Tillage Reversal and Nitrogen Fertilization Affected Greenhouse Gas Emissions and Soil Carbon Stability Differently in a Black Chernozem and a Gray Luvisol

by

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Abstract

Improving soil carbon (C) sequestration through land management practices is of great interest due to concerns over global climate change caused by increased atmospheric greenhouse gas (GHG) concentrations. Soil disturbance by conventional tillage (CT) generally accelerates soil organic carbon (SOC) mineralization, and changing from CT to no tillage (NT) has been shown to reduce GHG emissions and increase soil C sequestration in western Canada. However, long-term NT may cause crop residue accumulation and weed infestation. Reversing NT to CT, a process called tillage reversal, may be needed to address those issues but it may markedly alter soil C dynamics in agricultural ecosystems.

The effects of tillage reversal and nitrogen (N) fertilization on soil GHG emissions during the growing season, soil C and N concentrations, and C stability in top- and subsoils were studied in two long-term field experimental sites: a Malmo silty clay loam (an Orthic Black Chernozem) at Ellerslie and a Breton loam (an Orthic Gray Luvisol) at Breton. This study used a split-plot design with two levels of N (since 1979) - 0 (N0) vs. 100 kg N ha^{-1} yr^{-1} (N100) and two levels of tillage - long-term NT (since 1979) vs. tillage reversal (TR) (since 2009 at Ellerslie and 2010 at Breton) - treatments. Straw was retained in each plot. The results are: (1) tillage reversal increased area-scaled GHG emissions but decreased yield-scaled GHG emissions at Ellerslie while N fertilization increased area-scaled GHG emissions but decreased yield-scaled GHG emissions at Breton; (2) soil heterotrophic respiration (R_h) was stimulated by tillage reversal only at Ellerslie but was stimulated by N fertilization only at Breton; (3) tillage reversal and N

fertilization only increased soil C and N concentrations in the topsoil at Breton; (4) nitrogen fertilization increased water-extractable organic carbon (WEOC) concentrations at both sites but the stability of WEOC was increased by N fertilization only at Breton; (5) Nitrogen fertilization increased soil aggregation and aggregate-associated C in the topsoil at both sites; and (6) physical protection for C in the subsoil was decreased by N fertilization and tillage reversal only at Ellerslie. In conclusion, with straw retention, long-term N fertilization with short-term tillage reversal is recommended to increase soil C sequestration, improve soil aggregation, and decrease yield-scaled GHG emissions in the Gray Luvisol. In the Black Chernozem, short-term tillage reversal is recommended to improve soil aggregation and decrease yield-scaled GHG emissions.

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List of Symbols and Abbreviations

AR: aboveground residue

BD: Bulk density

BR: belowground residue

C: carbon

CT: conventional tillage

ECD: electron capture detectors

FID: flame ionizer

GDW: grain dry weight

GHG: greenhouse gas

GWP: global warming potential

HIX: humification index

LM: large macroaggregates

MI: microaggregates

MWD: mean weight diameter

N: nitrogen

N0: no N fertilization

N100: 100 kg N ha^{-1} yr^{-1} applied

ND: nitrifier denitrification

NT: no tillage

PD: soil particle density

R_a: autotrophic respiration

R_h: heterotrophic respiration

R_s: soil total respiration

SC: silt and clay particles

SM: small macroaggregates

SOC: soil organic carbon

SOM: soil organic matter

$SUVA_{280}$: ultraviolet absorbance at 280 nm

T: tillage

TCD: thermal conductivity detector

TN: total nitrogen

TOC: total organic carbon

TP: total porosity

TR: tillage reversal; one of the tillage levels

WEOC: water-extractable organic carbon

WEOM: water-extractable organic matter

WEON: water-extractable organic nitrogen

WFPS: water-filled pore space

WSA: water-stable aggregate

θ_v: volumetric water content

Chapter 1 General introduction

1. Introduction

1.1 Global warming and soil organic carbon pools

The increasing concentration of atmospheric greenhouse gases (GHG) since preindustrial times has led to warming of the earth's surface (IPCC, 2007). Agriculture is one of the major sources of anthropogenic GHG emissions, with up to 12% of the total anthropogenic GHG emissions derived from agricultural activities (Smith et al., 2007).

Soil is one of the most important carbon (C) reservoirs in terrestrial ecosystems (Jobbágy and Jackson, 2000). The total soil C stock is estimated at 2500 Pg C, compared with 560 Pg C in vegetation and 780 Pg C in the atmosphere (Lal, 2004). Soil processes directly affect climatic change through the production and consumption of GHGs (CO_2, CH_4 and N_2O). A proposed method to reduce atmospheric GHGs is to increase the global storage of C and N in soils as soil organic matter (SOM) (Batjes, 1998). Soil organic carbon (SOC) consists of various functional pools with different turnover rates. Current conceptual models differentiate functional SOC pools into labile, intermediate and passive pools (Amundson, 2001; Smith et al., 1997). Typically, the turnover time for the labile pool is days to a few years whereas for fractions in intermediate and passive pools, the turnover times range from a few years to centuries (Stevenson and Cole, 1999; Wander, 2004).

Soil water-extractable organic carbon (WEOC) is one of the most labile fractions of SOC (Marschner and Kalbitz, 2003). Soil WEOC is produced through SOM decomposition, release of microbial metabolites, and plant root exudates. Although WEOC is considered to be one of the most labile fractions, only 10-44% of WEOC is microbially degradable (Qualls and Hainers, 1992). The biodegradation of WEOC depends on its intrinsic properties, which in turn, influence the formation of stable SOC (Kalbitz et al., 2003).

Soil organic carbon in the intermediate and passive pools can be formed through physical protection within soil aggregates (Six et al., 2000), chemical interaction with soil mineral particles (Christensen, 1996), and biochemical recalcitrance (van Veen and Paul, 1981). Aggregate fractionation is a physical method used to separate labile from intermediate and passive SOC pools (von Lützow et al., 2007). Soil organic carbon associated with different aggregate fractions such as macroaggregates (>250 μm) and microaggregates (<250 μm) (Oades, 1984; Tisdall and Oades, 1982) has different turnover times (15-50 years for macroaggregate-associated C and 100-300 years for microaggregate-associated C) as revealed by ^{13}C natural abundance (John et al., 2005; Monreal, et al., 1997). Macroaggregates are mainly formed by biogenic aggregation (von Lützow et al., 2006) and are sensitive to land management (Tisdall, 1994). Microaggregates are formed mainly by abiotic clay flocculation and might be the most important aggregate fraction for long-term C stabilization (Six et al., 2000).

1.2 Impacts of N fertilization and tillage reversal on soil C dynamics

Soil C balance refers to the difference between C input into the soil and C output from the soil (Post and Kwon, 2000). Thus, an increase in soil C storage can be achieved by increasing C input into the soil or decreasing C output from soil. Soil C inputs are derived either from plant debris (e.g., straw, chaff and roots) or from root exudates and other organic substances released into the rhizosphere (Kuzyakov and Domanski, 2000). Soil C outputs are dominated by the efflux of CO_2 from the soil surface and leaching of dissolved and particulate C (Davidson and Janssens, 2006).

Land management has been shown to affect SOC storage by altering SOC inputs and outputs in agricultural systems (De Gryze et al., 2004; Yallop and Clutterbuck, 2009). It is commonly accepted that soil disturbance by conventional tillage (CT) causes SOC loss, and that C sequestration can be accomplished by changing from CT to no tillage (NT). The NT practice has been documented to reduce C loss in the form of CO_2 and the total C cost of cropping (e.g., by reducing the need for machine use). However, N_2O and CH_4 emissions are usually increased by NT because of increased soil moisture content and decreased soil porosity in NT. Additionally, long-term NT management practice can cause problems such as accumulation of crop residues, weed infestation (Baan et al., 2009; Grant and Bailey, 1994), and pesticide accumulation (Baker and Saxton, 2007). Long-term NT management practice may also cause a higher soil bulk density and, correspondingly, a greater soil strength (Martino and Shaykewich, 1994).

Reversing NT to CT, a process called tillage reversal, may be a way to deal with those problems and the possibility exists that landowners may change long-term NT to CT on a short-term basis (e.g. several years). In cropping systems with straw retention, tillage reversal helps to increase C inputs by incorporating crop residues into the soil

profile, which might contribute to the production of WEOC and the formation of soil aggregates. However, tillage reversal likely stimulate CO_2 emissions (Shahidi et al., 2014) due to the increased decay of labile C (e.g., crop residue inputs and WEOC), which has a more rapid turnover rate than SOC (De Gryze et al., 2004; Swanston et al., 2002; Wander et al., 1994). Despite this, the increased global warming potential by increased CO_2 emissions under tillage reversal may be offset by reduced N_2O and CH_4 emissions (Chatskikh and Olesen, 2007; Hutsch et al., 1994). Since only a few studies have focused on tillage reversal practice (e.g., Shahidi et al., 2014), the influence of tillage reversal on GHG emissions is poorly understood.

Soil total CO_2 emissions (R_s) can be partitioned into soil autotrophic (R_a) and heterotrophic (R_h) respirations. Soil R_a is from root growth and activity (Johnson-Flanagan and Owens, 1986), and the decomposition of root exudates and rhizodeposits. Soil R_h is from microbial decomposition of SOC. Thus, both R_a and R_h are related to the decomposition of soil organic compounds derived from above- and belowground biomass (Morell et al., 2011). The crop residues incorporated by tillage reversal can release nutrients for plant growth, which would likely increase root activity. Tillage reversal might also stimulate R_h due to the enhancement of soil aeration and destruction of soil aggregates, which would expose labile or fresh organic matter that exists in aggregates for microbial decomposition (De Gryze et al., 2004; Grandy and Robertson, 2007; Six et al., 1999). In agricultural ecosystems, although numerous studies have investigated the responses of R_s to different tillage management (Buysse et al., 2013; Zhang et al., 2013), there have been fewer attempts to partition R_s into R_a and R_h, which is critical to understand the underlying processes under different land management practices.

Nitrogen (N) fertilizers are widely used to enhance crop production in agricultural ecosystems. Nitrogen fertilization increases crop biomass (Malhi and Lemke, 2007), which is likely to increase soil C input. In addition, N fertilization alters belowground C allocation (Liu and Greaver, 2010), which also affects soil C input. However, the response of C output in the form of CO_2 to N fertilization is not consistent (Alvarez, 2005; Campbell et al., 2000; Halvorson et al., 2002). After partitioning R_s into R_a and R_h, N fertilization affects SOC mineralization (R_h) by changing the microbial community composition (Ågren et al., 2001). In addition, N fertilization alters soil pH (Aber et al., 1989), which might affect the production and consumption of WEOC, thus influencing R_h. Oikeh et al. (1999) found that N fertilization influencs the growth and distribution of plant roots, which might affect R_a. Root exudation patterns might be changed (Liljeroth et al., 1990) by N fertilization, which is likely to affect R_a as well.

It has been well documented that N application can increase N_2O emissions (Chen et al., 2008; Sainju et al., 2012a). Shahidi (2012) found that N fertilization stimulates N_2O emissions during the growing season but the magnitude of stimulation depends on NT vs. TR (tillage reversal) and soil type. Although growing season CO_2 and N_2O emissions under tillage reversal and long-term N fertilization have been studied by Shahidi (2012) in the first and second tillage years at Ellerslie and in the first tillage year at Breton, CH_4 fluxes were not considered in that research. Nitrogen fertilization decreased CH_4 uptake in dry-land cropping systems (Robertson and Vitousek, 2009; Sainju et al., 2012b), however, the response of soil CH_4 uptake to N fertilization combined with tillage reversal has not been studied. The 100-yr global warming potential (GWP) of CH_4 is 25. Therefore, potential CH_4 sinks in dry-land agricultural systems should also be

investigated when studying GHG emissions from soils and the influence on global climate change under various land management practices.

Additionally, most of the studies on soil GHG emissions were reported on an area-scaled basis. Land management practices such as N fertilization and tillage likely affect both grain yield and soil GHG emissions. Yield-scaled GHG emissions, expressed as soil GHG emissions per unit of yield produced (e.g., a ton of grain), are more important in evaluating the GHG cost of producing a crop (Grassini and Cassman, 2012; Mosier et al., 2006; van Kessel et al., 2013). The consideration of using a yield-scaled approach is that if a certain tonnage of food is needed to feed the world's population, management practices should be focused on producing crops with the lowest GHG emissions per unit yield (van Kessel et al., 2013).

1.3 Knowledge gap in subsoil C dynamics under different agricultural management practices

Studies on soil C storage and stabilization under different land management practices have mainly focused on the topsoil (A horizon) (Harrison et al., 2011; Rumpel and Kögel-Knabner, 2011; Syswerda et al., 2010). Despite low C concentration, subsoil is a significant reservoir of C, and approximately 46-63% of SOC stored in the first meter is located below 30 cm (Batjes, 1996). Carbon dynamics in this lower layer has not been studied to the same extent as that in the top 30 cm. Soil organic C in the subsoil is considered to be more stable as its radiocarbon age is much older than that in the topsoil (Eusterhues et al., 2003; Paul et al., 1997).Destabilization of the 'old' C in the subsoil

will markedly contribute to the increase of atmospheric CO_2 concentrations. In most studies where NT was found to enhance C sequestration, soils were only sampled down to a maximum of 30 cm depth, although crop roots often extend much deeper (Baker et al., 2007; Sapkota et al., 2012). Conversely, studies with deeper sampling depths generally show no C sequestration advantage for NT systems, or even show more C sequestrated in CT systems (Plaza-Bonilla et al., 2010; VandenBygaart et al., 2003; West and Post, 2002). Thus, the shallow sampling depths used in those studies may cause a bias when calculating soil C sequestration.

Tillage reversal might alter the subsoil C dynamics by changing C inputs (plant residues, root exudates and WEOC produced by pathways other than root exudates leaching from upper soil horizons to the subsoil) (Rumpel and Kögel-Knabner, 2011). Nitrogen fertilization could decrease soil pH, which might increase WEOC leaching from the topsoil to the subsoil (Muñoz et al., 2010; Shevtsova et al., 2003). Fresh OM input may stimulate microbial activity to degrade very 'old C' in deeper soil layers (Fontaine et al., 2007). Nitrogen fertilization may alter root distribution (Svoboda, 2006), which would also influence subsoil C input. Since only a few studies have focused on tillage reversal (e.g., Shahidi et al., 2014), the response of subsoil C dynamics to tillage reversal, and in combination with N fertilization, remains poorly understood.

2 Research objectives

The overall goal of this research was to study the responses of growing season GHG emissions, C and N concentrations, and C stability to tillage reversal and long-term N fertilization in two contrasting soils.

The specific objectives were to: 1) investigate area- and yield-scaled GHG emissions with tillage reversal and long-term N fertilization during the growing seasons; 2) investigate the responses of R_a and R_h to tillage reversal and long-term N fertilization; 3) assess soil C and N concentrations, and the quality and quantity of soil WEOC under tillage reversal and long-term N fertilization during the growing seasons; and 4) assess growing season crop residue inputs (both above- and belowground), soil aggregation and the distribution of C in aggregate fractions in top- and subsoils under tillage reversal and long-term N fertilization.

3 Hypotheses

Four experiments were conducted in this thesis research to test the following hypotheses (Fig. 1-1):

1. Nitrogen fertilization and tillage reversal will increase both area- and yield-scaled soil GHG emissions.
2. Nitrogen fertilization and tillage reversal will increase both R_a and R_h.
3. Nitrogen fertilization and tillage reversal will increase WEOC concentration but decrease its stability due to increased crop residue incorporation.
4. Nitrogen fertilization will improve soil aggregation and C sequestration because of the increased crop residue input under N fertilization. However, when combined with

tillage reversal, tillage reversal will diminish the benefit of N fertilization on soil aggregation and C sequestration due to the soil disturbance.

3 Thesis structure

This thesis includes six chapters. Chapter 1 provides a general introduction to soil C pools and C stability, and introduces the background of this thesis research. Chapter 2 evaluates grain yield, soil bulk density, soil temperature, soil water-filled pore space (WFPS), and area- and yield-scaled GHG emissions under tillage reversal and long-term N fertilization. Chapter 2 addresses the first hypothesis. Chapter 3 studies the responses of soil respiration components (R_a and R_h) to tillage reversal and long-term N fertilization, and the relationship of soil respiration with soil temperature and WFPS. Chapter 3 addresses the second hypothesis. Chapter 4 studies tillage reversal and long-term N fertilization effects on quality and quantity of WEOC (representing labile C pool) in the growing season. Chapter 4 addresses the third hypothesis. Chapter 5 evaluates crop residue inputs (both above- and belowground), soil pH, mean weight diameter (MWD) of soil aggregates, size distribution of sand-free water-stable aggregates, and aggregate associated C (representing intermediate and passive C pools) in top- and subsoils under tillage reversal and long-term N fertilization. Chapter 5 addresses the fourth hypothesis. Chapter 6 provides a summary of key findings and general conclusions. In addition, suggested future research is described in this chapter.

Each of the data chapters (2 to 5) constitutes a manuscript that has already been published, under review or will be submitted for publication:

Chapter 2, “Tillage reversal and N fertilization affected greenhouse gas emissions differently in a Black Chernozem and a Gray Luvisol”, will be submitted for publication.

Chapter 3, “Tillage reversal and nitrogen fertilization affected autotrophic and heterotrophic soil respirations differently in a Black Chernozem and a Gray Luvisol”, will be submitted for publication.

Chapter 4, “Nitrogen fertilization and tillage reversal affected water-extractable organic carbon and nitrogen differently in a Black Chernozem and a Gray Luvisol”, has been published in Soil and Tillage Research (Sun et al., 2015).

Chapter 5, “Nitrogen fertilization and tillage reversal effects on soil aggregation and carbon pools in top- and subsoils in a Black Chernozem and a Gray Luvisol”, has been submitted to Agriculture, Ecosystems and Environment for publication.

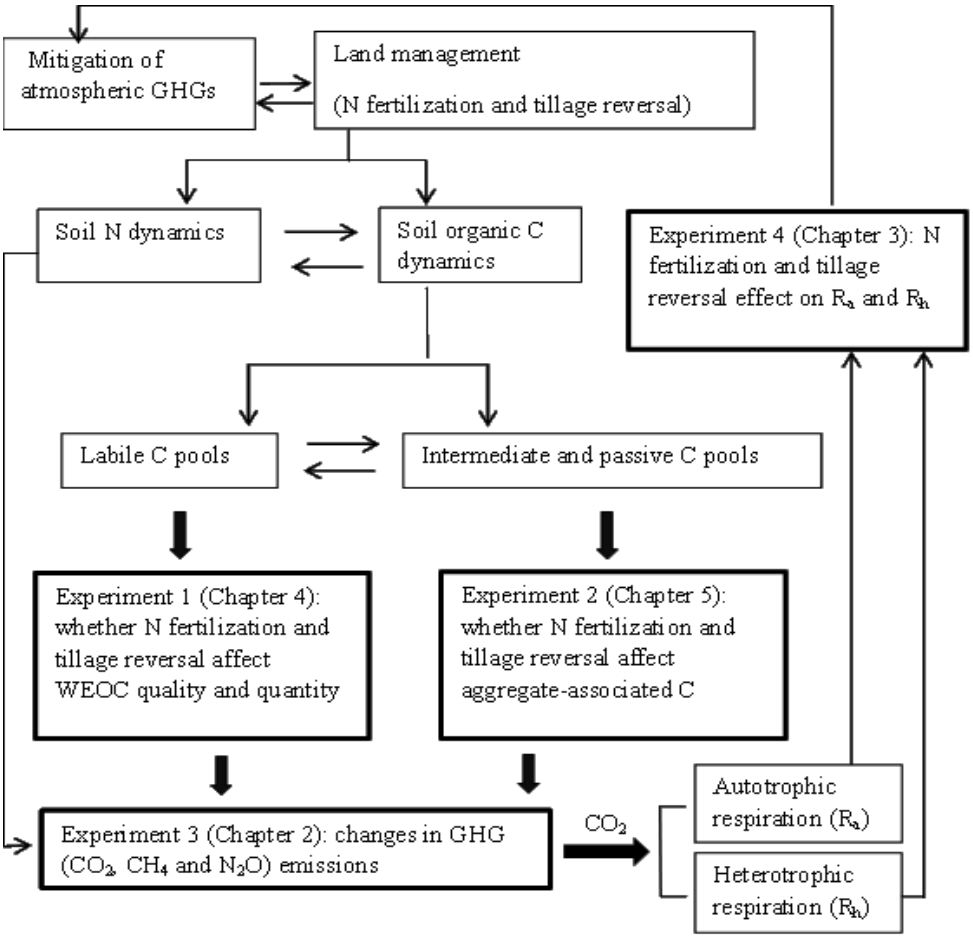

Fig.1-1 Flow chart of the study

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Chapter 2 Tillage reversal and N fertilization affected greenhouse gas emissions differently in a Black Chernozem and a Gray Luvisol

1 Introduction

Global warming caused by increased levels of atmospheric greenhouse gas (GHG) concentrations has been a concern for human well-being and the environment (Vitousek, 1994; Wisser et al., 2011). Atmospheric carbon dioxide (CO_2), methane (CH_4) and nitrous oxide (N_2O) concentrations have increased by 36, 148 and 18%, respectively, since the pre-industrial era (IPCC, 2007). Agriculture is one of the major sources of anthropogenic GHG emissions, with up to 12% of the total anthropogenic GHG emissions derived from agricultural activities (Smith et al., 2007).

Soil organic matter (SOM) represents a large store of C and N in terrestrial ecosystems. Changes in the size of this reservoir can substantially influence GHG concentrations in the atmosphere (Janzen et al., 1998). It is commonly accepted that changing from conventional tillage (CT) to no tillage (NT) is beneficial for SOM accumulation and decreases CO_2 emissions to the atmosphere (Bruce et al., 1999; Kahlon et al., 2013; West and Post, 2002). This benefit, however, should be balanced against a more complete budget of soil GHG emissions (e.g., CO_2, N_2O and CH_4) (Lal et al., 2004). In addition, long-term NT management practice can cause problems such as accumulation of crop residues, weed infestation, nutrient stratification on the soil surface (Baan et al., 2009; Grant and Bailey, 1994), and pesticide accumulation (Baker and Saxton, 2007). Long-term NT management practice may also increase soil bulk density,

and correspondingly, lead to greater soil strength (Martino and Shaykewich, 1994). It is documented that N_2O emissions are higher under NT because oxygen diffusion and air-filled porosity are usually reduced (Chatskikh and Olesen, 2007). Soil compaction caused by long-term NT may reduce CH_4 oxidation in uplands by half (Hansen et al., 1993).

Reversing NT to CT, a process called tillage reversal, may be a way to deal with some of those problems caused by long-term NT and the possibility exists that landowners may switch to tillage from long-term NT. In addition, tillage reversal can improve soil aeration, release CO_2 dissolved in soil solution (Jackson et al., 2003), and destroy soil aggregates. Destruction of soil aggregates exposes labile or fresh organic matter that exists in aggregates, making it susceptible to decomposition (DeGryze et al., 2004; Grant and Robertson, 2007; Six et al., 1999) and increased soil CO_2 emissions to the atmosphere. However, the increased global warming potential resulting from increased CO_2 emissions under tillage reversal may be offset by reduced N_2O and CH_4 emissions (Chatskikh and Olesen, 2007; Hutsch et al., 1994). Since only a few studies have focused on tillage reversal practices (e.g., Shahidi et al., 2014), the influence of tillage reversal on GHG emissions is not fully understood.

The effect of N fertilization on CO_2 emissions has not been consistent across various agroecosystems. Some studies reported no change (e.g., Castro et al., 1994) in CO_2 emissions after applying N fertilizers but others reported an increase (Morell et al., 2011; Sainju et al., 2012a) or a decrease (Al-Kaisi et al., 2008) in CO_2 emissions under N fertilization. A recent meta-analysis by Liu and Greaver (2010) showed that N fertilization increases aboveground litter inputs but at the same time decreases carbon loss by inhibiting heterotrophic respiration. Nitrogen fertilization has been reported to

stimulate soil N_2O emissions and decrease CH_4 uptake in dry-land cropping systems (Robertson and Vitousek, 2009; Sainju et al., 2012b). The addition of N fertilizers will likely affect microbial activities, which will influence soil C and N cycling, and thus GHG emissions. Moreover, at the field scale, the interaction between various agricultural management practices such as N fertilization and tillage reversal makes it difficult to predict the cumulative impact of these activities on soil GHG emissions. Few studies have evaluated the interactive effects of tillage reversal and N fertilization on GHG emissions.

Although some studies have measured both soil GHG emissions and grain yield, there have been fewer attempts to report GHG emissions on a yield-scaled basis (van Kessel et al., 2013). When assessing GHG emissions under specific agricultural practices such as tillage reversal and N fertilization, which are likely to affect both grain yield and GHG emissions, the yield-scaled approach, expressed as soil GHG emissions per unit of product (e.g., a ton of grain), appears to be more appropriate (Grassini and Cassman, 2012; Mosier et al., 2006; van Kessel et al., 2013). The rationale for using a yield-scaled approach is that if a certain tonnage of food is needed to feed the world population, management practices should be focused on producing crops with the lowest GHG emissions per unit yield (van Kessel et al., 2013).

The objective of this study was to investigate area- and yield-scaled GHG emissions during the growing seasons with tillage reversal and long-term N fertilization on two different soil types in a similar ecological region. We hypothesized that NT without N fertilization will mitigate both area- and yield-scaled soil GHG emissions as compared to NT with N fertilization and TR with or without N fertilization. This research represents

one of the first attempts to understand the interactive effects of tillage reversal and N fertilization on soil GHG emissions in agricultural soils in western Canada. Shahidi et al. (2014) reported the first two years' data and this paper focuses on the longer term effects of tillage reversal, reporting the third and fourth years' data.

2 Materials and methods

2.1 Site description

Gas samples were collected from two long-term research sites studying the effects of zero tillage, straw retention and N fertilization (Tillage-Straw-Nitrogen Plots) near Ellerslie (53°25′N, 113°33′W; elevation 692 m), with the soil classified as an Orthic Black Chernozem (Typic Cryoboroll) of the Malmo silty clay loam series, and Breton (53°07′N, 114°28′W; elevation 830 m), with the soil classified as an Orthic Gray Luvisol (Typic Cryoboralf) of the Breton loam series. Both sites are located in central Alberta, Canada. The Black Chernozem had greater soil fertility and better soil structure than the Gray Luvisol (Singh and Malhi, 2006). These two sites are ~70 km apart but represent two major and distinctly different soils found in central Alberta (Table 2-1). More detailed descriptions of the study sites can be found in Sun et al. (2015).

2.2 Experimental design

Long-term experimental plots were established at each site in 1979 with a randomized completely block design, which included 10 treatments with various combinations of tillage, N fertilization and straw retention (Nyborg et al., 1995; Fig. 2-1). In this study, we used a split-plot design with the whole plots (N fertilization, N0 vs. N100 kg N ha^{-1} yr^{-1}) completely randomized in each of four blocks, with tillage - NT vs. tillage reversal (TR) - arranged in subplots (Fig. 2-2). Plots for two of the treatments - T4 (No Till-straw-No N) and T6 (No Till-Straw-100N) - were split into 2 equal subplots (6.85 × 1.37 m). One of the subplots was subjected to TR on June 3, 2009 for the Black Chernozem and on June 4, 2010 for the Gray Luvisol (Shahidi et al., 2014). The other subplot was maintained as NT, except for the disturbance caused by the plot seeder used each spring to seed the plots. The TR subplots were tilled each spring prior to seeding using a rotary tiller to a depth of approximately 8-10 cm. In both NT and TR subplots with N fertilization, urea (100 kg N ha^{-1} yr^{-1}) was mid-row banded (46 cm apart) among seed rows placed 23 cm apart. Phosphorus (20 kg P $ha^{-1}yr^{-1}$) was applied with seed to each subplot in each year. The crop grown at both sites was spring barley (*Hordeum vulgare* L.) throughout the experiment, and after harvesting, straw was retained on each subplot.

2.3 Field instrumentation and gas sampling

Soil greenhouse gas (GHG) emissions were determined by measuring the increase in GHG concentrations within the headspace of the chamber (12.5 cm i.d. by 13.5 cm tall) placed over a pre-installed collar in each subplot. Details of the chamber were described

in Hutchinson and Mosier (1981). One collar per subplot was installed at each site (between seed rows). Pre-installed collars were inserted 5 cm into the soil in each of the subplots and maintained throughout the whole growing season; such a design avoids soil disruption caused by frequent installation of the collars. As tillage reversal at Breton began one year after Ellerslie, gas sampling was conducted in 2011 and 2012 at Ellerslie, and in 2012 and 2013 at Breton to be consistent on years since tillage reversal. The measurement of CO_2 emissions immediately after tillage reversal (the first and second years at Ellerslie, and the first year at Breton) was conducted by Shahidi et al. (2014). Seeding was conducted on the same day as tillage and N fertilization. Crop was harvested in early September at both sites. Gas samples were collected between 10:00 am and 12:00 pm, which represented the daily mean gas emissions (determined with diurnal emission measurement), at 7- to 14-d intervals from June to early September in each year, depending on weather conditions and the dates of seeding and crop harvest.

On each measurement day, after each chamber was placed onto its collar, air within the chamber was carefully mixed with the syringe used for gas sample collection to ensure representative sampling (Chatskikh and Olesen, 2007). Gas samples (20 mL) were collected 0, 10, 20 and 30 min after closing the chamber using a BD Plastipak polypropylene syringe (20 mL) and stored in pre-evacuated 12 mL Exetainers (Labco Ltd., High Wycombe, UK) that were capped with gray butyl rubber stoppers. Plants that grew above the height and circumference of the chamber were trimmed regularly before collecting gas samples when needed to reduce gas leakage associated with connecting the chamber and collar (Sainju et al., 2012a). The gas samples were analyzed within 48 h with a gas chromatograph (Model 3800, Varian, Palo Alto, CA) equipped with thermal

conductivity detector (TCD), a flame ionizer (FID) and electron capture detectors (ECD) for analyzing the concentrations of CO_2, CH_4 and N_2O, respectively.

Soil temperature at the 5 cm depth near the chamber in the central area of the subplots was monitored at 1 h intervals during the growing season with HOBO U10 temperature dataloggers (Doc# 1195-A, Onset Computer Corporation, MA). Mean soil volumetric water content in the 0-30 cm depth in the growing season was measured with CSC616 water content reflectometers, which were inserted into the soil vertically near the chamber in the central area of each subplot. The CSC616 water content reflectometers were connected to CX10 dataloggers (Campbell Scientific, INC.) and data was recorded at 1 h intervals. Soil bulk density of the 0-10 cm soil layer was measured after tillage in 2013 at each site using a steel core sampler (5 cm i.d.).

2.4 Calculations

Soil GHG emission rates during the chamber closure period were calculated from the increase of GHG concentrations in the headspace (Hutchinson and Mosier, 1981; Liebig et al., 2010) with respect to time. Volumetric gas emissions were converted to molarity by the ideal gas law using equation [1]:

$$F_{gas}=\frac{P}{R_vT}\times\beta\times\frac{V_c}{A} \quad [1]$$

where F_{gas} is gas emissions in mmol m^{-2} s^{-1}; P is atmospheric pressure of 1.01325 atm, R_v is the universal gas constant for volumetric conversion at 0.0831 L atm mol^{-1} K^{-1}, T is air temperature in K, V_c is the chamber volume (m^3), A is the soil area covered by the chamber (m^2), and β is the gas emission rate (ppm s^{-1}).

Bulk density (BD) and volumetric water content (θ_v) were used to determine water-filled pore space (WFPS) using equation [2]:

$$\%WFPS = (\theta_v / TP) \times 100 \quad [2]$$

where TP (total porosity) = (1 – PD/BD) ×100, and PD is soil particle density assumed to be 2.65 g cm^{-3}.

Cumulative GHG emissions in the growing season of each year were calculated by linearly interpolating the daily emission rates of GHG and integrating the underlying area (Gilbert, 1987). When calculating the cumulative GHG emissions using trapezoidal integration of emissions versus time, we assumed that emissions changed linearly between measurement dates. As the gas sample collection was started two weeks (June 18, 2012) after the date of tillage (June 3) in 2012, the mean GHG emissions during the missed two weeks in 2012 were estimated using the mean GHG emissions during the similar period in 2011 at Ellerslie and in 2013 at Breton. Area-scaled GHG emissions were calculated by adding soil CO_2, N_2O and CH_4 emissions in units of CO_2-C equivalents (CO_2-C eq.) for a 100-year horizon, with mass emissions of N_2O and CH_4 multiplied by their respective global warming potential (298 for N_2O and 25 for CH_4) (IPCC, 2007). Yield-scaled GHG emissions (CO_2-C eq.) were calculated by dividing area-scaled GHG emissions (CO_2-C eq.) by grain yield (Venterea et al., 2011).

2.5 Statistical analysis

Data from the two sites were analyzed separately since the main purpose was to evaluate the differences in GHG emissions due to different management regimes within

each soil type and the soil type was not replicated. Analysis of variance was conducted using the PROC MIXED procedure in SAS v8.01 (SAS Institute Inc., 2003) to test for treatment effects. Before analysis of variance, data were tested for normality and homogeneity of variance assumptions. Soil CO_2, CH_4 and N_2O emission data were log-transformed for statistical analysis. All the data shown in this chapter were the original data. Analysis of variance was based on N fertilization (N0 and N100) as the main plot factor, tillage (NT and TR) as the split-plot factor, and sampling data as a repeated measures variable when analyzing daily mean CO_2, CH_4 and N_2O emissions, soil temperature and WFPS. For analysis of cumulative CO_2, CH_4 and N_2O emissions, area- and yield-scaled GHG emissions, mean soil temperature and WFPS in a year, N fertilization and tillage reversal were considered as fixed effects, and year as a repeated measures variable. Means were separated using the least square means test when treatments and interactions were significant. All results were considered significant if p values were less than 0.05.

3 Results

3.1 Grain yield

The historical (1981-2008) mean grain yield in the N0 plots before tillage reversal were 1800±138 and 936±76 kg ha^{-1} for Ellerslie and Breton, respectively, which were significantly lower than that in N100 plots (2917±59 and 2129±72 kg ha^{-1} for Ellerslie and Breton, respectively; Fig. 3-a).

After tillage reversal, the N fertilization × tillage reversal effects on grain yield were not significant at either site. Grain yield was significantly higher in N100 than in N0 only in 2011 (Fig. 2-3b), and in TR compared to NT (Fig. 2-3c) in both years at Ellerslie. At Breton, the grain yield data was only available for 2012, when it was significantly higher in N100 than in N0 (Fig. 2-3d) but was not different between tillage treatments (Fig. 2-3e).

3.2 Carbon dioxide emissions

Peak daily mean CO_2 emissions were reached 6-8 weeks after applying N fertilizer and tillage (Fig. 2-4). There was no interaction effect between N fertilization and tillage reversal on CO_2 emissions (both mean and cumulative) at either site. At Ellerslie, both mean and cumulative CO_2 emissions were not different between N treatments but they were significantly higher in TR than in NT in both years (Table 2-2). However, at Breton, both mean and cumulative CO_2 emissions were significantly higher in N100 than in N0 in both years but were not different between tillage treatments. The mean growing season CO_2 emissions were significantly higher in 2012 (50.34±1.70 kg C ha^{-1} d^{-1}) than in 2011 (33.17±1.25 kg C ha^{-1} d^{-1}) (p<0.001) at Ellerslie but they were not different between years at Breton.

3.3 Nitrous oxide emissions

Soil GHG emission measurement was delayed (gas sample collection started two weeks after tillage reversal) in 2012. Applying N fertilizer or tillage reversal did not immediately induce a N_2O flush at Ellerslie in 2011(Fig. 2-5a), although a flush of N_2O emissions occurred within 2 weeks after applying N fertilizer at Breton in 2013 (Fig. 2-5b). The N fertilization × tillage reversal effects on N_2O emissions were significant in both years and sites (Table 2-3). Soil N_2O emissions (both mean and cumulative) were the highest in N100-TR and lowest in N0-NT at both sites except that the lowest emissions occurred in N0-TR in 2012 at Breton. Soil N_2O emissions (both mean and cumulative) were significantly higher in N100 than in N0. The magnitude of increase was greater in TR than in NT. Soil N_2O emissions were greater in TR than in NT, and higher emissions were observed in N100 than in N0.

3.4 Methane emissions

In contrast to CO_2 and N_2O emissions, CH_4 was taken up by the soil from the atmosphere on all measurement dates (Fig. 2-6). Neither tillage reversal nor N fertilization × tillage reversal affected CH_4 uptake at either site (Table 2-4). Mean CH_4 uptake was significantly higher in N0 than in N100 at both sites. Both soil mean and cumulative growing season CH_4 uptake were significantly lower in 2012 than the other year at both sites ($p<0.01$ for both mean and cumulative uptake).

3.5 Area- and yield-scaled GHG emissions

Mean area-scaled GHG emissions ranged from 3.10±0.09 to 4.50±0.19 Mg CO_2-C eq. ha^{-1} at Ellerslie and from 3.34±0.43 to 6.81±0.39 Mg CO_2-C eq. ha^{-1} at Breton. The N fertilization × tillage reversal effects were not significant on area- or yield-scaled GHG emissions at either site (Table 2-5). At Ellerslie, area-scaled GHG emissions were significantly lower in NT than in TR but were not different between N treatments. However, at Breton, the area-scaled GHG emissions were significantly lower in N0 than in N100 but were not different between tillage treatments (Fig. 2-7a and 2-7b).

Yield-scaled GHG emissions ranged from 1.36±0.17 to 5.84±1.46 kg CO_2-C eq. kg^{-1} grain at Ellerslie and from 5.74±3.07 to 62.33±21.86 kg CO_2-C eq. kg^{-1} grain at Breton. At Ellerslie, yield-scaled GHG emissions were not different between years or N treatments (Table 2-5) but were significantly lower in TR than in NT (Fig. 2-7c and 2-7d). At Breton, yield-scaled GHG emissions were significantly lower in N100 than in N0 but were not different between tillage treatments.

4 Discussion

4.1 Tillage reversal effects on area-scaled GHG emissions

This study was a continuation and a complementary investigation to that of Shahidi et al. (2014) who studied CO_2 emissions in the first and second tillage years on long-term no-tilled plots in 2009-2010 at Ellerslie and the first tillage year at Breton. This study showed that the magnitude of increase in CO_2 emissions induced by tillage reversal did not diminish even after 3-4 years of tillage reversal. At Ellerslie, the increase in total

growing season CO_2 emissions averaged across N treatments was 0.65 Mg C ha^{-1} in the first tillage year and 0.63 Mg C ha^{-1} in the second tillage year (Shahidi et al., 2014). In the third and fourth tillage years, the increase were 0.46 and 0.72 Mg C ha^{-1} (averaged across N treatments), respectively. Gaseous C losses 3 and 4 years following tillage reversal also did not decrease at Breton. Soil carbon is potentially conserved in the surface layer of the soil with NT practices (Lal, 1997). After tillage on long-term NT soils, the decomposition of conserved labile C in NT was likely accelerated in these soils due to increased soil aeration. Alvarez et al. (1995) found that soil organic carbon (SOC) under NT is more readily degradable than under CT.

Tillage reversal affected CO_2 emissions differently at Ellerslie and Breton, which might be attributed to the different soil initial SOM content. Balesdent et al. (2000) showed that the decomposition of soil organic matter (SOM) due to tillage is more rapid with high initial TOC and TN contents than when they are low. We suggest that tillage reversal helped to increase litter incorporation/input into the soil, which was greater than increased SOM mineralization caused by tillage reversal at Breton; this resulted in the higher TOC content in TR than in NT (Sun et al., 2015).

Studies showed that nitrifier denitrification (ND) is the preferential source of N_2O emissions from well-aerated soils (Bateman and Baggs, 2005; Mathieu et al., 2006). Nitrifier denitrification is the oxidation of NH_3 to NO_2^-, followed by the reduction of NO_2^- to N_2O and N_2 (Wrage et al., 2001). The contribution of ND to total N_2O emissions may be related to soil moisture content. Kool et al. (2011) found that ND contributes more to N_2O emissions than denitrification at 50% and 70% WFPS while denitrification is the dominant contributor to N_2O emissions at 90% WFPS. In this study, soil WFPS in

both soils was between 50-70% in the growing seasons except in 2011 at Ellerslie, in which soil WFPS was lower than 50% from August to September. Thus, we suggest that ND might be a dominant pathway of N_2O emissions in the soils studied.

Tillage has been reported to increase soil organic N mineralization and nitrification, and thus the NO_3-N content, resulting in higher daily N_2O emissions (Aulakh et al., 1982; Lemke et al., 1999), especially when combined with N fertilization, which can further increase soil inorganic N content. The enhanced CO_2 and N_2O emissions under tillage reversal at Ellerslie might indicate that tillage reversal increased microbial activities overall and those associated with nitrification enhanced N_2O emissions. The lower soil bulk density (7.8 and 1.5% lower in TR than in NT at N0 and N100 levels, respectively) and WFPS (2.1 and 3.4% lower in TR than in NT at N0 and N100 levels, respectively) in TR than in NT might indicate better soil aeration, enhancing N_2O diffusion to the atmosphere before being denitrified to N_2 (Arah et al., 1991; Elmi et al., 2003). The greater cumulative growing season N_2O emissions in TR than in NT was in agreement with the results of Shahidi (2012) who studied N_2O emissions in the same region. The cumulative growing season N_2O emissions were below 2 kg N ha^{-1} in the soils studied, which was similar to Lemke et al. (1999) but far less than that reported in Shahidi (2012).

Increased CO_2 emissions immediately after tillage and that may last for a few hours have been reported (Kessavalou et al., 1998; Reicosky et al., 1997). We found that short-term (first 30 min) CO_2 emissions were not changed by tillage reversal at Breton in 2013. The emissions tended to increase after our first measurement (within 30 min after N fertilization and tillage reversal). Soil temperature was usually low (10-12 °C) at the time of tillage (towards the end of May), which may limit the short-term enhancement of

microbial activity by tillage. Shahidi et al. (2014) reported a very small (0-0.5 kg CO_2 ha^{-1} h^{-1}) flush of CO_2 release within the first week after tillage at Ellerslie and Breton, suggesting low microbial activity at the time of tillage. Based on our measurement just after tillage reversal at Breton in 2013, tillage reversal did not change the short-term N_2O emissions, consistent with Regina and Alakukku (2010). Daily mean CH_4 emission rate recorded at these sites were in the range (1.87-0.62 g C ha^{-1} d^{-1}) reported for arable soils in Europe (Boeckx and van Cleemput, 2001). Some studies reported that tillage decreases CH_4 oxidation because of disturbance (Ball et al., 1999; Kessavalou et al., 1998). However, the enhanced soil aeration (lower soil bulk density and WFPS) under tillage reversal might increase CH_4 diffusivity (Boeckx and van Cleemput, 2001), which could lead to a non-significant effect of tillage reversal on CH_4 oxidation in the soils studied.

4.2 Nitrogen fertilization effects on area-scaled GHG emissions

Soil water-extractable organic carbon (WEOC; <0.2 μm) was higher in N100 than in N0 (Chapter 4), which might contribute to the increased CO_2 emissions in N100 at both sites. Another reason may be the greater amount of crop residue returned to the soil in N100 than in N0 (Chapter 5) at both sites. However, N fertilization effects on CO_2 emissions were only significant at Breton. The Luvisolic soil at Breton has lower available N compared to the Chernozemic soil at Ellerslie (Malhi et al., 2011; Shahidi et al., 2014). The stimulation effect on microbial activity and root growth upon N fertilization might be greater at Breton than at Ellerslie.

Previous studies showed that peak flushes of N_2O emissions occur immediately after mineral N application that can last for four weeks at the longest (Pelster et al., 2011). This stimulation effect was confirmed at Breton in 2013. However, we did not find the flush of N_2O emissions at Ellerslie when we took the first measurement three days after N fertilization in 2011, which was consistent with the results reported in Shahidi (2012) under similar conditions.

Nitrogen fertilization inhibited CH_4 oxidation in this study, in agreement with Bronson and Mosier (1994), Castro et al. (1994), and Sainju et al. (2012a). The decreased CH_4 uptake after N (urea) fertilization was likely caused by a shift of CH_4 oxidizing bacteria to nitrifying bacteria after applying N fertilizer (Castro et al., 1994; Le Mer and Roger, 2001). Soil pH was significantly lower in N100 than in N0 (5.1 and 4.6 for N0 and N100, respectively, at Ellerslie; 5.5 and 4.6 for N0 and N100, respectively, at Breton), which might affect the activity of methanotrophs (Le Mer and Roger, 2001).

4.3 Nitrogen fertilization and tillage reversal effects on yield-scaled GHG emissions

According to our study, land management decisions made to reduce GHG emissions on an area basis might be counterproductive because they may reduce crop production. Our study found that the influence of N fertilization and/or tillage reversal on yield-scaled GHG emissions was opposite to that on area-scaled GHG emissions at both sites. Nitrogen fertilization and/or tillage reversal increased both area-scaled GHG emissions and grain yield. The significantly higher grain yield under N fertilization and/or tillage reversal reduced the yield-scaled GHG emissions at both sites. This study provides the

first dataset on yield-scaled GHG emissions in relation to tillage reversal and N fertilization practices in Western Canada. Our study revealed that tillage reversal decreased yield-scaled GHG emissions from the Black Chernozem at Ellerslie. However, at Breton, N fertilization was the main factor reducing yield-scaled GHG emissions. All these findings highlight the need to consider grain yield when evaluating GHG emissions and global warming potential under specific agricultural management practices.

5 Conclusions

Agricultural management practices play an important role in mitigating rising atmospheric GHG concentrations. Although C sequestration can be achieved through NT and N fertilization, the sequestered atmospheric C is reversible. Nitrogen fertilization increased CO_2 emissions only at Breton but decreased CH_4 uptake at both sites. Tillage reversal increased CO_2 emissions only at Ellerslie. Nitrogen fertilization with tillage reversal largely stimulated N_2O emissions at both sites. Area-scaled GHG emissions were reduced by NT practice at Ellerslie and by N0 at Breton. However, under the cropping system and climate regime studied, tillage reversal decreased GHG emissions per unit grain produced at Ellerslie. Nitrogen fertilization decreased yield-scaled GHG emissions at Breton. Therefore, considering yield-scaled GHG emissions is likely more appropriate when making management decisions as the responses of area- and yield-scaled GHG emissions to management practices are not always the same.

Table 2-1 Descriptive characteristics (mean±SE) of topsoil (0-10 cm) at Ellerslie and Breton

Soil properties	Ellerslie	Breton
Total soil organic carbon (g C kg^{-1})	55.5±0.8	15.1±1.7
Total nitrogen (g N kg^{-1})	4.9±0.1	1.6±0.2
C:N ratio	11.4±0.2	9.2±0.4
Clay content (%)	38±2.0	24±0.8
Soil pH	4.9±0.1	5.1±0.2
Air temperature (°C)[§]	13.9	13.7
Cumulative precipitation (mm)[§]	275.3	330.9

Data are means of all treatments (n=16).

Soil pH was measured in 0.01 M $CaCl_2$ with a solid to liquid ratio of 1:2 (v:v).

[§], long-term average data (air temperature and cumulative precipitation from June to September in 1980-2010) at each site were from the official website of Agriculture and Rural Development, Government of Alberta (http://www.agric.gov.ab.ca/app116/stationview.jsp).

Table 2-2 Effects of nitrogen (N) fertilization and tillage of long-term no-till plots on soil CO_2 emissions (mean±SE) in the growing seasons at Ellerslie and Breton.

Site	Year	CO_2 emissions							
		NT	TR	*p* value	n	N0	N100	*p* value	n
		Cumulative CO_2 emissions (Mg C ha^{-1})							
Ellerslie	2011	3.42±0.17	3.88±0.04	0.04	4	3.64±0.09	3.67±0.13	0.84	4
	2012	3.42±0.04	4.14±0.27	0.04	4	3.57±0.21	4.00±0.32	0.08	4
		Mean CO_2 emissions (kg C ha^{-1} d^{-1})							
	2011	30.82±1.47	35.47±0.24	0.04	128	33.15±0.48	33.15±1.04	1.00	128
	2012	45.62±0.45	54.95±3.96	0.03	88	47.52±3.17	53.05±1.19	0.07	88
		Cumulative CO_2 emissions (Mg C ha^{-1})							
Breton	2012	4.61±0.35	4.76±0.90	0.87	4	3.55±0.32	5.82±0.88	0.02	4
	2013	4.95±0.13	5.47±0.23	0.14	4	3.98±0.24	6.44±0.33	0.01	4
		Mean CO_2 emissions (kg C ha^{-1} d^{-1})							
	2012	49.96±5.27	51.85±3.78	0.79	88	43.97±4.19	61.81±3.49	0.02	88
	2013	51.71±3.26	57.15±2.87	0.25	128	43.69±2.58	65.17±4.27	0.02	128

The N fertilization × tillage reversal effects on cumulative and mean CO_2 emissions were not significant at either site.

N0, no N fertilization; N100, 100 kg N ha^{-1} yr^{-1} applied; NT, no tillage; TR, tillage reversal. *p*, probability; n, number of observations

Table 2-3 Effects of nitrogen (N) fertilization and tillage (T) of long-term no-till plots on soil N_2O emissions (mean±SE) in the growing seasons at Ellerslie and Breton.

N	T	Ellerslie		Breton	
		2011	2012	2012	2013
		kg N ha^{-1}			
Cumulative emissions					
N0	NT	0.27±0.03a	0.16±0.02a	0.11±0.01a	0.32±0.04a
	TR	0.34±0.03a	0.21±0.04a	0.10±0.01a	0.43±0.08a
N100	NT	0.82±0.06b	1.15±0.11b	0.60±0.08b	0.80±0.13b
	TR	1.47±0.13c	2.01±0.17c	1.20±0.05c	1.24±0.14c
Significance					
N		**	***	***	***
T		**	***	***	*
N × T		**	**	***	*
Mean emissions		g N ha^{-1} d^{-1}			
N0	NT	2.29±0.25a	2.19±0.20a	1.45±0.06a	3.42±0.33a
	TR	3.11±0.27a	2.77±0.52a	1.29±0.16a	4.46±0.85a
N100	NT	6.69±0.55b	15.35±0.78b	8.29±0.92b	8.47±1.43b
	TR	12.02±1.09c	25.59±2.63c	16.62±0.57c	13.45±1.16c
Significance					
N		**	***	***	**
T		**	***	***	*
N × T		*	**	***	*

N0, no N fertilization; N100, 100 kg N ha^{-1} yr^{-1} applied; NT, no tillage; TR, tillage reversal.

Different letters indicate significant differences between treatment combinations within the same year at each site ($p<0.05$).

*, ** and *** indicate significance at the 0.05, 0.01 and 0.001 probability levels, respectively.

Table 2-4 Effects of N fertilization and tillage of long-term no-till plots on soil CH_4 uptake (mean±SE) in the growing seasons at Ellerslie and Breton.

Site	Year	CH_4 uptake							
		NT	TR	*p value*	n	N0	N100	*p value*	n
		Cumulative CH_4 uptake (kg C ha^{-1})							
Ellerslie	2011	0.40±0.02	0.38±0.03	0.39	4	0.46±0.04	0.31±0.02	0.03	4
	2012	0.28±0.01	0.27±0.01	0.46	4	0.30±0.01	0.25±0.01	0.04	4
		Mean CH_4 uptake (g C ha^{-1} d^{-1})							
	2011	3.40±0.19	3.30±0.17	0.15	128	3.98±0.33	2.72±0.12	0.01	128
	2012	3.96±0.12	3.71±0.16	0.30	88	4.21±0.16	3.45±0.16	0.02	88
		Cumulative CH_4 uptake (kg C ha^{-1})							
Breton	2012	0.17±0.01	0.16±0.00	0.19	4	0.18±0.01	0.15±0.01	0.05	4
	2013	0.37±0.02	0.34±0.02	0.13	4	0.40±0.02	0.31±0.02	<0.01	4
		Mean CH_4 uptake (g C ha^{-1} d^{-1})							
	2012	2.05±0.10	1.91±0.03	0.27	88	2.17±0.07	1.79±0.09	0.04	88
	2013	3.92±0.05	3.65±0.18	0.21	128	4.28±0.11	3.29±0.011	<0.01	128

The N fertilization × tillage reversal effects on cumulative and mean CH_4 uptake were not significant at either site.

N0, no N fertilization; N100, 100 kg N ha^{-1} yr^{-1} applied; NT, no tillage; TR, tillage reversal. *p*, probability; n, number of observations.

Table 2-5 The ANOVA data on area- and yield-scaled greenhouse gas (GHG) emissions.

Source of variation	Ellerslie			Breton		
	2011	2012	Over the experimental period	2012	2013	Over the experimental period
			Area-scaled GHG emissions			
N	n.s.	*	*	*	**	*
T	*	*	**	n.s.	n.s.	n.s.
N × T	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Year			n.s.			n.s.
Year × N			n.s.			n.s.
Year × T			n.s.			n.s.
Year × N × T			n.s.			n.s.
			Yield-scaled GHG emissions			
N	n.s.	n.s.	n.s.	*	n.d.	
T	n.s.	*	*	n.s.	n.d.	
N × T	n.s.	n.s.	n.s.	n.s.	n.d.	
Year			n.s.			
Year × N			n.s.			
Year × T			n.s.			
Year × N × T			n.s.			

N, nitrogen fertilization, and T, tillage.

* and ** indicate significance at the 0.05 and 0.01 probability levels, respectively.

n.s., not significant ($p>0.05$); n.d., not determined

B1	B2	B3	B4
(T10) Tillage, No straw, 50 N	(T9) Tillage, No straw, 50 N broadcast	(T8) Tillage, Straw, 50 N	(T6) No till, Straw, 100 N
(T7) Tillage, Straw, 100 N	(T4) No till, Straw, No N	(T6) No till, Straw, 100 N	(T9) Tillage, No straw, 50 N broadcast
(T6) No till, Straw, 100 N	(T5) Tillage, Straw, No N	(T3) No till, Straw, 50N	(T1) No till, No straw, No N
(T3) No till, Straw, 50N	(T8) Tillage, Straw, 50 N	(T7) Tillage, Straw, 100 N	(T3) No till, Straw, 50N
(T2) Tillage, No straw, No N	(T6) No till, Straw, 100 N	(T9) Tillage, No straw, 50 N broadcast	(T4) No till, Straw, No N
(T8) Tillage, Straw, 50 N	(T3) No till, Straw, 50N	(T10) Tillage, No straw, 50 N	(T8) Tillage, Straw, 50 N
(T1) No till, No straw, No N	(T10) Tillage, No straw, 50 N	(T4) No till, Straw, No N	(T5) Tillage, Straw, No N
(T9) Tillage, No straw, 50 N broadcast	(T7) Tillage, Straw, 100 N	(T1) No till, No straw, No N	(T7) Tillage, Straw, 100 N
(T5) Tillage, Straw, No N	(T2) Tillage, No straw, No N	(T5) Tillage, Straw, No N	(T2) Tillage, No straw, No N
(T4) No till, Straw, No N	(T1) No till, No straw, No N	(T2) Tillage, No straw, No N	(T10) Tillage, No straw, 50 N

Fig. 2-1 Experimental setup of TSN Plots at Ellerslie and Breton in 1979. Gray color indicates the plots used in this study. N, nitrogen; 50N, 50 kg N ha^{-1} yr^{-1} applied; 100N, 100 kg N ha^{-1} yr^{-1} applied; B, block.

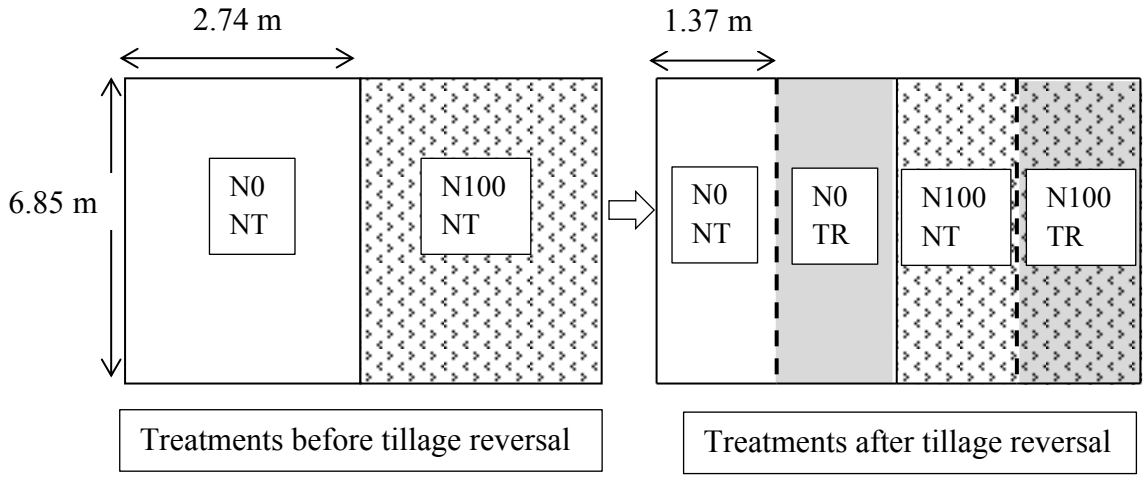

Fig. 2-2 Tillage reversal set up at Ellerslie and Breton. Straw was retained in all plots. Gray color indicates tillage reversal subplots; pattern indicates N fertilized subplots. There are 4 blocks at each site. N0, no N fertilization; N100, 100 kg N ha^{-1} yr^{-1} applied; NT, no tillage; TR, tillage reversal.

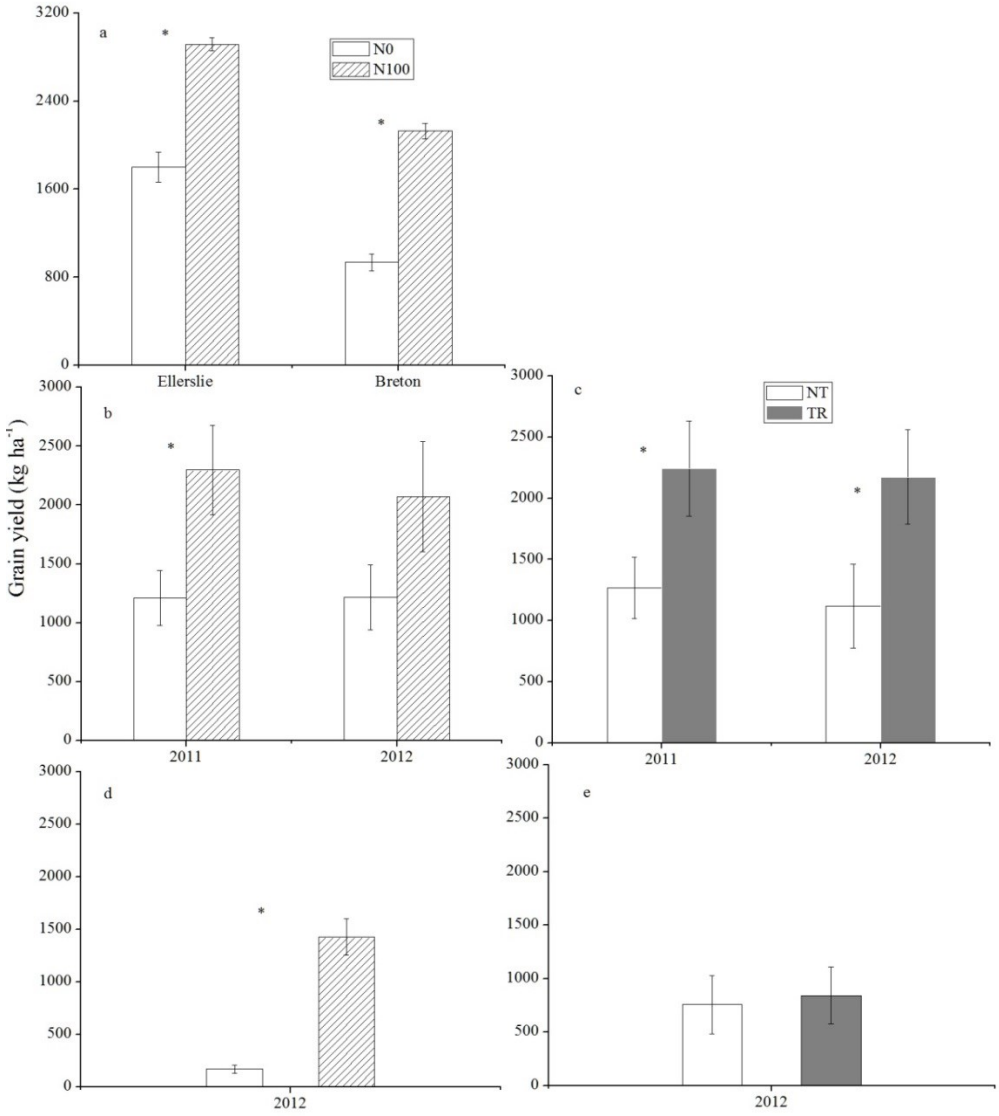

Fig. 2-3 Nitrogen (N) fertilization effects on historical (1981-2008) grain yield at (a) Ellerslie and Breton before reversing tillage, N fertilization effects on grain yield at (b) Ellerslie and (d) Breton, and tillage reversal effects on grain yield at (c) Ellerslie and (e) Breton. N0, no N fertilization; N100, 100 kg N ha^{-1} yr^{-1} applied; NT, no tillage; TR, tillage reversal. * indicates a significant difference between two bars in each graph (p<0.05). The N fertilization × tillage reversal effects on grain yield were not significant at either site.

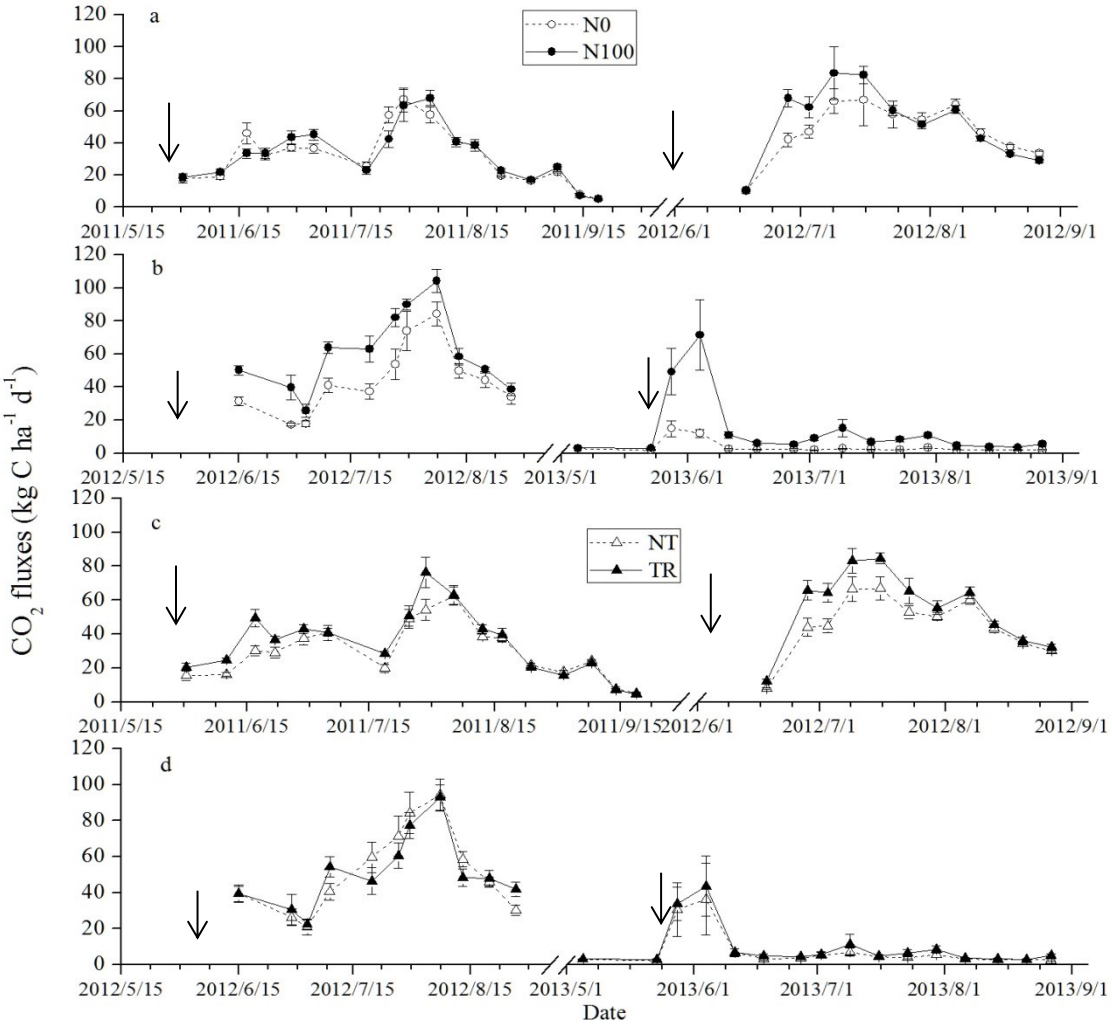

Fig. 2-4 Nitrogen (N) fertilization effects on soil daily mean CO_2 emissions in the growing seasons at (a) Ellerslie and (b) Breton, and tillage reversal effects on soil daily mean CO_2 emissions in the growing seasons at (c) Ellerslie and (d) Breton. N0, no N fertilization; N100, 100 kg N ha^{-1} yr^{-1} applied; NT, no tillage; TR, tillage reversal. Nitrogen fertilization effects were significant at Breton (b). The N fertilization × tillage reversal effects on CO_2 emissions were not significant at either site. Tillage reversal effects were significant at Ellerslie (c). Arrow indicates the date of tillage at each site and year.

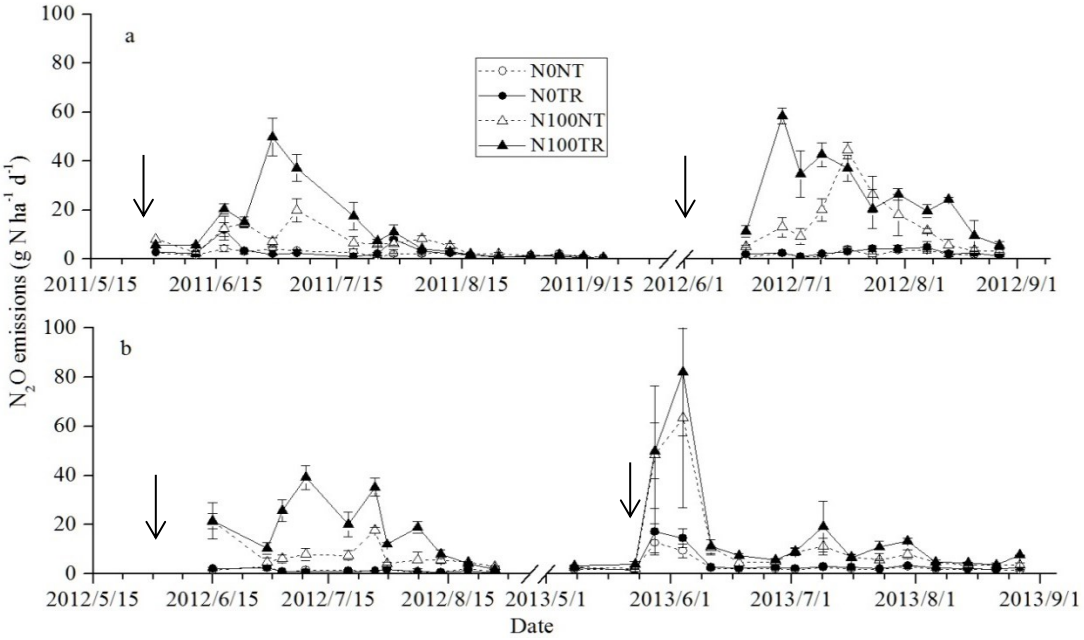

Fig. 2-5 Effects of N fertilization and tillage reversal on soil daily mean N_2O emissions in the growing seasons at (a) Ellerslie and (b) Breton. N0, no N fertilization; N100, 100 kg N ha^{-1} yr^{-1} applied; NT, no tillage; TR, tillage reversal. The N fertilization × tillage reversal effects on N_2O emissions were significant at both sites. Arrow indicates the date of tillage at each site and year.

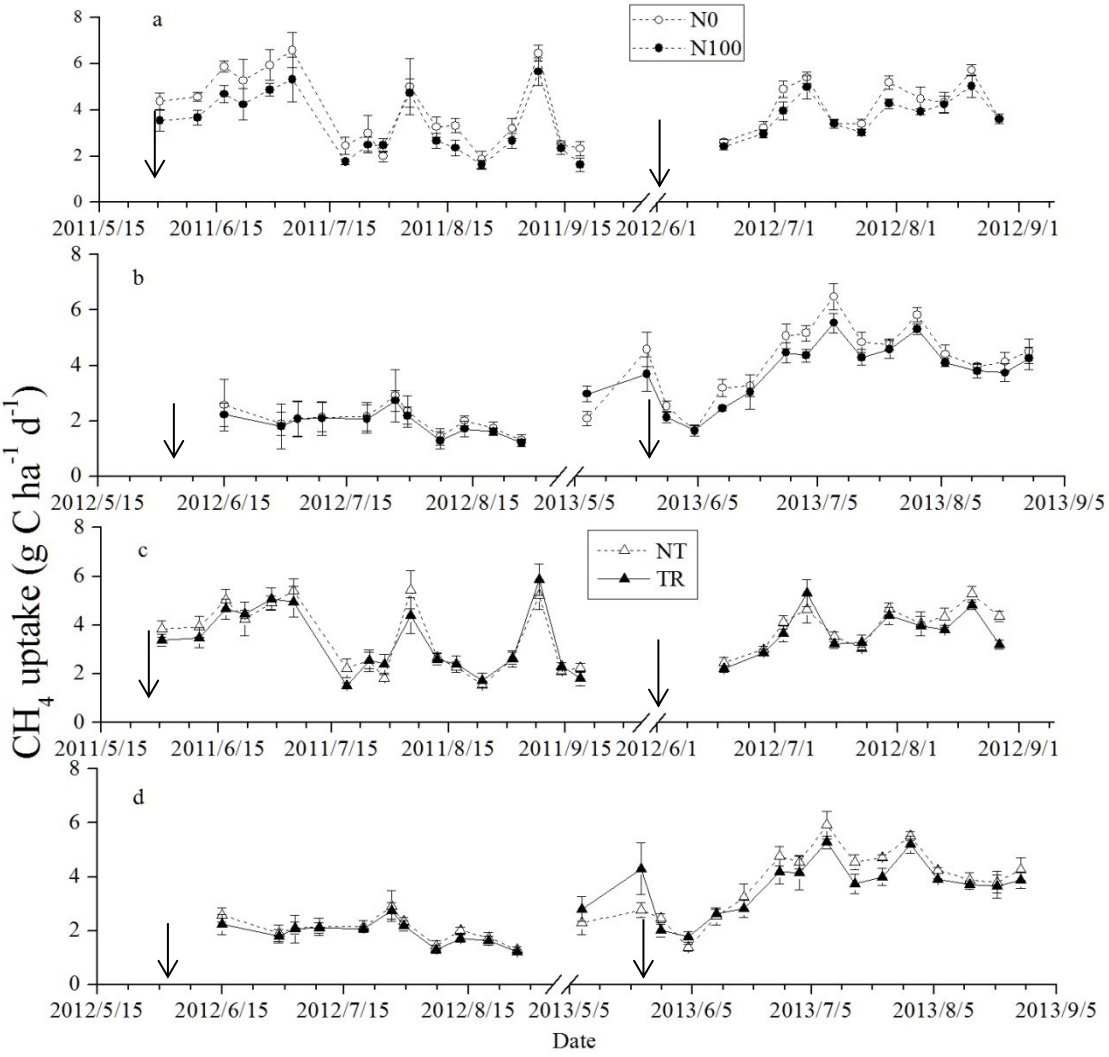

Fig. 2-6 Effects of nitrogen (N) fertilization on soil daily mean CH_4 uptake at (a) Ellerslie and (b) Breton, and effects of tillage reversal on soil daily mean CH_4 uptake at (c) Ellerslie and (d) Breton. N0, no N fertilization; N100, 100 kg N ha^{-1} yr^{-1} applied; NT, no tillage; TR, tillage reversal. The N fertilization × tillage reversal effects on CH_4 uptake were not significant at either site. Arrow indicates the date of tillage at each site and year.

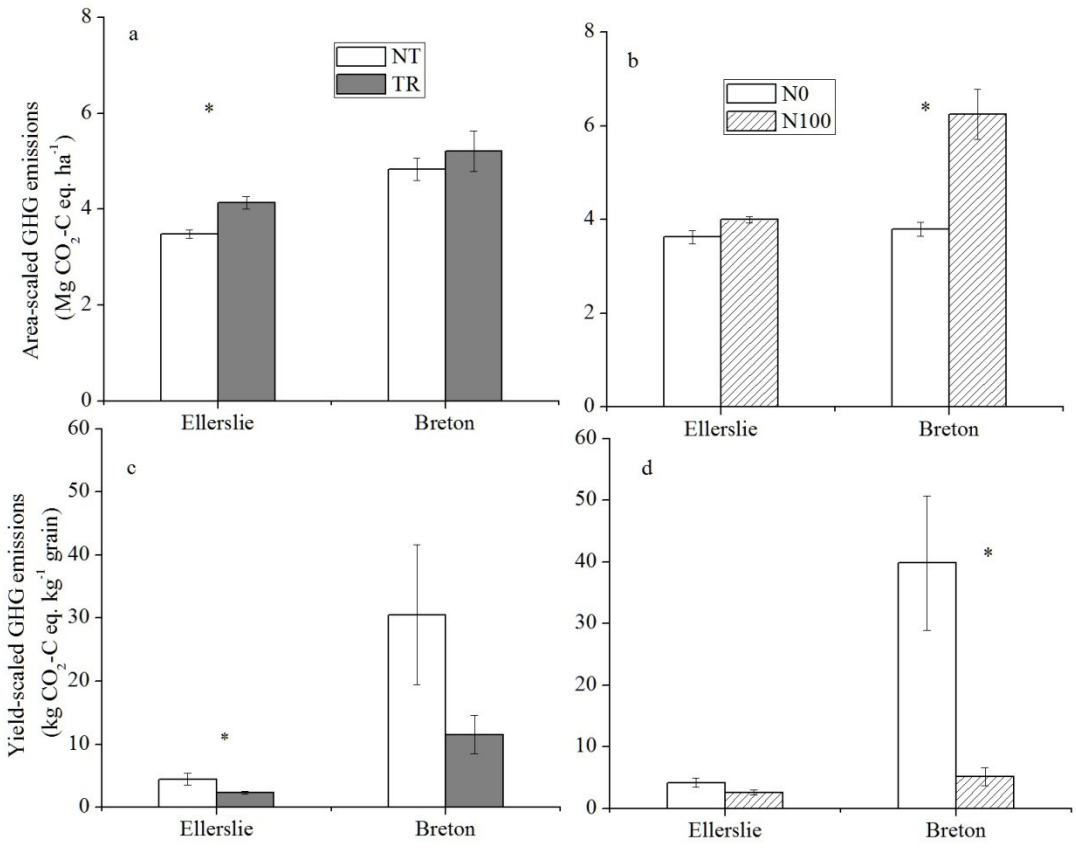

Fig. 2-7 Tillage reversal (T) effects on (a) area- and (c) yield-scaled greenhouse gas (GHG) emissions, and nitrogen (N) fertilization effects on (b) area- and (d) yield-scaled GHG emissions in the growing seasons at Ellerslie and Breton. N0, no N fertilization; N100, 100 kg N ha^{-1} yr^{-1} applied; NT, no tillage; TR, tillage reversal. * indicates a significant difference between two bars at each site (p<0.05). Year (Y), N × T, N × Y, T × Y and N × T × Y effects were not significant at either site.

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Chapter 3 Tillage reversal and nitrogen fertilization affected autotrophic and heterotrophic soil respirations differently in a Black Chernozem and a Gray Luvisol

1 Introduction

Atmospheric carbon dioxide (CO_2) concentration has increased by 36% since the pre-industrial era (IPCC, 2007). On an annual basis, the amount of CO_2 emitted to the atmosphere through soil respiration (R_s) accounts for over two-thirds of ecosystem respiration (Valentini et al., 2000). Agricultural systems are often considered to be net atmospheric carbon (C) sources (Schulze et al., 2009). Soil respiration can be altered by agricultural management practices such as nitrogen (N) fertilization (Kowalenko et al., 1978; Sainju et al., 2012), straw retention (Mueller et al., 1998), liming (Brumme and Beese, 1992), and tillage (Chatskikh and Olesen, 2007; Shahidi et al., 2014). Soil respiration consists of autotrophic (R_a) and heterotrophic respirations (R_h). Autotrophic respiration is from root growth and activity, and can be affected by morphological and metabolic changes (Johnson-Flanagan and Owens, 1986). Microbial decomposition of root exudates and rhizodeposits belongs to R_a (Morell et al., 2011). Heterotrophic respiration is from microbial decomposition of soil organic matter (SOM) that can be influenced by many factors such as microbial community composition, initial SOM content (Thomson et al., 2006), soil structure (van Veen and Kuikman, 1990), soil temperature, and soil moisture content (Kirschbaum, 1995; Leirós et al., 1999). Thus, both R_a and R_h are related to the decomposition of soil organic compounds from above- and belowground biomass (Morell et al., 2011). Although numerous agricultural studies

have examined the responses of R_s to different land management practices (Buysse et al., 2013; Zhang et al., 2013), there have been fewer attempts to partition R_s into R_a and R_h, which is critical to understand the underlying processes.

No-till (NT) practices are widely accepted as beneficial for SOM accumulation and soil organic carbon (SOC) conservation through reducing soil respiration (Bruce et al., 1999; Kahlon et al., 2013; West and Post, 2002). However, because of disadvantages such as weed infestation, nutrient stratification (Baan et al., 2009), pesticide accumulation, (Baker and Saxton, 2007) and increased soil strength (Ball-Coelho et al., 1998; Martino and Shaykewich, 1994) under long-term NT, landowners may revert to conventional tillage (tillage reversal) after long-term NT. The adoption of tillage reversal might stimulate R_a by enhancing root activities due to the decreased soil strength, and stimulate R_h because of enhanced soil aeration and destruction of soil aggregates, which would expose labile or fresh organic matter that exists in aggregates (De Gryze et al., 2004; Grandy and Robertson, 2007; Six et al., 1999). Since only a few studies have focused on tillage reversal practices (Shahidi et al., 2014; Sun et al., 2015), the responses of R_a and R_h to tillage reversal are not well understood.

Oikeh et al. (1999) found that N fertilization influences the growth and distribution of plant roots, which might affect R_a. In addition, N fertilization has been reported to change microbial community composition (Ågren et al., 2001) and enhance physical protection for C by improving surface soil structure (Blanco-Canqui et al., 2014), which might alter R_h. Tillage reversal and N fertilization may alter soil temperature and moisture content (Cook and Orchard, 2008; Pregitzer et al., 2000), which would indirectly influence R_a and R_h.

The objective of this study was to investigate the responses of R_a and R_h to long-term (~30 years) N fertilization and short-term (3-4 years) tillage reversal during the growing season on two different soil types in similar ecological regions. We hypothesized that N fertilization and tillage reversal will increase both R_a and R_h, regardless of the soil type.

2 Materials and methods

2.1 Sites description

A detailed description of the area studied can be found in Chapter 2. Briefly, gas samples were collected from two long-term research sites studying the effects of no tillage, straw retention and N fertilization (Tillage-Straw-Nitrogen Plots) near Ellerslie (53°25′N, 113°33′W; elevation 692 m), with a soil classified as an Orthic Black Chernozem (Typic Cryoboroll) of the Malmo silty clay loam series, and Breton (53°07′N, 114°28′W; elevation 830 m), with a soil classified as an Orthic Gray Luvisol (Typic Cryoboralf) of the Breton loam series. The Black Chernozem has greater soil fertility and better soil structure than the Gray Luvisol (Singh and Malhi, 2006). These two sites are ~70 km apart but represent two major and distinctly different soils found in north-central Alberta (Table 3-1).

2.2 Experimental design

Long-term experimental plots were established at each site in 1979 with a randomized completely block design, which included 10 treatments with various combinations of tillage, N fertilization, and straw retention (Nyborg et al., 1995). In this study, we used a split-plot design with the whole plots (N fertilization, N0 vs. N100 kg N ha^{-1} yr^{-1}) completely randomized in each of four blocks, with tillage - NT vs. tillage reversal (TR) - arranged in subplots. Plots for two of the treatments - T4 (No Till-straw-No N) and T6 (No Till-Straw-100N) - were split into 2 equal subplots (6.85 × 1.37 m). One of the subplots was subjected to TR on June 3, 2009 for the Black Chernozem and on June 4, 2010 for the Gray Luvisol (Shahidi et al., 2014). The other subplot was maintained as NT, except for the disturbance caused by the plot seeder used each spring to seed the plots. The TR subplots were tilled each spring prior to seeding using a rotary tiller to a depth of approximately 8-10 cm. In both NT and TR subplots with N fertilization, urea (100 kg N ha^{-1} yr^{-1}) was mid-row banded (46 cm apart) among seed rows placed 23 cm apart. Phosphorus (20 kg P $ha^{-1}yr^{-1}$) was applied with seed to each subplot in each year. The crop grown at both sites was spring barley (*Hordeum vulgare* L.) throughout the experiment, and after harvesting, the straw was retained on each subplot.

2.3 Field instrumentation and gas sampling

Soil respiration was determined by measuring the increase in CO_2 concentration within the headspace of the chamber (12.5 cm i.d. and 13.5 cm tall) placed over a pre-installed collar in each subplot. Details of the chamber were described in Hutchinson and Mosier (1981). One collar per subplot was randomly installed between rows near the

central area of each subplot for R_s measurement. Heterotrophic respiration was determined by a similar procedure, the only difference being that the collar was installed within a PVC pipe (20 cm i.d. and 30 cm length) completely inserted into the soil (within 50 cm from the collar for R_s) (Suseela et al., 2012). Pre-installed collars were inserted 5 cm into the soil in each of the subplots and maintained throughout the whole growing season; such a design avoids soil disruption caused by frequent installation of the collars. The inserted depth of collar was calculated based on the duration of deployment according to Rochette and Eriksen-Hamel (2007). Autotrophic respiration was calculated as the difference between R_s and R_h. As tillage reversal at Breton began one year after Ellerslie, gas sampling was conducted in 2011 and 2012 at Ellerslie and in 2012 at Breton.

Gas samples were collected between 10:00 am and 12:00 pm, which represented the daily mean gas emissions (determined with diurnal emission measurement), at 7- to 14-d intervals from June to early September in each year, depending on weather conditions and the dates of seeding and crop harvest. Gas samples (20 mL) were collected 0, 10, 20 and 30 min after closing the chamber using a BD Plastipak polypropylene syringe and stored in pre-evacuated 12 mL Exetainers (Labco Ltd., High Wycombe, UK), which were capped with butyl rubber stoppers. Plants that grew above the height and circumference of the chamber were trimmed regularly before collecting gas samples (Sainju et al., 2012). Gas samples were analyzed within 48 h with a gas chromatograph (Model 3800, Varian, Palo Alto, CA) equipped with thermal conductivity detector (TCD), a flame ionizer (FID) and electron capture detectors (ECD).

Soil temperature at the 5 cm depth near the chamber in the central area of the subplots was monitored at 1 h intervals during the growing season with HOBO U10 temperature dataloggers (Doc# 1195-A, Onset Computer Corporation, MA). Mean soil volumetric water content in the 0-30 cm depth in the growing season was measured with CSC616 water content reflectometers, which were inserted into the soil vertically near the chamber in the central area of each subplot. The CSC616 water content reflectometers were connected to CX10 dataloggers (Campbell Scientific, INC.) and data was recorded at 1 h intervals. Soil bulk density of the 0-10 cm soil layer was measured after tillage in 2013 at each site using a steel core sampler (5 cm i.d.).

2.4 Calculation

Soil R_s and R_h were calculated from the increase of CO_2 concentrations in the headspace (Hutchinson and Mosier, 1981; Liebig et al., 2010) with respect to time. Volumetric CO_2 emissions were converted to molarity by the ideal gas law using equation [1]:

$$F_{gas} = \frac{P}{R_v T} \times \beta \times \frac{V_c}{A} \quad [1]$$

where F_{gas} is gas emissions in mmol m^{-2} s^{-1}; P is atmospheric pressure of 1.01325 atm, R_v is the universal gas constant for volumetric conversion at 0.0831 L atm mol^{-1} K^{-1}, T is air temperature in K, V_c is the chamber volume (m^3), A is the soil area covered by the chamber (m^2), and β is the gas emission rate (ppm s^{-1}).

Bulk density (BD) and volumetric water content (θ_v) were used to determine water-filled pore space (WFPS) using equation [2]:

$$\%WFPS = (\theta_v / TP) \times 100 \quad [2]$$

where TP (total porosity) = (1 – PD/BD) ×100, and PD is soil particle density, which is assumed to be 2.65 g cm^{-3}.

2.5 Statistical analysis

Data from the two sites were analyzed separately since the main purpose was to evaluate the differences between R_h and R_a due to different management regimes within each soil type and soil type was not replicated. Analysis of variance was conducted using the PROC MIXED procedure in SAS v8.01 (SAS Institute Inc., 2003) to test for N fertilization and tillage reversal effects on R_h and R_a. Before analysis of variance, R_s, R_a and R_h data were log-transformed to meet normality and homogeneity of variance assumptions. All the data shown in this chapter were the original data. Analysis of variance was based on N fertilization (N0 and N100) as the main plot factor, tillage reversal (NT and TR) as the split-plot factor, and sampling date as a repeated measures variable. Means were separated using the least square means test when treatments and interactions were significant. To examine which soil environmental factor had the most important effect on soil respiration and its components, stepwise multiple regression analysis was conducted with R_s, R_a and R_h as the dependent variables, and soil temperature and WFPS as the independent variables. All tests with a probability value of 0.05 or less were considered significant.

3 Results

3.1 Climatic conditions

Cumulative growing season precipitation amounts (June - September; Table 3-1) at Ellerslie were 275.9 and 278.1 mm in 2011 (the third tillage year) and 2012 (the fourth tillage year), respectively, which were similar to the 30-yr (1980-2010) average (275.3 mm; Table 3-1). At Breton, cumulative growing season precipitation from June to September in 2012 (the third tillage year) was 335.1 mm, which was similar to the long-term average (330.9 mm; Table 3-1). Annual maximum monthly rainfall occurred in July at both sites (142 and 117 mm in 2011 and 2012, respectively, at Ellerslie, and 161 mm in 2012 at Breton). At Ellerslie, the mean air temperature in 2011 (June - September) was 14.0 °C, which was similar to the long-term average (13.9 °C; Table 3-1). However, the mean growing season air temperature in 2012 at Ellerslie and Breton was 15.2 and 14.7 °C, 1.3 and 1.0 °C higher than the long-term average data (13.9 and 13.7 °C at Ellerslie and Breton, respectively).

3.2 Hetero- and autotrophic respirations

The highest rate of R_h occurred in July at Ellerslie (Fig. 3-1a) and Breton (Fig. 3-2a). There was no significant N fertilization × tillage reversal effect on R_h at either site (Table 3-2). Heterotrophic respiration was higher in N100 than in N0 (p<0.001) only at Breton

(Fig. 3-3a). Heterotrophic respiration was higher in TR than in NT in both years (p=0.02 and 0.01 for 2011 and 2012, respectively) at Ellerslie (Fig. 3-3b).

The highest rate of R_a occurred in July at Ellerslie (Fig. 3-1b) and Breton (Fig. 3-2b). There was no N fertilization, tillage reversal or N fertilization × tillage reversal effect on R_a in 2011 at Ellerslie and in 2012 at Breton. However, in 2012, there were significant N fertilization × tillage reversal effects on R_a at Ellerslie (Table 3-2, Fig. 3-4a and 3-4c). In the subplots without N fertilization, R_a was higher in TR than in NT but was not different between tillage treatments in the subplots with N fertilization (Fig. 3-4b). Autotrophic respiration was higher in N100 than in N0 only in the NT subplots at Ellerslie.

The mean growing season contribution of R_h to R_s (expressed as R_h/R_s) was not influenced by N fertilization or tillage reversal in 2011 at Ellerslie, and in 2012 at Breton. However, in 2012 at Ellerslie, there were significant N fertilization × tillage reversal effects on R_h/R_s (Table 3-2). Heterotrophic to total respiration ratio was significantly higher in N100 than in N0 only at Breton (Fig. 3-5a and 3-5c). At Ellerslie, in the NT subplots, the ratio was significantly higher in N0 than in N100. However, in the TR subplots, the ratio was significantly higher in N100 than in N0 in 2012 (Fig. 3-5b).

3.3 Relationships between soil respiration and soil temperature and WFPS

There were no N fertilization × tillage reversal effects on soil temperature and WFPS at either site. Soil temperature was lower in 2011 than in 2012 at Ellerslie (p<0.001). Soil temperature was significantly lower in N100 than in N0 in 2012 at both sites (Table 3-3). Soil WFPS was lower in 2011 than in 2012 at Ellerslie (p<0.001). Soil WFPS was not

affected by N fertilization or tillage reversal at either site. Stepwise multiple regression analysis showed that in 2011 at Ellerslie, respiration rates were influenced by both soil temperature and WFPS (explaining 85% of the variation in R_s, 70% in R_a, and 85% in R_h; Table 3-4). However, in 2012, the respiration rates were only affected by soil temperature, which explained 40% of the variation in R_s, 30% in R_a, and 20% in R_h. At Breton, the respiration rates were affected by both WFPS and soil temperature (explaining 53% of the variation in both R_s and R_a) in 2012 while R_h was only affected by WFPS (explaining 30% of the variation in R_h); $p<0.01$ for all regression models.

4 Discussion

4.1 Partitioning of soil respiration components

Soil R_h/R_s were 52 to 61% at Ellerslie and 46 to 61% at Breton in the growing seasons, which were within the range reported in several studies conducted in agricultural ecosystems (Prolingheuer et al., 2014; Rochette et al., 1999). Although both R_h and R_a were enhanced by tillage reversal in the N0 subplots in 2012 at Ellerslie, the decreased R_h/R_s in the N0 subplots might indicate that the enhancing effect of tillage reversal on root activity was more than that on microbial decomposition rate without applying N fertilizer at Ellerslie. Tillage reversal incorporated crop residue (C/N = 75 for barley straw) into the soil profile. Decomposition of barley straw with a high C/N ratio might require a large amount of N in the absence of N fertilizer, R_h/R_s was increased due to the alleviation of N-limitation for microbial decomposition. The stable R_a and enhanced R_h

increased R_h/R_s at Breton. Soil temperature and WFPS are usually considered to be the two dominant factors controlling soil respiration (Ceccon et al., 2011; Yan et al., 2010). The responses of soil respiration to soil environmental factors were different between years at Ellerslie. In 2011, which had lower mean growing season soil temperature and WFPS than in 2012, both soil temperature and WFPS affected respiration rates. However, in 2012 with a higher mean growing season soil WFPS, soil temperature (with a range of 15-21 °C) was the only factor affected respiration rates. Soil moisture might not be a limiting factor for plant root and microbial activity in 2012 at Ellerslie as soil moisture content was higher compared to 2011. As with other root exclusion methods, soil moisture is usually higher within than outside the PVC pipes because of a lack of water uptake by plant roots (Schindlbacher et al., 2009). In this study, we did not measure soil moisture content within the PVC pipes, which somewhat limits our ability to interpret the data.

4.2 Effects of N fertilization on R_h and R_a

Soil C balance is the difference between C input to soil and C output from soil (Post and Kwon, 2000). In the soils studied, C input came mainly from crop residue (both above- and belowground) while C output was mainly due to CO_2 emissions. Our results showed that R_h was higher in N100 than in N0 at Breton but not at Ellerslie, which was consistent with the effect of N fertilization on soil CO_2 emissions (Chapter 2). At Breton, although crop residue C input increased with N fertilization (Chapter 5), the stimulated R_h under N100 might indicate that N fertilization also potentially caused an increased soil

C output from the soil to the atmosphere. At Ellerslie, N fertilization did not affect microbial decomposition of SOC in the growing seasons of either year.

Soil water-extractable organic carbon (WEOC; belongs to the labile C pool) was higher in N100 than in N0 (Sun et al., 2015), suggesting that the increased C availability for microbial decomposition might contribute to the increased R_h in the N100 subplots. However, N fertilization effect on R_h was significant only at Breton. The Gray Luvisol at Breton might be N deficient for optimum microbial activity because of the low available N concentration (below 10 mg kg^{-1} soil). Thus, microbial activity might be largely stimulated with N fertilization. Soil R_h was significantly higher in 2012 than in 2011 at Ellerslie, which might be attributed to the significantly higher mean growing season soil temperature and WFPS in 2012; higher temperature and WFPS might be beneficial for plant root and microbial activity.

The positive effect of N fertilization on R_a was observed only in the NT subplots in 2012 at Ellerslie, which was consistent with the results reported by Morell et al. (2011). This may indicate that in the NT subplots, the straw retained on the soil surface was slow to release nutrients for plant growth. Nitrogen fertilization might alleviate the N-limitation to plant growth due to the significantly increased crop biomass (both above- and belowground) from 2009 to 2011 by applying N fertilizer (p<0.01; Chapter 5), which might stimulate R_a. However, on the tilled subplots, root growth might be enhanced due to the decreased soil strength under tillage reversal, which might have confounded the effect of N fertilization on R_a in those plots. In addition, tillage reversal significantly decreased soil WFPS, which might also influence the response of R_a to N fertilization (Morell et al., 2011).

4.3 Effects of tillage reversal on R_h and R_a

Our study showed that soil C output through R_h was higher in TR than in NT at Ellerslie but not at Breton, which was consistent with the effect of tillage reversal on soil CO_2 emissions (Chapter 2). We speculated that the different responses of R_h to tillage reversal in the two soils were due to the different initial SOM concentration. The Black Chernozem at Ellerslie had a higher initial SOM than the Gray Luvisol at Breton. Balesdent et al. (2000) found that with higher initial TOC and TN concentrations (as was the case at Ellerslie), the decomposition of SOM due to tillage is more rapid than at a site with lower initial TOC and TN concentrations (as was the case at Breton), and this is consistent with our findings. The higher TOC concentration in TR than in NT at Breton (Sun et al., 2015) might also suggest that tillage reversal helped to increase litter incorporation/input into the soil, which was greater than the increase of SOM mineralization by tillage reversal at Breton. Thus, adopting tillage reversal might be beneficial for atmospheric C mitigation at Breton.

The positive effect of tillage reversal on R_a was only found in the N0 subplots in 2012 at Ellerslie. In the subplots with NT-N0 (>30 years), available N might have been one of the limiting factors for plant growth although SOC was high (55.5 ±0.8 g kg^{-1}) at Ellerslie. Tillage reversal would incorporate crop residue retained on the soil surface into soil profile, where it can be decomposed releasing N for plant growth (Nyborg et al., 1995). However, after applying N fertilizer, the N limitation was alleviated (crop residue

input was higher in N100 than in N0), thus diminishing the effect of tillage reversal on increasing R_a.

5 Conclusions

Agricultural management practices such as N fertilization and tillage reversal can affect R_s by altering its components (R_a and R_h); however, the responses of R_a and R_h to N fertilization and tillage reversal were site-specific. In both soils without N fertilization and tillage (>30 years), N availability might have been a limiting factor for plant growth. Thus, N from N fertilizer application or the decomposition of crop residues incorporated by tillage reversal, enhanced crop productivity and thus R_a. Soil respiration and its components were affected by soil temperature and WFPS at both sites. However, when soil WFPS was high and its variation was low in 2012, soil respiration rates were only affected by soil temperature at Ellerslie.

The N limitation at Breton directly limited soil microbial activity due to the enhanced R_h by N fertilization. Thus, long-term N fertilization at Breton will result in elevated rate of R_s, particularly R_h. This effect discounted the potential for increased atmospheric CO_2 mitigation by increasing crop residue C input into soil under N fertilization because that C input will likely be respired by N-stimulated microbial decomposition. At Ellerslie, the enhanced R_h by tillage reversal potentially offset the atmospheric C mitigation by increasing crop residue C input into the soil due to the unaffected SOM by tillage reversal.

Table 3-1 Total soil organic carbon, total nitrogen, bulk soil C/N ratio, clay content, soil pH (mean ± SE) in the growing season of 2013 , and long-term average air temperature and cumulative precipitation in a Black Chernozem at Ellerslie and a Gray Luvisol at Breton.

Variables	Ellerslie	Breton
Total soil organic carbon (g C kg^{-1})	55.5±0.8	15.1±1.7
Total nitrogen (g N kg^{-1})	4.9±0.1	1.6±0.2
Bulk soil C/N ratio	11.4±0.2	9.2±0.4
Clay content (%)	38.4±2.0	24.2±0.8
Soil pH	4.90±0.10	5.12±0.23
Air temperature (°C)†	13.9	13.7
Cumulative precipitation (mm)†	275.3	330.9

Data of soil properties are means of all treatments (n=16) in 0-10 cm soil depth.

Soil pH was measured in 0.01 M $CaCl_2$ with a solid to liquid ratio of 1:2 (v:v).

†, Long-term average data (soil temperature and cumulative precipitation from June to September in 1980-2010) at each site were from the official website of Agriculture and Rural Development, Government of Alberta (http://www.agric.gov.ab.ca/app116/stationview.jsp).

Table 3-2 ANOVA *p* values on soil autotrophic (R_a) and heterotrophic (R_h) respirations (kg C ha^{-1} d^{-1}), and the ratio of heterotrophic to total soil respiration (R_h/R_s) as affected by nitrogen (N) fertilization, tillage reversal (T) and their interactions at Ellerslie and Breton.

Source of variation	Ellerslie		Breton
	2011	2012	2012
R_a			
N	n.s.	n.s.	n.s.
T	n.s.	n.s.	n.s.
N×T	n.s.	**	n.s.
R_h			
N	n.s.	n.s.	**
T	**	**	n.s.
N×T	n.s.	n.s.	n.s.
R_h/R_s (%)			
N	n.s.	n.s.	*
T	n.s.	n.s.	n.s.
N×T	n.s.	*	n.s.

n.s., not significant ($p>0.05$)

* and ** indicate significance at the 0.05 and 0.01 probability levels, respectively.

Table 3-3 Effects of N fertilization and tillage reversal on soil temperature (°C) at 5 cm depth, and average soil water-filled pore space (WFPS; %) in the top 30 cm depth (mean±SE) in the growing seasons at Ellerslie (the third and fourth years of tillage in 2011 and 2012, respectively) and Breton (the third year of tillage in 2012).

Site		Year	NT	TR	*p* value	N0	N100	*p* value
Ellerslie	Soil temperature	2011	16.1±0.5	16.4±0.5	0.594	16.5±0.4	15.4±0.1	0.163
		2012	17.7±0.3	18.0±0.2	0.458	18.1±0.2	17.2±0.2	0.033
	Soil WFPS	2011	49.1±1.8	48.6±3.9	0.915	50.5±2.9	47.0±3.4	0.623
		2012	66.4±1.4	65.2±2.2	0.395	65.6±2.9	64.1±1.7	0.730
Breton	Soil temperature	2012	19.1±0.3	19.5±0.2	0.316	19.7±0.1	19.0±0.2	0.048
	Soil WFPS	2012	66.3±2.5	65.1±1.0	0.676	67.6±3.8	65.2±2.0	0.689

N0, no nitrogen fertilization; N100, 100 kg N ha^{-1} yr^{-1} nitrogen fertilizer applied; NT, no tillage; TR, tillage reversal. *p*, probability.

The N fertilization × tillage reversal effects on soil temperature and WFPS were not significant at either site or year.

Table 3-4 Dependence of growing season soil total (R_s), autotrophic (R_a) and heterotrophic (R_h) respirations (μmol m^{-2} s^{-1}) on soil temperature (temp) at 5 cm depth and water-filled pore space (WFPS) averaged for the top 30 cm soil depth at Ellerslie and Breton.

Site	Years since tillage	Respiration	Regression equation	$n^{\S}$
Ellerslie	3	R_s	$\ln(R_s)$=-0.96+0.06temp+0.03WFPS; R^2=0.85***	40
		R_a	$\ln(R_a)$=-0.90+0.05temp+0.02WFPS; R^2=0.70***	40
		R_h	$\ln(R_h)$=-0.77+0.03temp+0.03WFPS; R^2=0.85***	40
	4	R_s	$\ln(R_s)$=-0.40+0.12temp; R^2=0.40**	32
		R_a	$\ln(R_a)$=-2.02+0.17temp; R^2=0.30**	32
		R_h	$\ln(R_h)$=-0.47+0.05temp; R^2=0.20**	32
Breton	3	R_s	$\ln(R_s)$=-5.05+0.05temp+0.09WFPS; R^2=0.53***	44
		R_a	$\ln(R_a)$=-5.21+0.07temp+0.08WFPS; R^2=0.53***	44
		R_h	$\ln(R_h)$=-4.22+0.09WFPS; R^2=0.30**	44

**, p<0.01; ***, p<0.001.

§: n, number of observations

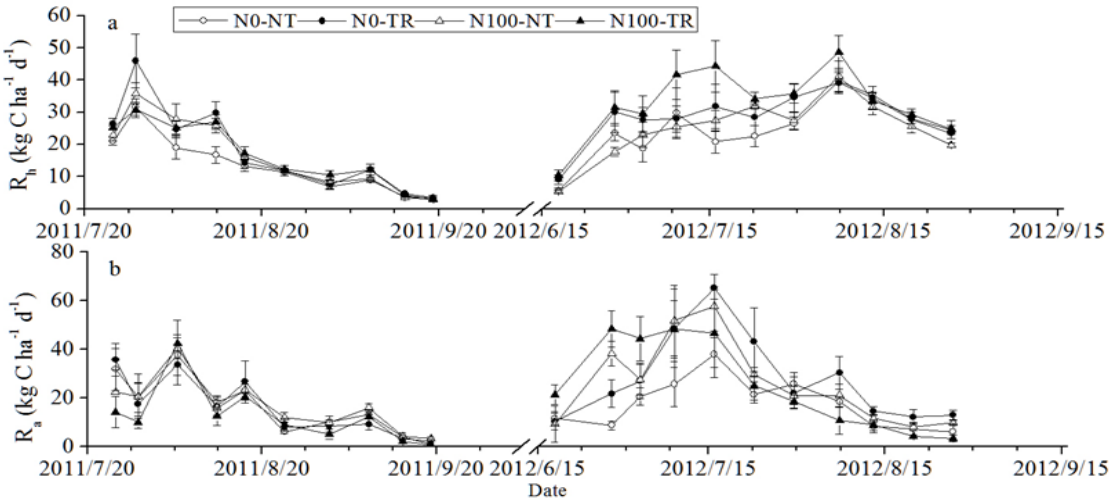

Fig. 3-1 Seasonal variability of (a) heterotrophic (R_h) and (b) autotrophic (R_a) respirations as affected by nitrogen (N) fertilization and tillage reversal in 2011 and 2012 at Ellerslie. N0, no N fertilization; N100, 100 kg N ha^{-1} yr^{-1} applied; NT, no tillage; TR, tillage reversal. Plots were tilled on May 26, 2011 and June 1, 2012. Error bars indicate standard errors of the mean (n=4).

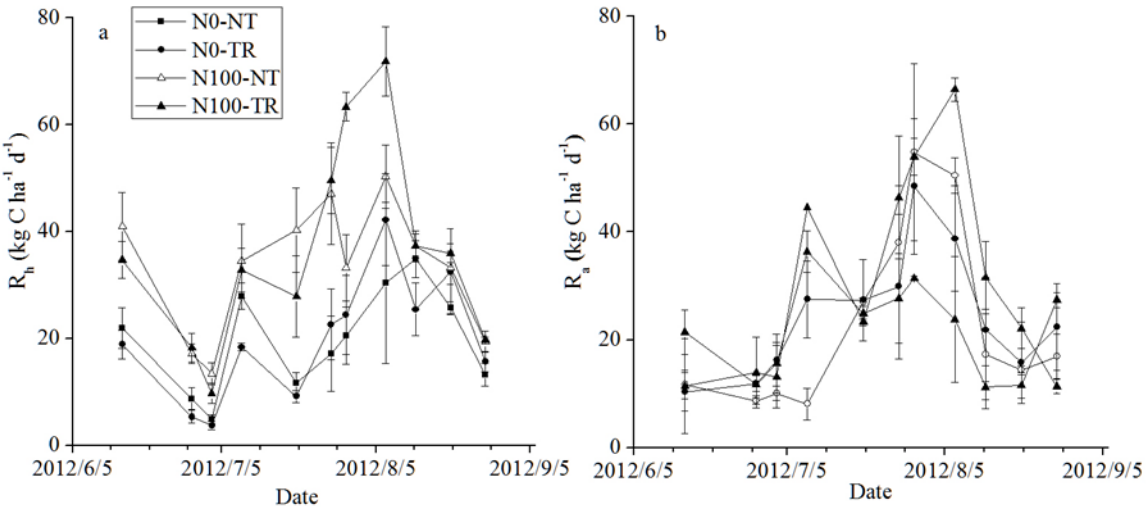

Fig. 3-2 Seasonal variability of (a) heterotrophic (R_h) and (b) autotrophic (R_a) respirations as affected by nitrogen (N) fertilization and tillage reversal in 2012 at Breton. N0, no N fertilization; N100, 100 kg N ha^{-1} yr^{-1} applied; NT, no tillage; TR, tillage reversal. Error bars indicate standard errors of the mean (n=4).

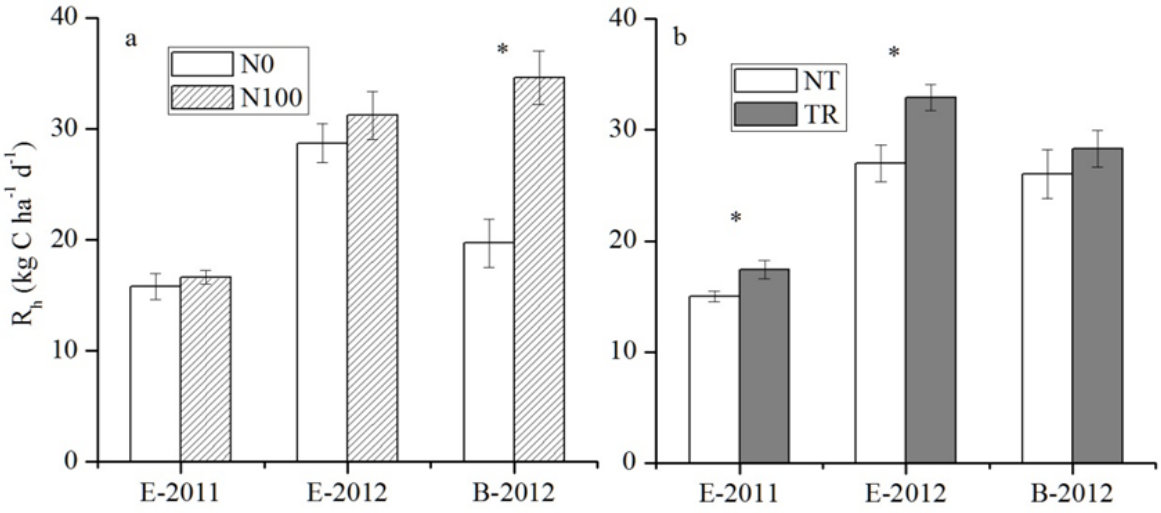

Fig. 3-3 Soil heterotrophic respiration (R_h) as affected by (a) N fertilization and (b) tillage reversal in 2011 and 2012 at Ellerslie, and in 2012 at Breton. N0, no N fertilization; N100, 100 kg N ha^{-1} yr^{-1} applied; NT, no tillage; TR, tillage reversal; E, Ellerslie; B, Breton. * indicates a significant difference between two bars in each year or graph (p<0.05). Error bars indicate standard errors of the mean.

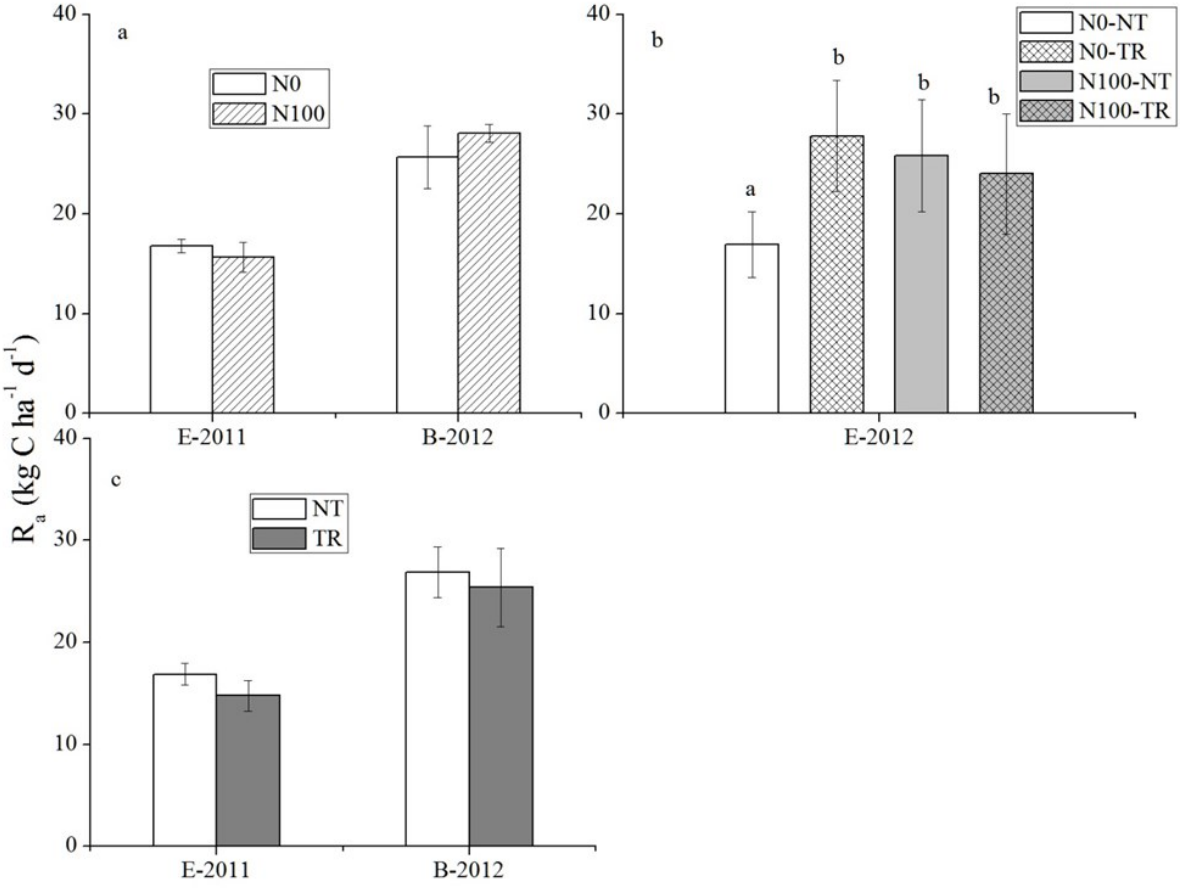

Fig. 3-4 Soil autotrophic respiration (R_a) as affected by (a) N fertilization in 2011 at Ellerslie and in 2012 at Breton, by (b) N fertilization ×tillage reversal in 2012 at Ellerslie, and by (c) tillage reversal in 2011 at Ellerslie and in 2012 Breton. N0, no N fertilization; N100, 100 kg N ha^{-1} yr^{-1} applied; NT, no tillage; TR, tillage reversal; E, Ellerslie; B, Breton. * indicates a significant difference between two bars in each year or graph (p<0.05). Error bars are standard errors of the mean. Different lowercase letters indicate significant differences in R_a between treatments (p<0.05).

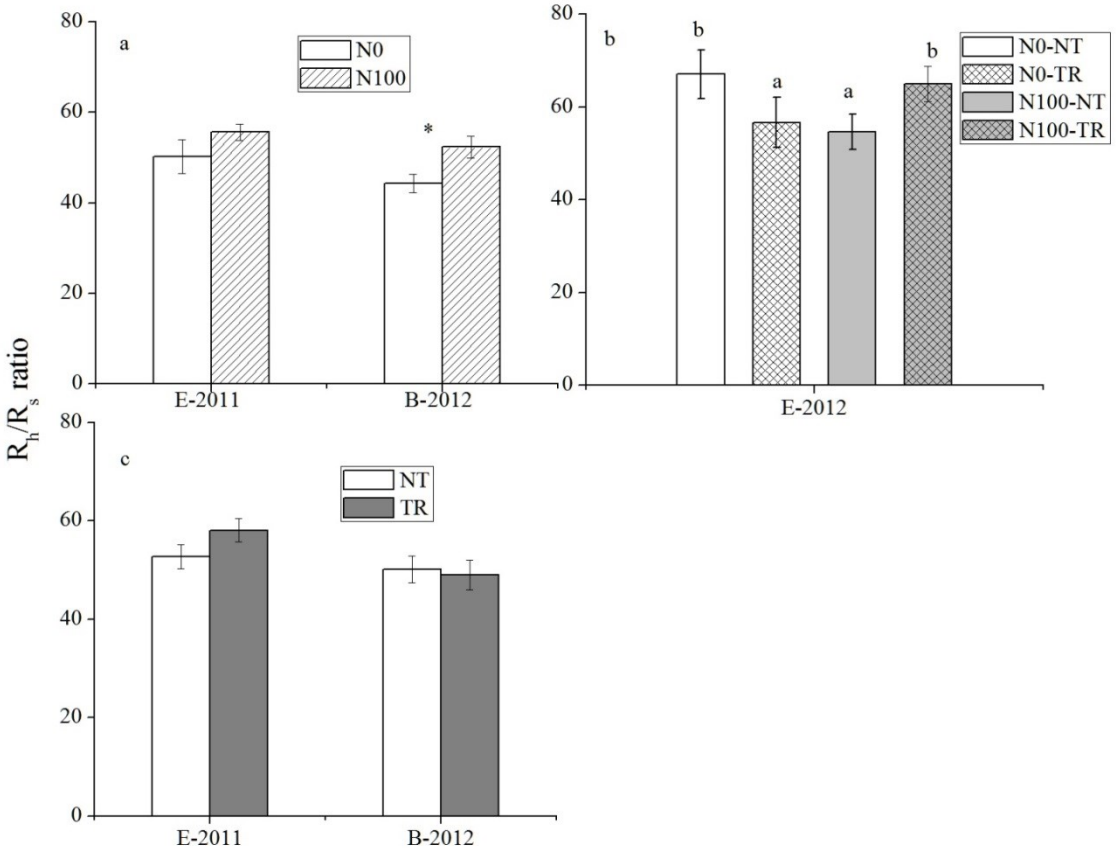

Fig. 3-5 Soil heterotrophic to total respiration ratio (R_h/R_s) as affected by (a) N fertilization in 2011 at Ellerslie and in 2012 at Breton, by (b) N fertilization ×tillage reversal in 2012 at Ellerslie, and by (c) tillage reversal in 2011 at Ellerslie and in 2012 at Breton. N0, no N fertilization; N100, 100 kg N ha^{-1} yr^{-1} applied; NT, no tillage; TR, tillage reversal; E, Ellerslie; B, Breton. * indicates a significant difference between two bars in each year or graph (p<0.05). Error bars are standard errors of the mean. Different lowercase letters indicate significant differences in R_h/R_s between treatments (p<0.05).

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Chapter 4 Nitrogen fertilization and tillage reversal affected water-extractable organic carbon and nitrogen differently in a Black Chernozem and a Gray Luvisol*

1 Introduction

Water-extractable organic carbon (WEOC) and nitrogen (WEON) are important components of water-extractable organic matter (WEOM), which is considered to be the most labile and biodegradable fraction of soil organic matter (Chantigny, 2003). The dynamics of WEOM may reflect changes in soil conditions (Akagi et al., 2007). Water-extractable organic matter plays a key role in nutrient cycling in terrestrial ecosystems because all microbial uptake mechanisms require an aqueous environment (Metting, 1993). Water-extractable organic matter can provide a source of energy for microbial activities and alter soil biogeochemical processes (Hassouna et al., 2010).

Previous incubation studies showed that 10-44% of WEOM are microbial degradable (Kalbitz et al., 2000; Sachse et al., 2001). Qualls and Hainers (1992) found rapid and slow degradable WEOM in soil solutions. The biodegradation of WEOM is dependent on its intrinsic properties, which in turn influence the formation of stable organic C in the soil (Kalbitz et al., 2003). Controls on WEOM quality to a large extent are still poorly understood (Fellman et al., 2008; Kalbitz et al., 2000).

In agricultural ecosystems, land management practices (e.g., tillage and N fertilization) can alter soil physical and chemical properties such as soil structure and pH.

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Such changes in soil properties are likely to influence the quantity and quality of WEOM. However, most studies on N fertilization effects over the past decade have been focused on forest and grassland ecosystems, with little effort spent on investigating WEOM fluxes in intensively managed systems such as in agricultural soils (McDowell, 2003). Some studies report no significant effect from N fertilization (Rochette and Gregorich, 1998; Zsolnay and Görlitz, 1994), while others report decreases (Chantigny et al., 1999; Liang et al., 1998) or increases in WEOC after N fertilization (McTiernan et al., 2001). Those contradictory results highlight a need for improved understanding of the effects of intensive agricultural management practices on WEOM dynamics.

It is commonly accepted that C sequestration can be accomplished by changing from conventional tillage (CT) to no tillage (NT) (Bruce et al., 1999; Kahlon et al., 2013; West and Post, 2002). However, long-term NT can cause problems such as accumulation of crop residues, weed infestation, nutrient stratification on the soil surface (Baan et al., 2009; Grant and Bailey, 1994), and pesticide accumulation (Baker and Saxton, 2007). Long-term NT may also reduce water infiltration due to surface soil compaction. Reversing NT to CT (tillage reversal) may be a way to deal with those problems and the possibility exists that landowners may revert to tillage from long-term NT. Tillage reversal can disrupt soil aggregates and expose labile or fresh organic matter that was once protected with aggregates, making it susceptible to microbial decomposition (DeGryze et al., 2004; Grandy and Robertson, 2007; Six et al., 1999), which may change the quantity and quality of WEOC. Tillage reversal may also result in the incorporation of fresh crop residue into the soil profile, which would also change the quantity and quality of WEOC. Since only a few studies have focused on tillage reversal practices

(e.g., Shahidi et al., 2014), the influence of tillage reversal on C sequestration is poorly understood. Moreover, the interactions between various agricultural management practices such as N fertilization and tillage management make the prediction of the cumulative impact of these activities on the quality and quantity of WEOC even more complicated.

We studied the dynamics of WEOC and WEON after long-term (34 years) N fertilization and 4-5 years of tillage reversal (after about 30 years of NT) during the growing season on two different soil types in a similar ecological region. We hypothesized that N fertilization and tillage reversal will increase WEOC concentrations and decrease its stability. The objective of this study was to assess the effects of N fertilization and tillage reversal on soil C and N concentrations, and the quality and quantity of soil WEOC.

2 Materials and methods

2.1 Field sites and soil sampling

Soil samples were collected from two long-term research sites studying the effects of zero tillage, straw retention, and N fertilization (Tillage-Straw-Nitrogen Plots) established on an Orthic Black Chernozem (Typic Cryoboroll) of the Malmo silty clay loam series near Ellerslie (53°25′N, 113°33′W; elevation 692 m), and on an Orthic Gray Luvisol (Typic Cryoboralf) of the Breton loam series near Breton (53°07′N, 114°28′W; elevation 830 m). Both sites are located in central Alberta, Canada. The Black

Chernozem had greater soil fertility and better soil structure than the Gray Luvisol (Singh and Malhi, 2006). These two sites are ~70 km apart but represent two major and distinctly different soils found in central Alberta (Table 4-1). The mean air temperature from May to August in 2013 was 13.9 °C at Ellerslie and 13.6 °C at Breton. The mean annual precipitation in 2013 was 452 mm at Ellerslie and 555 mm at Breton.

Long-term experimental plots were established at each site in 1979 with a randomized completely block design, which included 10 treatments with various combinations of tillage, N fertilization and straw retention (Nyborg et al., 1995; Fig. 2-1). In this study, we used a split-plot design with the whole plots (N fertilization, N0 vs. N100 kg N ha^{-1} yr^{-1}) completely randomized in each of four blocks, with tillage - NT vs. tillage reversal (TR) - arranged in subplots (Fig. 2-2). Plots for two of the treatments - T4 (No Till-straw-No N) and T6 (No Till-Straw-100N) - were split into 2 equal subplots (6.85 × 1.37 m). One of the subplots was subjected to TR on June 3, 2009 for the Black Chernozem and on June 4, 2010 for the Gray Luvisol (Shahidi et al., 2014). The other subplot was maintained as NT, except for the disturbance caused by the plot seeder used each spring to seed the plots. The TR subplots were tilled each spring prior to seeding using a rotary tiller to a depth of approximately 8-10 cm. In both NT and TR subplots with N fertilization, urea (100 kg N ha^{-1} yr^{-1}) was mid-row banded (46 cm apart) among seed rows placed 23 cm apart. Phosphorus (20 kg P $ha^{-1}yr^{-1}$) was applied with seed to each subplot in each year. The crop grown at both sites was spring barley (*Hordeum vulgare* L.) throughout the experiment, and after harvesting, straw was retained on each subplot.

The first soil sampling was conducted within one week after N application in May 2013. After that, soil samples were collected monthly until the end of the growing season (August 2013). On each sampling, seven soil cores (3 cm diameter) were collected from the 0-10 cm depth (Ap horizon) of each plot and composited. In the laboratory, visible roots, earthworms, and straw residue were removed. The moist soil was passed through a 2 mm sieve. A subsample was taken from each soil sample and air-dried for a week to determine soil total organic C (TOC) and TN concentrations. The remaining fresh soil was frozen for further analyses.

2.2 Soil total organic C, total N and WEOM extraction

Air-dried soil samples were used to determine soil TOC and TN concentrations using a Carlo Erba NA 1500 elemental analyzer (Carlo Erba Instruments, Milan, Italy).

Organic matter was extracted from fresh soil samples using deionized water according to a modification of the procedure of Roehm et al. (2009). Fresh soils were added to ultrapure water (1/2, w/w) and shaken for 2 h on a reciprocal shaker at 20 °C. After being centrifuged at 12500×g on a high speed centrifuge (Thermo IEC MultiRF) for 20 min, the supernatant was filtered with a 0.2 μm syringe filter (Fisherbrand, Nylon) to remove microbial biomass (Fellman et al., 2008; Xu et al., 2013). The filtrates were then analyzed for WEOC and total water-extractable N by a Shimadzu TOC-V CSH/CSN analyzer (Shimadzu Corporation, Kyoto, Japan). Nitrate in the extract was determined by the method of Miranda (2001) at room temperature with a UV spectrometer (Thermo Spectronic Genesys 10S) in 1 cm quartz cuvettes. Ammonium in the extract was

determined with the indophenol blue method with the same UV spectrometer (Keeney and Nelson, 1982). Soil WEON was then calculated by subtracting mineral N (nitrate plus ammonium) from total water-extractable N.

The chemical characteristics of WEOC were determined through measurement of ultraviolet (UV) absorbance and fluorescence spectroscopy. UV absorbance at 280 nm is used to estimate the aromaticity of WEOC (Kalbitz et al., 2003; McKnight et al., 1997) while fluorescence spectra provides additional information on the complexity and condensation of the organic molecules (Don and Kalbitz, 2005; Zsolnay et al., 1999). Subsamples of the WEOC were used for analyses of UV absorption at 280 nm with a UV spectrometer (Thermo Spectronic Genesys 10S) in 1 cm quartz cuvettes. The specific UV absorbance at 280 nm ($SUVA_{280}$) was calculated by dividing the absorbance at 280 nm by the WEOC concentration and reported in L mg C^{-1} m^{-1}.

Fluorescence excitation-emission matrices were obtained with a Photon Technologies International (PTI) Fluorimeter (Birmingham, NJ, USA) using quartz cuvettes. Emission and excitation slits were set at a 5 nm bandwidth. Fluorescence emission spectra were collected at an excitation wavelength of 254 nm and an emission wavelength range of 280 to 500 nm. As fluorescence is influenced by molecular concentrations, all filtrates were diluted to a WEOC concentration of 10 mg L^{-1} before measurement (Kalbitz et al., 2003). Humification index (HIX) for WEOC was then calculated as the ratio of the emission intensity integrated from 435 to 480 nm (fluorescence emission from condensed unsaturated systems, such as humic substances) to the integration from 300 to 345 nm (fluorescence emission from relatively fresh, non-humified organic matter) at a fixed excitation wavelength of 254 nm (Zsolnay et al.,

1999). The HIX has been used as an indicator of the degree of humification (Akagi et al., 2007; Zsolnay et al., 1999). The emission intensity integration was calculated with ORIGIN software (OriginPro 8.5.1 SR2). Generally, high UV absorbance and HIX indicate high C stability.

2.3 Statistical analyses

Data from the two sites were analyzed separately since the main purpose was to evaluate the differences in WEOC and WEON due to different management regimes within each soil type. Analysis of variance was conducted using the PROC MIXED procedure in SAS v8.01 to test for treatment effects. Before analysis of variance, data were tested for normal distribution and log-transformed where appropriate. The analysis was based on N fertilization (N0 and N100) as the main plot factor, tillage reversal as the split-plot factor, and sampling time (4 times) as a repeated measures variable. Means were separated using the least square means test when interactions were significant. All results were considered significant if p values were less than 0.05.

3 Results

3.1 Soil TOC and TN concentrations

There was no interaction effect of N fertilization and tillage on soil TOC, TN or C/N ratio of bulk soil in either soil. At Ellerslie, N fertilization and tillage reversal did not

change soil TOC or TN (Fig. 4-1a and 4-1c). However, at Breton, soil TOC and TN were higher under N100 than under N0 (Fig. 4-1a and 4-1c). Soil TOC was higher under TR than under NT only at Breton (Fig. 4-2b). Soil TN was not affected by tillage reversal at Breton (Fig.4-2d). Bulk soil C/N ratio was not influenced by N fertilization or tillage reversal at either site (Fig. 4-1e and 4-1f).

3.2 Water-extractable organic C and N concentrations

There was a significant interaction effect between N fertilization and sampling time on WEOC concentrations at both sites (Table 4-2). Water-extractable organic C concentrations were markedly higher under N100 than under N0 in May, June and July but soil WEOC concentrations of the same N treatment were not different between sampling time at Ellerslie (Fig. 4-2a). However, soil WEOC concentrations were influenced by N fertilization × sampling time and tillage reversal × sampling time at Breton (Table 4-2). Soil WEOC concentrations were significantly higher in N100 than in N0 at all sampling times (Fig. 4-2c). Soil WEOC concentrations were lower in TR than in NT only in May at Breton (Fig. 4-2d). There were significant interaction effects between N fertilization, tillage reversal and sampling time on WEON concentrations at both sites (Table 4-2). A significant interaction effect of N fertilization and tillage reversal was observed on WEON concentrations in May at both sites (Fig. 4-3). At Ellerslie, there were significant N fertilization × tillage reversal effects on the WEOC/WEON ratio (Table 4-2). Soil WEOC/WEON ratio was not different between treatments (Table 4-2 and Fig. 4-2b).

3.3 Spectroscopic properties of WEOC

There was a significant interaction effect between N fertilization and sampling time on $SUVA_{280}$ at both sites (Table 4-3). The $SUVA_{280}$ was significantly lower in N100 than in N0 only in May at Ellerslie (Fig. 4-4a). At Breton, the $SUVA_{280}$ was significantly lower in N100 than in N0 in July and August (Fig. 4-4b). Nitrogen fertilization or tillage reversal had no influence on HIX at Ellerslie (Table 4-3). At Breton, there was a significant N fertilization× sampling time effect on HIX in both soils. The HIX of WEOC at Breton was significantly higher in N100 than in N0 in June, July and August (Fig. 4-4c).

4 Discussion

4.1 Nitrogen fertilization and tillage reversal on soil TOC and TN concentrations

Agricultural management practices such as N fertilization and NT are being used to increase soil C sequestration (Alvarez, 2005; López-Fando and Pardo, 2011; Malhi et al., 2011b). However, at Ellerslie, soil TOC was less in 2013 than at the onset of the experiment in 1979 except in N100-TR where soil TOC was unchanged. Soil TN was not changed by long-term N fertilization at Ellerslie. At Breton, where the soil was much lower in TOC and TN in 1979 (13.8 and 1.2 g kg^{-1} for TOC and TN, respectively), the trend was reversed. Soil TOC and TN were increased by adopting N fertilization and

tillage reversal compared with the values at the onset of the experiment in 1979. Our results suggested that the soil ecosystems studied reacted differently to N fertilization and tillage reversal. The greater soil TOC and TN in N100 than in N0 at Breton might be attributable to the differences in soil texture (a silty clay loam texture in Ellerslie and a loam texture in Breton). The effect of N fertilization might be greater in coarser soils than in fine textured ones (Alvarez, 2005) because coarser soils may have lower fertility, thus leading to larger crop responses to N fertilization which might contribute to greater soil C input.

Conventional tillage management has been found to decrease soil TOC in many studies (Plante et al., 2006; Wright et al., 2007). However, our results showed an increasing trend after 4-5 years of tillage on long-term no-till soils at Breton. A rotary tiller was used in this experiment for seedbed preparation and breaking up compacted soil. Increases in soil TOC when plowing with rotary tiller maybe caused by plant residues being more fully incorporated into the plow layer (Hajabbasi and Hemmat, 2000). We suggested that tillage reversal helped to increase litter incorporation/input into the soil, which was greater than increased SOM mineralization caused by tillage reversal at Breton.

4.2 Nitrogen fertilization and tillage reversal effects on WEOC and WEON concentrations

The production and consumption of WEOC are driven by a complex series of biological and physicochemical processes that are still poorly understood (Hagedorn et

al., 2012; Kalbitz and Kaiser, 2008; Kalbitz et al., 2006). These processes include abiotic leaching of soil organic matter and crop residues, production of WEOC during decomposition of soil organic matter and crop residue, rhizosphere processes such as root exudation, and consumption of WEOC through microbial respiration (McDowell, 2003; McDowell et al., 2004). Nitrogen fertilization increases crop residue C input in both soils (Malhi et al., 2011a). This might subsequently increase the production of WEOC through abiotic leaching. Soil pH was significantly lower in N100 than in N0 at both sites (pH=4.9 and 5.2 for N100 and N0, respectively, at Ellerslie, and 4.9 and 5.5 for N100 and N0, respectively, at Breton). In low pH conditions, dissolution of organo-metal complexes can contribute to leaching of WEOM (Kalbitz et al., 2000). Tillage reversal effects on WEOC were different in these two soils. Tillage reversal might enhance soil aeration, which can increase soil microbial decomposition rate, especially in soil with relative poor structure, which might be a reason for the significant decrease of WEOC concentrations after applying tillage reversal at Breton in May.

In this study, we found a significant interaction effect between N fertilization and tillage reversal on WEON concentrations in both soils. Nitrogen fertilization accompanied with tillage yielded the highest WEON among all the treatments in both soils. Applying N fertilizer can change microbial community structure (e.g., fungal/bacterial ratio), and subsequently alter organic N mineralization (McDowell et al., 2004; Raubuch and Beese, 1998; Ros et al., 2009). Tillage can break up soil aggregates (Beare et al., 1994; Page et al., 2013), which releases WEON occluded within aggregates. Moreover, tillage reversal enhances soil aeration (Sun et al., 2011) and incorporates fresh OM into the soil profile. All these processes influence the dynamics of WEON and led to

the highest WEON concentration in the N100-TR treatment in May in both soils. However, this effect was temporary and WEON concentrations decreased as mineral N concentration was reduced by crop uptake in the following months.

The C/N ratio of WEOM in the soils studied ranged from 6 to 9, which means that WEOM is very labile and can be readily decomposed by microbes. Although the value was much lower than that reported by some studies in agricultural soils (Embacher et al., 2008; Xu et al., 2013), it is consistent with those observed in other studies (Ghani et al., 2007; Tian et al., 2010; Zhang et al., 2011). One explanation for the narrow C/N ratio was that WEON is a proportionally more labile component of soil organic matter than WEOC in the soils studied, and the proportionally greater WEON in the WEOM decreased the C/N ratio in the WEOM (Ghani et al., 2007).

4.3 Nitrogen fertilization and tillage reversal effects on spectroscopic properties of WEOC

Soil WEOC quality revealed by spectroscopic properties was changed by N fertilization. This is consistent with reports by Aitkenhead-Peterson and Kalbitz (2005), Michel et al., (2006), and Ohno et al. (2009). However, the responses of WEOC quality to N fertilization were different in the soils studied. Unfortunately, it is still not clear which factor drives the different responses of WEOC quality to N fertilization in these two soils. The following potential processes may explain our results. The decreased aromatic C (lower $SUVA_{280}$ on treatments receiving N fertilizer) at Ellerslie and Breton might be caused by the contribution of active WEOC from crop residues, dead roots and

root exudates, which have relative low aromatic C (Zhang et al., 2011). Urea fertilization has been found to change soil microbial community structure (McDowell et al., 2004; Zhang et al., 2008). Shahidi et al. (2014) conducted a field experiment in 2009 and 2010 to evaluate urea fertilization effect on soil respiration rate with the same plots used in this study. She found that in the Black Chernozem, N fertilization has no effect on total soil respiration rate during the growing season but in the Gray Luvisol, soil respiration rate is significantly increased, indicating that N fertilization has different effects on microbial and root activities between these two soils. Previous studies showed that N input alters decomposition processes (Knorr et al., 2005). Nitrogen fertilization had been reported to stimulate microbes to use fresh substrates such as root exudates, dead root or more labile fractions of WEOC, which have low N content instead of recalcitrant compounds with high N content (Berg and Matzner, 1997; Hagedorn et al., 2012). Rutherford and Juma (1989) found that the Gray Luvisol at Breton has a more rapid N turnover rate than the Black Chernozem at Ellerslie with applying ^{15}N labelled urea. Thus, with N fertilization, the stimulation of humification processes might be greater at Breton than at Ellerslie, which increased the HIX at Breton. The decreased aromaticity and increased condensed structure of WEOC at Breton might indicate an accumulation of non-aromatic compounds of WEOC.

5 Conclusions

In this study, cultivation increased soil TOC and TN concentrations. However, the magnitude of the increase was site-specific. With straw retention, N addition and tillage

reversal increased soil C and N concentrations in the soil with poor structure and low fertility. Nitrogen fertilization rather than tillage reversal was the main factor affecting the quality and quantity of WEOC. The N effect was much more complicated at Breton than Ellerslie. Sampling time within the growing season influenced the effect of N fertilization on the quality and quantity of WEOC. Nitrogen fertilization appears to have increased the stability of WEOC at Breton, but not at Ellerslie. Nitrogen fertilization and tillage reversal had an interaction effect on WEON cycling in the soils studied. Future studies should focus on comparing the chemical structure of WEOC under N100 to that under N0 to reveal how N fertilization affects the stability of WEOC in these two soils. Soil microbial community and activity are important aspects that affect WEOC production and decomposition, which are worthwhile evaluating in N100 as well as in N0 at both sites.

Table 4-1 Total soil organic carbon, total nitrogen, bulk soil C/N ratio, clay content, soil pH (mean ± SE) in the growing season of 2013 , and long-term average air temperature and cumulative precipitation in a Black Chernozem at Ellerslie and a Gray Luvisol at Breton.

Variables	Ellerslie	Breton
Total soil organic carbon (g C kg^{-1})	55.5±0.8	15.1±1.7
Total nitrogen (g N kg^{-1})	4.9±0.1	1.6±0.2
Bulk soil C/N ratio	11.4±0.2	9.2±0.4
Clay content (%)	38.4±2.0	24.2±0.8
Soil pH	4.90±0.10	5.12±0.23

Data were means of all treatments (n=16).

Data are mean±SE.

Table 4-2 ANOVA *p* values on soil water-extractable organic C (WEOC), N (WEON) and WEOC/WEON ratio in the 0-10 cm soil layer as affected by nitrogen (N) fertilization, tillage reversal (T), sampling time and their interactions at Ellerslie and Breton.

Site	Source of variation	WEOC	WEON	WEOC/WEON
Ellerslie	N	0.02	n.s.	n.s.
	T	n.s.	<0.01	n.s.
	N × T	n.s.	<0.01	0.04
	Time	n.s.	<0.01	<0.01
	N × Time	0.046	<0.01	n.s.
	T × Time	n.s.	<0.01	n.s.
	N × T× Time	n.s.	<0.01	n.s.
Breton	N	0.01	0.01	n.s.
	T	0.03	n.s.	n.s.
	N × T	n.s.	0.02	n.s.
	Time	<0.01	<0.01	<0.01
	N × Time	<0.01	<0.01	n.s.
	T × Time	<0.01	<0.01	n.s.
	N × T × Time	n.s.	<0.01	n.s.

Data are mean±SE (n=16).

n.s., not significant (p>0.05).

Table 4-3 ANOVA *p* values on spectroscopic characteristics of water-extractable organic carbon (WEOC) in the 0-10 cm soil layer as affected by nitrogen (N) fertilization, tillage reversal (T), sampling time, and their interactions in a Black Chernozem at Ellerslie and a Gray Luvisol at Breton.

Site	Source of variation	$SUVA_{280}$ (L mg C^{-1} m^{-1})	HIX
Ellerslie	N	<0.01	n.s.
	T	n.s.	n.s.
	N × T	n.s.	n.s.
	Time	n.s.	<0.01
	N × Time	0.05	n.s.
	T × Time	n.s.	n.s.
	N × T × Time	n.s.	n.s.
Breton	N	0.02	0.02
	T	n.s.	n.s.
	N × T	n.s.	n.s.
	Time	<0.01	<0.01
	N × Time	0.02	0.05
	T × Time	n.s.	n.s.
	N × T × Time	n.s.	n.s.

Data are mean±SE (n=16).

n.s., not significant (p>0.05)

$SUVA_{280}$, specific UV absorbance at 280 nm; HIX, humification index.

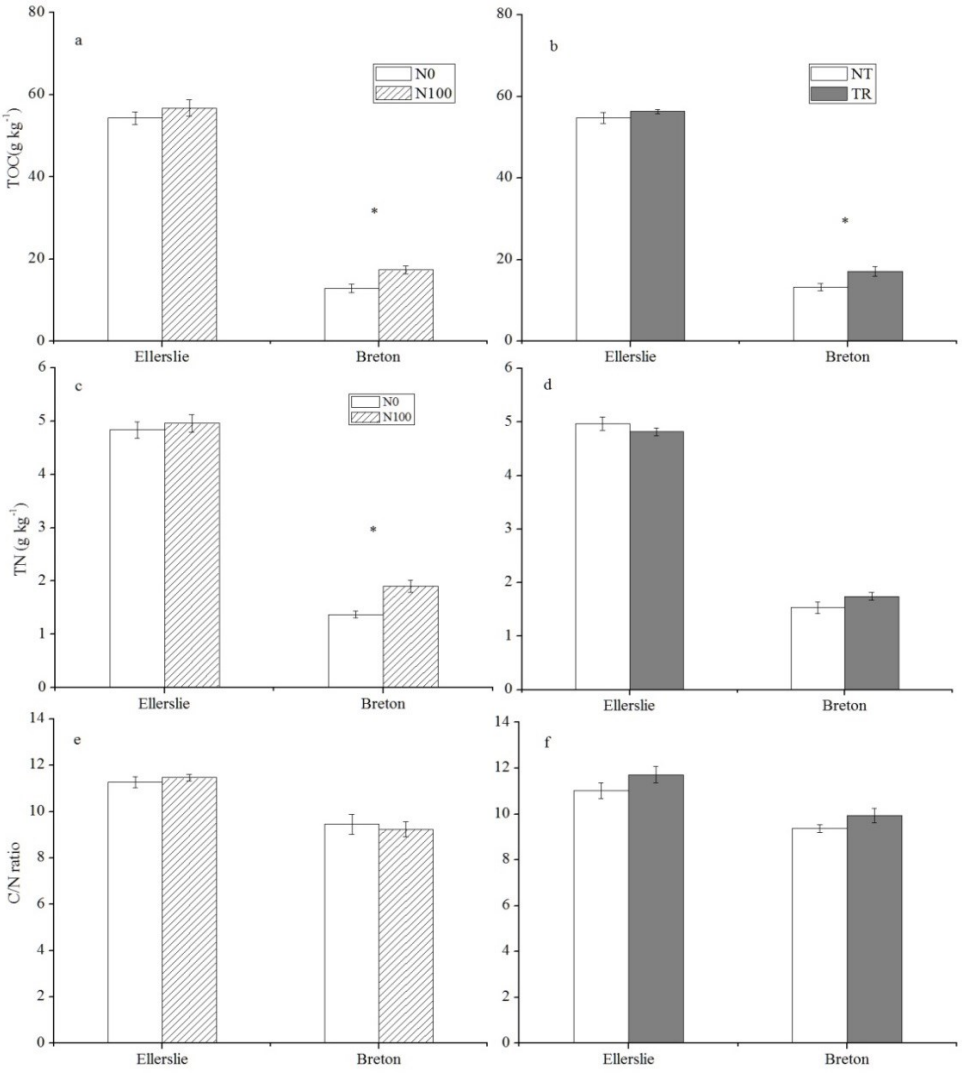

Fig. 4-1 Soil total organic carbon (TOC) as affected by (a) N fertilization and (b) tillage reversal, total nitrogen (TN) as affected by (c) N fertilization and (d) tillage reversal, and C/N ratio as affected by (e) N fertilization and (f) tillage reversal at Ellerslie and Breton. N0, no N fertilization; N100, 100 kg N ha^{-1} yr^{-1} applied; NT, no tillage; TR, tillage reversal. Error bars are standard errors of the mean (n=8). * indicates a significant difference between two bars within each site. The N fertilization × tillage reversal effects on TOC, TN and C/N ratio were not significant at either site.

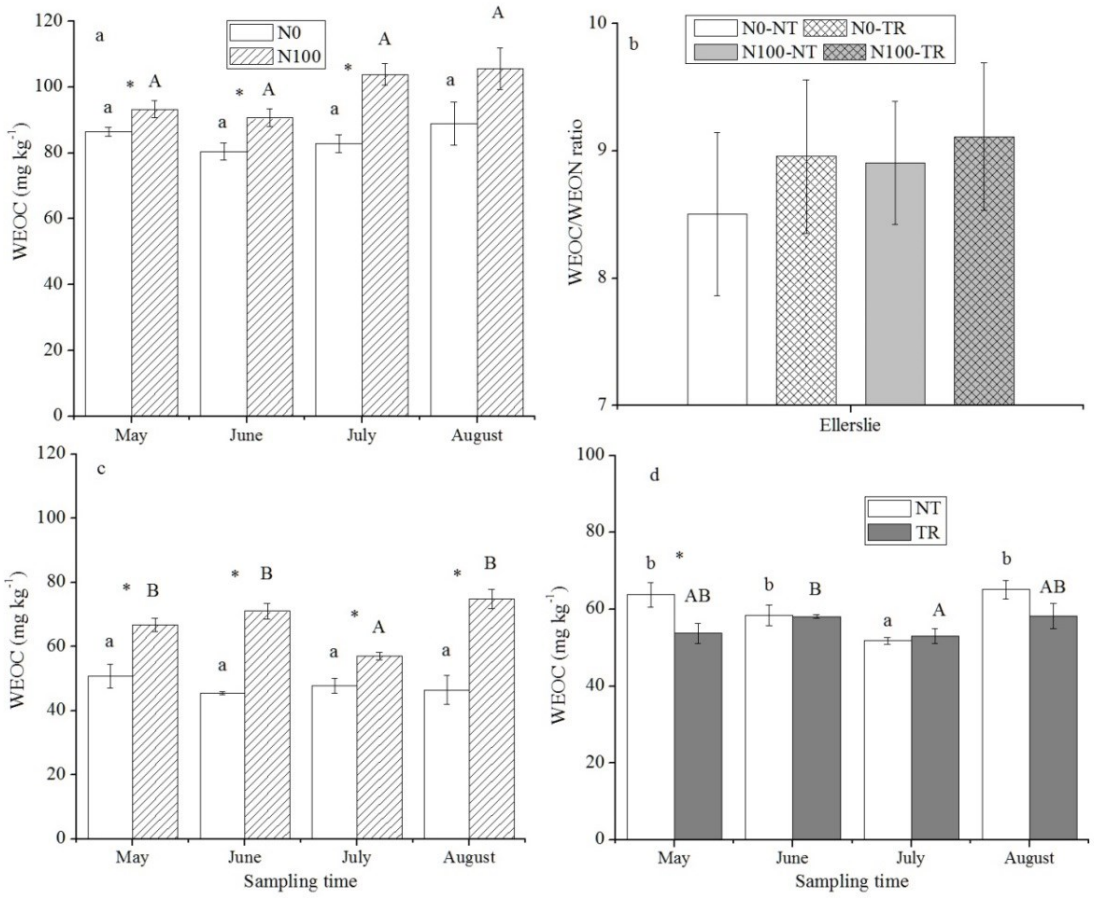

Fig. 4-2 Water-extractable organic carbon (WEOC) concentrations in 0-10 cm soil layer from May to August as affected by (a) nitrogen fertilization at Ellerslie, by (c) N fertilization and (d) tillage reversal effects at Breton, and (b) WEOC/WEON ratio as affected by N fertilization and tillage reversal interaction effects at Ellerslie. N0, no N fertilization; N100, 100 kg N ha^{-1} yr^{-1} applied; NT, no tillage; TR, tillage reversal. Error bars are standard errors of the mean (n=8). Different lowercase and uppercase letters indicate significant differences between sampling time in N0 and N100 (or in NT and TR), respectively; * indicates a significant difference between treatments on each sampling time. All results were considered significant if p values were ≤ 0.05.

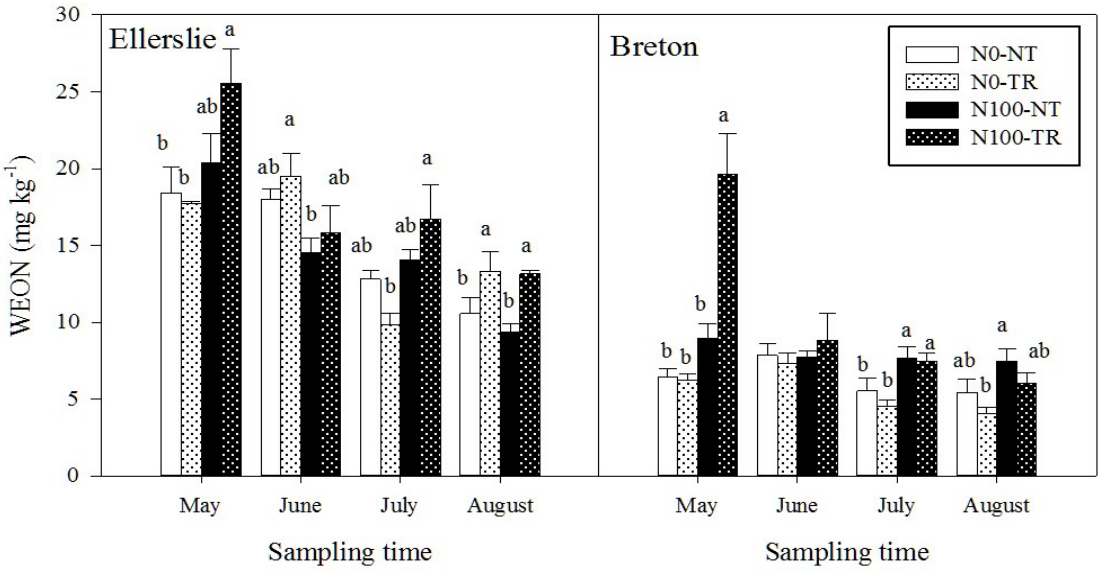

Fig. 4-3 The dynamics of water-extractable organic nitrogen (WEON) concentrations in 0-10 cm soil layer from May to August as affected by nitrogen fertilization and tillage reversal at Ellerslie and Breton; N0, no N fertilization; N100, 100 kg N ha^{-1} yr^{-1} applied; NT, no tillage; TR, tillage reversal. Error bars are standard errors of the mean (n=4). Different letters indicate significant differences between treatments on each sampling time at the 0.05 probability level.

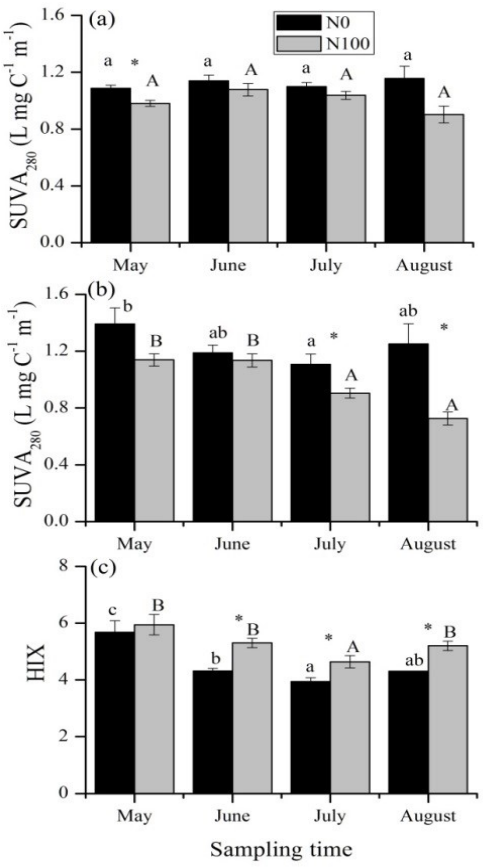

Fig. 4-4 Spectroscopic characteristics of water-extractable organic carbon (WEOC) in 0-10 cm soil layer from May to August as affected by nitrogen fertilization (N) at (a) Ellerslie and (b and c) Breton; N0, no N fertilization; N100, 100 kg N ha^{-1} yr^{-1} applied. Error bars are standard errors of the mean (n=8). Different lowercase and uppercase letters indicate significant differences between sampling times in N0 and N100, respectively; * indicates a significant difference between treatments on each sampling time. All results were considered significant if p values were ≤ 0.05.

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Chapter 5 Nitrogen fertilization and tillage reversal effects on soil aggregation and carbon pools in top- and subsoils in a Black Chernozem and a Gray Luvisol

1 Introduction

The dynamics of carbon (C) in the subsoil has received increasing attention in recent years. Despite the low C concentration, subsoil is a significant reservoir of C and understanding the dynamics of this C is critical (Batjes, 1996). However, studies on soil C stabilization have focused mainly on topsoil (A horizon) (Harrison et al., 2011; Rumpel and Kögel-Knabner, 2011; Syswerda et al., 2010). More research is needed to evaluate the stabilization of C in the subsoil in order to fully understand global C dynamics (Salomé et al., 2010).

Soil C stabilization can be achieved through physical protection within soil aggregates (Six et al., 2000), chemical interaction with soil particles (Christensen, 1996) and biochemical recalcitrance (van Veen and Paul, 1981). Carbon dynamics in top- and subsoils may be controlled by different mechanisms. Fierer et al. (2003) found that C in the subsoil is more susceptible to nutrient input than that in the topsoil. Salomé et al. (2010) reported that disrupting the structure of the subsoil causes a 75% increase in mineralization while the topsoil samples remain unaffected. In agricultural ecosystems, land management practices (e.g., tillage, nitrogen (N) fertilization, liming and straw retention) can alter soil properties such as soil structure and pH. Thus, the response of C dynamics in top- and subsoils may be different under different land management practices in agricultural systems.

Plant residues, root exudates and dissolved organic carbon (DOC, produced by pathways other than root exudates) leaching from upper soil horizons are considered to be the main sources of subsoil C input (Rumpel and Kögel-Knabner, 2011). Land management that favors the production of these C sources may influence the dynamics of C in top- and subsoils (Lorenz and Lal, 2005). It is commonly accepted that changing from conventional tillage (CT) to no tillage (NT) is beneficial for soil organic matter (SOM) accumulation in the topsoil (Bruce et al., 1999; Kahlon et al., 2013; West and Post, 2002). However, long-term NT can cause problems such as accumulation of crop residues, weed infestation, nutrient stratification (Baan et al., 2009; Grant and Bailey, 1994), and pesticide accumulation (Baker and Saxton, 2007). Long-term NT may also increase soil bulk density and, correspondingly, soil strength (Martino and Shaykewich, 1994). Reversing NT to CT, a process called tillage reversal, may be a way to deal with those problems and the possibility exists that landowners may revert to short-term CT after long-term NT (Sun et al., 2015). Tillage reversal can enhance the decomposition of SOM by disrupting soil aggregates, and exposing labile or fresh OM that is occluded within aggregates, making it susceptible to microbial decomposition (De Gryze et al., 2004; Grandy and Robertson, 2007; Six et al., 1999). Thus, there might be a high risk of soil C loss when adopting tillage reversal in a long-term NT system. Since only a few studies have been focused on tillage reversal practices (Shahidi et al., 2014; Sun et al., 2015), the influence of tillage reversal on C dynamics, especially in the subsoil, is poorly understood. Also, nitrogen fertilization generally increases crop yields, and is expected to increased soil organic carbon (SOC) and aggregation stability (Blanco-Canqui et al., 2014). Our previous study (Sun et al., 2015) showed that N fertilization increases WEOC

concentrations in the topsoil; however, the previous study did not investigate the effects of N fertilization on C dynamics in the subsoil.

The objective of this study was to assess the effects of N fertilization and tillage reversal on growing season crop residue inputs (both aboveground and belowground), soil aggregation, and the distribution of C in aggregate fractions in top- and subsoils. We studied the dynamics of aggregate-associated C in top- and subsoils under N fertilization and tillage reversal on two different soil types in a similar ecological region. We hypothesized that 1) N fertilization will improve soil aggregation and C sequestration in both top- and subsoils, and 2) tillage reversal will offset the benefit of N fertilization on soil aggregation and C sequestration.

2. Materials and methods

2.1 Soils and experimental design

Soil samples were collected from long-term research sites studying the effects of zero tillage, straw retention and N fertilization (Tillage-Straw-Nitrogen Plots) established on an Orthic Black Chernozem (Typic Cryoboroll) of the Malmo silty clay loam series near Ellerslie (53°25′N, 113°33′W; elevation 692 m), and on an Orthic Gray Luvisol (Typic Cryoboralf) of the Breton loam series near Breton (53°07′N, 114°28′W; elevation 830 m). Both sites are located in central Alberta, Canada. The Black Chernozem had a greater soil fertility and better soil structure than the Gray Luvisol (Singh and Malhi, 2006). These two sites are ~70 km apart but represent two major and distinctly different soils

found in central Alberta (Table 5-1). The mean air temperature from May to August in 2013 was 13.9 °C at Ellerslie and 13.6 °C at Breton. The mean annual precipitation in 2013 was 452 mm at Ellerslie and 555 mm at Breton.

Long-term experimental plots were established at each site in 1979 with a randomized completely block design, which included 10 treatments with various combinations of tillage, N fertilization and straw retention (Nyborg et al., 1995; chapter 2). In this study, we used a split-plot design with the whole plots (N fertilization, N0 vs. N100 kg N ha^{-1} yr^{-1}) completely randomized in each of four blocks, with tillage - NT vs. tillage reversal (TR) - arranged in subplots. Plots for two of the treatments - T4 (No Till-straw-No N) and T6 (No Till-Straw-100N) - were split into 2 equal subplots (6.85 × 1.37 m; chapter 2). One of the subplots was subjected to TR on June 3, 2009 for the Black Chernozem and on June 4, 2010 for the Gray Luvisol (Shahidi et al., 2014). The other subplot was maintained as NT, except for the disturbance caused by the plot seeder used each spring to seed the plots. The TR subplots were tilled each spring prior to seeding using a rotary tiller to a depth of approximately 8-10 cm. In both NT and TR subplots with N fertilization, urea (100 kg N ha^{-1} yr^{-1}) was mid-row banded (46 cm apart) among seed rows placed 23 cm apart. Phosphorus (20 kg P $ha^{-1}yr^{-1}$) was applied with seed to each subplot in each year. The crop grown at both sites was spring barley (*Hordeum vulgare* L.) throughout the experiment, and after harvesting, straw was retained on each subplot.

2.2 Soil sampling, aggregation fractionation and soil analyses

Soil samples were taken from each subplot at two different soil horizons-A horizon (0-10 cm) vs. B horizon (50-60 cm) at both sites in August 2012, corresponding to the 4th and 3rd cropping season after tillage reversal at Ellerslie and Breton, respectively. The Black Chernozem at Ellerslie has a thicker A horizon (around 40 cm) than the Gray Luvisol at Breton (around 30 cm) (Cairns, 1978). The sampling depth of B horizon was decided based on the bottom rooting zone of barley (Dwyer et al., 1988). Representative subsamples of approximately 300 g were gently passed through an 8 mm sieve by breaking the soil along natural planes of weakness. After air-drying, the soils were separated into 4 fractions according to the wet sieving method described by Elliott (1986): (a) large macroaggregates (LM; >2000 μm), (b) small macroaggregates (SM; 250-2000 μm), (c) microaggregates (MI; 53-250 μm), and (d) silt and clay-sized particles (SC; <53 μm). A series of three sieves (2000, 250 and 53 μm) were used. A 100 g sample of air-dried soil was placed on the top of the 2 mm sieve and submerged for 10 min in deionized water at room temperature to allow slaking (Kemper and Rosenau, 1986). The 2 mm sieve was manually moved up and down 3 cm 50 times in 2 min. The material passed through 2 mm sieve was collected and the same procedure was followed to pass the material to 250 μm and then the 53 μm sieve. Floating OM (density<1 g cm^{-3}) was removed from the 2000 μm aggregate size fraction as it was mostly plant debris. Soil aggregates retrieved from each sieve were carefully backwashed into an aluminum pan. The water and soil particles that passed through 53 μm, together with soil aggregate fractions on different sieves, were oven-dried (60 °C), weighed and stored in plastic bags at room temperature for analysis.

Sand-free water-stable aggregates (WSA) were measured using a 5 g subsample of aggregates retained on each sieve (> 53 μm). Organic matter from the subsample was first removed by adding H_2O_2. Aggregates were dispersed by adding 50 mL sodium hexametaphosphate (5 g L^{-1}), and shaking for 4 hours after resting overnight. Sand was collected on a 53 μm sieve, washed with deionized water, and dried at 105 °C for 24 h.

The weight of aggregate size fractions and sand particles of the same size aggregate fraction were recorded for estimating the sand-free correction as:

$$\text{Sand-free aggregate (g 100 g}^{-1}\text{ soil)} = \frac{(\text{total fraction weight} - \text{same-sized sand weight in fraction})}{\sum \text{sand corrected aggregate weight}}$$

where total fraction weight is the weight of each aggregate fraction (LM, SM, MI and SC) obtained by wet sieving; same-sized sand weight in fraction was the weight of sand in the corresponding aggregate fraction; and ∑sand corrected aggregate weight is the sum of the weight of each sand-free aggregate fraction (Plaza-Bonilla et al., 2013; Six et al., 2002).

Mean weight diameter (MWD) of aggregates was calculated as:

$$MWD = (M_{LM} \times 5\text{ mm}) + (M_{SM} \times 1.125\text{ mm}) + (M_{MI} \times 0.151\text{ mm}) + (M_{SC} \times 0.0265\text{ mm})$$

where M is the proportion of the soil sample weight in the aggregate class with the size given in the subscript. The assumption is that soil aggregates have a unique distribution

in the same size class. Here, 5 mm, 1.125 mm, 0.151mm and 0.0265 mm are the mean diameter of LM (2-8 mm), SM (2-0.25 mm), MI (0.25-0.053 mm) and SC (0.053-0 mm), respectively (Zotarelli et al., 2005).

Soil total organic carbon (TOC) and total N (TN) were analyzed in whole soil and aggregate fractions with a Carlo Erba NA 1500 elemental analyzer (Carlo Erba Instruments, Milan, Italy). Soil pH was measured in a 1/2 (w/v) mix of soil to 0.01 M $CaCl_2$ solution (Kalra and Maynard, 1991) with an Orion 3-Star portable pH meter (Thermo Fisher Scientific Inc., Waltham, MA).

2.3 Determination of crop residue inputs

The amount of annual crop residue inputs after tillage reversal (from 2009 to 2011) were estimated as aboveground residue (AR) plus belowground residue (BR) returned to the soil. Aboveground residue was determined from straw yield of barley. Belowground residue was estimated from grain dry weight (GDW) and AR, using the formula: BR=a (GDW+AR) (Malhi et al., 2011) where 'a' is 0.22 for barley (IPCC, 2006).

2.4 Statistical analysis

Data from the two sites were analyzed separately since the main purpose was to evaluate the differences in SOC pools due to different management regimes within soil type and each soil type was not replicated. Analysis of variance was conducted using the PROC MIXED procedure in SAS v8.01 (SAS Institute Inc., 2003) to test for treatment

effects. All data conformed to a normal distribution and equal variance and so no transformation was performed. For analyzing bulk soil C and N concentrations and MWD, we used split-plot analysis with N fertilization and tillage reversal treated as the fixed effects and block as the random effect. When comparing C or N concentrations of different aggregates size classes, N fertilization (N0 and N100) was considered as the main plot effect, tillage reversal as the split plot effect and different size classes were considered as further split plots effect. All results were considered significant if p values were less than 0.05.

3. Results

3.1 Crop residue inputs and bulk soil properties

All crop residues were returned to the plots; the average crop residue inputs between 2009 and 2011 were 3.0 Mg ha^{-1} at Ellerslie and 2.0 Mg ha^{-1} at Breton (Fig. 5-1). There was no interaction effect between N fertilization and tillage reversal on the amount of crop residue inputs at either site. Crop residue inputs were higher under N100 than under N0 at both sites (Fig. 5-1b; $p<0.01$ and $p=0.02$ for Ellerslie and Breton, respectively). Crop residue inputs were not different between tillage treatments at either site (Fig. 5-1a).

Subsoil TOC and TN concentrations were not influenced by N fertilization or tillage reversal or their interaction at either site (Table 5-2). Soil pH was not influenced by N fertilization × tillage reversal effect at either site or depth. Soil pH was significantly lower in N100 than in N0 ($p<0.01$ for both sites) but was not influenced by tillage

reversal in the topsoil at either site (Table 5-2). Subsoil pH was not influenced by N fertilization, tillage reversal or their interactions at either site (Table 5-2).

3.2 Aggregate-size distribution

Soil MWD was not influenced by N fertilization × tillage reversal effects at either site or depth. Soil MWD in the topsoil was significantly higher in TR than in NT and in N100 than in N0 at both sites (Table 5-2). Soil MWD was significantly lower in TR than in NT in the subsoil only at Breton (Table 5-2).

In the topsoil, there were significant N fertilization × size and tillage × size effects on the size distribution of sand-free WSA at both sites (Table 5-3). The proportion of LM was significantly higher while the proportions of MI and SC were significantly lower in N100 than in N0 in the topsoil at both sites (Fig. 5-2a and 5-2c). The proportion of SM in the topsoil increased by N fertilization at both sites but the increase was only significant at Breton. A significantly higher proportion of LM was observed in TR than in NT in the topsoil at both sites (Fig. 5-2b and 5-2d). The proportion of SM decreased under tillage reversal at both sites but the decrease was only significant at Ellerslie. The proportions of MI and SC were not different between tillage treatments in the topsoil at either site.

In the subsoil, there were significant N fertilization × size and tillage × size effects on the size distribution of sand-free WSA only at Breton (Table 5-3). The proportion of MI was greater in N100 than in N0 (p=0.02; Fig. 5-3a). Tillage reversal had no effect on sand-free WSA in the subsoil at Ellerslie (Fig. 5-3b).

3.3 Aggregate-associated carbon

At Ellerslie, there was a significant N fertilization × size effect on aggregate-associated C in the topsoil (Table 5-3). The MI-associated C concentration was significantly higher in N100 than in N0 while C associated with the other three aggregate-size fractions was similar between N treatments in the topsoil (Fig. 5-4a). At Breton, organic C concentrations in all aggregate size fractions were higher in N100 than in N0 (Fig. 5-4c). Tillage reversal had no effect on aggregate-associated C in the topsoil at either site (Table 5-3 and Fig. 5-4d).

In the subsoil, there was a significant nitrogen × tillage × size effect on aggregate-associated C concentrations at Ellerslie (Table 5-3). Organic C concentration in the LM fraction was significantly higher in N0 than in N100 (Fig. 5-4b). The MI-associated C was lower in TR than in NT in the N0 subplot ($p<0.001$), and in N100 than in N0 in the NT subplot. Nitrogen fertilization and tillage reversal had no effect on aggregate-associated C in the subsoil at Breton (Table 5-3).

4. Discussion

4.1 Soil aggregation

Nitrogen fertilization improved topsoil structure by significantly increasing MWD of soil aggregates and the proportion of LM. The amount of plant residue input is a vital factor in the formation and stabilization of aggregates (Haynes and Beare, 1996).

Previous studies suggested that incorporated fresh crop residue first helps to form macroaggregates through binding microaggregates (Golchin et al., 1994; Olchin et al., 2008). The significantly increased crop residue inputs by N fertilization likely provided more OM for binding MI and SC into LM. This is corroborated by the greater MWD in N100 than in N0 at both sites. Tillage was commonly found to decrease soil aggregation by breaking down LM (Mikha and Rice, 2004; Zotarelli et al., 2005). However, some other researchers found that MWD is smaller in NT than in other tillage practices (Hamblin, 1980; Unger, 1997), which is consistent with our finding. In this study, tillage reversal improved soil aggregation in the topsoil by increasing LM proportion and MWD at both sites. Meanwhile, we found that tillage reversal likely increased macroaggregates (> 250 μm) rather than microaggregates (<250 μm), similar to that observed by Tisdall and Oades (1982). Straw was retained onsite and was subsequently incorporated into soil by tillage reversal. The incorporation of crop residues might improve soil aggregation. In addition, root growth and root exudates might be altered by tillage reversal and N fertilization (Anderson, 1987), which may have influence on soil aggregation.

The influence of N fertilization on soil aggregation was also found in the subsoil at Breton. Nitrogen fertilization improved soil structure by increasing the proportion of MI in the subsoil. Freshly incorporated residues play a major role in forming and stabilizing macroaggregates (Sollins et al., 1996). Fierer et al. (2003) reported that nutrient input can influence subsoil C dynamics. Nitrogen fertilization can increase NO_3-N content in the topsoil (Aulakh et al., 1982; Lemke et al., 1999), which may subsequently be leached to the subsoil and accelerate the decomposition of fragmented substrate within LM, causing the release of MI under N fertilization.

However, N fertilization influenced the aggregate dynamics in the subsoil at Breton but not at Ellerslie. The microbial activities and community were different between Ellerslie and Breton, with the latter favoring a more rapid C turnover (Rutherford and Juma, 1989). The subsoil texture is coarser at Breton (clay loam) than at Ellerslie (clay). Ladd et al. (1985) reported that a fine texture could reduce the decomposition of plant residue. The normal annual precipitation was greater at Breton than at Ellerslie (555.9 and 452.8 mm for Breton and Ellerslie, respectively), which may increase nutrient leaching from the topsoil to the subsoil, resulting in a larger treatment influence at Breton than at Ellerslie.

4.2 Soil C sequestration

In the topsoil, N fertilization improved long-term physical C protection at both sites. However, in the subsoil, N fertilization only improved long-term physical C protection at Breton. The significant nitrogen × tillage × size effects on aggregate-associated C in the subsoil at Ellerslie might indicate that the response of aggregate-associated C to N fertilization depended on tillage treatments and aggregate sizes.

The amount of C sequestrated in soil is controlled by the balance between C inputs (e.g., crop residues and root exudates) and C outputs (e.g., decomposition and erosion) (Blanco-Canqui and Lal, 2010). A previous study (Sun et al., 2015) found that soil TOC and TN concentrations in the topsoil are increased by N fertilization and/or tillage reversal at Breton. This is corroborated by the higher crop residue inputs in N100 than in N0 found in this study. However, at Ellerslie, the increased crop residue inputs under

N100 did not result in an increased soil TOC in the topsoil. This is consistent with the results reported by Malhi et al. (2011). The reason for this observation may be attributed to the different initial SOM in those two soils (Havlin et al., 1990; Nyborg et al., 1995). The Black Chernozem at Ellerslie had higher initial SOM than that in the Gray Luvisol at Breton. The Black Chernozem at Ellerslie is developed in the Aspen Parkland ecoregion of the Prairies (transitional fescue grassland interspersed by trembling aspen groves) while the Gray Luvisol at Breton is in the Boreal Transition ecoregion of the Boreal Plain, which is predominantly aspen (Plante et al., 2010). The higher amount of root biomass of grasses than trees contributes to the higher OM content in the surface layer of the Black Chernozem than the Gray Luvisol. In addition, most of the C in the Gray Luvisol is in leaf litter, which was not mixed into surface soil layer and was thus lost when the land was cleared for agriculture (Dyck et al., 2012). The SOC levels at Ellerslie were not affected by long-term (~30 years) N fertilization, which might indicate that this Chernozemic soil has probably reached C saturation level at high crop residue inputs (Gulde et al., 2008).

In general, trapping C in aggregates can slow mineralization and result in net C gain over time (Mikha and Rice, 2004; Zibilske and Bradford, 2007). Aggregation reduces the accessibility of OM to microbes and fauna, rates of diffusion of reactants, and products of extracellular synthesis reactions (Sollins et al., 1996). The greater TOC under N100 than under N0 in the topsoil at Breton (Sun et al., 2015) can be partly explained by the increased aggregate-associated C concentrations. However, at Ellerslie, although MI-associated C concentration was increased under N100, soil TOC was not changed by N fertilization in the topsoil (Sun et al., 2015). This may indicate that although the total

amount of SOC was not increased by N fertilization, N fertilization improved long-term physically protected C in the topsoil at Ellerslie. In the subsoil, long-term N fertilization did not influence soil TOC and TN concentrations at Ellerslie but the decreased LM-associated C concentration under N fertilization indicated that applying N fertilizer decreased C stability. Tillage management practices have been reported to decrease LM-associated C in the topsoil by many studies (Mikha and Rice, 2004; Zotarelli et al., 2005). However, the lack of differences in aggregate-associated C concentrations between tillage treatments in this study may indicate that reversing long-term NT to CT in a short-term basis had little influence on physical protection for C in the topsoil at those two sites (Paul et al., 2013).

5. Conclusions

Agricultural management practices influence soil C and N concentrations, soil aggregation, and the distribution of C in aggregate fractions in the soils studied. The influence of N fertilization and tillage reversal was more pronounced in the topsoil than in the subsoil. In soils with straw retained, N fertilization and tillage reversal favored a better topsoil structure by forming macroaggregates. In the subsoil, only N fertilization improved soil structure by increasing MI proportion at Breton with relatively low initial SOM. As aggregate formation is beneficial for physical protection of C, and C in the microaggregates is more stable than that in the macroaggregates, short-term tillage reversal did not influence the improved long-term physical C protection by N fertilization in the topsoil studied. In the subsoil, N fertilization and tillage reversal decreased

physical protection of C only at Ellerslie. The Gray Luvisolic soil at Breton had a greater potential for physical C sequestration in the topsoil under N fertilization in comparison with the Black Chernozemic soil at Ellerslie. However, N fertilization and/or tillage reversal had a risk of decreasing physical protection of C in the subsoil of Black Chernozemic soil at Ellerslie. This is important in the context of making management decisions for long-term mitigation of atmospheric CO_2 on different soil types.

Table 5-1 Total soil organic carbon (TOC; g C kg^{-1}), total nitrogen (TN; g N kg^{-1}), bulk soil C to N ratio (C/N), clay content (%) and soil pH of the top- (0-10 cm) and subsoils (50-60 cm) in a Black Chernozem at Ellerslie and a Gray Luvisol at Breton.

Soil properties	Ellerslie		Breton	
	0-10 cm	50-60 cm	0-10 cm	50-60 cm
TOC	55.5±0.8	6.5±0.3	15.1±1.7	4.1±0.1
TN	4.9±0.1	0.8±0.02	1.6±0.2	0.4±0.02
C/N	11.4±0.2	8.1±0.6	9.2±0.4	11.7±0.6
Clay content	38.4±2.0	48.5±0.5	24.2±0.8	34.0±0.1
Soil pH	4.90±0.10	5.73±0.05	5.12±0.23	6.04±0.04

Data were means of all treatments (n=16).

Data are mean ± SE.

Table 5-2 Soil pH, mean weight diameter (MWD; mm) in top- (0-10 cm) and subsoils (50-60 cm), and total organic carbon (TOC; g C kg^{-1}) and total nitrogen (TN; g N kg^{-1}) in the subsoil (50-60 cm) as affected by nitrogen (N) fertilization and tillage reversal (T) at Ellerslie and Breton.

Site	Treatment	Soil pH		MWD		TOC	TN
		0-10 cm	50-60 cm	0-10 cm	50-60 cm	50-60 cm	50-60 cm
Ellerslie	N0	5.08	5.73	1.42	1.18	6.30	0.78
	N100	4.73	5.73	1.73	1.21	6.65	0.82
	p value	0.003	0.964	0.046	0.429	0.189	0.292
	NT	4.94	5.66	1.33	1.23	6.09	0.82
	TR	4.86	5.76	1.82	1.19	6.41	0.79
	p value	0.080	0.498	0.010	0.286	0.362	0.311
Breton	N0	5.52	6.00	1.11	0.98	4.01	0.38
	N100	4.66	6.08	1.32	0.91	4.24	0.40
	p value	0.006	0.254	0.046	0.627	0.175	0.188
	NT	5.14	6.01	1.04	1.03	4.10	0.41
	TR	5.04	6.07	1.39	0.86	4.16	0.39
	p value	0.873	0.360	0.005	0.013	0.101	0.163

The N fertilization × tillage reversal effects on soil pH, MWD, TOC and TN were not significant at either site or depth.

N0, no N fertilization; N100, 100 kg N ha^{-1} yr^{-1} applied; NT, no tillage; TR, tillage reversal. *p*, probability.

Table 5-3 ANOVA *p* values showing significant differences in the proportion of sand-free water-stable aggregates (WSA) and aggregate-associated carbon (C) concentrations in two soil layers (0-10 cm and 50-60 cm) as affected by nitrogen (N) fertilization, tillage reversal (T), size class (S) and their interactions in a Black Chernozem at Ellerslie and a Gray Luvisol at Breton.

Variables	Source of variation	Sand-free WSA		Aggregate-associated C concentrations	
		0-10 cm	50-60 cm	0-10 cm	50-60 cm
Ellerslie	N	n.s.	n.s.	n.s.	n.s.
	T	n.s.	n.s.	n.s.	n.s.
	N × T	n.s.	n.s.	n.s.	n.s.
	S	***	***	***	**
	N × S	***	n.s.	**	n.s.
	T × S	***	n.s.	n.s.	n.s.
	N × T × S	n.s.	n.s.	n.s.	**
Breton	N	n.s.	n.s.	**	n.s.
	T	n.s.	n.s.	n.s.	n.s.
	N × T	n.s.	n.s.	n.s.	n.s.
	S	***	***	***	***
	N × S	***	*	n.s.	n.s.
	T × S	***	*	*	n.s.
	N × T × S	n.s.	n.s.	n.s.	n.s.

*, **and *** indicate significance at the 0.05, 0.01 and 0.001 probability levels, respectively.

n.s., not significant (p>0.05)

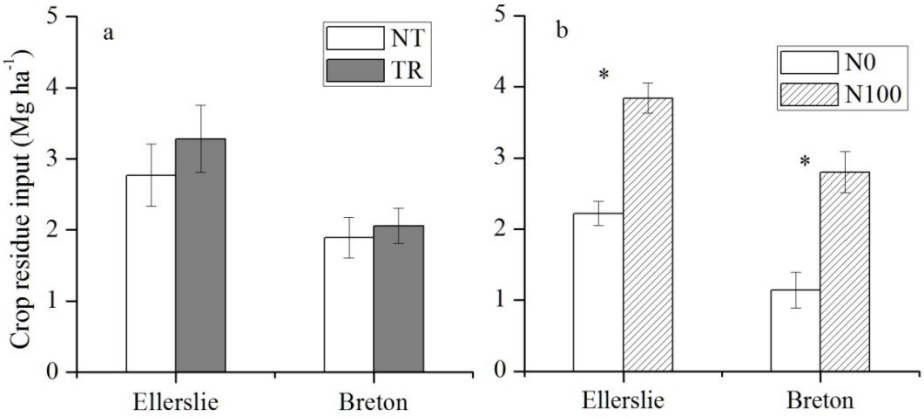

Fig. 5-1 Mean crop residue input (aboveground and belowground) as affected by (a) tillage reversal and (b) nitrogen fertilization at Ellerslie (2009-2011) and Breton (2010-2011). N0, no N fertilization; N100, 100 kg N ha^{-1} yr^{-1} applied; NT, no tillage; TR, tillage reversal. * indicates a significant difference between two bars at each site at the 0.05 probability level. The error bars indicate standard errors of the mean (n=24 and 16 for Ellerslie and Breton, respectively). The N fertilization × tillage reversal effects were not significant at either site.

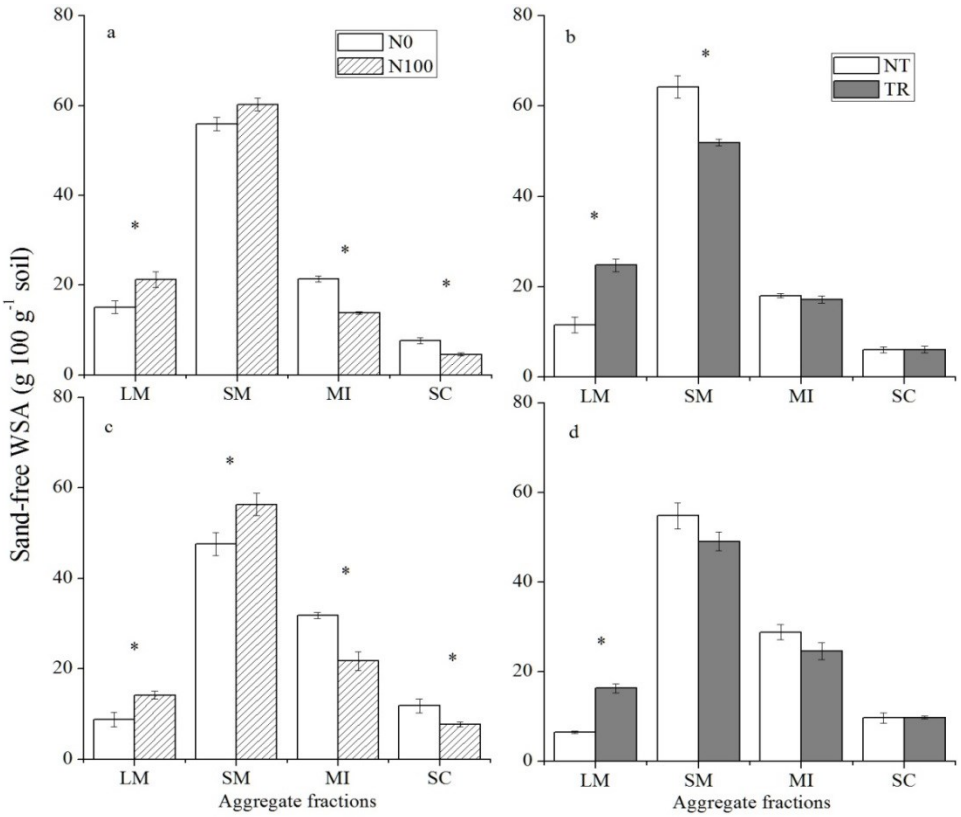

Fig. 5-2 Sand-free water-stable aggregates (WSA) in the topsoil (0-10 cm) as affected by nitrogen fertilization at (a) Ellerslie and (c) Breton, and by tillage reversal at (b) Ellerslie and (d) Breton. N0, no N fertilization; N100, 100 kg N ha^{-1} yr^{-1} applied; NT, no tillage; TR, tillage reversal. LM, large macroaggregates; SM, small macroaggregates; MI, microaggregates; SC, silt and clay; * indicates a significant difference between two bars in the same size fraction at the 0.05 probability level. The error bars indicate standard errors of the mean (n=8).

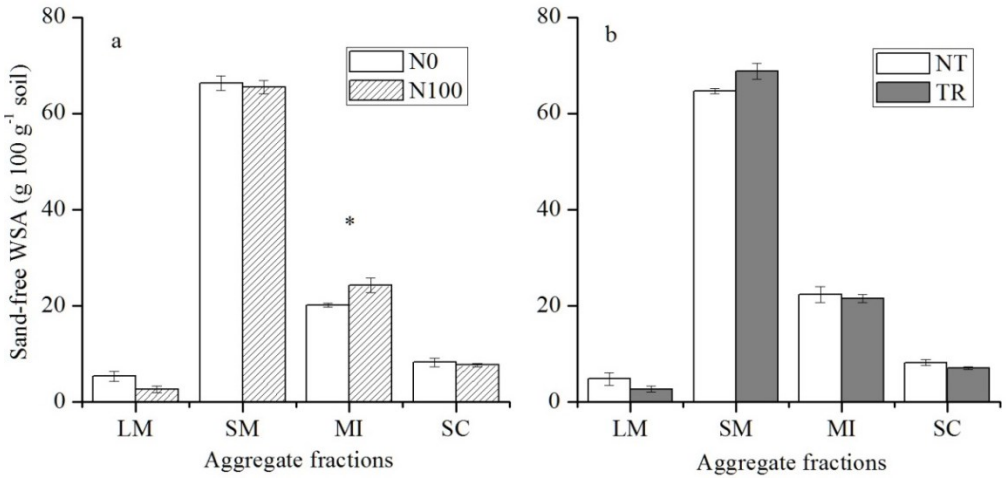

Fig. 5-3 Sand-free water-stable aggregates (WSA) as affected by (a) N fertilization and (b) tillage reversal in the subsoil (50-60 cm) at Breton. N0, no N fertilization; N100, 100 kg N ha^{-1} yr^{-1} applied; NT, no tillage; TR, tillage reversal; LM, large macroaggregates; SM, small macroaggregates; MI, microaggregates; SC, silt and clay. * indicates a significant difference between two bars in the same size fraction at the 0.05 probability level. The error bars indicate standard errors of the mean (n=8).

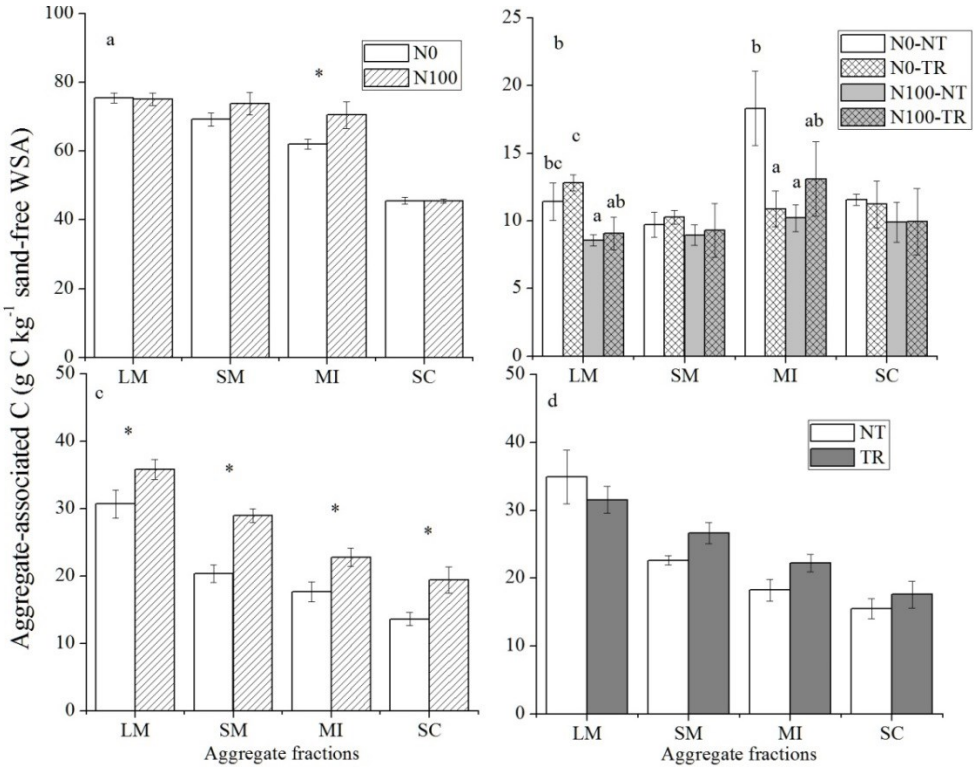

Fig. 5-4 Aggregate-associated C as affected by N fertilization at (a) Ellerslie and (c) Breton, by tillage reversal at (d) Breton in the topsoil (0-10 cm), and by N fertilization × tillage reversal effect at (b) Ellerslie in the subsoil (50-60 cm). N0, no N fertilization; N100, 100 kg N ha^{-1} yr^{-1} applied; NT, no tillage; TR, tillage reversal; LM, large macroaggregates; SM, small macroaggregates; MI, microaggregates; SC, silt and clay. The error bars indicate standard errors of the mean (n=8). * indicates a significant difference between two bars in the same size fraction at the 0.05 probability level. Different lowercase letters indicate significant differences between four bars in the same aggregate fraction.

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Chapter 6 Conclusions and Future Research

1 Concluding remarks and management implications

This doctoral dissertation covers a range of interrelated subjects that together improve our understanding of soil carbon (C) dynamics under tillage reversal (reversing long-term no tillage to conventional tillage) and long-term nitrogen (N) fertilization with straw retention in agricultural ecosystems. In particular, soil greenhouse gas (GHG) emissions, soil C and N concentrations, and C stability are addressed with the overall aim of improving our ability to mitigate atmospheric GHGs under different agricultural management practices. In addition to assessing effects of tillage reversal and N fertilization on GHG emissions (CO_2, N_2O and CH_4), this study also aims to enhance our understanding of area- and yield-scaled GHG emissions (Chapter 2). Focusing on the yield-scaled GHG emissions under N fertilization and tillage reversal is very important since if a certain tonnage of food is needed to feed the world's population, management practices should be focused on producing crops with the lowest GHG emissions per unit yield (van Kessel et al., 2013).

My dissertation complements existing knowledge about the effects of short-term tillage reversal on CO_2 and N_2O emissions from agricultural soils (Shahidi et al., 2014), and improves our understanding of the impacts of tillage reversal and N fertilization on C and N concentrations and soil structure in agricultural soils. In addition, new knowledge gains in my research on different responses of aggregate-associated C to long-term N fertilization and short-term tillage reversal between top- and subsoils and should inspire

further thinking about differences in C dynamics in top- and subsoils as mechanisms controlling C turnover in top- and subsoils might be different (Fierer et al., 2003; Fontaine et al., 2007).

It is worth mentioning here that inferences drawn throughout my dissertation about the responses of C dynamics to tillage reversal and N fertilization are most pertinent to the specific time frame and soil types considered. The experiments were conducted during the growing season after ~30 years of N fertilization and 3-4 years of tillage reversal in a Black Chernozem and a Gray Luvisol. Thus, recommendations to agricultural producers are mainly relevant to this relative short-term research on tillage reversal in the two soils studied. Nonetheless, with the extensive history of NT, its drawbacks such as accumulation of crop residues, weed infestation, nutrient stratification (Baan et al., 2009; Grant and Bailey, 1994) and pesticide accumulation (Baker and Saxton, 2007) are being more pronounced. I strongly believe that my results can guide improvement of management practices to sequester more C in agricultural soils. Furthermore, because my suggestions are offered in the context of the long-term No Tillage-Straw-Nitrogen plots, this research provides a valuable baseline for further research designed to extend these results.

It is well-known that tillage and/or N fertilization stimulate GHG emissions in agricultural soils (Halvorson et al., 2008; Morell et al., 2011; Sainju et al., 2012; Shahidi et al., 2014). Results from Chapter 2 confirm this observation for area-scaled GHG emissions and extend its significance in at least two ways as elaborated below. First, my results provide the first dataset on yield-scaled GHG emissions in relation to tillage reversal and N fertilization practices in Western Canada. The responses of area-scaled

GHG emissions to N fertilization and tillage reversal were different from that of yield-scaled GHG emissions in each soil. Although area-scaled GHG emissions were stimulated by tillage reversal in the Black Chernozem and by N fertilization in the Gray Luvisol, yield-scaled GHG emission was decreased by tillage reversal in the Black Chernozem and by N fertilization in the Gray Luvisol. Therefore, management decisions will have to consider whether the objective is to reduce the total GHG emission rate on an area basis or to minimize GHG emissions per crop yield (Chapter 2).

Second, I partitioned soil total respiration (R_s) into autotrophic (R_a) and heterotrophic (R_h) respirations (Chapter 3). My results showed that the responses of R_h and R_a to N fertilization and tillage reversal were different. The responses of R_h to N fertilization and tillage reversal at each site were consistent with that of R_s. The responses of R_a were less sensitive than R_h to N fertilization and tillage reversal. The contribution of R_h to R_s was around 50% in the growing season. Our results provide further insight of the pathways of CO_2 production under tillage reversal and/or N fertilization.

Another important aspect in this dissertation is improved understanding of C stabilization under different N and tillage management practices. This is not only related to the intermediate and passive C pools, but also the stable C fraction in the labile C pool. Water-extractable organic carbon (WEOC) is considered to be one of the most labile and biodegradable fractions of soil organic matter (Chantigny, 2003). However, only 10-44% of WEOC in soil solutions is microbial degradable (Qualls and Hainers, 1992). I found that N fertilization increased the concentrations of WEOC in both soils. However, the stability of WEOC was increased by N fertilization only in the Gray Luvisol through increasing non-aromatic compounds. Tillage reversal had no influence on the quality of

WEOC in both soils (Chapter 4). Thus, I conclude that N fertilization was the main factor controlling the quantity and quality of WEOC in both soils.

Studies on soil C stabilization under different land management practices have focused mainly on the topsoil (A horizon) (Harrison et al., 2011; Rumpel and Kögel-Knabner, 2011; Syswerda et al., 2010). Our study extended the sampling depth to B horizon and found that management practices affected C dynamics in different soil horizons. Our results showed that these influences were not the same for all components in the soil system, because the responses of C accumulation to tillage reversal and N fertilization differed with soil depth, and among the physically separated fractions (Chapter 5). Interestingly, our results showed that with straw retention, both tillage reversal and N fertilization increased soil C and N concentrations in the topsoil but not in the subsoil in the Gray Luvisol that had low initial soil organic matter. The Gray Luvisol might have a greater potential for C sequestration than the Black Chernozem. Nitrogen fertilization improved long-term physical protection of C in aggregates in the topsoil at both sites. However, N fertilization decreased the physical protection of C in the subsoil of the Black Chernozem but not that of the Gray Luvisol. In conclusion, with straw retention, long-term N fertilization with short-term tillage reversal is recommended to increase soil C sequestration, improve soil aggregation and decrease yield-scaled GHG emissions in the Gray Luvisol. In the Black Chernozem, short-term tillage reversal is recommended to improve soil aggregation and decrease yield-scaled GHG emissions.

2 Suggestions for future Research

2.1 Soil microbial community

One of the issues relevant to C sequestration that I believe deserves further exploration is related to understanding shifts in soil microbial community under different N and tillage regimes in those two soils. Rutherford and Juma (1989) indicated that microbial activity and community are different between Black Chernozem and Gray Luvisol, with the latter favoring a more rapid C turnover. As suggested in this research, the responses of R_h to N fertilization and tillage reversal were quite different in those two soils. Microbes play an important role in soil C cycling. The degradation of SOC is performed by two major groups of microorganisms: fungi and bacteria (Bailey et al., 2002). The ratio of fungal to bacterial biomass has been used to indicate microbial community structure shift under different management practices (Ågren et al., 2001; Frey et al., 1999). Fungal to bacterial ratio has also been used to determine the most active group of organisms that is responsible for the decomposition of plant residues (Beare et al., 1990). Soil organisms such as fungi and bacteria are important labile C pools in soils. Polymers (e.g., melanin and chitin; components of fungal cell wall) are more resistant to degradation than phospholipids (main component of bacterial membranes) (Bailey et al., 2002). Thus, understanding the relative dynamics of fungal and bacteria populations may help explain C sequestration potential and the fate of added crop residues to soil under different management practices such as N fertilization and tillage reversal employed in this research.

2.2 Annual C balance

As suggested in this research, N fertilization increased crop residue C inputs into the soil while soil C accumulation and CO_2 emissions were not affected by N fertilization in the Black Chernozem. This indicates that soil C appears to be lost by pathways other than CO_2 emissions during the growing season (June - September). The processes of freeze-thaw and short-term flooding following snowmelt occurring in the region of this research have been reported to affect the leaching of SOC (e.g., WEOC) to underground water (Howitt and Pawluk, 1985; Wang and Bettany, 1993). This might be one way for soil C to be lost. In addition, short-term flooding following snowmelt was reported to increase CH_4 emissions from soil, which might be another way for C loss (Wang and Bettany, 1997). Annual C dynamics are still unclear in the Gray Luvisol. Thus, annual soil C dynamics are worthy of study in order to better understand the fate of added crop residue C.

2.3 Study of subsoil C stabilization

The stabilization of C in the subsoil is also worth studying in order to fully understand global C dynamics (Salomé et al., 2010). As suggested in this research, physically protected C was altered by N fertilization and tillage reversal in top- and subsoils at both sites. Evidence showed that C in fine silt and clay particles are more stable than that in sand particles (Eusterhues et al., 2003; Quideau et al., 2001). Thus, chemical interaction with soil mineral particles plays an important role in soil C stabilization. Soil texture is

different between Black Chernozem (silty clay loam and clay for top- and subsoil, respectively) and Gray Luvisol (loam and clay loam for top- and subsoil, respectively) with more silt and clay particles in the Black Chernozem. The responses of chemically stabilized SOC in silt and clay particles to N fertilization and tillage reversal are still unclear in the soils studied.

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