomposition as the climate changes (Gorham, 1991). To a point, increased soil moisture can increase microbial activity through increased diffusion of substrates to microbial cells, increased movement of extracellular enzymes to substrates, or decreased dehydration of microbial

cells (Stark and Firestone, 1995). As demonstrated in studies of short term water table manipulation, soil moisture can exert strong, often immediate, control over the magnitude of biological process rates (Freeman et al., 1993; Mikha et al., 2005; Yurova et al., 2007). As soil moisture increases and conditions become increasingly anaerobic, rates of microbial respiration tend to decline in both surface soils and at depth (Gorham, 1991; Oechel et al., 1998; Freeman et al., 2001).

In low slope-high moisture landscapes, the negative effect of high soil moisture on C mineralization rates may represent a fourth mechanism leading to increased C and MRT in downslope positions. Only a few hillslope studies have occurred at sites with a high water table or high soil moisture conditions. In low slope positions where soil moisture is high, low decomposition rates for litter (Hobbie et al., 2000) and large C pool sizes (Bedard-Haughn et al., 2006) have been attributed to increased moisture levels as opposed to variation in erosion and burial rates, soil texture, or N availability. Many other studies, and some along hillslopes, have suggested the importance of hydrologic characteristics as a control over SOC pool dynamics (Torn et al., 1997; Gregorich et al., 1998; Stallard, 1998; Harden et al., 1999; McCarty and Ritchie, 2002; Berhe et al., 2007). Despite this, few studies of mineral soils have quantified the importance of hydrology on the C dynamics of hillslope soils (Castellano et al., 2011).

Estimating the total soil organic C pool size is an inaccurate method of evaluating the soil C storage potential of a landscape (Trumbore, 2000). The dynamics of soil C storage include not only how much C is stored in the soil, the pool size, but also the time that an average atom of C in that pool will remain in the soil, the MRT (pool size divided by the flux rate into or out of the pool) (Schlesinger, 1997). Pool size

and MRT can be estimated for the total C pool or for smaller, functionally defined C pools. Labile, slow, and resistant C pools are defined broadly by their MRTs which range from days to years, decades to centuries, and centuries to millennia respectively (Parton et al., 1987). The large variation in C pool MRT is due to biological, physical and chemical SOC protection processes which interact with C input and loss rates to determine the net balance of C in landscapes. The importance of looking at SOC as multiple distinct pools lies in their unique turnover times and unique ecosystem controls (a fast turnover time corresponds to a short mean residence time) (Trumbore, 2000). Though low slope positions show larger soil C pools and longer MRTs across a broad range of ecosystems, few studies have examined the relative sizes of the distinct C pools or their unique MRTs along hillslopes. While a few studies suggest that topography exerts greater control on the dynamics of the slow and resistant C pools than on the labile C pool (Burke et al., 1999; Sherrod et al., 2005), the variability in both the size and MRT of individual C pools, as it relates to topography, has not been determined across a broad range of landscapes.

To develop a complete understanding of C pool distribution and dynamics along hillslopes, a deep soil profile must be examined. There is a strong negative relationship between C and soil depth across soil types (Baisden et al., 2002; Rasse et al., 2006). Although most C is stored in the upper soil horizons (~ upper 20 cm) (Garten and Ashwood, 2002), the MRT estimated in surface soils can greatly underestimate the C storage potential of soils. This miscalculation is due to large stores of very slowly cycled C buried at depth (Jobbagy and Jackson, 2000; Trumbore, 2000; Paul et al., 2001b; Harden et al., 2002). Examination of C distribution and dynamics within a deep soil

profile is necessary to achieve a complete understanding of the controls on soil C cycling (Angers et al., 1997; Dolan et al., 2006; Baker et al., 2007).

The prairie pothole region that extends from southern Minnesota northwards into southern Canada is characterized by high quality agricultural soils, rolling topography, and an abundance of low slope, seasonally saturated, "marginal" farming sites within otherwise prime farmland. With climate change, winter precipitation in Minnesota is expected to increase by 15–40% by 2099, while summer precipitation is expected to decrease by 15% (Kling et al., 2003). Combined with warmer temperatures, these precipitation changes are expected to result in an overall decrease in soil moisture and water table levels in the state (Kling et al., 2003). In addition to potential declines in soil moisture and water table levels due to climate change, the recent push for biofuel production puts economic pressure on farmers to return marginal farmland enrolled in the Conservation Reserve Program (CRP) and other conservation set-aside programs back into active crop production (Wilhelm et al., 2010). In recent years, CRP lands in this region have shown increased rates of C sequestration over cultivated land (Johnson et al., 2005). This conversion back to active crop production will likely result in long term declines in soil C beyond those expected with climate change (Jacinthe et al., 2001a; Bedard-Haughn et al., 2006). Further declines in soil C could be due in part to altered hydrology if new areas of land are drained. Improving understanding of the sensitivity of soil C to changes in soil moisture along low slope, seasonally saturated hillslopes is a first step toward understanding how soil C pools and MRTs may respond to long-term soil moisture and water table level shifts due to change in climate or land use management.

In low erosion, seasonally saturated landscapes, understanding of the controls on carbon pool size and turnover is limited. The first objective of this research was to estimate both the patterns of C pool size and MRT along a low slope, seasonally saturated hillslope. The second objective was to quantify the effects of changing moisture conditions, ranging from near the wilting point to saturation, on these C pool sizes and MRTs throughout soil profiles along a hillslope. The C pool size and MRT were determined using long term incubations combined with hydrolysis of soil from three hillslope positions, with each horizon subjected to a series of soil moisture treatments encompassing the range of moisture conditions along the slope. We hypothesized that in each horizon pool sizes and MRT of C in each pool would increase downslope, but that the labile fraction of total C would show the least effects of hillslope position. This follows patterns for the MRT of total C in surface soils (Kravchenko and Robertson, 2007) and evidence that topography exerts greater control over the slow and resistant C pools than over the labile C pool (Burke et al., 1999; Sherrod et al., 2005). Others have observed that soils from different hillslope positions, when exposed to uniform climate conditions, show no variation in soil respiration rates when normalized by soil C content (Conant et al., 2000). We hypothesized that the MRT of labile C pools across hillslope positions, but within horizons, would converge when exposed to a uniform moisture treatment. By identifying the patterns of soil C pool size and MRT across this hillslope, our results represent a case study of the importance of soil moisture and position as controls of soil C dynamics in this landscape. As such, our results will contribute to a broader understanding of the interactions among topography, hydrology, and soil C cycling, an understanding that is necessary in order to predict responses of

soil C to climate change, and to inform models used for C credit allocation (e.g., Bricklemyer et al., 2007).

MATERIALS AND METHODS

Research Site Description and Sample Collection

Soil cores were collected from the Agricultural Ecology Research Farm (AERF) at the University of Minnesota's Southern Research and Outreach Center in Waseca, MN (44°03'62"N, 93°32'32"W). Mean annual precipitation at the site is 88 cm, with a mean January temperature of -12°C and a mean July temperature of 22°C (1971–2000 average, NRCS, 2000). Soils are derived from Des Moines Lobe glacial till and developed under native tall-grass prairie (Reuter and Bell, 2003). Soil series sampled were part of the Clarion–Nicollet–Webster sequence typical of soil catenas in southern Minnesota and northern Iowa. The average water table depth along a transect from ridge to toesplope positions ranged from 151 cm to 33 cm respectively, with alternating discharge and recharge hydrology (Reuter and Bell, 2003). These depths likely represent the depth to a perched water table, resting at the boundary of the basal till (below) and ablation till (above) (Bettis et al., 1996; Reuter and Bell, 2003). Compared to the region around our site, one atypical facet of the management was the absence of tile drainage along the sampled transect. The transect is surrounded by drained fields, and drainage tile has been placed above the water table at the summit position (not sampled for this study). Further information on the soils and hydrology are described by Reuter and Bell

(2003). Site management practices are typical for corn-soybean rotations throughout this region (Huggins et al., 2007).

Experimental Design

C pool sizes and MRTs were determined through long term incubations of soil from three hillslope positions. Intact soil core sections from four horizons at each position were subjected to a series of soil moisture treatment levels: 50, 75, 90, and 100% water filled pore space (WFPS) (Fig. 4.1). The four treatments extended from roughly the wilting point to saturation, and spanned the range of field moisture conditions (Reuter and Bell, 2003, unpublished data, D. Wheeler and J. Bell, 2008). Long-term incubations combined with acid hydrolysis have frequently been used to determine soil C pool sizes and turnover rates in mid-western soils (e.g., Collins et al., 2000). Non-linear models of respiration data from incubations, with variable numbers of C pools, have been shown to be effective in separation of functionally distinct C pools (Paul et al., 2006).

Intact cores were collected along a 55 m hillslope transect with a change in elevation of less than 4 m (Fig. 4.1). Thirty-nine cores were collected, thirteen from each of three positions: the upper shoulder, lower backslope, and footslope (positions described by Reuter and Bell, 2003). We used a random cluster sampling approach to capture soil variability along the hillslope, similar to that of Lin et al. (2005), with two to four cores collected from each of four clusters at each position. All soils were fine-loams varying in drainage characteristics from somewhat poorly drained Nicollet soils (mesic Aquic Hapludoll) at the upper shoulder (US), to poorly drained Webster soils (mesic

Typic Endoaquoll) at the lower backslope (LB), and very poorly-drained Glencoe soils (mesic Cumulic Endoaquoll) at the footslope (F).

Along the hillslope, sampling extended through the B horizon(s) and into the C horizon(s) where possible, reaching an average depth of 90 cm, but exceeding 140 cm depth in the lower slope locations. Sampling depth constrained primarily by our ability to collect samples with minimal compaction. Cores were collected in the late fall, after harvest but before fall tillage, using a 3.8 cm inner diameter Giddings probe. Cores were wrapped in plastic film, transported in PVC tubing, and stored at 12°C until processed. Horizons are referred to throughout by a horizon number, 1–4, for clarity. Horizon depth, soil series names and select characteristics are presented in Fig. 4.1.

Moisture Treatments

Within 48 hours of core collection, sections of the upper four horizons (Ap or A through the B horizon) were taken from each horizon in each core for the following: C mineralization incubations, net N mineralization incubations, field soil moisture, bulk density (BD), and initial inorganic N content determination. The remaining soil from each horizon was air dried, sieved, and stored in glass vials for total C (TC), non-hydrolyzable C (NHC), and total N (TN) analysis.

Core sections for C and N incubations remained field moist until four days prior to the start of the incubations. The soil moisture (105°C for 48 hours) and BD were measured on two 30 g sections (n=230) collected immediately above and below the C and N incubation sections from each core. The average moisture and BD of these samples was assumed equal to the field moisture and BD of the C and N incubation

sections. Based on field soil moisture, BD, field moist weight of the incubation sections, and an assumed particle density of 2.65 g/cm^3, we calculated the changes in weight needed to bring soils from field moist conditions to the target moisture treatment levels. We randomly assigned moisture treatments to soils prepared for C and N incubations. When WFPS treatment levels (%) exceeded field WFPS, incubation WFPS levels were established incrementally over four days using a spray bottle containing deionized water that had been degassed with N_2. When WFPS treatment levels were lower than field WFPS levels, incubation jars were covered loosely with plastic wrap to allow a slow rate of drying until the target weight was reached. During the period of treatment establishment, all jars remained in the dark at 12°C.

Nitrogen Mineralization

Short duration N mineralization incubations were conducted at the same treatment levels used for C mineralization incubations. Net N mineralization rates are an index of available N in high N soils (Schimel and Bennett, 2004) and are useful for interpretation of C mineralization data (Weintraub and Schimel, 2003). Intact sections of all cores (15 g dry soil equivalent) were incubated in plastic specimen cups at room temperature in the dark for an average of 35 days, the range was 32–39 days (6 replicates x 4 treatments x 4 horizons x 3 positions, *n*=288). Initial and final soil inorganic N concentrations, ammonium (NH_4^+) and nitrate (NO_3^-), were determined by extraction in a 2M KCl solution and flow injection colorimetric analysis (Quickchem 8000, Lachat, Loveland, CO). Net N mineralization was calculated as the change in total inorganic N concentration between the end (NH_4^+-N_{final} and NO_3^--N_{final}) and start (NH_4^+-

$N_{initial}$ and NO_3^--$N_{initial}$) of the incubation and scaled to the average 35 day incubation period. Negative net N mineralization values indicate net N immobilization (Binkley and Hart, 1989).

Carbon Mineralization Incubations

To determine hillslope position and soil moisture effects on C pool sizes and turnover rates, year-long incubations were conducted at four moisture treatment levels. Intact core sections at field moisture (25 g dry soil equivalent) were placed in 50 mL glass beakers within 1 L glass jars (4 replicates x 4 treatments x 4 horizons x 3 positions, *n*=192). To minimize structural changes in the soil and minimize aeration via the sides of core samples, the core diameter collected was chosen such that core sections fit tightly within the beakers. Headspace samples were collected through a Hungate septum (Bellco Glass, Inc., Vineland, NJ) inserted into each jar lid. No moisture adjustments were made between day 0 (defined as 24 hours after all moisture treatments had been established) and day 13. Soil moisture was monitored at two to four week intervals and was adjusted based on soil wet weight one week prior to each sampling period. Jars were closed between samplings so this frequency was sufficient to maintain moisture levels within 10% of the target WFPS level (within 1% of target wet weight). Samples were incubated in the dark at room temperature for 355 days; air temperature was monitored with iButtons (Maxim Integrated Products, Sunnyvale, CA).

Incubations were sampled with decreasing frequency as flux rates decreased and stabilized over the course of the nearly year-long incubation. Sampling occurred on the following days: 1, 3, 6, 10, 16, 37, 85, 130, 175, 217, 259, and 352. Sampling events

beginning on these dates will be referred to as sampling periods, to distinguish between events occurring each time a jar was sampled and events occurring one time per sampling period. Flux rates were determined from the regression slope of the headspace concentrations over time; sampling periods were 24 hours until day 80 after which point low flux rates required longer, 48–96 hour, sampling periods.

Simultaneous analyses for CO_2, N_2O, and CH_4 concentrations were completed using a gas chromatograph equipped with thermal conductivity, electron capture, and flame ionization detectors, respectively (HP 8580, Foster City, CA) and a headspace autosampler (Model 7000/7050, Teledyne Tekmar, Mason, OH). Gas standards (Scott Specialty Gas, Plumsteadville, PA) were used for calibration: three for CO_2 and two for N_2O and CH_4. The headspace volume of each jar (jar volume - soil volume) and room air temperature (18–20°C, data not shown) were used to calculate headspace gas concentrations using the ideal gas equation. We used linear interpolation between sampling dates to calculate cumulative fluxes.

At each sampling period, jar lids were removed, and jars were covered loosely with plastic wrap for one hour to release built up headspace gases and establish ambient atmospheric concentrations. Jars were then capped and remained closed for one hour prior to sampling. Before collecting each sample, a 20 mL syringe and needle were used to mix the headspace gas. Syringes were flushed five times, 10 mL per flush, prior to collection of a 15 mL sample. Samples were then injected into 9 mL glass vials capped with butyl rubber septa. Over-pressurizing reduced the possibility of leakage into the sample vials before analysis. Samples were analyzed within 96 hours of collection. Jars

remained capped between sampling periods except during monitoring of the WFPS level.

The watering and sampling procedures for the 100% WFPS treatment differed slightly from the other moisture treatments. After soil moisture was adjusted, each 100% WFPS incubation jar was flushed at 1 L/min for three minutes with pure N_2 in order to decrease the headspace O_2 that accumulated while jars were opened. As with the three other treatments, jars were opened prior to sampling for one hour, but the 100% treatment jars were opened in an N_2-filled glove bag to minimize O_2 introduction.

Determination of Carbon Pool Size and Turnover

A two-pool model was fit to the cumulative respiration data from C incubations in order to quantify the size and turnover rate of the labile C pool and a constant respiration rate of non-labile C, representing losses from the slow and resistant C pools (Dijkstra et al., 2006). Constrained two-pool models have been shown in other studies to better fit incubation data from soils in this region (Paul et al., 2006). However, for a subset of our incubation data, model comparisons using Akaike's Information Criterion, indicated that the model in Eq. 4.1 fit the data better than the more complex model described by Paul et al. (2006). Unique parameter values were determined for each sample:

$$C_t = C_l * (1 - e^{-k_l * t}) + c * t, \qquad \text{(Equation 4.1)}$$

where C_t is the cumulative amount of C emitted as CO_2 (mg C/g soil) at time t (d), C_l is the size of the labile C pool (mg C/g soil), k_l is the decomposition constant for the labile

C pool (1/d), c is the constant, non-labile C emission rate (mg C/g soil/d). To constrain the curve-fitting for Eq. 4.1, c was first determined using daily respiration rate data (Dijkstra et al., 2006):

$$R_t = a * e^{-k*t} + c, \qquad \text{(Equation 4.2)}$$

where R_t is the daily rate of C emission as CO_2 (mg C/g soil), and a is the initial respiration rate (day 1). C pools are presented primarily as a fraction of the TC pool (% TC). When the absolute C pool sizes are presented, values obtained from Eq. 4.1 are converted using BD to a volumetric basis (mg C/cm^3).

We used acid hydrolysis to isolate the size of a resistant fraction of soil organic C; in soils of this region, the MRT of the NHC fraction has been consistently longer than the MRT of the hydrolyzed C, averaging 1400 years (Paul et al., 2001a; Paul et al., 2006). The size of the NHC pool was examined across the hillslope positions and horizons, but the dynamics of the NHC pool were not estimated in models used to quantify the effects of moisture treatments on C pool sizes and MRT. We assumed that the contributions of NHC emissions to c (in Eq. 4.1 and 4.2) were negligible, meaning we assumed non-labile C emissions (c) came exclusively from the slow C pool. The size of the slow C pool was determined by difference: TC - NHC - C_l. Using a two-pool model we estimated the effect of moisture treatments on C pool size and MRT along the hillslope. The MRT of the labile pool is the inverse of k_l from Eq. 4.1, and is reported in units of days. The MRT of slow pool C was estimated by dividing the pool size, C_s, by the flux rate out of the pool, c and is reported in units of years.

The TC and the resistant fraction of total C were determined for soils from each horizon and position. All total and non-hydrolyzable C and N analyses were completed on an Elementar VarioMAX C/N Analyzer (Elementar Americas Inc., Mt. Laurel, NJ). To determine the size of the NHC pool, ten mL of 6N HCl were added to one gram of air-dried soil in digestor tubes and placed in a digestion block (Digestion System 40, 1016 Digestor, Tecator, Höganäs, Sweden) at 110°C. Samples were mixed with a vortex after 6–8 hours. After 16 hours, samples were filtered (Whatman 42 ashless filters, Fisher Scientific, Pittsburg, PA), rinsed with 75 mL of deionized water, and oven dried (90°C, 48 hours). The samples and filters were weighed pre- and post-drying. Dry samples were transferred from the filters to glass vials for storage until analysis. The NHC fraction was determined according to the following equation which accounts for mass loss from the mineral portion of samples during acid hydrolysis (Paul et al., 2006; Plante et al., 2006b).

$$\% \text{ NHC} = \frac{\left(\frac{\text{gC}}{\text{kg sample}}\right)_{\text{after}} \times \frac{\text{mass}_{\text{after}}}{\text{mass}_{\text{before}}}}{\left(\frac{\text{gC}}{\text{kg sample}}\right)_{\text{before}}}, \qquad \text{(Equation 4.3)}$$

Acid hydrolysis was performed on both density fractionated (adapted from Dijkstra et al., 2004) and non-fractionated samples; this comparison yielded no significant differences. With the high clay content of these soils, density fractionation methods did not result in clear separation between the heavy and light fractions; clay particles did not settle even 72 hours after separation with NaI and subsequent rinsing. Therefore, whole soil acid hydrolysis was used to estimate the NHC pool size (Paul et al., 2006; Pendall and King, 2007; Adair et al., 2008).

Data Analysis

ANOVA analyses were used to examine the main effects of position, moisture treatment, and their interaction on soil characteristics, C pool sizes, and turnover rates. ANOVA analyses were completed using R (version 2.10.1) (R Development Core Team, 2009) with the "nlme" package (Pinheiro et al., 2009). Where needed transformations were selected using the "car" (Fox and Weisberg, 2010) package. The estimates of C pool size and MRT were made using JMP 9.0.2 (SAS Institute, Cary, NC). Differences were considered significant when $p \leq 0.05$ and marginally significant when p-values were between 0.1 and 0.05. Tukey HSD comparisons are used to identify significant differences among positions and treatment levels.

As sampling was completed along a single hillslope, samples from each position and horizon are pseudoreplicates, not true replicates (Hurlbert, 1984). As a result, this study represents a case study of a single hillslope within this landscape and it must be recognized that this experiment is intended to be an initial test of the role that soil moisture and hillslope position play as controls over soil C dynamics within this landscape. Results should not however be extrapolated beyond this hillslope without further research.

RESULTS

Soil Respiration Data and Treatment Effectiveness

CO_2 emission data were used for determination of the labile and slow C pool sizes and turnover rates, while N_2O and CH_4 data were primarily used as indicators of treatment effectiveness. N_2O and CH_4 emissions would be expected to increase under anaerobic conditions. Cumulative emissions of all three greenhouse gases showed strong effects of hillslope position in most soil horizons (Table 4.1). As expected, CO_2 emissions were highest in the US position and lowest in the F position. Position effects were detected in all horizons except horizon 1. N_2O emissions similarly were highest from the US position and lowest from the F position. However, these patterns were only observed in horizons 1 and 2 and were driven by very high N_2O emissions from the US 100% treatment. CH_4 emissions were similarly highest in the 100% treatment from the US position and lowest from the F position, but only for horizon 1. From horizons 2 and 3, the pattern reversed with the highest emissions from the F position and the lowest emissions from the US position.

Cumulative emissions of all three greenhouse gases increased with increasing moisture treatment levels in all horizons (Table 4.1). When examined across hillslope positions and horizons, CO_2, N_2O, and CH_4 emissions decreased from the 100% WFPS treatment to the 50% WFPS treatment. Cumulative emissions of CO_2 reflect C loss from both the labile and slow C pools with losses from the latter assumed to be constant during the incubation period (Eq. 4.1). As such, cumulative emissions reflect treatment effects on C losses from all pools, whereas the size and turnover of pools, discussed later, reflect unique treatment effects on each of these two pools. Differences in C

emissions as CO_2, within horizon and position, primarily existed between either the 50% or 100% treatment and the remaining three treatments (horizon 1 shown, Fig. 4.2).

Positive N_2O and CH_4 emissions confirmed that conditions were more anaerobic in the 100% WFPS treatment than in the three drier treatments (Table 4.1). N_2O emissions within each hillslope position were significantly higher from the 100% WFPS treatment than the other three treatments. CH_4 uptake occurred in the three drier treatments in most horizons and from all hillslope positions. CH_4 production was observed in all 100% treatment incubations except from horizon 2 in the F position.

Landscape Position Effects on Soil Characteristics, Carbon Pool Sizes and Turnover Rates

Hillslope position significantly influenced C pool size across horizons, with the most consistent position effects on the TC pool size and the slow and resistant C fractions of TC (%) (Fig. 4.3). At any given depth, TC was greatest in the F position and lowest in the US position, as expected. However, when analyzed by horizon, due to the greater thickness of F horizons (Fig. 4.1), the TC content of F soils only exceeded LB or US soils in horizon 1. In horizons 2 and 3, US soils had the greatest TC content, while in horizon 4, LB soils had the greatest C content. On average, labile C represented less than 1% of TC across all soils and treatments, ranging from a low of 0.33% in horizon 2, to a high of 1.0% in horizon 4. Within each horizon, the labile C fraction of TC varied little among positions (Fig. 4.3, Table 4.3). NHC represented the largest fraction of TC; across all positions and horizons, the NHC fraction of TC was 64%. The NHC fraction was greatest in the F position for all horizons, averaging 52%, 59%, and 82% of TC in

the US, LB, and F positions respectively. Slow C represented the second largest fraction (35%) of TC. Conversely, the slow C fraction of TC was consistently greatest in the US soils (48%), smallest in the F soils (17%), and intermediate in LB soils (41%), in all but horizon 4. As C_s was determined by difference, the slow and resistant fractions of TC are necessarily inversely related. The size of the labile, slow, and resistant C pools (mg C/cm^3) decreased with depth with the exception of the LB horizon 4 soils which showed a slight increase in the size of both the labile and slow C pools between horizons 3 and 4. The effects of hillslope position on the size of C pools did not differ significantly from the position effects on C pool fractions (data not shown).

The MRT of C pools also varied by position (Table 4.3). The MRT of labile C was longest in the US and LB positions in horizon 1, the LB position in horizons 2 and 3, and the US and F in horizon 4. A more consistent pattern emerged across horizons for the MRT of the slow C pool; the MRT of the slow C pool was shortest in the F position in all but horizon 1. The MRT of the labile pool ranged from 53 days in horizon 1 (averaged across position and treatment) to 108 days in horizon 4. The MRT of the slow pool ranged from 63 years in horizon 1 (averaged across position and treatment) to 10 years in horizon 4.

Other soil characteristics along the hillslope showed differing effects of position by horizon. For example, BD did not differ by position in horizons 1 or 2, but at greater depth, in horizons 3 and 4, BD was higher in the low slope positions (Table 4.2). Both TC and TN (mg/cm^3) increased downslope, with the highest TC and TN content at any given depth found in the F position (TC: Fig. 4.3; TN: Table 4.2). The relatively high clay content of the LB and F soils, as compared to the sandier US soils, suggest that

erosional transport of clay particles and associated organic matter are at least in part responsible for greater soil C and N content downslope (Table 4.2). The soil C to N ratio (C:N) did not differ among positions in horizon 1. The soil C:N in horizon 2 was higher in the F than in either the LB or US positions, while in horizons 3 and 4 C:N was much higher in the US and LB soils than in the F soils.

One reason for expecting longer MRT of C downslope is the potential for changes in soil texture to influence C protection. Aggregation, an important mechanism of C protection from microbial degradation, typically increases with increased silt and clay content and decreased sand content. Throughout the profile, the percent sand content at the site did decrease in the downslope positions (US > LB > F) (Table 4.2). Across positions, the sand content increased with depth (1 < 2, 3 < 4). The silt and clay content both increased downslope (silt: F > LB, US; clay: F, LB > US) and decreased with depth (silt: 1, 2 > 3 > 4; clay: 1, 2 > 3, 4) (analysis based on data from Reuter and Bell, 2003). The resistant C pool size showed strong positive relationships with soil clay content (R^2=0.35) and negative relationships with sand (R^2=0.45) content.

Moisture Treatment Effects on Carbon Pool Size and Turnover Rates

Moisture treatments were expected to exert the greatest impact on the turnover time of the labile C pool and a smaller impact on the turnover time of the slow pool. The TC pool size, the NHC fraction of TC, and the NHC pool turnover rate were assumed to be unaffected by moisture treatments in this experiment. The effects of moisture treatments on the labile fraction of TC and the MRT of the labile C fraction were consistent across most horizons (Table 4.3). The labile fraction of TC in all but horizon

3 was greatest in the 100% WFPS treatment. Both the labile fraction of TC and the turnover time of the labile C pool were highest in the 100% WFPS treatment (Table 4.3). Interestingly, the slow C fraction of TC showed no effect of moisture treatment, though the MRT of slow C was consistently high in the 90% treatment followed by the 75 and 50% treatments. The shortest slow pool MRT values (fastest turnover) were measured in the 100% WFPS treatment.

Within position and horizon, we determined the percent change across moisture treatments for the size (mg C/cm^3) and MRT of the labile and slow C pools. This was calculated as the difference between the minimum treatment response and the maximum treatment response, and then divided by the maximum treatment response. The size of the labile C pool changed by roughly 75% across all horizons in response to the moisture treatment (0.14 mg C/cm^3). This change was greatest in the F position and in horizons 3 and 4. Changes in the MRT of the labile C pool with moisture treatments ranged from 66% in horizon 1 to 54% in horizon 3. These changes were greatest in the F position in horizons 1 and 4, and in the LB position in horizons 2 and 3. The greatest change in response to moisture treatments observed for the slow C pool size (mg C/cm^3) was in horizon 3, 44%, while the lowest change measured was in horizon 1, 11%. The MRT of the slow pool showed minimal variation in terms of the percent change across horizons, 86–90%. As with the labile C pool, the greatest changes in the size and MRT of the slow pool were observed in the F soils.

Nitrogen Mineralization

Net N mineralization rates were greater in the US and F positions than in the LB position in horizons 2 and 3; there were no significant effects of position on N mineralization rates in horizons 1 or 4. Rates ranged from -0.58 to +0.73 μg N/g soil/d, with a mean net N mineralization rate of 0.05 μg N/g soil/d. In contrast net N mineralization rates were significantly affected by soil moisture in horizons 1 and 4, but not horizons 2 and 3. In horizon 1, net N mineralization rates from the 100, 90, and 75% WFPS treatments were greater than rates in the 50% WFPS treatment. In horizon 4, net N mineralization rates were greater in the 50% WFPS treatment than any of the other three treatments. Net immobilization in horizon 1 only occurred in the 50% treatment, while in lower horizons, particularly in horizon 3, net immobilization occurred in most of the moisture treatments (Table 4.4).

The relationship between C and net N mineralization was horizon dependent. In horizon 1, there was a clear positive relationship between rates of C and N mineralization per gram of C and per gram of N respectively. In horizons 2 and 3, there were no relationships between C and net N mineralization. At greater depth (horizon 4), the relationship was clearly negative, with increased C mineralization corresponding to decreased N mineralization (Fig. 4.4). Within each position, C and N mineralization in horizon 1 each increased with moisture treatment level. In horizon 4, while C mineralization often increased with moisture treatment, N mineralization rates tended to decrease with increasing moisture.

DISCUSSION

In contrast to most studies of hillslope C dynamics, the total elevation change in this landscape was low, less than 4 m over a 55 m transect. Nevertheless, we found significant effects of hillslope position on C pool sizes and turnover rates even in this low slope landscape. Patterns of finer textured soils and greater TC and TN pools in the lowest landscape position suggest a contribution of erosion to downslope accumulation of C and N along the hillslope, as others have observed. As hypothesized, soil moisture also significantly affected C pool sizes and turnover rates. Also as hypothesized, saturated treatments did result in longer MRT estimates for the labile C pool. The MRT of the slow C fraction was longest at intermediate moisture levels (90 and 75% WFPS). The surprising observation that the MRT of the slow C pool was shortest under saturated conditions is likely a function of the model dynamics. Accordingly, we conclude that there is significant potential for increased soil moisture to act as a control over C dynamics along this hillslope.

Landscape Position Effects on Carbon Pools and Turnover Rates

The measured rates of C mineralization and corresponding C pool sizes were similar to measurements made by others for this region (Collins et al., 2000; Paul et al., 2006). On average, labile C represented less than 1% of TC in this study with MRTs increasing with depth from 51–107 days. Slow C represented 35% of TC with MRTs decreasing with depth from 62–9 years. The resistant C represented 64% of TC. Paul et al. (2006) report MRTs for the labile pool increasing with depth from 11–148 days, and MRTs for the slow pool decreasing with depth from 41–28 years. These MRT values

were estimated using very similar soil incubation procedures for a cropped field with similar soils in southern Minnesota (Paul et al., 2006). Across a broader range in climate, topography, and land use, the approximate C pool fractions of TC and MRTs do not differ as much as might be expected. In a cropped field in eastern Colorado, Sherrod et al. (2005) determined that the labile, slow, and resistant C pools represented 7, 28, and 71% of TC. Berhe et al. (2008) measured much higher labile C contributions, ranging from 5–45% of TC in a high slope, more arid, grassland system in California. However, the range of MRTs measured for the labile C pools was very similar between the California grassland site and our Minnesota cropland site. The range throughout the horizons in both studies (to a depth of 75 cm for Berhe et al., 2008) was roughly 15–190 days, with one value of greater than 300 days in this study.

Our expectation, based on prior studies of hillslope C dynamics, that TC would be greater in soils from the lower slope positions was only upheld in horizon 1. If viewed by depth (as depicted in Fig. 4.3), as opposed to comparing within horizon (statistics in Fig. 4.3), TC at any given depth was in fact greatest in the F position throughout the profile. The NHC fraction of TC reflected the expected pattern across positions and was significantly greater in the F than in the US position in all horizons. Contrary to our expectations, the slow fraction of TC was largest (in both relative and absolute terms) in the US position and smallest in the F position; the labile C fraction showed no consistent position effect across horizons. As observed for horizon 1 soils in this study, Jacinthe et al. (2001a) measured smaller labile C pool sizes in the upper 45 cm of depressional area soils adjacent to agricultural fields. Similarly, Sherrod et al. (2005) measured a greater labile fraction of TC in drier sites. In contrast, in horizons 2

and 4, we measured the greatest labile C as a fraction of TC in the F position. Similarly, Berhe et al. (2008) found that across three horizons, reaching a depth of 75 cm, the labile fraction of TC increased downslope. Our results also support a suggestion of Jacinthe et al. (2001a) that the resistant fraction of TC is higher in depositional areas than in erosional areas (in this study, the F and US positions respectively).

Moisture Treatment Effects on Carbon Pools and Turnover Rates

Especially viewed in combination with the high CH_4 and N_2O emissions in the 100% WFPS treatment, the CO_2 emissions were unexpectedly high. The effects of moisture treatments on the slow and labile C fractions of TC as well as the MRT of these two pools were driven primarily by the response of the 100% WFPS treatment. In other studies, the use of intact cores has resulted in both high CO_2 and N_2O emissions under very wet conditions (Ball et al., 2008; Castellano et al., 2010; Castellano et al., 2011). In the wetter treatments (90 and 100% WFPS) we also observed increasing rates of CO_2 emission in some of the jars over the first few days of the incubation. This may reflect increased CO_2 production via anaerobic pathways (fermentation) compensating for decreased CO_2 production via aerobic respiration limited by the low oxygen conditions (Weier et al., 1993).

Increased C diffusion in wetter soils (Stark and Firestone, 1995; Zak et al., 1999) could explain the unexpectedly high CO_2 emissions from the wettest treatments in this and other studies (Franzluebbers, 1999). Increased pore connectivity and transport of dissolved C could result in increased C availability for microbes. Fitting non-linear models to almost linear cumulative respiration curves of the 100% treatment resulted in

larger estimates of the labile C fraction as compared with the drier treatments. This suggests that increased moisture may indeed have caused an increase in the accessibility of soil C. Changes in the decomposition rate constant, not the C pool size, are most often expected with altered soil moisture or temperature treatments. However, in similar experiments with imposed temperature, or temperature and moisture treatments, others have also measured changes in the size of the slow and labile C pools among treatments (Townsend et al., 1997; Zak et al., 1999). Zak et al. (1999) found that the substrate pool size decreased at decreasing water potentials between -0.01 and -0.3 MPa. For comparison, we derived soil tension curves (data not shown) to estimate the matric potential at our moisture treatment levels. We observed the greatest decline in the labile C pool size, across all horizons and positions, between 0 and -0.02 MPa (corresponding to a decline between the 100 and 90% WFPS treatments and the 75% WFPS treatment). Labile C pool sizes were roughly stable between -0.2 and -3 MPa for all horizons and positions (50% WFPS treatment, data not shown).

Greater C availability could also be caused by decreased aggregate stability in the wetter treatments (Six et al., 2004). The creation and maintenance of the 100% WFPS treatment required additions of greater volumes of water over the incubation period. These additions could have contributed decreased aggregate stability. However, analysis of the soil BD at the end of the incubation suggested that the greatest change in BD during the incubation occurred in the 50% WFPS treatment (increased BD), while the smallest change occurred in the 100% WFPS treatment. Therefore, it seems unlikely reduced aggregate stability caused the unusually high respiration measurements and the increased size of the labile C pool in the saturated treatments.

A result of the increased size of the labile C pool was a longer labile pool MRT and a shorter slow pool MRT. As determination of the slow pool size, and therefore the MRT, is not independent of labile C pool size, it seems probable that the increased moisture resulted in a reduction of the physical or chemical protection of slow pool C. Reallocation of C between the slow and labile pools could explain the changes in both the size and MRT of these two C pools. Further field and laboratory studies of moisture effects on C availability are needed to identify a mechanism leading to the unexpectedly high C losses from saturated and nearly-saturated soils and to examine how soil C protection mechanisms are influenced by both long- and short-term changes in soil moisture. As stated, the results from this study should be viewed as a test of the role that soil moisture and hillslope position have as controls over C dynamics along this hillslope. Extrapolation from this experiment to other hillslopes, or from laboratory incubations to expected field observations must be made with caution.

Nitrogen Mineralization and Carbon-Nitrogen Coupling

Net N mineralization reflects the balance of mineralization and immobilization occurring in a given time period (Binkley and Hart, 1989). The greatest occurrence of N immobilization measurements was in horizon 3. N immobilization can be a result of soil environmental conditions such as high temperature, low moisture, or high substrate C:N ratios (Manzoni and Porporato, 2009). Rates of N mineralization observed, -0.58 to +0.73 µg N/g soil/d, are comparable to rates measured in a range of ecosystems across the Upper Midwest. In a sandy old field site in Minnesota, Dijkstra et al. (2006) measured rates of -0.2–0.8 µg N/g soil/d. In cultivated soils in Michigan, Robertson et

al. (1997) measured rates of -0.1–1.4 µg N/g soil/d. We observed a strong positive relationship between C and N mineralization rates in horizon 1, where results indicate that rates of both C and N mineralization were higher in US soils and in soils at higher moisture treatment levels.

We found opposing moisture treatment effects on N mineralization rates in horizons 1 and 4 (respectively, increased and decreased N mineralization rates with increased soil moisture), and no treatment effects in horizons 2 or 3. Positive effects of increased soil moisture on N mineralization rates, found in this study and by others (Zak et al., 1999), could be a function of increased N mobility in soils. The negative relationship between moisture and net N mineralization rates is unlikely to have been a function of decreased oxygen availability at depth. Redox values (Eh, corrected mV, data not shown) in the saturated horizon 4 incubations were higher than redox values in the saturated horizon 1 incubations, suggesting oxygen availability was slightly higher in horizon 4 than horizon 1.

The reversal in the N mineralization rate response to moisture treatment effects is very likely related to the reversal of the relationship between C and N mineralization also observed between horizons 1and 4. Often a positive relationship is expected between C and N mineralization, per gram C and per gram N, (Weintraub and Schimel, 2003). Based on observations made during soil processing, the high C:N ratios of horizon 4 soils in the US and LB positions were likely a result of black C which was only noted in horizon 4 at these two positions. Until recently black C was thought to be highly resistant to decomposition (Paul et al., 2006). Recent work suggests that this resistance may be a function of interactions with mineral soil rather than biochemical

recalcitrance (Marschner et al., 2008). In support of this, black C is more often found at depth in this region, and the fraction of organic C bound to mineral surfaces increases with depth (Kögel-Knabner et al., 2008). If black C is the cause of the high C:N ratios in horizon 4 soils, and if it is indeed accessible to microbial attack, the high C:N ratio of horizon 4 soils could result in elevated gross rates of N immobilization by microbes in these soils (e.g., Manzoni and Porporato, 2009) as compared to horizon 1 soils with lower C:N ratios. However, as the reversal in treatment effects on N mineralization rates and the reversal of the relationship between C and N mineralization were observed at all positions, effects of the C:N ratio are unlikely to be the sole cause of these two reversals with depth.

Conclusions

Here we show that patterns of downslope C accumulation typically associated with higher relief landscapes are also observed along a very low slope and highly managed hillslope. However, along this slope, patterns of individual C pools did not necessarily match those of the total C pool, highlighting the benefit of quantifying individual C pool characteristics. Emissions of CO_2 and N_2O per gram soil, the slow C fraction of TC, and net N mineralization rates each tended to be greater in upslope positions than lower slope positions. Increased moisture significantly increased greenhouse gas emissions from all positions and horizons, as well as increasing the MRT of labile C, decreasing the MRT of slow pool C, and generally increasing net N mineralization rates. These results suggest that the potential exists along low relief hillslopes for increased rates of C and N mineralization with increased soil moisture,

particularly in the US position. Extrapolation of these results to a broader landscape requires additional understanding of the mechanisms driving soil moisture effects on C pool size and turnover rates.

Perhaps most importantly, our results suggest that increased soil moisture may increase labile C fractions of TC and increase the MRTs for the slow C pool. Whether the effects of saturation on the MRT of the slow C pool are simply a function of the models used needs further investigation. The duration of this incubation experiment makes our results most representative of how long-term changes in soil moisture or water table levels could impact C dynamics along this hillslope. Climate change models predict that winter precipitation in the Upper Midwest region will increase and summer precipitation will decrease leading to more frequent occurrences of very wet conditions in the short-term, but reduced average soil moisture and water table levels in the long-term (Kling et al., 2003). Investigation of the relative importance of short-term versus long-term soil moisture changes to soil C pool sizes and MRTs would improve our ability to predict whether these soils will shift toward greater labile C fractions of TC and increased slow C MRT.

Table 4.1 Cumulative emissions: CO_2 (mg C/g soil), N_2O (µg N/g soil), and CH_4 (µg C/g soil), means shown, *n*=4. Different lower-case letters indicate significant differences among moisture treatments within each horizon by position, based on Tukey HSD results. Tukey comparisons across positions are not made within any horizon. Two-way ANOVA results show effects of hillslope position (P), treatment (WFPS) and their interaction on emissions by horizon.

	Position	WFPS (%)	Horizon 1		Horizon 2		Horizon 3		Horizon 4	
CO_2	Upper Shoulder	50	1.05 (0.06)	a	0.35 (0.07)	a	0.14 (0.01)	b	0.16 (<0.01)	b
		75	1.22 (0.02)	a	0.40 (0.04)	a	0.25 (0.02)	a	0.18 (0.01)	ab
		90	1.41 (0.30)	a	0.60 (0.02)	a	0.26 (0.01)	a	0.18 (0.02)	b
		100	1.86 (0.66)	a	0.57 (0.14)	a	0.27 (0.03)	a	0.27 (0.02)	a
	Lower Backslope	50	0.68 (0.12)	b	0.23 (0.01)	a	0.13 (0.01)	b	0.16 (0.01)	b
		75	1.11 (0.13)	ab	0.21 (0.01)	a	0.18 (0.01)	b	0.16 (0.01)	ab
		90	1.17 (0.02)	ab	0.32 (0.04)	a	0.15 (0.02)	b	0.22 (0.01)	ab
		100	1.39 (0.10)	a	0.28 (0.03)	a	0.27 (0.03)	a	0.24 (0.01)	a
	Footslope	50	0.68 (0.06)	b	0.10 (<0.01)	b	0.10 (0.01)	b	0.08 (0.01)	c
		75	1.18 (0.03)	a	0.20 (0.02)	a	0.13 (<0.01)	b	0.09 (0.01)	bc
		90	1.28 (0.12)	a	0.22 (<0.01)	a	0.14 (0.01)	b	0.15 (0.02)	ab
		100	1.47 (0.05)	a	0.23 (0.02)	a	0.24 (0.01)	a	0.20 (0.01)	a
N_2O	Upper Shoulder	50	0.10 (0.04)	b	0.09 (<0.01)	b	0.05 (0.03)	a	0.05 (0.04)	b
		75	0.12 (<0.01)	b	0.02 (0.01)	b	0.07 (0.04)	a	0.01 (0.01)	b
		90	1.12 (0.32)	b	0.07 (0.01)	b	-0.02 (0.06)	a	0.07 (0.01)	b
		100	20.78 (6.25)	a	4.31 (0.58)	a	0.18 (0.06)	a	0.25 (0.04)	a
	Lower Backslope	50	0.03 (0.03)	b	0.05 (<0.01)	a	0.08 (0.03)	a	0.07 (0.03)	a
		75	0.14 (0.02)	b	0.05 (0.01)	a	0.05 (<0.01)	a	0.08 (0.03)	a
		90	0.13 (0.04)	b	0.04 (0.04)	a	0.03 (0.01)	a	0.09 (0.02)	a
		100	1.60 (<0.01)	a	0.05 (0.01)	a	0.10 (<0.01)	a	0.12 (0.03)	a
	Footslope	50	0.06 (0.04)	a	-0.06 (0.01)	c	0.04 (0.03)	b	0.06 (0.01)	b
		75	0.14 (0.02)	a	0.07 (0.01)	b	0.07 (<0.01)	b	0.01 (0.01)	b
		90	0.14 (0.08)	a	0.04 (<0.01)	b	0.02 (0.02)	b	0.05 (0.04)	b
		100	0.28 (0.10)	a	0.19 (0.03)	a	0.20 (<0.01)	a	0.19 (0.04)	a
CH_4	Upper Shoulder	50	0.05 (0.04)	ab	-1.36 (0.23)	b	-0.27 (0.04)	c	0.03 (0.08)	a
		75	0.06 (0.05)	ab	-1.02 (0.05)	b	-0.02 (0.04)	ab	0.08 (0.04)	a
		90	-0.05 (0.07)	b	-0.31 (0.15)	a	-0.19 (0.07)	bc	0.06 (0.03)	a
		100	0.25 (0.02)	a	0.10 (0.04)	a	0.05 (0.05)	a	0.16 (0.03)	a
	Lower Backslope	50	-0.42 (0.14)	b	-0.32 (0.01)	ab	-0.07 (0.06)	ab	-0.01 (0.09)	a
		75	-0.26 (0.10)	ab	-0.53 (0.06)	b	-0.06 (0.02)	ab	-0.06 (0.08)	a
		90	-0.16 (0.05)	ab	-0.38 (0.15)	b	-0.17 (0.04)	b	-0.10 (0.08)	a
		100	0.27 (0.21)	a	0.05 (0.03)	a	0.12 (0.08)	a	0.14 (0.09)	a
	Footslope	50	-0.21 (0.02)	b	-0.09 (0.07)	a	0.08 (0.01)	a	0.04 (0.02)	a
		75	-0.01 (0.03)	ab	0.02 (0.05)	a	0.07 (0.05)	a	0.03 (0.07)	a
		90	<0.01 (0.09)	ab	-0.03 (0.05)	a	<0.01 (0.08)	a	0.10 (0.06)	a
		100	0.14 (0.08)	a	-0.04 (<0.01)	a	0.12 (0.04)	a	0.12 (0.03)	a
CO_2		P		†	US > LB > F	***[9]	US > LB > F	***	US, LB > F	***
		WFPS	100, 90 > 50	***	90 > 75 > 50; 100 > 50	***	100 > 90, 75 > 50	***	100 > 90 > 50; 100 > 75	***
		P x WFPS		n/s		n/s		**		n/s
N_2O		P	US > LB, F	***	US > LB, F	***		n/s		n/s
		WFPS	100 > 90, 75, 50	***	100 > 90, 75, 50	***	100 > 90, 75, 50	***	100 > 90, 75, 50[†]	***
		P x WFPS		***		***		n/s		†
CH_4		P	US > LB, F	*	F > LB > US	***	F > LB, US	***		†
		WFPS	100 > 90, 75, 50	***	100 > 90 > 75, 50	***	100 > 90, 50	***	100 > 75, 50[†]	*
		P x WFPS		n/s		***		n/s		n/s

*Significant at the $p \leq 0.05$ level.

**Significant at the $p \leq 0.01$ level.

***Significant at the $p \leq 0.001$ level.

[†] Marginally significant, $0.1>p>0.05$.

[§] Data were log-transformed to normalize residuals.

Table 4.2 Soil bulk density (BD), total N (TN), % clay, and % sand by position and horizon. Mean and SE shown, *n*=24 for BD, *n*=16 for TN and C:N; mean only shown for texture data. Differences between positions, within horizons, were tested with one-way ANOVA and Tukey HSD comparisons (shown).

Position	Horizon	BD	TN‡	C:N	% clay§	% sand§
		– g soil/cm^3 –	– mg N/cm^3 –	– –	– % –	– % –
Upper	1	1.23 (0.04)	2.62 (0.03)	12.90 (0.14)	28.9	31.6
Shoulder	2	1.21 (0.03)	2.08 (0.05)	13.53 (0.20)	29.9	31.0
	3	1.27 (0.03)	0.85 (0.05)	14.82 (0.66)	32.1	33.3
	4	1.32 (0.03)	0.12 (0.01)	32.38 (1.51)¶	32.9	31.6
Lower	1	1.23 (0.03)	3.13 (0.09)	13.00 (0.20)	37.5	23.8
Backslope	2	1.31 (0.03)	1.78 (0.12)	14.73 (0.43)	35.9	26.7
	3	1.39 (0.03)	0.69 (0.03)	14.83 (0.55)	32.8	30.4
	4	1.34 (0.04)	0.27 (0.04)	46.22 (7.12)¶	28.6	34.5
Footslope	1	1.21 (0.03)	3.22 (0.09)	12.68 (0.16)	36.7	19.5
	2	1.27 (0.03)	1.13 (0.07)	17.10 (0.43)	36.3	22.5
	3	1.40 (0.03)	0.66 (0.02)	12.54 (0.40)	32.9	21.2
	4	1.47 (0.03)	0.52 (0.03)	7.88 (0.41)	31.4	35.0

ANOVA	Horizon	BD	TN	C:N
Position	1	n/s	F, LB > US***	n/s
(within	2	†	US > LB > F***	F > LB, US***
Horizon)	3	F, LB > US*	US > LB, F***	LB, US > F**
	4	F > LB, US**	F > LB > US***	LB, US > F***

*Significant at the $p \leq 0.05$ level.
**Significant at the $p \leq 0.01$ level.
***Significant at the $p \leq 0.001$ level.
† Marginally significant at the $p \leq 0.1$ level.
‡ Data were square-root transformed for statistical analysis.
§ Texture data from J. Bell (*personal communication* 2008).
¶ Samples contained black C, particularly in the LB position.

Table 4.3 Labile and slow C pool size as a fraction of total C (TC) and mean residence time (MRT) by treatment by position and horizon (H1–H4). Means shown, *n*=4. Two-way ANOVA results show effects of position (P), treatment (WFPS) and their interaction on C pools and MRT. When both main effects and their interaction were significant, lower case letters indicate results of a Tukey HSD comparison; values connected by the same letter are not significantly different. When only main effects were significant, Tukey HSD comparisons are shown with the main effect significance.

		C_l (% TC)[§]				MRT_l (d)[§]			
Position	**WFPS (%)**	**H1**	**H2**	**H3**	**H4**	**H1**	**H2**	**H3**	**H4**
Upper Shoulder	50	0.38	0.23	0.13 a	1.18 bc	50 abc	51	27 c	118 bcd
	75	0.39	0.09	0.18 a	0.60 bc	37 abc	21	20 c	183 ab
	90	1.25	0.22	0.29 a	0.74 bc	35 bc	38	21 c	132 bc
	100	1.20	0.15	1.17 a	2.15 ab	129 a	54	41 bc	103 bcd
Lower Backslope	50	0.44	0.19	0.34 a	0.87 bc	53 abc	108	131 ab	46 d
	75	0.89	0.45	0.50 a	0.35 bc	41 bc	132	176 a	51 cd
	90	0.69	0.26	0.15 a	0.28 bc	64 abc	54	122 ab	41 cd
	100	1.44	0.28	0.90 a	1.28 bc	89 ab	187	58 bc	100 bcd
Footslope	50	0.21	0.20	0.52 a	0.26 c	20 c	48	39 bc	81 cd
	75	0.59	0.38	0.39 a	0.65 bc	50 abc	37	33 c	67 cd
	90	0.19	0.43	0.53 a	1.10 bc	14 c	37	60 abc	64 cd
	100	0.72	0.85	1.17 a	3.55 a	51 abc	82	44 bc	308 a
ANOVA	P	LB>F *	F>US *	n/s	F,US>LB *	US,LB>F *	LB>F,US ***	LB>F,US ***	US,F>LB ***
	WFPS	100>50 *	n/s	100>all ***	100>all ***	100>all ***	100>90 **	n/s	100>all ***
	P x WFPS	n/s	n/s	*	*	†	n/s	*	***
Position	**WFPS (%)**	**C_s (% TC)**				**MRT_s (y)[§]**			
Upper Shoulder	50	41.67	44.34	53.18	51.97	22	49 abcd	12	3 efg
	75	42.29	45.84	56.55	50.37	55	89 ab	30	10 cd
	90	40.09	45.26	55.13	53.65	93	103 a	36	12 bcd
	100	42.17	42.60	46.50	51.47	13	12 cde	4	1 fg
Lower Backslope	50	31.96	27.27	32.32	74.35	33	47 abcd	15	24 a
	75	30.94	26.08	27.53	71.30	74	58 ab	11	20 abc
	90	29.66	29.63	30.39	73.07	65	55 abc	29	27 ab
	100	30.67	24.91	30.23	76.33	11	6 e	3	4 ef
Footslope	50	27.53	17.56	10.49	18.18	121	15 cde	3	3 efg
	75	24.70	11.96	8.19	16.51	103	14 de	5	3 efg
	90	28.86	16.21	8.45	16.81	143	36 bcd	11	6 de
	100	29.15	15.26	7.43	13.48	17	3 e	1	1 g
ANOVA	P	US>LB,F ***	US>LB>F ***	US>LB>F ***	LB>US>F ***	F>LB,US ***	US>LB>F ***	US,LB>F ***	LB>US>F ***
	WFPS	n/s	n/s	n/s	n/s	90>50>100; 75>100 ***	90>50>100; 75>100 ***	90>75,50>100 ***	90>50>100; 75>100 ***
	P x WFPS	n/s	n/s	n/s	n/s	n/s	†	n/s	**

*Significant at the $p \leq 0.05$ level.
**Significant at the $p \leq 0.01$ level.
***Significant at the $p \leq 0.001$ level.
† Marginally significant, $0.1>p>0.05$.
§ Data were square-root transformed to normalize residuals.

Table 4.4 Net N mineralization rates (µg N/g soil/d), mean and SE shown, *n*=6. ANOVA results show effects of position (P), treatment (WFPS), and their interaction on net N mineralization rates. When both main effects and their interaction were significant, Tukey HSD comparisons are shown with lower case letters, otherwise Tukey comparison data is indicated with the main effect significance. Values connected by the same letter are not significantly different.

Position	WFPS (%)	Horizon 1	Horizon 2	Horizon 3	Horizon 4	
Upper	50	-0.161 (0.114)	0.004 (0.049)	-0.004 (0.006)	0.003 (0.002)	abc
Shoulder	75	0.285 (0.113)	0.147 (0.018)	-0.003 (0.010)	0.003 (0.002)	abc
	90	0.321 (0.054)	0.053 (0.016)	0.017 (0.007)	-0.007 (0.003)	cd
	100	0.341 (0.087)	0.004 (0.029)	0.017 (0.016)	0.011 (0.006)	abc
Lower	50	0.056 (0.074)	0.004 (0.023)	-0.017 (0.014)	0.041 (0.016)	a
Backslope	75	0.153 (0.059)	-0.082 (0.005)	-0.011 (0.008)	0.009 (0.010)	abcd
	90	0.122 (0.062)	-0.007 (0.021)	-0.043 (0.021)	0.023 (0.007)	ab
	100	0.240 (0.064)	-0.029 (0.041)	-0.020 (0.016)	-0.002 (0.001)	bcd
Footslope	50	-0.075 (0.044)	0.050 (0.022)	0.010 (0.004)	0.055 (0.023)	ab
	75	0.100 (0.025)	-0.013 (0.004)	-0.008 (0.002)	0.014 (0.004)	abc
	90	0.248 (0.032)	0.011 (0.005)	-0.017 (0.002)	0.020 (0.009)	abc
	100	0.280 (0.085)	0.076 (0.040)	-0.005 (0.003)	-0.047 (0.008)	d

ANOVA	Horizon 1	Horizon 2	Horizon 3	Horizon 4
P	n/s	US,F>LB***	US,F>LB **	LB>F,US*
WFPS	100,90,75>50***	n/s	n/s	50>75,90,100***
P*WFPS	n/s	***	†	***

*Significant at the $p \leq 0.05$ level.
**Significant at the $p \leq 0.01$ level.
***Significant at the $p \leq 0.001$ level.
†Marginally significant at the $p \leq 0.1$ level.

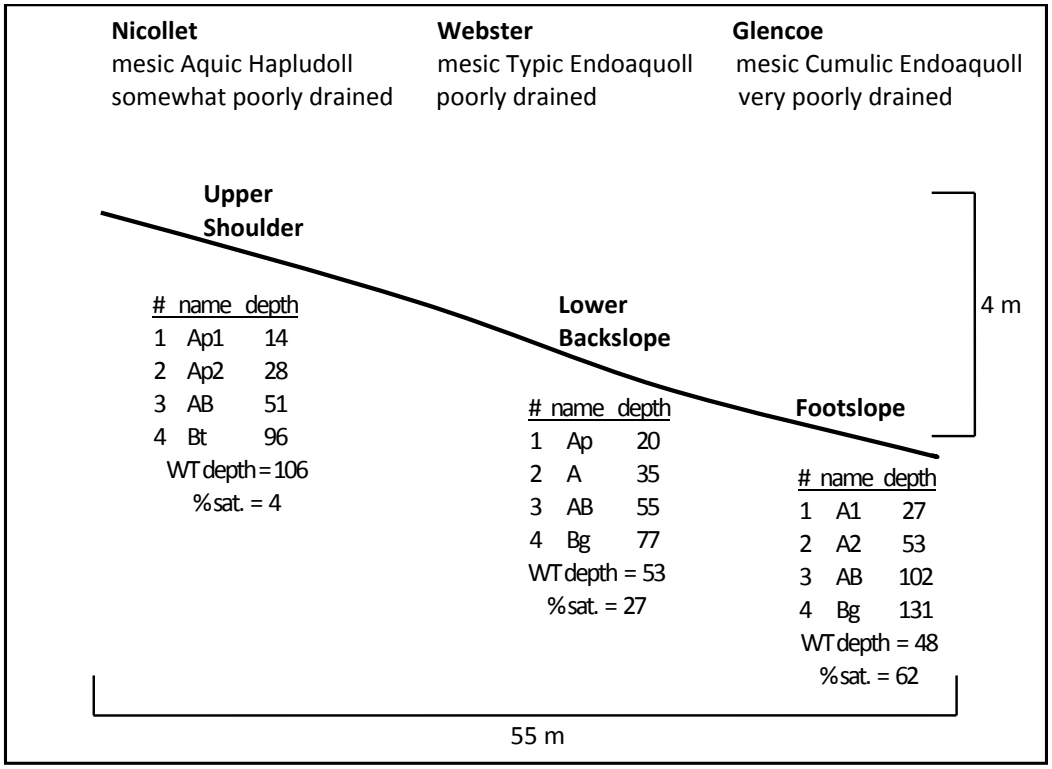

Figure 4.1 Hillslope positions with soil series names, soil types, and drainage class. For each horizon, the following information is shown: horizon number, horizon name, depth of each horizon base (cm). The mean water table depth (cm) at each position and the fraction of saturated piezometer readings at 50 cm taken throughout the year (water table depth and piezometer data from Reuter and Bell, 2003) are presented for each position. The overall change in elevation and transect length are shown (not to scale).

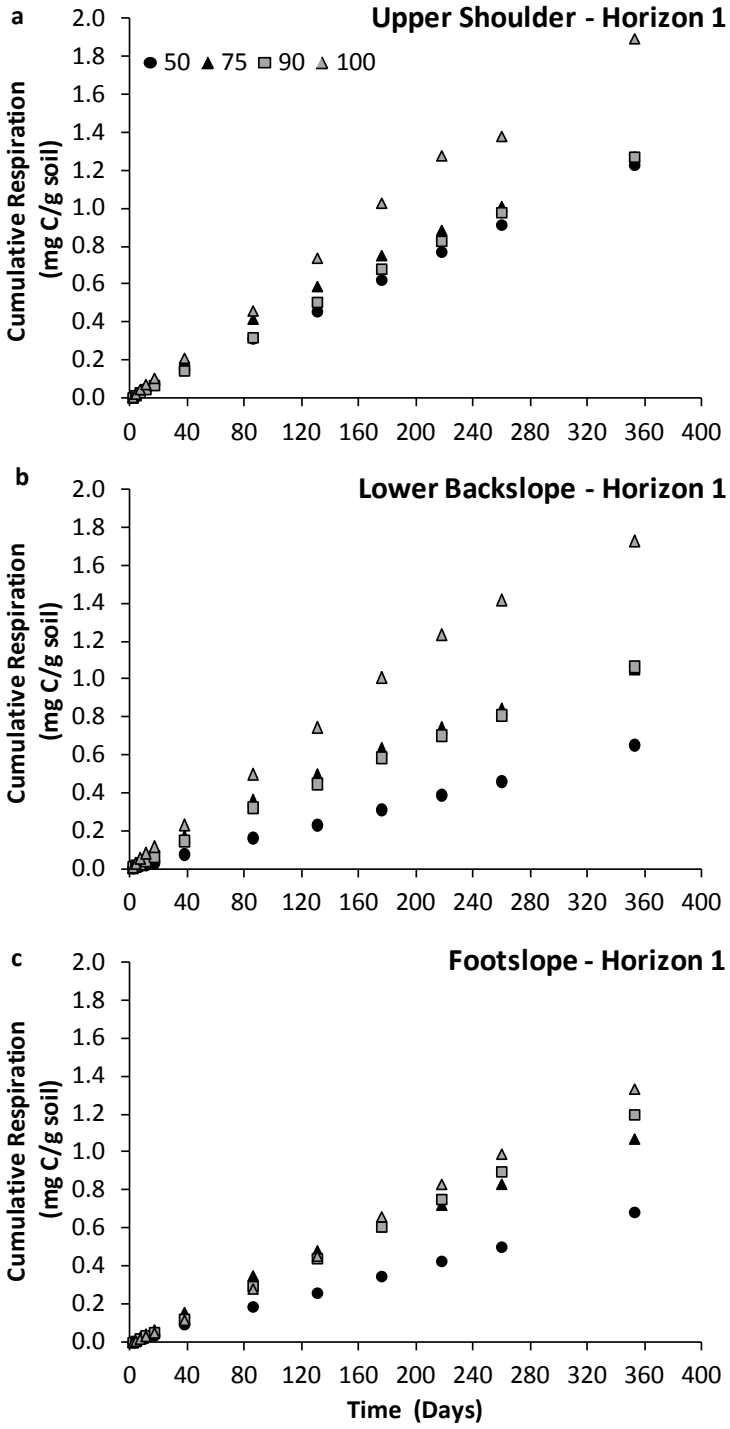

Figure 4.2 Moisture treatment (WFPS %) effects on cumulative CO_2 emissions from horizon 1 over the incubation period for each hillslope position. Treatment mean shown (*n* = 4).

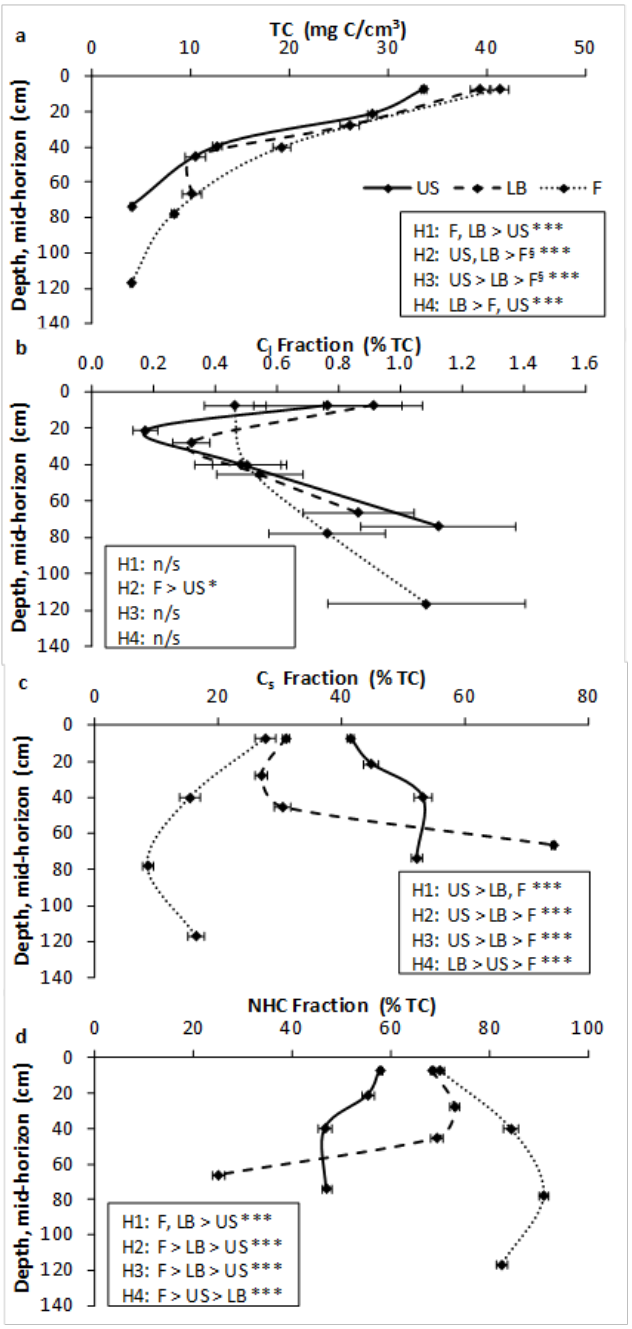

Figure 4.3 Effects of hillslope position on (a) total soil C content (TC), and the fraction of TC in the (b) labile (C_l), (c) slow (C_s), and (d) resistant (NHC) C pools in each horizon (H1 – H4), C_l and C_s data represent the average across moisture treatments, effects of moisture are shown in Table 4.3. Mean and SE shown (*n*=4). [§]Position effects, shown in the box on each figure panel, were tested within horizons. However, as horizon thickness varied significantly among positions, the TC analyses of H2 and H3 would have resulted in different conclusions regarding position effect if they had been based on soil depth. See Table 4.3 for an explanation of the statistical significance.

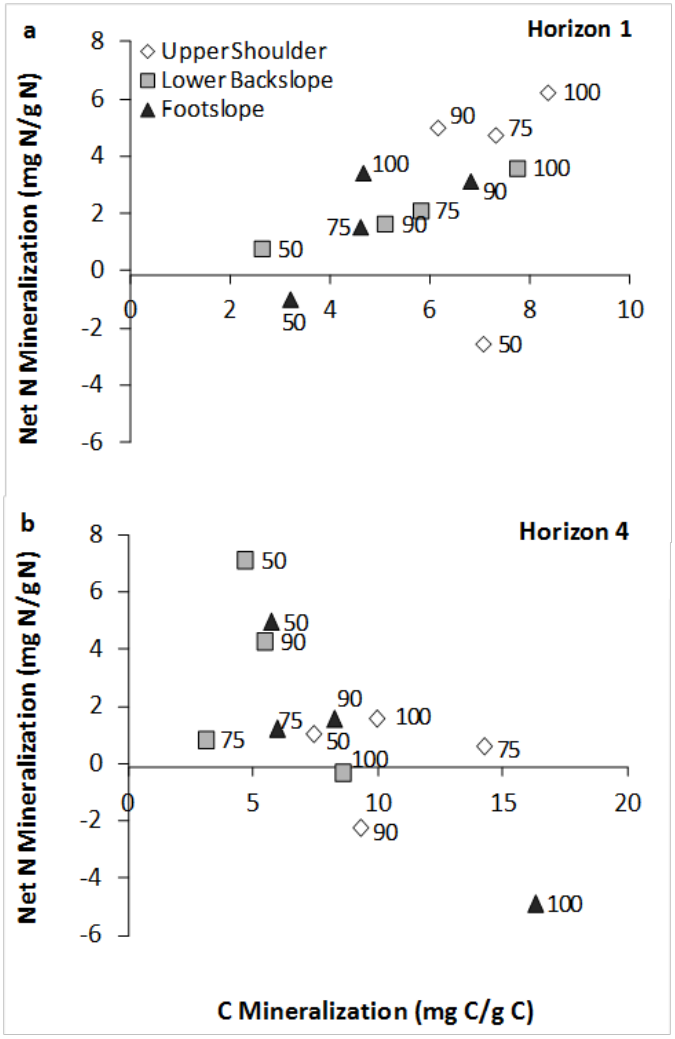

Figure 4.4 Cumulative C and N mineralization scaled to the average N incubation length, 35 days, for (a) Horizon 1 soils and (b) Horizon 4 soils from each position. Values next to the data points indicate the corresponding moisture treatment level. Mean shown, *n*=4 for C mineralization, *n*=6 for N mineralization.

CHAPTER 5

Conclusions

The research presented in this dissertation has shown the importance of considering the landscape context of even the most intensive land management practices. In the high carbon (C) soils of the Upper Midwest, USA, I found that effects of soil environmental conditions equaled or exceed the effects of agricultural management on loss of recent C inputs as carbon dioxide (CO_2). Through a field based study (Chapters 2 and 3) we examined the effects of five agricultural management systems on the loss of root-derived C as CO_2, and nitrogen (N) as nitrous oxide (N_2O), and how management systems affected the coupling of root-derived C and N emissions as CO_2 and N_2O. In Chapter 4 returning to the observed importance of soil environmental characteristics from Chapter 2, we showed that small changes in topography and changes in soil moisture can significantly alter soil C pool sizes and turnover rates even in highly managed systems. Combined, these results illustrate the benefit of examining the fate of individual C substrates or soil C pools in addition to quantification of total C (TC) inputs or the TC pool. Additionally, these results underscore the importance of examining environmental as well as management effects on C and N cycling rates in managed ecosystem soils. Finally, this work highlights the importance of considering the element cycle coupling by showing how both management and state factors can alter the relationship between rates of C and N mineralization.

Research Summary

The primary objective of the research presented in Chapter 2 was to determine the effects of agricultural management systems (varying in tillage and fertilization, and crop rotation diversity) on the emission of root-derived C as CO_2. Root C is an important contributor to the total soil C pool, particularly in agricultural systems. I showed that growing season losses of root-derived C averaged 35% over two seasons and that the average root-derived contribution to cumulative soil C emissions was 8.5% over two seasons (the emission factor, EF_C). Management effects on total C emissions were not evident in either season, but effects on root-derived C emissions were detectable. Organic fertilization reduced the root-derived C emissions and the root-derived contribution to total C emissions. Effects of tillage, fertilization and rotation diversity were not consistent among seasons. More intense tillage and fertilization did increase root-derived C emissions in 2005, while root-derived C emissions decreased with greater rotation diversity in 2006. I suggest that the observed negative effect of rotation diversity on root-derived C turnover in 2006 is a result of rotation diversity effects on root-derived C input quantity. An unexpected result of the research in Chapter 2, was the evident importance of soil environmental characteristics as controls over root-derived C cycling in this highly managed ecosystem. As the study was designed to account for, rather than quantify, variation in soil characteristics with topography, the research in Chapter 4 builds off these results to quantify the role of topography and hydrology as controls over soil C mineralization rates along a hillslope.

I used the same isotope tracer study to quantify the emission of corn root N as N_2O and the effects of management practices on this quantity. The primary objectives of

this research (Chapter 3) were to determine the quantity of corn root-derived N emitted as N_2O (EF_N), and to determine management effects on both EF_N and the relationship between EF_C and EF_N. As compared with the root-derived contribution to CO_2 emissions, the root-derived contribution to N_2O emissions and EF_N were much lower, on average 7% and less than 1% over the two seasons. These results support suggestions that the IPCC's use of 1% as the EF for all N sources overestimates emissions from organic N sources, and that belowground N, like belowground C, likely exhibits greater retention in soils than aboveground sources. Management effects on EF_N were limited to a negative effect of crop rotation diversity. Rotation diversity reduced both EF_C and EF_N resulting in no rotation diversity effect on the slope of the EF_C-EF_N relationship, only an effect on the magnitude of EF_C and EF_N. In contrast, management systems differing in tillage and fertilization did significantly change the EF_C-EF_N slope. High losses of root-derived N relative to C in organically fertilized systems suggest either greater N "leakiness," or tighter C-N coupling in organic and low tillage-low fertilization systems, as compared to high tillage and high synthetic fertilization systems.

Along a low-slope and high soil moisture hillslope I examined the effects of topography and soil moisture on both the distribution and turnover of soil C pools in agricultural soils (Chapter 4). This study represents an initial step toward determination of the importance of topography and moisture as controls over soil C cycling in low slope–high moisture landscapes. The effects of topography in this landscape were most evident on the size of the TC pool and the resistant fraction of TC. The resistant fraction of TC showed strong positive, and negative relationships with soil clay (R^2=0.35) and sand (R^2=0.45) content respectively. As is typical of higher slope landscapes, this

suggests a role for erosional transport of C and/or fine particles downslope contributing to the amount and protection of both the TC and the resistant C downslope. The size of the slow C pool tended to be greatest in the upslope position soils, regardless of soil moisture.

Effects of soil moisture were most evident on the size and turnover of labile C and the turnover of slow C. Increased soil moisture resulted in greater labile fractions of the TC across most horizons and positions, and longer mean residence time (MRT) of labile C. Specifically, the change in the size of the labile C pool (mg C/cm^3) across moisture treatments was 75% on average, with little variation among horizons. The change in the MRT of labile C in response to moisture treatments was 61% on average across positions and horizons. The MRT of slow C pool was longest in the 90% and 50% water filled pore space (WFPS) treatments and was shortest in the 75 and 100% WFPS treatments. Whereas changes in the size of the slow C pool in response to moisture were relatively small, 27% across positions and horizons, the MRT of the slow C pool appeared highly responsive to soil moisture treatments, exhibiting an 88% change across positions and horizons. Overall, my results indicate strong differences in soil C pool distribution and turnover along hillslopes, with greater total and resistant C downslope. This result suggests the potential for greater C storage downslope in this landscape. Manipulation of soil moisture levels showed that the potential for moisture to influence the labile fraction of TC is greatest in the lower horizons (AB and B, or horizons 3 and 4) and the footslope position. Accordingly, whether the importance of burial as a means of C storage decreases with increased moisture should be explored. Combined with higher than expected C emissions from the 100% WFPS treatment, the

effects of moisture treatments on C pool size suggest that, in this landscape, soil moisture may have a strong effect on the availability of C to microbes. These results reflect a potential for significant shifts in C pool dynamics across this landscape with changing soil moisture regimes due to climate change or land management.

A primary goal of my research has been to determine the importance of land management decisions on rates of biogeochemical cycling in soils. Through field (Chapters 2 and 3) and laboratory (Chapter 4) studies I have shown that even in highly impacted agricultural ecosystems, processes commonly associated with more pristine landscapes play a key role in determining rates of C and N mineralization in soils. Effects of agricultural management practices on cumulative root-derived C and N emissions were evident (Chapters 2 and 3), but inconsistent between seasons. In contrast, soil environmental characteristics showed significant relationships with daily rates of root-derived C and N cycling while management practices showed little to no effect on daily cycling rates. This research furthers our understanding of the contribution of the belowground C and N sources to emissions of important greenhouse gases and our understanding of how management systems affect both rates of C and N mineralization individually, as well as C and N coupling. Results of my research could be used to improve soil C storage models used for C credit allocation, and model of N_2O emissions used for compliance with international climate treaties such as the Kyoto Protocol. Research from Chapter 4 tested our understanding of hillslope patterns of soil C pools and MRTs in a lower slope, managed, landscape. Results suggest the key patterns observed in higher slope landscape can be found in low slope-high moisture landscapes. Additional work is needed to bring the same mechanistic understanding of hillslope C

processes achieved in high slope, erosion-dominated landscapes to lower slope landscapes.

Directions for Future Research

Though I have shown the importance of examining management and environmental impacts on both TC and fractions of the TC pool, there are some key limitations to extrapolation of the results presented here. Primarily, each study could only be conducted on one site. The relative importance of management versus soil environment varies regionally. Thus, the sensitivity of root-derived C and N to management needs to be studied in other regions, especially those differing in soil texture and moisture. Similarly, before hillslope position or soil moisture effects on C pool sizes and turnover rates can be either extrapolated geographically or used to predict effects of management or climate induced moisture changes, parallel studies must be conducted across a wide variety of landscapes and soil types.

Corn roots are both larger and more resistant to decomposition that most other common crops. Consequently, the overall magnitude of root-derived C and N losses, as well as the management effects on root-derived C and N cycling, need to be studied for additional crops. In this process, the negative effect of crop rotation diversity on losses of root-derived C and N as CO_2 and N_2O should be further examined. Studies incorporating an analysis of root-derived C and N input quantity on both priming effects and loss rates could help determine what mechanism drive the relationship between inputs and losses, as well as the relationship between rotation diversity and losses of root-derived C and N.

While losses of root-derived N as N_2O over a growing season represented less than 0.2% of the initial root-derived N, total root-derived N losses from the soil ranged from 0–65% (D. Allan, *personal communication*, 2010). Assessment of management effects on other, larger, N loss pathways would broaden our understanding of management effects on root-derived N cycling. Though a similar argument can be made for studying other root-derived C loss pathways, unlike N_2O, CO_2 is one of the primary forms of C lost from agricultural ecosystems.

My research has shown that the potential exists along low relief hillslopes for increased rates of C and N mineralization with increased soil moisture, particularly in the upper slope positions. Most importantly, increased soil moisture may result in a shift toward greater labile fractions of TC pool and decreased MRT of slow pool C. If found across a broad geographic scale, this result indicates that, where average soil moisture is expected to increase, the vulnerability of soil C to decomposition may also increase. Further research is needed on how the duration of changes in soil moisture influence C pool size and MRT and to further understand the geographic patterns determining the relative importance of state factors such as soil moisture versus management control over C and N mineralization rates.

In their recent review, Gärdenäs et al. (2011) critique our understanding of C–N coupling from the molecular to the global scale and outline key research priorities. I expanded our knowledge of coupled root-derived C and N cycling rates, and management effects on C–N coupling. Further studies examining management effects on the coupling of belowground C and N could provide valuable information with the potential to guide land management and policy decisions as well as future scientific

research. Extending my research on topography and soil moisture effects on soil C pool size and turnover, further study of moisture effects on C protection mechanisms, and C and N availability could improve the accuracy of biogeochemical models and our ability to predict climate change effects on soil C pools. More broadly, a theoretical framework is needed to help identify factors, and the context in which those factors (climate, soils, land use type, etc.) are most likely to influence C and N cycling individually, or weakly influence C–N coupling (e.g., physical processes), versus those which are likely to have a strong influence on C–N coupling (i.e. biological processes).

Conclusions

My dissertation research has advanced our understanding of management and soil environmental effects on individual sources and pools of C and N in the soil and factors influencing C–N coupling. This research has important implications for biogeochemical models, and for management and regulatory decisions in the face of climate change. The methods used, in particular the use of stable isotope labeling to trace root-derived CO_2 and N_2O emissions, could be applied to a wide variety of future biogeochemical and applied research questions. Through contributions to our understanding of the interrelationships between agricultural management, landscape features, and soil environmental factors as they influence rates of biogeochemical cycles my research has highlighted important directions for future research.

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