CARBON DIOXIDE AND NITROUS OXIDE PRODUCTION FROM CORN AND SOYBEAN AGROECOSYSTEMS

by

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October, 2006

A thesis submitted to
McGill University
in partial fulfillment of the requirement for
the degree of Doctor of Philosophy

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Your file Votre référence
ISBN: 978-0-494-27838-3
Our file Notre référence
ISBN: 978-0-494-27838-3

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ABSTRACT

Globally, an estimated 25% of the CO_2 and 90% of the N_2O is believed to come from agroecosystems. The objective of this study was to investigate the dynamics of the below-ground CO_2 and N_2O concentrations and efflux in corn and soybean systems. In our field study, changes in the below-ground concentrations of CO_2 and N_2O were closely related to seasonal changes in soil moisture, with the first two months of the growing season being particularly critical to the production of these gases. Tillage significantly increased CO_2 content in the soil profile, however, this effect was greater in the soybean plots than in the corn plots. In our greenhouse studies, an average of about 79% of the soil respiration in corn came from rhizosphere respiration, compared to an estimated 58% in the case of soybean. Specific rhizosphere respiration was significantly higher in soybean (0.29 mg C g^{-1} root h^{-1}) than corn (0.09 mg C g^{-1} root h^{-1}), which supports previous observations made with regards to slower-growing plants (e.g. soybean) having relatively higher root respiration than faster growing plants. We observed a non-significant difference between N_2O efflux in the soybean-planted soil and unplanted bulk soil, which is in contrast to the perception that legumes could stimulate more N_2O production from the soil by increasing the N pool through N_2 fixation. While corn had the greatest uptake of fertilizer N, N_2O efflux in corn pots was higher (2.84 µg N pot^{-1} h^{-1}) than the soybean pots (0.06 µg N pot^{-1} h^{-1}). In the laboratory setting, denitrification in the microaggregates proceeded at about 4.4 to 39.6 times higher rate than in large macroaggregates, small macroaggregates or the bulk soil, and showed the greatest response to high moisture levels (80% WFPS).

RÉSUMÉ

Globalement, on estime 25% du CO_2 et 90% du N_2O proviennent des agro-écosystèmes. L'objectif de cette étude était d'examiner la dynamique souterraine des concentrations et efflux de CO_2 et N_2O dans les systèmes de maïs et soja. Dans notre étude sur le terrain, les changements dans les concentrations souterraines de CO_2 et N_2O étaient reliés de près aux changements saisonniers dans l'humidité du sol, les deux premiers mois de la période de croissance étant particulièrement critiques. Le labourage a augmenté de façon significative la quantité de CO_2 contenue dans les terres cultivées; cependant, cet effet était plus grand dans les terrains de culture de graines de soja que dans ceux de culture de maïs. Dans nos études de serre, une moyenne d'environ 79 % de la respiration du sol pour le maïs provenait de la respiration de la rhizosphère, comparativement à une moyenne de 58 % dans le cas du soja. La respiration caractéristique de la rhizosphère était significativement plus élevée pour le soja (0,29 mg C g^{-1} racine h^{-1}) que pour le maïs (0,09 mg C g^{-1} racine h^{-1}), ce qui soutient des observations effectuées auparavant à l'effet que la respiration de la racine des plantes à croissance lente (par exemple, le soja) est plus élevée que celle des plantes à croissance plus rapide. Nous avons observé une différence non significative entre les émanations de N_2O du sol où a été plantée le soja et d'une masse de sol non planté. Cette observation s'oppose à la perception que les légumineuses pourraient stimuler la production de plus de N_2O du sol en augmentant le niveau de N par la fixation du N_2. Bien que le maïs ait la plus grande absorption d'engrais azotés, les émanations de N_2O dans les pots de maïs était plus grandes (2,84 µg N pot^{-1} h^{-1}) que celles dans les pots de soja (0,06 µg N pot^{-1} h^{-1}). Notre expérience en laboratoire a démontré que la dénitrification des micro-agrégats s'est effectuée à un taux environ 4,4 à 39,6 fois plus élevé que pour les autres fractions du sol; notre étude a également démontré une plus grande réponse à des taux d'humidité élevés (80 % WFPS).

PREFACE

Greenhouse gases such as carbon dioxide (CO_2) and nitrous oxide (N_2O), produced and emitted from agricultural soils can contribute significantly to the atmospheric pools of such greenhouse gases. In order to develop effective strategies to mitigate the production of these gases, there is a need to identify and understand the processes that underline the production of these gases in the different agroecosystems. This present study investigated the processes and dynamics in the production of CO_2 and N_2O from two different agroecosystems; corn and soybean.

This dissertation consists of six chapters preceeded by an Introduction. The first chapter is the literature review and provides the scientific background and context of this study. The next four chapters constitute the body of the thesis and are presented in paper format suitable for submission to scientific journals for publication. Chapter 2 is a field study investigating how management practices act to affect the below-ground content of CO_2 and N_2O. Chapter 3 investigates the dynamics and the processes affecting rhizosphere respiration in corn and soybean. Chapter 4 examines the dynamics in N_2O efflux from the root-associated soil of corn and soybean in a greenhouse setting. Chapter 5 investigates how N_2O efflux varies between various soil aggregate fractions and soil moisture. Chapter 6 gives the general conclusions of this study and suggestions for future research. Connecting paragraphs are inserted between chapter 2 and 3, between chapter 3 and 4 and lastly between chapter 4 and 5.

In accordance with the requirements set by the Faculty of Graduate Studies and Research of the McGill University, the following remark concerning the authorship of papers is excerpted from Guidelines Concerning Thesis Preparation:

“In general, when co-authored papers are included in a thesis the candidate must have made a substantial contribution to all papers included in the thesis. In addition, the candidate is required to make an explicit statement in the thesis as to who contributed to such work and to what extent. This statement should appear in a single section entitled "Contributions of Authors" as a preface to the thesis. The supervisor must attest to the accuracy of this statement at the doctoral oral defence. Since the task of the examiners is made more difficult in these cases, it is in the candidate's interest to clearly specify the responsibilities of all the authors of the co-authored papers.”

The first paper in this thesis (Chapter 2) was co-authored by the candidate, Dr. Joann Whalen (co-supervisor), Dr. Ed. Gregorich (co-supervisor), Dr. Philippe Rochette (Agriculture and Agri-food Canada, Ste-Foy) and Dr. Roger Cue (Department of Animal Science, McGill University). The remaining three papers were co-authored by the candidate, Dr. Joann Whalen, Dr. Ed. Gregorich, and Dr. Philippe Rochette. The candidate was fully responsible for both conducting the original research and for the preparation of the manuscripts. Assistance from the co-authors took the form of general guidance and editorial comments during the preparation of the manuscripts.

ACKNOWLEDGEMENTS

I would like to thank God for the help and support through the many difficult times in the course of this study.

I wish to sincerely thank my co-supervisors Dr. Joann Whalen and Dr. Ed Gregorich for all the wonderful support and guidance they provided me throughout the entire study and during the preparation of the manuscripts. I would also like to express my appreciation to Dr. Philippe Rochette and Normand Betrand of Agriculture and Agri-Food Canada, Ste-Foy for help with analyzing gas samples. I wish to also thank Dr. Roger Cue for his tremendous help with the statistical analyses.

I am grateful also to Ms. Valerie Campeau and Ms. Carine Desjardins for helping me collect gas samples on the field. I would like to thank Mr. Peter Kirby for help with setting up the field plot and Mr. Khosro Mousavi for the assistance he gave me in the greenhouse.

I wish to also express my sincere gratitude to all the students of the Department of Natural Resources, especially the students and workers of the Soil Ecology Lab for making my stay on campus a lively and pleasant one.

I am deeply grateful for the funding I received for this study from the Biological Greenhouse Gas Sources and Sinks (BGSS) program of the Canadian Agri-Food Research Council.

I would like to dedicate this work to my lovely wife, Abena. I am most grateful for the love, prayers, encouragement and support you gave me through it all.

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INTRODUCTION

It is generally accepted that the increasing concentrations of greenhouse gases in the earth's atmosphere have the potential to adversely effect global climatic and weather patterns. According to the Intergovernmental Panel on Climate Change (IPCC, 1995) anthropogenic emissions of CO_2 account for about 63.5% whilst N_2O accounts for about 4.5% of global warming contributions. About 25% of the CO_2 and 90% of the N_2O is believed to come from agroecosystems (Duxbury, 1994).

Emissions of CO_2 and N_2O from agricultural soils are mostly due to the activities of soil microorganisms and micro-fauna. Soil production of CO_2 results from microbial decomposition of organic substrate and to a lesser extent, from plant root respiration, whilst N_2O production is mostly due to anaerobic respiration by certain chemoautotrophic bacteria (Foth and Ellis, 1988). The accuracy of greenhouse gas production and emission estimates at the field scale is complicated by the large temporal and spatial variability that exist in the surface fluxes of these gases (Grant and Pattey, 2003).

Soil microbial activity fluctuates temporally with soil temperature and moisture (Petersen et al., 2002) and is spatially distributed in zones of concentrated microbial activity which we refer to as "hotspots". The rhizosphere constitutes the first of these hotspots. The C-rich environment produced by root rhizodeposition, provides favorable conditions for high decomposer activity, increases mineralization of organic N and subsequently depletes O_2 near the root surfaces, thereby stimulating denitrification (Foth and Ellis, 1988). The second of these hotspots are newly incorporated plant/crop residues because they contain organic C and N that can be readily mineralized by decomposer microorganisms. They are also a source of soluble organic C and NO_3-N that can stimulate denitrifier activity, by creating microsites in the soils where O_2 concentration is depleted through higher decomposer activities (Foth and Ellis, 1988). Leguminous and non-leguminous crops have different compositions of rhizodeposits and crop residues, thereby have different contributions to the production of CO_2 and N_2O from agroecosystems. Soil aggregates constitute the third of these hotspots. This is because soil aggregates are the habitat for soil microflora and fauna and are likely to be hotspots for bacterial activity due to increased aeration and moisture retention in well-aggregated soils, which may directly affect the nutrient and O_2 availability in soils, and ultimately affect decomposer and nitrifier activity (Tomasella and Hodnett, 1996; Weitz et al., 2001).

Agricultural practices such as tillage and fertilizer application affects distribution of these hotspots in diverse ways. Tillage may disrupt soil aggregates mechanically, changing the soil climate (temperature, moisture and aeration), accelerating the decomposition of organic matter and crop residues and effecting changes in the nutrient cycling in the soil ecosystem (Balesdent et al., 2000; Yeates et al. 1997). Fertilizer use can increase crop production, leading to higher inputs of crop residues and greater levels of water stable aggregates than in unfertilized soil (Campbell et al., 2001). In addition, the application of organic fertilizers also increase the levels of soil organic matter as well as water soluble aggregates (Haynes and Naidu, 1998). Although these agricultural management practices significantly affect soil heterogeneity, routine procedures for measuring greenhouse gas production seldom consider how they interact on the farm scale to affect CO_2 and N_2O production.

This thesis will address the following research questions:

1. How do management factors such as tillage type, crop and fertilizer types interact to affect the production of CO_2 and N_2O?
2. How does the production of CO_2 and N_2O differ between the rhizosphere-associated soil of corn and soybean?
3. What are the dynamics in the production of CO_2 and N_2O in soil aggregation and how is it affected by soil temperature and moisture?

CHAPTER 1: LITERATURE REVIEW

1.1. CO_2 IN AGRICULTURAL SOILS

Although cultivation of agricultural soils is not as obvious a source of CO_2 as direct fossil fuel combustion, net cumulative CO_2 emissions from agriculture have been estimated at between 81 and 191 Pg C as compared to 240 and 300 Pg C from fossil fuel combustion between the period of 1850 to 1998 (Houghton, 1997, 1999; Houghton et al., 1999). According to Cihacek and Ulmer (2002), sources of CO_2 in agricultural soils include:

(1) CO_2 dissolved in rainfall as a weak solution of carbonic acid (H_2CO_3).

$H_2O_{(l)} + CO_{2\,(g)} \leftrightarrow H_2CO_{3\,(aq)}$

(2) CO_2 produced in the soil by decomposition of soil organic matter and organic residues, including soil micro-, meso-, and macrofauna.

(3) CO_2, CO_3^{2-} or HCO_3^- evolved by plant root respiration or nutrient transport processes and dissolved in the soil solution.

(4) Dissolution of soil carbonate minerals releases CO_2 in the form of carbonic acid (H_2CO_3).

Under prolonged reducing conditions when the oxygen (O_2) and nitrates (NO_3-N) have been exhausted as potential electron acceptors, some microorganisms are capable of using energy stored in organic compounds by reducing hydrogen and hydrogen ions and by fermenting organic matter to CO_2, organic acids and alcohols and ultimately methane and hydrogen gases (Strong, 1995). This occurs at redox potentials between –250 mV and -300 mV (Strong, 1995).

1.2. MINERALIZATION OF ORGANIC N IN SOILS

1.2.1. Ammonification

Ammonification occurs when organic nitrogenous compounds are enzymatically transformed to ammonium (NH_4-N). Organic N exists in the form of amino acids, amino sugars, purines and pyrimidines in organic matter/plant material and urea (Stevenson, 1982). Enzymes originating from plants, animal or microbial sources, act on the substrate in different ways, depending on the enzyme type/class (Ladd and Jackson, 1982). Proteinases and peptidases are responsible for the first step in the ammonification process, hydrolysing peptide bonds in the proteins to produce shorter chain amino acids. Although there is an important contribution of microorganisms to this pool (Ladd and Paul 1973), seasonal changes in the proteinases activity in the soil are not correlated to changes in the microbial populations, suggesting the enzymes may originate from other sources (Franz, 1973). Plant and animal sources have been identified as probable contributors (Ladd and Jackson, 1982).

1.2.2. Nitrification

Nitrification is the process by which NH_4-N is oxidized to NO_3-N. This process is largely attributed to the activities of some chemoautotrophic bacteria and to a lesser extent by some heterotrophs in the soil (Schmidt, 1982). The first step in the nitrification process is the conversion of NH_4-N to NO_2-N. Although the conversion is attributed to Nitrosomonas activity, other bacteria in the genera Achromobacter and Corynebacter may participate and dominate the process (Belser and Schmidt, 1978).

The final process in nitrification is the conversion of NO_2-N to NO_3-N carried on mostly by bacteria of the genus Nitrobacter. Heterotrophs capable of nitrification include bacteria belonging to the genus Achromobacter and Corynebacterium, which are capable of oxidizing NH_4-N to NO_2-N (Quastel et al., 1950) and the fungus Aspergillus flavus, which in laboratory cultures, can oxidize NO_2-N to NO_3-N (Schmidt, 1954).

1.3. N_2O PRODUCTION IN SOILS

N_2O production in agricultural soils results from simultaneous nitrification and denitrification in soil, and is often associated with N fertilizer applications and high soil moisture content (Petersen et al., 1991). Denitrification is the main biological process by which fixed nitrogen is lost from the soil to the atmosphere and is often catalyzed by facultative heterotrophic bacteria (Hassett and Banwart, 1992) as well as some eukaryotes such as yeasts (Tsuruta et al., 1985) and filamentous fungi (Bollag and Tung, 1972). It occurs when the microorganisms cannot obtain enough O_2 to meet their metabolic requirements and use alternate sources of oxidized materials such as NO_3-N and NO_2-N as electron acceptors to produce NO, N_2O and N_2 (Firestone, 1982) in reactions catalyzed by the enzymes nitrate reductase, nitrite reductase, nitric oxide reductase and nitrous oxide reductase (Fig. 1). However, since many fungi lack the enzyme nitrous oxide reductase, the main product for fungal denitrification is N_2O (Shoun et al., 1992). In addition, under anaerobic conditions, interactions between the enzymes hydroxylamine oxidoreductase and nitrite reductase result in the production of N_2O instead of NO_2^-. Hydroxylamine oxidoreductase forms the intermediate product NOH which may dismutate chemically to produce to yield N_2O (Nicholas 1978).

Quantitative estimates of N loss through denitrification range from 0 to 70% of applied fertilizer (Rolston et al., 1976, 1979; Craswell 1978; Kissel and Smith 1978; Kowalenko, 1978). Although, exact controls for the denitrification process are not yet fully understood, it has been shown that reduced oxygen availability enhances the production of N_2O by NH_4-N oxidizers in laboratory cultures and in the bulk soil as well (Blackmer et al 1980; Goreau et al., 1980). It has therefore been suggested that nitrifying bacteria shift into a form of anaerobic respiration (or denitrification) when O_2 is scarce (Payne 1973).

However, the production of N_2O is not limited to anaerobic soils and can occur in apparently aerobic soils (Denmead et al., 1979). Some bacteria are able to convert NH_4-N and other reduced nitrogen compounds to N and nitrogen oxides in combined nitrification/denitrification processes (Kuenen and Robertson, 1994). In such cases nitrification and denitrification occur simultaneously even at high O_2 tension (Firestone, 1982). The nitrification-denitrification process may occur in close proximity as first described by Knowles (1978), in microsites within a soil aggregate (Stevens et al., 1997) or simultaneously in wet soil where both aerobic and anaerobic zones exist (Lindau et al., 1988). Soil particles, especially the colloidal surfaces, adsorb and concentrate both bacteria and organic substrates and serve as microsites of biological activity. Given that the rate of diffusion of O_2 in water is about 0.0001 of its diffusion in the atmosphere, the presence of water in the soil creates the right anaerobic conditions to favor denitrification even in aerobic soils (Hassett and Banwart, 1992).

1.4. FACTORS AFFECTING CO_2 AND N_2O PRODUCTION

The factors that influence the production of CO_2 and N_2O take effect on different scales/levels within the soil ecosystem. On the cellular level, changes in the temperature, moisture and pH affect the activity of the individual microorganisms. This is because microorganisms are intimately associated with their physical and chemical environment. As a result, temporal dynamics in the soil such as temperature, moisture and pH status will partly reflect the adaptation to those environmental variables rather than competition between different components of the microbial biomass (Petersen et al., 2002). On the community level, factors such as the rhizosphere effect, organic matter residues and soil aggregates effect changes in the activities and structure of these microbial communities (Bending et al. 2000). On the ecosystem level, cultivation practices such as tillage, fertilization and crop rotations affect the soil temperature and moisture regimes, structure, pore distribution and nutrient availability, which in turn affects the dynamics of relationships such as predation or competition in the microbial communities (Petersen et al., 2002). Any resulting change in the microbial communities, example fungal:bacterial ratio, may affect soil properties such as litter decomposition and nutrient cycling, which are underlining factors in the production of CO_2 and N_2O (Bardgett et al. 1996).

1.4.1. Soil temperature and moisture

Temperature is a primary rate determinant of microbial processes such as C mineralization and is a central input to models simulating the effects of mineralisation of soil C pools on global warming (Parton et al., 1987). Normally, there is an exponential increase in soil respiration with respect to temperature increases observed for biological systems (O'Connel, 1990). At temperatures above the optimal (9 to 30°C) however, the sensitivity of soil respiration to temperature increments may be reduced due to enzymes being deactivated or killed (Fang and Moncrieff, 2001). The optimal temperature for nitrification is reported to range from 20 to 40°C (Keeney and Bremner, 1967). Temperatures above the optimal range often leads to reduced solubility of O_2 and also to an increase in the demand for O_2 by other soil heterotrophic microorganisms (Schmidt, 1982) resulting in higher rate of denitrification. However, the production response of CO_2 and N_2O within the optimal range are very similar, increasing with temperature from 3 to 15°C (Sitaula and Bakken, 1993).

Water is essential for microbial survival and activity. Moisture level and its effect on CO_2 production was described by Skopp et al. (1990) as a delicate balance between having sufficient water for substrate diffusion and microbial requirements and adequate oxygen for respiration. Soil moisture affects the availability of substrate such as organic C, ammonium and nitrate that are essential for microbial functions (Weitz et al., 2001). Microbial respiration is maximized when water-filled pore spaces is from 50 to 75% (Olness et al., 2002). The soil water-filled pore space is a function of soil water content and is largely controlled by the texture, especially the clay and soil organic matter content (Hudson, 1994).

The potential for denitrification in soils on the other hand, is a complex interaction between aeration, NO_3-N availability, C substrate availability and other intrinsic "soil factors" (Firestone, 1982). Although high levels of N_2O production have been reported in some soils after a heavy downpour, in flooded organic soils, the N_2O production in most soils is limited by the NO_3-N availability (Firestone 1982). It is well established that denitrification enzymes are synthesized and active only when O_2 availability is low or O_2 is absent. While water content, matric potential and water-holding capacity all serve as relative predictors of microbial activity in soil, the expression of water content as water-filled pore space encompasses the role of water as a barrier to O_2 diffusion and as such is a better predictor of aeration-dependent microbial processes such as denitrification than any other factor (Sommers et al., 1981).

1.4.2. The rhizosphere effect

The rhizosphere, soil in the immediate vicinity of plant roots, is known to support a large number of microbes and high microbial activity due to the nutrient rich environment created by root exudates through the process of rhizodeposition (Casey et al., 1998). The products of rhizodeposition include root exudates, root secretions and root residues such as sloughed cells, dead roots and root hairs (Kuzyakov and Siniakina, 2001). Root exudates generally contain carbohydrates, amino acids, vitamins, organic acids and other miscellaneous compounds in minute concentrations (Casey et al., 1998).

Greater microbial activity induced by the presence of the readily degradable C in the rhizosphere leads to greater CO_2 production in rhizosphere soils than non-rhizosphere soils. The presence of plant roots may also induce a higher level of N_2O production in the rhizosphere because active microorganisms create a localized zone of lowered O_2 concentration (Woldendorp 1962). Root respiration also depletes the levels of O_2 in the rhizosphere, and it is estimated that two-third of the O_2 consumed in the rhizosphere is by plant roots with one-third resulting from root-associated microbes (Woldendorp 1962). Smith and Tiedje (1979) reported that the potential for denitrification was much higher near corn roots (Zea mays L.) and decreased rapidly in the first few millimeters of soil away from the roots. Stefanson (1972) observed that the presence of wheat roots enhanced the denitrification at soil moisture levels below the field capacity, however, above field capacity, denitrification was limited by NO_3-N availability. This is because the low oxygen availability limits nitrification, which provides the substrate for N_2O production. In some cases however, introduction of plant roots does not lead to an increase in the denitrification, probably due to the root competing with the denitrifying bacteria for NO_3-N (Guenzi et al 1978).

1.4.3. Organic matter and plant residue decomposition

Plant residue decomposition in soils involves the mineralization of the labile C fractions by microorganisms, resulting in the formation of microbial by-products and accumulation of recalcitrant organic compounds that become part of the stable SOC pool (Jacinthe et al., 2002). The conversion of residue-C into stable SOC (humification) and the mineralization of SOC are accompanied by CO_2 production.

Residue application has a significant effect on annual and seasonal fluxes of CO_2, being proportional to the amount of residue added (Jacinthe et al., 2002). Decomposition is generally slower for surface applied residues than for residues incorporated into the soil (Schomberg et al., 1994; Thonnissen et al., 2000). This is because surface-applied residues are subject to greater fluctuations in water and temperature regimes, reduced availability of soil nutrients and reduced soil contact, which adversely affects microbial colonization and activity, hence slowing decay rates and CO_2 production (Schomberg et al., 1994; Thonnissen et al., 2000).

The distribution of denitrifier activity in aerobic soils is extremely heterogeneous, and is probably to a large extent associated with the amount and location of decomposable organic matter (Christensen et al., 1990). This promotes O_2 consumption by heterotrophic microbes, and therefore the maintenance of anoxic conditions (Firestone, 1982). In addition, higher organic matter levels may induce higher levels of nitrification, which may also increase the production of N_2O (Schmidt, 1982). As a result, application of manure, which is a readily decomposable C source relatively high in N, may greatly enhance denitrification both in laboratory and field studies (Rolston et al., 1979; Guenzi et al., 1978). In laboratory studies, plant residues have been observed to enhance denitrification (Brar et al., 1978). However, the quality of the plant material added is an important factor in determining the response of denitrification to the amendment since soils amended with high C/N plant residue may immobilize mineral N, thereby limiting denitrification (Craswell 1978). Lower C/N ratio plant residues such as sorghum have been found to cause a more rapid turnover and release of immobilized N than maize residues, which may lead to higher levels of N_2O production (Varvel and Peterson, 1990). However, Bremner and Shaw (1958) reported that cellulose supported higher rates of denitrification than straw or grass most likely due to the formation of anaerobic microsites in the soil during the decomposition process. Burford and Bremner (1975) reported that water-soluble C or mineralizable C (determined by CO_2 evolution during a 7-day incubation) was highly correlated with denitrification activity. They concluded that mineralizable C or water soluble organic C was a good indicator of the denitrification potential of soils.

1.4.4. Soil aggregates

Several studies have shown that soil microorganisms and their activities are heterogeneously distributed across aggregate-size classes (Gupta and Germida, 1988). Whilst some studies show greater microbial biomass and higher activities in macroaggregates (>0.25 mm) compared with microaggregates (<0.25 mm) (Gupta and Germida, 1988), others report similar levels of activities between macroaggregates and microaggregates (Seech and Beauchamp, 1988; Miller and Dick, 1995). Still others have reported greater microbial biomass and activities in the intermediate size (0.25–1.00 mm) aggregates than in the >1.00-mm or <0.25-mm size class (Mendes et al., 1999). The heterogeneous distributions of microbial biomass and the associated activities among the different aggregate size classes may be conceptually explained in two ways. Firstly, it may be a reflection of their structural properties that selectively or generally restrict the transfer of nutrients and O_2 for respiration (Mendes et al., 1999). Alternately, variations in microbial biomass might imply that the structural variation among the aggregates restricts predatorial grazing to certain aggregate-size classes but not to others (Mendes et al., 1999).

An examination of microbial biomass and activities and C and N pools within aggregates will provide insight to better understand the growth of microbial communities, turnover of organic matter, N availability in soil and consequently the contribution of soil aggregates to the production of CO_2 and N_2O (Sainju et al., 2003).

1.4.5. Tillage and crop residue management

Land use and soil management practices significantly impact on the soil organic C dynamics and C flux from the soil (Paustian et al., 1995). Reduced or no-till farming systems that retain plant residues in the fields, rely more on chemical control of weeds and minimal disturbance of the soil and are becoming more prevalent (Peoples et al., 1995). Conversion of land from conventional tillage to reduced or no-till system has been reported to increase the C sequestration and reduce production and emission of CO_2 (Franzluebbers et al., 1995)

The effect of tillage on soil respiration and soil organic matter pools has been extensively studied in the field. Tillage increases the rate of organic-matter decomposition and mineralization by aerating the soil, burying surface residues, breaking soil aggregates, and thereby increasing the exposure of soil organic matter to microbial activity (Phillips and Phillips, 1984). However, the normal high initial flush of CO_2 experienced immediately after tillage has been attributed to the release of CO_2 in the soil pores or the rapid direct oxidation of C substrates rather than from residue incorporation and only partially explains the loss of C from agricultural soils

(Reicosky, 2002). Over time, however, studies indicate that tillage practices tend to increase soil respiration by increasing the availability of the soil organic matter previously protected in aggregates to decomposers (Wander and Bollero, 1999). Conversion of land from conventional tillage to reduced or no-till system has been reported to increase the C sequestration and reduce production and emissions of CO_2 (Franzluebbers et al., 1995). However, adoption of the no-till system does not guarantee an increase in the soil organic matter levels. The ability of no-till practices to increase SOM sequestration and reduce CO_2 production has been reported to be limited in poorly drained soils (Paustian et al., 1997), in cooler climates where cold temperatures slows SOM decay (Angers et al., 1997), and in agroecosystems where erosion rates are low (Alvarez et al., 1998).

Despite all the advances made to understand how tillage affects C cycling, the effect of tillage on N_2O production is one area that has not been adequately studied. However, conservation tillage and no-tillage have been reported to reduce N mineralization rates, increase soil organic matter content and lead to better retention of soil structure (Lal, 1989). Soil moisture is better conserved and biological activity of the soil microbes and fauna are enhanced (Lal, 1989). As a result, denitrification may be higher in no-till soils than in conventionally-tilled soils (Aulakh et al., 1992).

1.4.6. N fertilization

Expansion and intensification of N fertilization in agriculture is expected to be a major contributor to increasing atmospheric CO_2 and N_2O concentrations (Keller and Matson, 1994). Fertilization increases N availability for microbial processes such as organic matter decomposition and denitrification and may lead to an increase in

CO_2 and N_2O fluxes for different climate zones and land uses (Brumme and Beese, 1992). However, Persson and Wiren (1989) observed that the long-term addition (17 y) of NH_4NO_3, fertilizer at the rate of 74 kg N ha^{-1} y^{-1} resulted in a 30-40% reduction in CO_2 evolution rate from Scots pine forest soil. Possible explanations for such effects of high N-doses were changes in microbial communities, production of inhibitory substances or inhibition of the synthesis of certain enzymes, such as phenoloxidases (Persson and Wiren, 1989).

Post-fertilization N_2O production and fluxes is dependent on the N-release rate of the fertilizer (Weitz et al., 2001). Generally however, N_2O emissions are observed to be highest during the first weeks N fertilizer is applied and declines with elapsing time post-fertilization approaching background flux rates of a soil. Although N fertilization may significantly increase the soil-atmosphere flux of N_2O on soils (Matson et al., 1996), it has also been reported to reduce microbial activity by lowering the pH which may reduce nitrification and denitrification in the long-term (Katznelson and Stevenson, 1956).

1.5. CONCLUSIONS AND FUTURE DIRECTIONS

The literature review shows that the processes that define the production of CO_2 and N_2O can be complex and that agricultural management factors can affect these processes on various levels.

Consequently, the objective of the present study were:

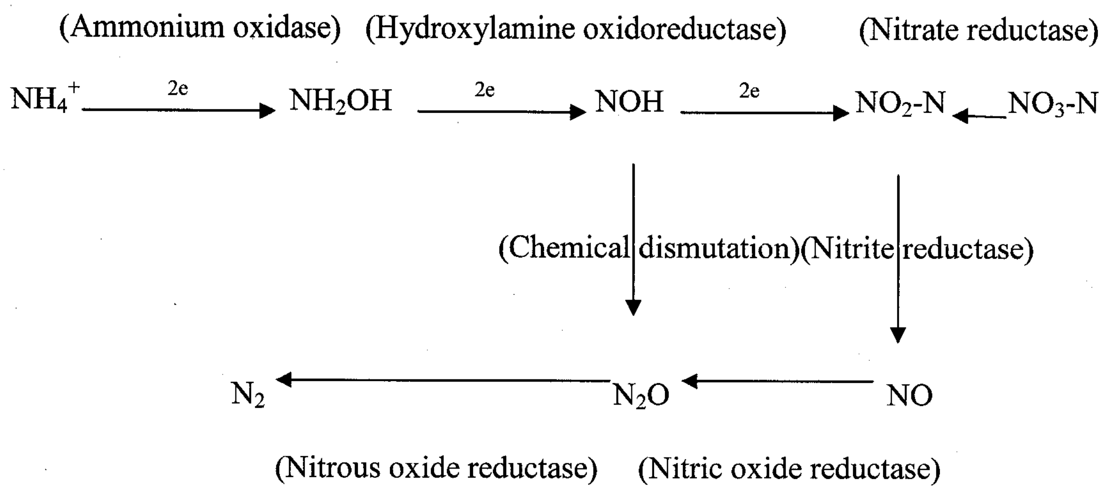
1- To determine the effects of tillage {conventionally-tilled (CT) and no-till (NT)}, and fertilizer sources (composted cattle manure and inorganic NPK fertilizer) on CO_2 and N_2O below-ground content in the soil profile of corn and soybean cropping systems.

2- To investigate rhizosphere and specific rhizosphere respiration in corn and soybean, as affected by plant phenology.

3- To investigate the N_2O production from rhizosphere-associated soil of corn and soybean and to investigate how partitioning of incorporated N between the roots and shoots affects the production of N_2O in rhizosphere-associated soil of corn and soybean at different growth stages.

4- To determine the effect of soil moisture on CO_2 and N_2O efflux from soil aggregate fractions

Fig. 1. Overall reaction scheme of denitrification adapted from Nicholas (1978).



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CHAPTER 2: FERTILIZER AND CROP EFFECTS ON BELOW-GROUND CARBON DIOXIDE AND NITROUS OXIDE CONTENT

2.1. ABSTRACT

Carbon dioxide (CO_2) and nitrous oxide (N_2O) produced in and emitted from agricultural soils are affected by management practices such as tillage, fertilizer type and crop type. The purpose of this two-year field study was to determine the effects of tillage [conventionally-tilled (CT) and no-till (NT)], and fertilizer sources (composted cattle manure and inorganic NPK fertilizer) on CO_2 and N_2O below-ground content in the soil profile of corn and soybean cropping systems. The soil atmosphere was sampled via plastic tubes installed within the crop row at three depths (10, 20 and 30 cm) in the soil profile and gas contents (i.e., mass of gas per unit soil volume) were derived from means of the three sampling depths. Above a WFPS of 60%, CO_2 and N_2O content declined as denitrification likely yielded more N_2 than N_2O gas as the final product. Nitrous oxide content declined rapidly as WFPS levels fell below 60%, as N_2O production in the soil profile denitrification was likely limited to anaerobic microsites and to the relative smaller amounts produced from nitrification. Conventionally-tilled plots had higher CO_2 content than NT plots, however, the effect of tillage was greater in the soybean than in the corn plots. Compost-treated soil had significantly higher below-ground content of CO_2 than the inorganic fertilizer-treated soil, however most of the differences observed in CO_2 contents occurred in the first half of the growing season (June and July), which indicates that mineralization processes are strongest during this time than at any other time later in the season.

2.2. INTRODUCTION

Rising greenhouse gas concentrations in the earth's atmosphere have the potential to adversely affect global climate (Houghton et al., 1995). An estimated 25% of CO_2 and 90% of N_2O emitted worldwide comes from agroecosystems (Duxbury, 1994). Reducing the amounts of CO_2 and N_2O released from agricultural soils into the atmosphere is possible, yet information is needed on how agricultural practices such as tillage, fertilizer applications and cropping systems influence the distribution and activity of the soil organisms that produce CO_2 and N_2O gases.

The activity of decomposer organisms is stimulated by tillage, which fragments and incorporates plant residues in the plow layer and disrupts soil aggregates, thus increasing the pool of readily-mineralizable organic substrates (Adu and Oades, 1978; Beare et al., 1994; Reicosky and Lindstrom, 1993; Rochette and Angers, 2000). This accelerated loss of soil carbon following tillage is also related to changes in soil porosity. Curtin et al. (2000) reported that the mean annual CO_2 fluxes were about 10% lower under no-till (NT) than conventionally tilled (CT) soils in continuous wheat and wheat-fallow cropping systems. NT soils have larger and more anaerobic soil aggregates than plowed soils, microsites where denitrifying bacteria may be active (Grevers and de Jong, 1982; Aulakh et al., 1984). Doran (1980) found denitrifier populations were 7.3 times higher in the profile of NT soils than CT soils, while Aulakh et al. (1984) reported denitrifier counts between 1.3 and 6.6 times higher in NT soils than CT soils.

Fertilization increases the amount of nitrogen (N) available for microbial processes such as organic matter decomposition or mineralization and denitrification, and may increase CO_2 and N_2O fluxes in diverse ecosystems and climate zones (Brumme and Beese, 1992; Crill et al., 2000). However, Kowalenko (1979) and Rochette and Gregorich (1998) found lower CO_2 from N fertilized plots than from unfertilized controls

Organic fertilizers may also stimulate N_2O production by increasing the soil NO_3-N content or by providing a readily mineralizeable C substrate for decomposers; the activity of decomposers can deplete the soil O_2 supply, creating microsites favorable for denitrifying bacteria (Flessa and Beese, 1995; Parkin, 1993). Inorganic N fertilizer applications that increase the mineral N content in soil solution may enhance the production of N_2O from nitrification (Bowman and Focht, 1974; Kohl et al., 1976) or denitrification when the soil water content is sufficiently high (Aulakh et al., 1984; Cates and Keeney, 1987; Sehy et al., 2003). In eastern Canada, Drury et al. (1998) found twice as much N_2O was evolved from soils receiving 16.8 kg N ha^{-1} from NH_4NO_3 fertilizer than non-fertilized soils.

The annual and seasonal fluxes of CO_2 are affected by the amount, type and timing of crop residue incorporation (Jacinthe et al., 2002). In their analysis of C sequestration rates using a global database of 67 long-term agricultural experiments, West and Post (2002) noted that the continuous corn system yielded more plant residue and hence a greater C input than the corn-soybean rotations, but changing from continuous corn to corn-soybean rotation did not change soil C sequestration, suggesting that more CO_2 was lost from the continuous corn system than the corn-soybean rotation. Leguminous plants such as soybeans may induce higher levels of N_2O production in the soil directly, by rhizobia denitrification (O'Hara and Daniel,

1985) or indirectly, by increasing inputs of N to the soil, thereby increasing the substrate pool for nitrification and denitrification (Ta et al., 1986). The N_2O production was greater in soils amended with leguminous plant residues than corn residue (McKenney et al., 1993). However, Rochette and Janzen (2005) determined that much of the increase in soil N_2O emissions in legume crops may be attributable to the N release from root exudates during the growing season and from decomposition of crop residues after harvest, rather than from biological N fixation (BNF)

The objective of this study was to investigate the effects of tillage and fertilizer sources on CO_2 and N_2O production in the soil profile of corn and soybean cropping systems. We also provide information on how the temporal variation in CO_2 and N_2O fluxes was related to changes in the soil environment (temperature and moisture) during the two growing seasons.

2.3. MATERIALS AND METHODS

2.3.1. Research Site

The study was conducted at the Macdonald Research Farm of McGill University located at Ste-Anne-de-Bellevue, Québec from June 2002 to September 2003. The soil was a mixed, frigid Typic Endoaquent, mostly of the St.-Damase Series (Lajoie and Baril, 1953), containing 700 g kg^{-1} of sand, 140 g kg^{-1} of silt and 160 g kg^{-1} of clay with 15.4 g organic C kg^{-1} and a pH 6.1 in the top 15 cm. The sandy loam layer (28 cm mean thickness) was underlain by sand (6 cm mean thickness) and clay starting at depths below 34 cm, on average.

2.3.2. Experimental Design

The plots selected for this study are a subset of a larger field experiment established in May 2000 that was described by Carefoot and Whalen (2003) and Whalen et al. (2003). The experimental design was a split-plot design consisting of twenty-four 20 by 24 m main plots arranged in four blocks (six main plots per block), with each main plot further split into four 20 by 6 m sub-plots. The main plot factors were two tillage treatments [conventional tillage (CT) or no-till (NT)] and three crop rotations [corn-soybean (CS), soybean-corn (SC) or continuous corn (CC)], for a total of six factorial treatments. At the subplot level, four fertilizer treatments were randomly applied. The fertilizer treatments provided the same amount of plant-available P (45 kg P ha^{-1}) from 100% inorganic fertilizer, 100% composted cattle manure, 66% composted cattle manure mixed with 34% inorganic fertilizer and 33% composted cattle manure mixed with 67% inorganic fertilizer. For the purposes of this study, only four main plots per block were considered for the main plot factors, whilst at the subplot level the mixed inorganic fertilizer plus compost treatments were excluded. Hence we provide information on the 100% inorganic fertilizer and 100% compost treatments only.

The conventionally tilled plots were tilled with a tandem disk (10-cm depth) each spring prior to seeding and with a moldboard plough (20-cm depth) each fall after harvest, but there was no disturbance of the no-till plots. Soybean (*Glycine max* L. Merr. 'Cargill A086TR'; 400,000 seeds ha^{-1}) and corn (*Zea mays* L. 'Cargill

2610'; 75,000 seeds ha^{-1}) plots were seeded in the first week of June 2002 and June 2003, and 45 kg P ha^{-1} was band applied at seeding to the inorganic fertilizer treatment. All corn plots received 50 kg N ha^{-1} as NH_4NO_3 banded at seeding, and the inorganic fertilizer plots received a side-dress application of 150 kg N ha^{-1} from NH_4NO_3 and 125 kg K ha^{-1} from potash at the four to five leaf stage, about one month after seeding. No inorganic N fertilizer was applied to the soybean plots, although the inorganic fertilizer plots received a side-dress application of 125 kg K ha^{-1} from potash about one month after seeding. Compost contained, on average, 401 g total C kg^{-1}, 20.7 g total N kg^{-1} (Carlo Erba Flash EA NC Soils Analyzer, Milan, Italy), 2.3 g total P kg^{-1} [H_2SO_4/H_2O_2 digestion (Parkinson and Allen, 1975)] and 0.66 kg of H_2O kg^{-1} (105°C for 48 h). The subplots with compost treatment received a surface application of 45 Mg ha^{-1} (wet weight basis), which was incorporated in the CT treatment or left on the surface of the NT treatment before seeding.

2.3.3. Environmental measurements

Bulk density (ρ_b) was determined separately for the conventionally-tilled and no-till plots as an average of measurements taken just prior to gas sampling tube installation in June 2002 and after harvest in September 2003. Undisturbed soil cores (3-cm long x 5.5-cm I.D.) were collected from the 0-10, 10-20 and 20-30 cm depths of the soil profile, in the row and inter-row positions of eight plots randomly selected to represent both tillage treatment (four conventionally-tilled plots and four no-till plots . Bulk density in each core was determined after drying at 105°C for 48-h. Subsequently, within the top 30-cm layer, we determined an average ρ_b of approximately 1.27 g cm^{-3} for the conventionally-tilled plots and 1.29 g cm^{-3} for the no-till plots. Average porosity, f in the top 30-cm was calculated as:

$$f = 1 - {\rho_b}/{\rho_s} \qquad [1]$$

where ρ_s is the density of soil particles ($\approx$2.65 g cm^{-3}). Soil temperature and moisture were recorded on each gas sampling date from eight randomly selected plots representing a replicate of each of the tillage x crop x fertilizer. Soil temperature in the top 30-cm of the soil profile was measured using thermocouples installed at three depths (10, 20 and 30 cm) of the row and inter-row positions. Soil cores (length 30 cm, i.d. 3 cm) were collected from the row and inter-row positions of these eight plots, at three soil depths (0-10, 10-20 and 20-30 cm), and the moisture content was measured gravimetrically (105°C for 48 h). Soil temperature and moisture were averaged over the top 30 cm for the conventionally-tilled plots and the no-till plots at each sampling date. We then converted gravimetric water content to water-filled pore space (WFPS, $\mathrm{cm}^3/\mathrm{cm}^3$), in order to normalize for differences in the bulk densities:

$$WFPS = \left(\frac{w\,{\rho_b}/{\rho_w}}{f} \right) \qquad [2]$$

where w is the gravimetric water content (g/g), and ρ_w is the density of water (1 g cm^{-3}). Air-filled porosity, f_a ($\mathrm{cm}^3/\mathrm{cm}^3$) is defined by:

$$f_a = f - WFPS \qquad [3]$$

2.3.4. Gas Sampling

Polystyrene tubes (0.3-cm I.D.) were installed on 19 June 2002 and 23 June 2003 at three depths (10, 20 and 30-cm) in the planted rows of each experimental plot (6 tubes per plot, with 96 tubes installed across the field site). The belowground end of the tube was covered with a plastic mesh (≤ 1 mm) to prevent soil from entering and blocking the tube. The aboveground portion of the tube was fitted with a one-way male slip stop-cock (Cole-Parmer Instrument Company, Vernon Hills, IL). After removing the air in the deadspace of tubes with a gas-tight syringe, 25 mL of air from the soil profile was transferred into previously evacuated 12 mL exetainers (LABCO, Wycombe, U.K.). Gas samples were taken at 7 to 12-d intervals from June to September, for a total of 10 sampling dates in 2002 and 9 sampling dates in 2003. Gas samples were analyzed with a gas chromatograph (Varian Model 3800, Walnut Creek, CA, U.S.A.) equipped with automated valve injectors to simultaneously quantify CO_2, N_2O and O_2 concentrations (expressed in ppm v/v units). A Haysep A column followed by a molecular sieve and He carrier at 46 ml min^{-1} were used to separate CO_2 from O_2 whereas N_2O was quantified on a Porapak Q column with Ar/CH_4 (90:10) carrier gas at 20 mL min^{-1} (Rochette and Hutchinson, 2005).

2.3.5. Soil profile gas content

Gas concentrations (µL/L) were averaged over the three sampling depths to obtain the average gas concentrations in the top 30 cm of the soil profile.

The soil temperature data were taken into account in order to determine the solubility of the gas in the soil water (Bunsen coefficients) for CO_2, N_2O and O_2

(Glinski and Stepniewski, 1985; Tiedje, 1994). The solubility in water of CO2 (β_{CO_2}), N2O (β_{N_2O}) and O2 (β_{O_2}) as a function of soil temperature were derived from the following functions:

$$\beta_{CO_2} = 0.0007T^2 - 0.0521T + 1.7031 \qquad [4]$$

$$\beta_{N_2O} = 0.0005T^2 - 0.0398T + 1.2407 \qquad [5]$$

$$\beta_{O_2} = 0.00001T^2 - 0.0011T + 0.048 \qquad [6]$$

Where T is the average temperature in the top 30-cm of the soil profile (°C). The method used to convert soil profile gas concentration from µL/L of air to total gas content in mg m^{-3} of soil is an adaption of that outlined by Christian and Cranston (1997). Based on the ideal gas equation, the number of moles in the gaseous state of the soil volume (n_g in moles) is given by:

$$n_g = 10^{-6}\left(\frac{C_g P V_g}{RT_1}\right) \qquad [7]$$

where C_g is the concentration of the gas in µL/L as determined by the gas chromatograph, P is the atmospheric pressure at which gas samples are analyzed (≈ 1 atm), T_1 (K) is the temperature in the soil profile, V_g is the volume of the gas sampled in the field (0.025 L) and R is the ideal gas constant (0.082 L.atm mol^{-1} K^{-1}). The number of moles of gas (n_w in moles) in the dissolved phase is defined by:

$$n_w = 10^{-6}\,\frac{CP\beta\, V_w}{RT_1} \qquad [8]$$

where β is the Bunsen coefficient or the solubility of the gas in the soil water and V_w is the volume of water in volume of soil sampled, expressed in litres:

$$V_w = {}^{V_g}\!/_{f} * \theta \qquad [9]$$

where θ is the mean volumetric water content in the top 30 cm (L/L) derived from the gravimetric water content measurements. The total mass of gas, M_g (g), dissolved in the volume of soil sampled was given by:

$$M_g = W_g(n_g + n_w) \qquad [10]$$

where W_g (g/mol) is the molecular weight of the gas in question (in grams). The total volume of soil sampled, V_t (L) is given by:

$$V_t = V_g / f_a \qquad [11]$$

Dividing equation [10] by equation [11] yields the mass of gas content per unit volume of the soil (mg m^{-3} of soil).

2.3.6. Statistical Analysis

Owing to the sequential nature of gas sampling, CO_2, N_2O, O_2 content data were analyzed by repeated measures analyses of mixed models method using the PROC MIXED feature of SAS (SAS Institute, 1999). The general procedure for mixed models is outlined by Littell et al. (1998). Covariance structures were objectively compared using the Bayesian information criterion (BIC) and the Akaike information criterion (AIC), based on which we determined that the AR (1) covariance structure provided the best fit for our model with the least complexity. This analysis yielded estimates of autocorrelation coefficients between sampling dates. Least square (LS) means were separated at a 95% confidence level using the Tukey-Kramer method.

2.4. RESULTS AND DISCUSSIONS

2.4.1. Temporal variations in CO_2 and N_2O content

Mean monthly air temperature and precipitation data for the site are shown in Table 1 (Environment Canada, 2006). Below-ground content of CO_2 and N_2O were disproportionately high during the first month of the growing seasons, with an estimated 45 and 91% of total content of CO_2 and N_2O respectively, measured in 2002, and 17% and 38% of total CO_2 and N_2O contents respectively, in 2003. These temporal variations may have been linked to differences in soil moisture levels within seasons.

Our results show that CO_2 and N_2O content declined as WFPS fell below 50% in both 2002 and 2003 (Figs. 2, 3, 4 and 6). That is because water is essential for microbial survival and activity. The effect of moisture level on CO_2 production was described by Skopp et al. (1990) as a delicate balance between having sufficient water for substrate diffusion and microbial requirements and adequate oxygen for respiration. Soil moisture affects the availability of substrate such as organic C, ammonium and nitrate that are essential for microbial functions (Weitz et al., 2001). Microbial respiration is maximized when WFPS is from 50 to 75% (Olness et al., 2002). Soil moisture is also perhaps the key controller of microbially-mediated gaseous N production because it affects microbial growth and metabolism, and because it affects the solubility and availability of substrates (organic carbon, ammonium and nitrate) for nitrification and denitrification reactions (Weitz et al., 2001). N_2O production levels decline rapidly when WFPS levels falls below 60% (Linn and Doran, 1984), as denitrification activity decreases with increasing O_2 availability (Davidson, 1991).

We estimate that average CO_2 contents in 2002 were approximately 1.4 times higher than in 2002 whilst for N_2O, this difference was even greater; 11 times more N_2O was produced in the soil profile in 2002 than in 2003. Considering that sampling started earlier in 2002, when soils were wetter (Fig. 1), likely from the effects of the spring snowmelt and/or higher precipitation (Table 1), our sampling in 2002 may have captured peaks in soil respiration and denitrification resulting from the higher soil moisture which were likely missed in 2003.

There is an exponential increase in soil respiration with respect to temperature increases observed for biological systems (O'Connel, 1990; Thierron and Laudelout, 1996; Winkler et al., 1996). Similarly, N_2O production has been reported to increase with temperature within the optimal range from 3 to 15°C (Sitaula and Bakken, 1993). Thus, soil temperature during both the 2002 and 2003, which ranged between 14 and 25°C (Fig. 1), was well in the range considered to favor microbial respiration and denitrification. Seasonally, although the 2003 data did not show a clear relationship between temperature and CO_2 or N_2O contents, we observed peaks in CO_2 content around 30 d and 60 d after seeding (DAS) in 2002 season (Figs. 2a and 5) that corresponded to peaks in soil temperature around the same time (Fig. 1). However, the CO_2 content during this period (around 60 DAS) was much less than the CO_2 content around 30 DAS. We attributed this to the lower soil moisture around

60 DAS which may have limited decomposer activity. Similarly, Rochette and Gregorich (1998) observed that soil respiration is strongly correlated with soil temperature only when organic substrates are abundant and soil moisture is optimal. N_2O content in soil, on the other hand did not seem to be related to soil temperature in this study, and we did not observe any peaks in N_2O production that coincided with increasing soil temperature in either growing season (Fig. 3). Similarly, Akiyama and Tsuruta (2003) found no correlation between soil temperature and N_2O production from agricultural soil, but noted that such a relationship might be observed if WFPS and mineral N levels were constant and optimal for denitrification.

2.4.2. Correlations between below-ground contents of CO_2, N_2O and O_2

CO_2 and O_2 were mostly were often negatively correlated ($P<0.001$) throughout both seasons (Tables 2 and 3). This confirms that aerobic respiration depleted the O_2 in the soil environment, however, in the latter half of each growing season, the correlations observed were less negative (Tables 2 and 3), suggesting that the decomposer organisms in the soil did not utilize or deplete the O_2 to the same extent as in the first half of the seasons. Thus, although aeration improved with the lower WFPS (Fig. 1), another factor was limiting aerobic respiration, as the season progressed. Based on our previous discussion, we speculate that soil moisture was this limiting factor.

In 2002, the correlation between N_2O and O_2 on 16 DAS was not significant in contrast to significantly negative correlation in the subsequent sampling days, up until the middle of the season (Table 2). We attribute this to the higher than optimum

(> 60%) WFPS in the soils at that time, given that the value of 60% WFPS is a critical level above which N_2O production tends to decline, as denitrification yields more N_2 than N_2O gas as the final product (Kiese and Butterbach-Bahl, 2002). This will serve to weaken the negative correlation between the N_2O content and O_2 content. Significant negative correlation between N_2O and O_2 for the rest of the first half of the 2002 season further suggests that denitrification still occurred to a significant degree in the soils. For the rest of the 2002 season however (i.e. after 50 DAS), as soil WFPS fell below 35%, nitrification likely became a more prominent source of N_2O in the soil (Sylvia et al., 1998), thereby introducing a positive relationship between N_2O and O_2, which likely compensated for the negative correlations between N_2O and O_2 resulting from denitrification. Thus, the resulting relationship was a non-significant correlation, as observed. Although these correlations in the latter half of the season were not significant, they were all negative, implying that even at lower moisture level, denitrification was still the dominant source of N_2O. Similarly, we observed significantly negative correlations between N_2O and O_2 in the first half of the 2003 season (up until 50 DAS) and insignificant correlations for the rest of the season, further supporting our observations in 2002 (Table 3). However, considering the relatively low WFPS observed in our soils, we can conclude that this denitrification process was mostly limited to anaerobic microsites in the soil volume. Positive correlations observed between CO_2 and N_2O in our soils, especially in the first half of the seasons, when denitrification was the major process in N_2O production (Tables 2 and 3), is consistent with other research (Abbasi and Adams, 1998; Firestone, 1982), which showed that aerobic respiration and denitrification can occur simultaneously in aerobic upland soils.

2.4.3. Effect of tillage, crop and fertilizer types on below-ground CO_2 and N_2O content

Tillage had a significant effect on CO_2 contents in the 2002 season (Table 4), with greater ($p<0.05$) CO_2 contents in CT (6.36 g C m^{-3}) than in NT soils (4.43 g C m^{-3}) (Fig. 2). This tillage effect on CO_2 was most dominant in the first month of the growing seasons (Fig. 2), suggesting that the greatest tillage-induced losses of soil C may occur shortly after tillage, and that this is the period was important for C mineralization processes. Although the tillage effect was not significant in 2003, our results in 2002 are consistent with those of Franzluebbers et al. (1995) who reported 9 to 12% higher rates of CO_2 emissions in the CT plots relative to NT plots under soybean-corn-wheat rotation. This is because the physical process of tillage incorporates plant residues and manure into the soil, and also makes previously-protected organic materials in the soil to become more accessible to the decomposers; these process would thereby indirectly affect O_2 levels in the soil. However, considering that we did not observe any tillage effects on O_2 content in the soil, we speculate that any effects of tillage on the O_2 content may have been limited to creating anaerobic microsites in the soil which would be difficult to estimate with the method we used.

Tillage may affect the soil temperature and moisture regimes, soil structure and pore distribution and nutrient availability, which in turn affects the dynamics of relationships such as predation or competition in the microbial communities (Petersen et al., 2002). Any resulting change in the microbial communities (e.g. fungal:bacterial ratio) may affect soil properties such as litter decomposition and nutrient cycling, which are underlining factors in the production of CO_2 and N_2O (Bardgett et al. 1996; Yeates et al. 1997). In no-till systems, the soil is more compact and more likely to have anaerobic zones that favor denitrification. Thus, the soil environment may be more favorable to denitrification in the NT than the CT systems (Grevers and de Jong, 1982; Aulakh et al., 1984; Lal, 1989). Although we did not observe any significant effect ($P<0.05$) of tillage on N_2O content (Tables 4 and 5), there was a significant tillage by sampling date interaction indicating that N_2O content in the tilled and no-till plots did not follow the same trend with time (Fig. 3). The results also show that differences between the two tillage systems were only significant during the first sampling event, likely due to the higher soil moisture in the soils at that sampling stage.

Although corn roots are estimated to input about 3400 kg C ha^{-1} into the soil as rhizodeposits, soybean roots contribute only a fraction of this amount at 1300 kg C m^{-2} (Kogel-Knabner, 2002). Thus, theoretically, decomposer and denitrifier activity in corn rhizosphere is expected to be higher than in the soybean rhizosphere, resulting from this difference in the substrate C pool. In addition, root respiration and root-derived respiration are generally higher in corn than in soybean due to a greater flow of photosynthates to the roots (Cardon et al., 2002; Chapter 3 of this thesis). However, the crop effect on CO_2 content was insignificant in 2003 (Tables 5). The CO_2 content in corn plots averaged 5.37 g C m^{-3} soil compared to 5.47 g C m^{-3} soil in soybean whilst in 2003 corn plots averaged 4.55 g C m^{-3} soil compared to 3.31 g C m^{-3} soil for soybean, however, there was no significant difference between the crop treatments in either growing season. Interaction between tillage and crop type was significant in 2002 (Table 4). Figure 5 shows that for 2002, tillage significantly increased CO_2 content in the soybean plots but not in the corn plots ($P<0.05$), suggesting that tillage affects CO_2 differently in corn and soybean rotations.

Fertilizer amendment significantly affected ($P<0.05$) the CO_2 content in 2002 (Table 4), with higher content of CO_2 in the compost-treated soil (6.48 g C m^{-3} soil) than the inorganic fertilizer-treated soil (4.3 g C m^{-3}). However, this trend did not persist in 2003. Figure 6 shows organic plots had a greater response to the changes in soil temperature (Fig. 1). The presence of readily decomposable C in the manure stimulated decomposer activity in the organic fertilizer treatment than in the inorganic fertilizer treatment as indicated by the significant effect of fertilizer treatment on O_2 content in 2002 (Table 4).

Increasing the labile organic C concentration in soils can increase the denitrification potential, leading to more N_2O production as long as NO_3-N, O_2 and moisture are not limiting (Drury et al., 1998). Similarly, Drury et al. (1998) reported a significant relationship ($r^2=0.76$, $P=0.001$) between CO_2 production and denitrification capacity. None of the main factors had a significant effect on below-ground content of N_2O production which highlights the complexities involved in studying N_2O in the field setting.

Although the autocorrelation (Box and Jenkins, 1976) is a useful tool that can be used to detect non-randomness in a dataset, as well to help identify an appropriate time-series model if data are not random, very few researchers have utilized this tool in past greenhouse gas studies. The autocorrelation is a correlation coefficient. However, instead of correlation between two different variables, the correlation is between two values of the same variable at times X_i and X_{i+k} (Engineering Statistics Handbook, 2006). When the autocorrelation is used to detect non-randomness, it is usually only the first autocorrelation that is of interest (Engineering Statistics Handbook, 2006). Thus, we observed high autocorrelation of 0.64, 0.47 and 0.16 for successive measures of CO_2, N_2O and O_2 respectively, whilst in 2003, the autocorrelation was 0.91, 0.90 and 0.78 for successive measures of CO_2, N_2O and O_2 respectively. These results indicate that CO_2 and N_2O content observed in our soils were not erratic or random, but were dependent on site specific factors. This may also be indications that for field studies like ours involving replicate measurements of CO_2 and N_2O in time; it may be possible to reduce the number of replicates in time without compromising excessively on the reliability of the results.

2.5. CONCLUSIONS

Above a WFPS of 60%, the CO_2 and N_2O contents declined as denitrification likely yielded more N_2 than N_2O gas as the final product. N_2O levels decline rapidly as WFPS levels falls below 60%, as N_2O production was limited to denitrification occurring in anaerobic microsites in the soil profile and the relative smaller amounts produced from nitrification. Generally, in the latter stages of the season as WFPS fell below 35%, denitrification process was limited to anaerobic microsite in the soil volume. Our results also show a significant positive correlation between the CO_2 and

N_2O contents in the soil at all sampling dates which indicated that denitrification and aerobic respiration occurred simultaneously in soils. Our results indicate that towards the end of the season, as soils became more aerobic, most of the N_2O content observed was most likely derived from nitrification. Although we observed some peaks in soil temperature corresponding to peaks in the soil temperature, the effect of soil temperature was limited by the depletion in soil moisture levels towards the end of the season.

Conventionally-tilled plots had higher levels of CO_2 contents than no-till plots, however, the effect of tillage in corn and soybean plots significantly varied, affecting below ground content of CO_2 in the soybean plots to a greater extent than in the corn plots due to the fact that soybean is more easily incorporated into the soil by the tillage action and also more readily decomposable.

Compost-treated soil had significantly higher below-ground contents of CO_2 than the inorganic fertilizer-treated soil, which could be attributed to the presence of readily decomposable C in the manure, stimulating more decomposer activity in the organic fertilizer treatment than the inorganic fertilizer treatment. That most of the differences observed in CO_2 contents among the different treatments occurred in the first half of the growing season (June and July), also indicates that the processes that encourage C mineralization are strongest during this time than at any other time later in the season.

Table 1. Monthly averages for temperature and precipitation for 2002 and 2003 field seasons and long-term (1971-2000) average temperature and precipitation (Environmental Canada Meteorological Service, unpublished data).

	Monthly Precipitation (mm)			Daily average air temperature (^{o}C)		
Month	2002	2003	30-yr average	2002	2003	30-yr average
June	106.0	70.0	87.5	17.5	18.8	19.3
July	55.0	54.0	106.2	22.1	21.6	22.3
August	11.0	79.0	100.6	21.8	21.6	20.8
September	86.5	104.0	100.8	18.3	17.7	15.7
Total	258.5	307	395.1			

Table 2. Pearson correlation coefficients (*r*) between CO_2, O_2 and N_2O content in the soil profile during the 2002 growing season (n=16). Significance levels were $P<0.05$ (*), $P<0.01$ (**) and $P<0.001$ (***).

	Days after seeding														
	16			23			30			37			44		
	CO_2	N_2O	O_2	CO_2	N_2O	O_2	CO_2	N_2O	O_2	CO_2	N_2O	O_2	CO_2	N_2O	O_2
CO_2		0.46 **	-0.85 ***		0.56 ***	-0.90 ***		0.64 ***	-0.95 ***		0.78 ***	-0.93 ***		0.77 ***	-0.90 ***
N_2O	0.46 **		-0.33 NS	0.56 ***		-0.58 ***	0.64 ***		-0.74 ***	0.78 ***		-0.74 ***	0.77 ***		-0.66 ***
O_2	-0.85 ***	-0.33 NS		-0.90 ***	-0.58 ***		-0.95 ***	-0.74 ***		-0.93 ***	-0.74 ***		-0.90 ***	-0.66 ***	

	Days after seeding														
	50			58			65			80			101		
	CO_2	N_2O	O_2	CO_2	N_2O	O_2	CO_2	N_2O	O_2	CO_2	N_2O	O_2	CO_2	N_2O	O_2
CO_2		0.59 ***	-0.70 ***		0.60 ***	-0.05 NS		0.60 ***	-0.63 ***		0.61 ***	-0.83 ***		0.63 ***	-0.47 **
N_2O	0.59 ***		-0.08 NS	0.60 ***		-0.16 NS	0.60 ***		-0.32 NS	0.61 ***		-0.46 *	0.63 ***		-0.23 NS
O_2	-0.70 ***	-0.08 NS		-0.05 NS	-0.16 NS		-0.63 ***	-0.32 NS		-0.83 ***	-0.46 *		-0.47 **	-0.23 NS	

Table 3. Pearson correlation coefficients (*r*) between CO_2, O_2 and N_2O content in the soil profile during the 2003 growing season (n=16). Significance levels were *P*<0.05 (*), *P*<0.01 (**) and *P*<0.001 (***).

	Days after seeding														
	27			37			42			49			56		
	CO_2	N_2O	O_2	CO_2	N_2O	O_2	CO_2	N_2O	O_2	CO_2	N_2O	O_2	CO_2	N_2O	O_2
CO_2		0.66 **	-0.77 ***		0.86 ***	-0.84 ***		0.83 ***	-0.84 ***		0.74 ***	-0.93 ***		-0.29 NS	-0.76 ***
N_2O	0.66 **		-0.94 ***	0.86 ***		-0.74 **	0.83 ***		-0.57 *	0.74 ***		-0.64 **	-0.29 NS		0.03 NS
O_2	-0.77 ***	-0.94 ***		-0.84 ***	-0.74 **		-0.84 ***	-0.57 *		-0.93 ***	-0.64 **		-0.76 ***	0.03 NS	

	Days after seeding											
	63			71			84			94		
	CO_2	N_2O	O_2	CO_2	N_2O	O_2	CO_2	N_2O	O_2	CO_2	N_2O	O_2
CO_2		0.53 *	-0.69 **		0.45 NS	-0.80 ***		0.45 NS	-0.76 ***		0.30 NS	-0.62 **
N_2O	0.53 *		-0.14 NS	0.45 NS		-0.21 NS	0.45 NS		-0.17 NS	0.30 NS		-0.05 NS
O_2	-0.69 **	-0.14 NS		-0.80 ***	-0.21 NS		-0.76 ***	-0.17 NS		-0.62 **	-0.05 NS	

Table 4. Analysis of variance table describing the significance of sampling date (Rep), main-plot effects [tillage (till) and crop treatments (crop)], sub-plot effects [fertilizer treatment (fert)] and their interactions on CO_2, N_2O and O_2 content in the top 30-cm of the soil profile during the 2002 growing seasons.

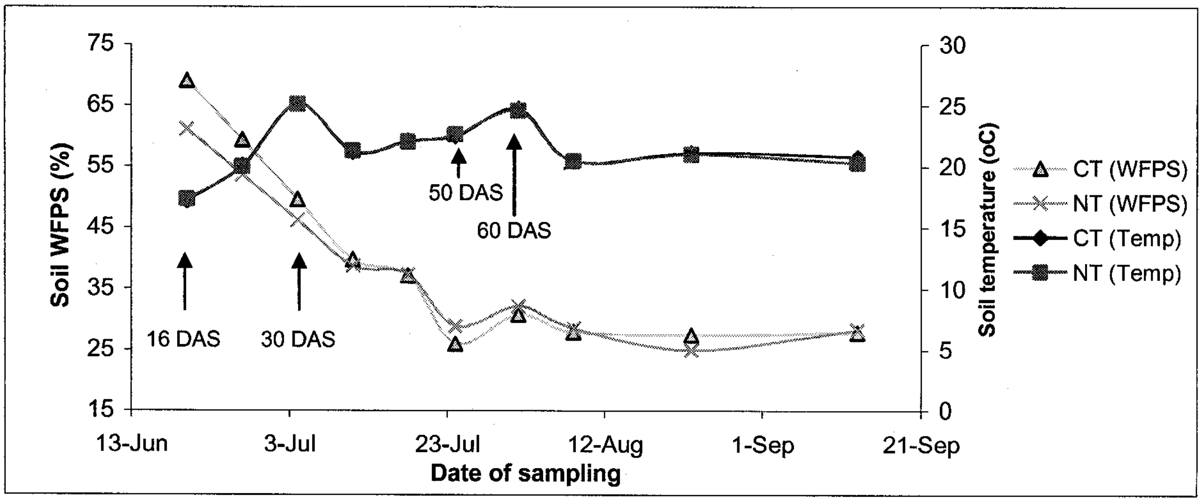
		CO_2		N_2O		O_2	
	DF	F	*P*>F	F	*P*>F	F	*P*>F
Rep (T)	9	22.18	<.0001	10.93	<.0001	8.81	<.0001
Till (A)	1	14.38	0.0006	1.34	0.2799	0.78	0.3852
Crop (B)	1	0.01	0.9313	0.47	0.5121	2.4	0.1344
Fert (C)	1	18.35	0.0002	2.19	0.1631	13.76	0.0011
A*B	1	5.83	0.0216	0.00	0.9533	1.36	0.2553
A*C	1	2.82	0.1027	0.47	0.5056	0.31	0.5846
B*C	1	0.14	0.7135	0.38	0.5486	0.39	0.5382
A*B*C	1	0.03	0.8539	0.68	0.423	0.01	0.9173
T*A	9	1.42	0.1834	1.68	0.0983	0.28	0.9783
T*B	9	0.93	0.5003	1.29	0.2463	2.03	0.0388
T*C	9	2.71	0.0053	1.02	0.4296	2.16	0.0264
T*A*B	9	1.10	0.3657	0.03	1.0000	0.19	0.9951
T*A*C	9	0.59	0.8014	0.25	0.9863	0.21	0.993
T*B*C	9	0.16	0.9975	1.11	0.3588	1.83	0.0648
T*A*B*C	9	1.06	0.3924	0.71	0.7023	0.14	0.9986

Table 5. Analysis of variance table describing the significance of sampling date (Rep), main-plot effects [tillage (till) and crop treatments (crop)], sub-plot effects [fertilizer treatment (fert)] and their interactions on CO_2, N_2O and O_2 content in the top 30-cm of the soil profile during the 2003 growing seasons.

		CO_2		N_2O		O_2	
	DF	F	*P*>F	F	*P*>F	F	*P*>F
Rep(T)	8	24.08	<.0001	10.43	<.0001	26.26	<.0001
Till (A)	1	1.49	0.2436	0.63	0.4393	1.38	0.2615
Crop (B)	1	3.88	0.0701	0.10	0.7534	4.28	0.0599
Fert (C)	1	2.27	0.1563	0.00	0.9455	2.71	0.1253
A*B	1	0.62	0.4448	0.00	0.9668	0.08	0.7879
A*C	1	0.04	0.8363	0.05	0.831	0.04	0.8422
B*C	1	1.10	0.3136	0.02	0.8771	1.24	0.2863
A*B*C	1	0.02	0.8975	0.05	0.8214	0.01	0.9353
T*A	8	11.38	<.0001	4.81	<.0001	6.41	<.0001
T*B	8	2.02	0.0457	0.94	0.4884	0.11	0.9987
T*C	8	0.88	0.5335	0.04	1.0000	0.52	0.8415
T*A*B	8	0.73	0.6691	0.66	0.7256	2.21	0.0294
T*A*C	8	3.38	0.0012	0.14	0.9975	0.47	0.8787
T*B*C	8	1.51	0.1559	0.37	0.933	0.59	0.7884
T*A*B*C	8	0.49	0.8626	0.29	0.9698	0.67	0.7143

Figure 1. Soil temperature and water-filled pore space (WFPS) in top 30-cm of conventionally-tilled (CT) and no-till soil (NT) in 2002 (a) and 2003 (b). Seeding occurred in the first week of June for both years. Arrows point to specific days after seeding (DAS).

a)


Soil WFPS (%)
75
65
55
45
35
25
15
Soil temperature (oC)
30
25
20
15
10
5
0
16 DAS
30 DAS
50 DAS
60 DAS
13-Jun
3-Jul
23-Jul
12-Aug
1-Sep
21-Sep
Date of sampling
CT (WFPS)
NT (WFPS)
CT (Temp)
NT (Temp)

b)

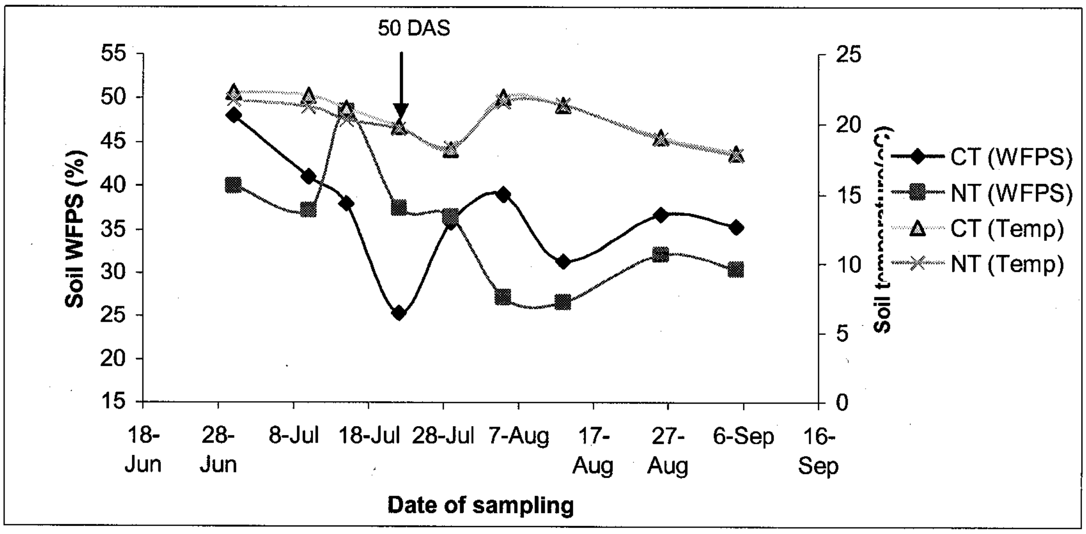
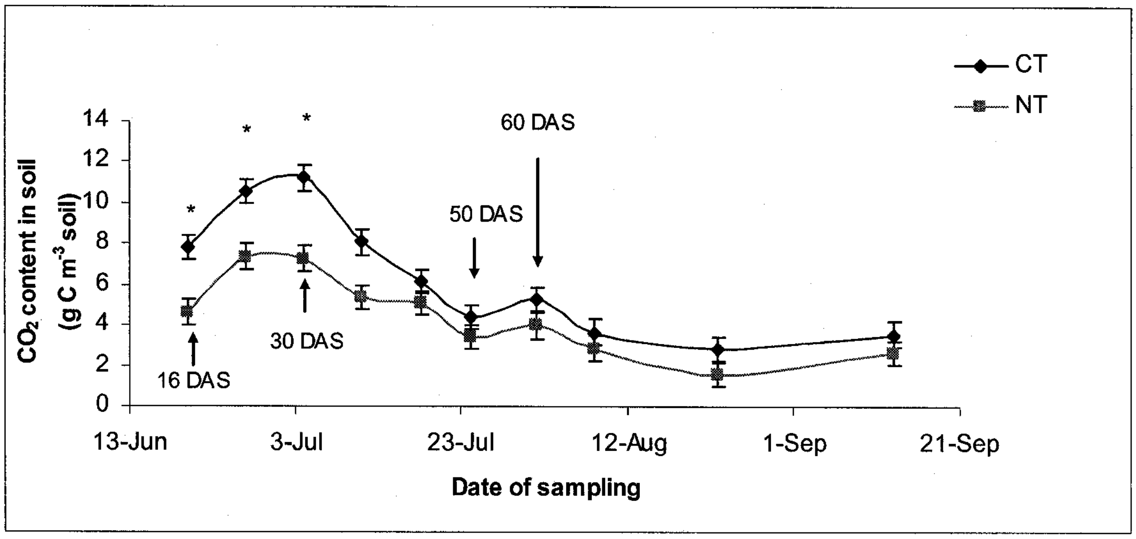
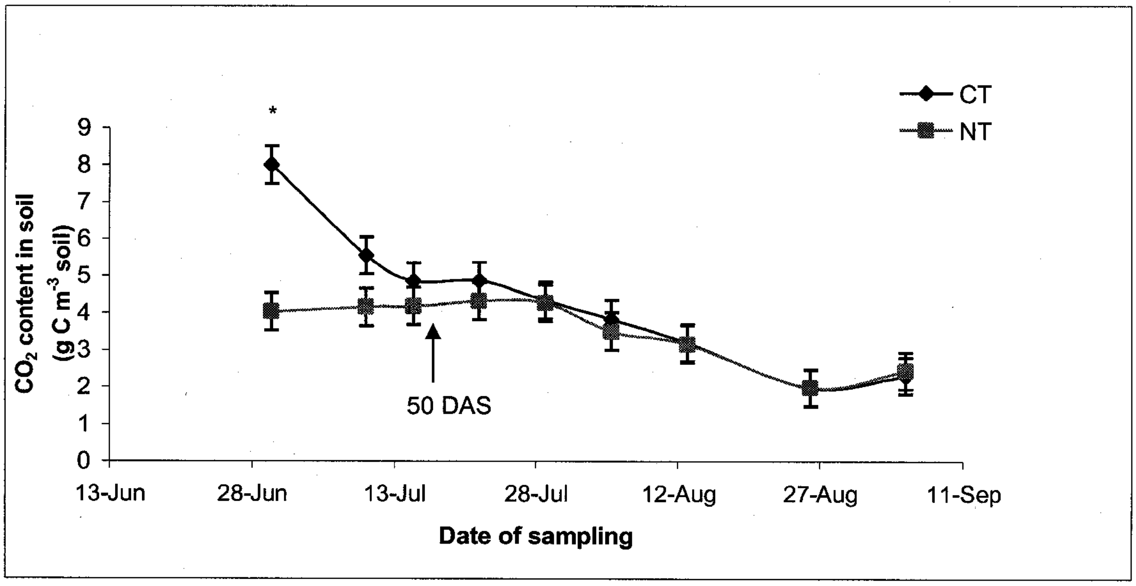

50 DAS
Soil WFPS (%)
55
50
45
40
35
30
25
20
15
Soil t
25
20
15
10
5
0
18-Jun
28-Jun
8-Jul
18-Jul
28-Jul
7-Aug
17-Aug
27-Aug
6-Sep
16-Sep
Date of sampling
CT (WFPS)
NT (WFPS)
CT (Temp)
NT (Temp)

Figure 2. Variation in soil content of CO_2 in 2002 (a) and 2003 (b) with sampling date in the conventionally-tilled (CT) and no-till soil (NT) plots. Vertical bars signify standard errors. Seeding occurred in the first week of June for both years. Arrows point to specific days after seeding (DAS).

a)

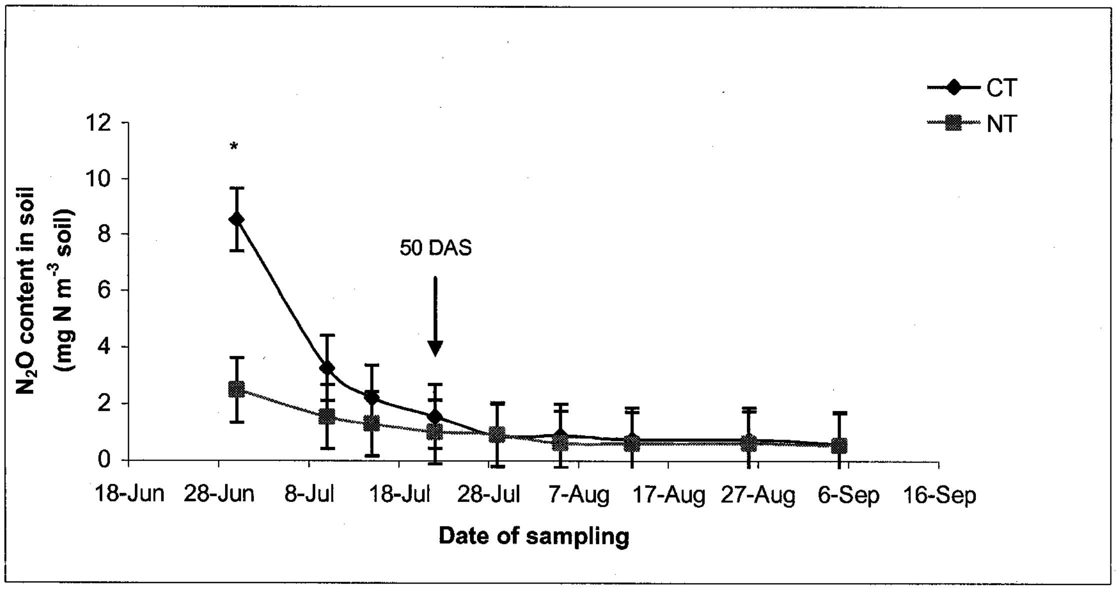


b)



* Significantly different at $p < 0.05$

Figure 3. Variation in soil N_2O content with sampling date in the conventionally-tilled (CT) and no-till soil (NT) plots during the 2003 growth season. Vertical bars signify standard errors. Seeding occurred in the first week of June. Arrows point to specific days after seeding (DAS).



* Significantly different at $P < 0.05$

Figure 4. Variation in soil CO_2 content with sampling date in the corn and soybean (soy) plots during the 2003 growth season. Seeding occurred in the first week of June for both years.Vertical bars signify standard errors. Arrows point to specific days after seeding (DAS).

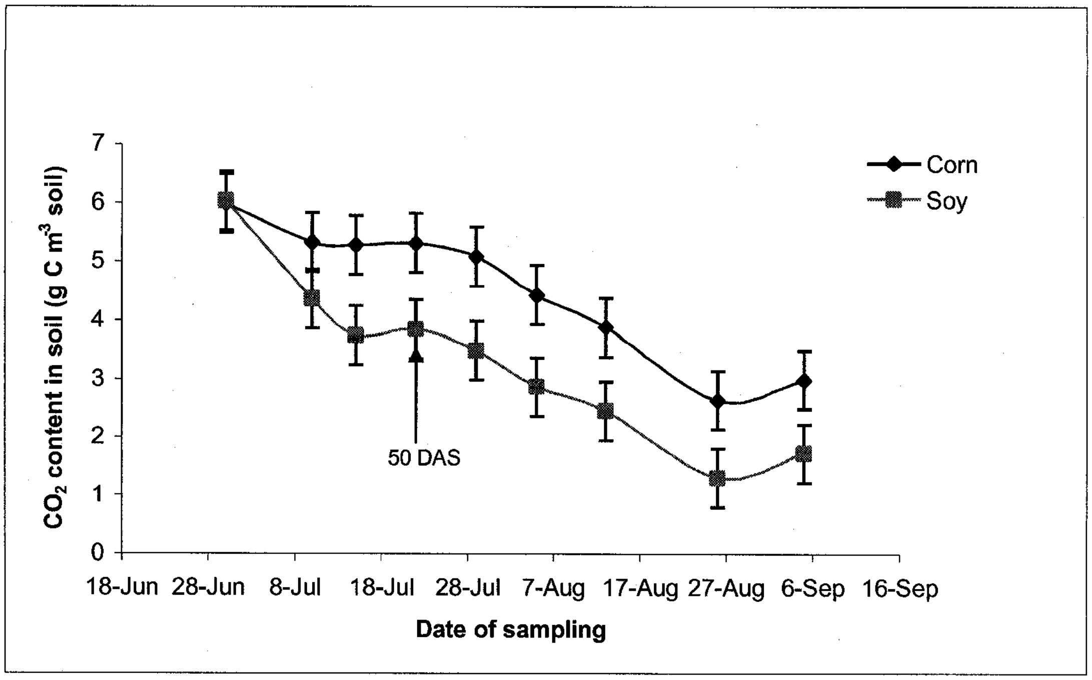

7
6
5
4
3
2
1
0
CO$_2$ content in soil (g C m^3 soil)
Corn
Soy
50 DAS
18-Jun
28-Jun
8-Jul
18-Jul
28-Jul
7-Aug
17-Aug
27-Aug
6-Sep
16-Sep
Date of sampling

Figure 5. Variation in the mean soil CO_2 content in corn and soybean plots by tillage type [conventionally-tilled (CT) and no-till soil (NT)] during the 2002 growth season. Seeding occurred in the first week of June for both years. Vertical bars signify standard errors, and bars with the same letter were not significantly different ($P<0.05$, Tukey test).

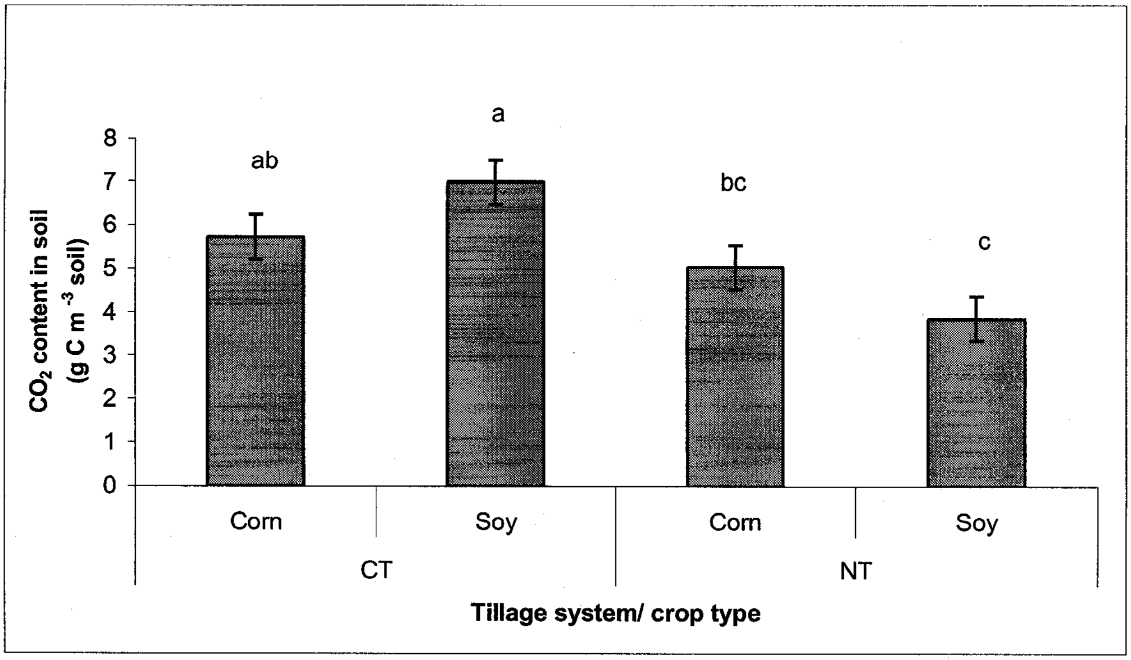
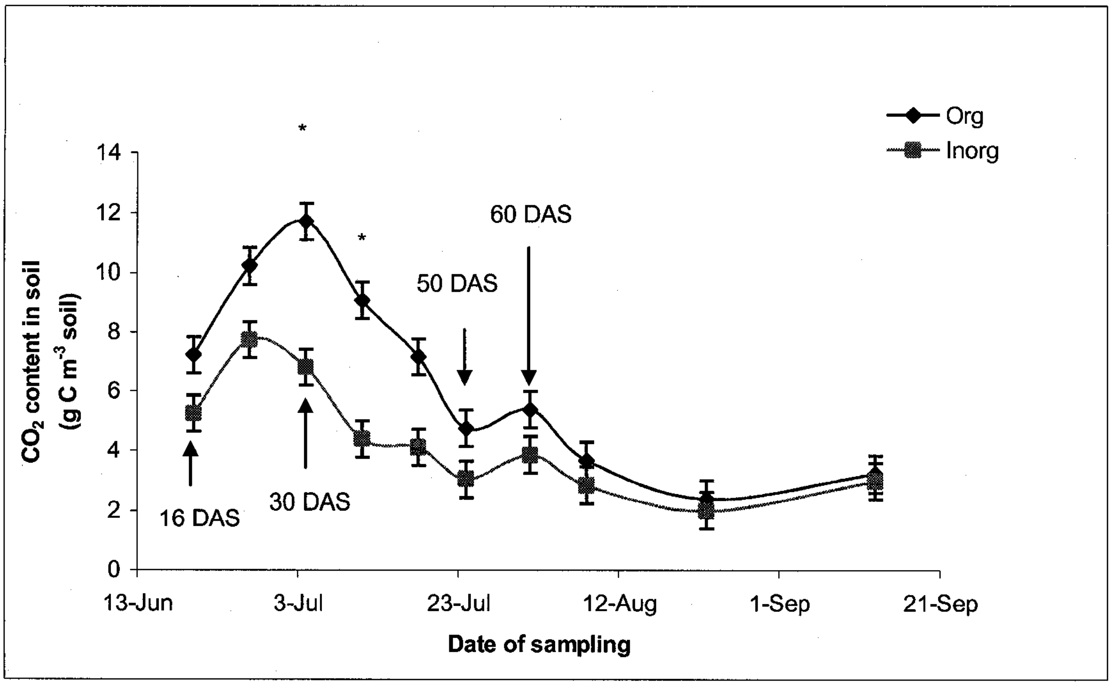

CO$_2$ content in soil (g C m^{-3} soil)
0
1
2
3
4
5
6
7
8
ab
a
bc
c
Corn
Soy
Corn
Soy
CT
NT
Tillage system/ crop type

Figure 6. Variation in soil CO_2 content with sampling date in the compost-treated plots (Org) and inorganic fertilizer-treated plots (Inorg) during the 2002 growth season. Vertical bars signify standard errors. Arrows point to specific days after seeding (DAS).



* Significantly different at $P < 0.05$

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CONNECTING PARAGRAPH

Chapter 2 showed the variations in the below-ground content of CO_2 and N_2O in a field setting. We identified that agricultural management factors such as tillage system and fertilizer type had the potential to increase the production in these agroecosystems by a significant amount. In view of the fact that crop effect was not significant in our field experiment, we set out to investigate the dynamics in CO_2 and N_2O efflux from the rhizosphere of corn and soybean in two greenhouse experiments. The next chapter discusses the first of these experiments, investigating CO_2 efflux from the rhizosphere of corn and soybean using the root exclusion method.

CHAPTER 3: RHIZOSPHERE AND SPECIFIC RHIZOSPHERE RESPIRATION IN CORN AND SOYBEAN

3.1. ABSTRACT

The rhizosphere is an important component of soil respiration, contributing about 30 to 80% of total belowground CO_2 efflux in various ecosystems. In order to better understand CO_2 production from these agroecosystems, it is important to understand the temporal variation in CO_2 produced by the rhizosphere. The objective of this study was to investigate rhizosphere and specific rhizosphere respiration in corn and soybean, as affected by plant phenology. The greenhouse study was conducted with grain corn (*Zea mays L.*) and soybean (*Glycine max*) under natural lighting. The experimental design consisted of a completely randomized design consisting of three treatments (pots seeded with corn, pots seeded with soybean and control unseeded pots). We estimate an average of about 79% of the soil respiration in the corn pots came from rhizosphere respiration whilst in the soybean pots rhizosphere respiration constituted 58% of the soil respiration. Although we observed a higher rate of rhizosphere respiration in corn than soybean, at all stages of growth, specific rhizosphere respiration was significantly higher in soybean (0.29 mg C g^{-1} root h^{-1}) than corn (0.09 mg C g^{-1} root h^{-1}), which supports previous observations made with regards to slower-growing plants (e.g. soybean) having relatively higher root respiration than faster growing plants (e.g. corn).

3.2. INTRODUCTION

Globally, agricultural soils have been estimated to contribute about 25% of the total CO_2 released into the atmosphere annually (Duxbury, 1994). Soil respiration is an index of soil biological activity and comprises of the soil microbial respiration (derived from the decomposition of soil organic matter) and the rhizosphere respiration (comprised of root respiration and microbial respiration of root-derived material) (Cheng et al., 1993). In agricultural soils under crop cultivation, part of the photosynthetically fixed C is translocated from the above-ground parts of the plant to the roots and subsequently respired or released into the surrounding soil as root exudates, mucilage, and sloughed cells and tissues (Qian et al., 1997; Kuzyakov and Siniakina, 2001).

Rhizosphere C flow represents the pump driving the majority of below-ground ecosystem processes (Killham and Yeomans, 2001) and creates a nutrient-rich environment that supports a large number of microbes and high microbial activity (Casey et al., 1998). As such, the rhizosphere could be a very important source of CO_2 in soils, ranging from 30 to 80% of total belowground CO_2 efflux in various ecosystems (Rochette and Flanagan, 1997; Hanson et al., 2000). Direct root respiration may contribute a significant portion of this rhizosphere respiration, with estimates ranging from 10 to 90% of rhizosphere respiration (Hanson et al., 2000). Much of the variability in these estimates reflects problems with measurement techniques that often disturb the roots and soils (Hogberg et al., 2001). Root respiration on the other hand has been observed to be closely related to the relative growth rate (RGR) (Garnier, 1991) of plant species, estimated between 1.4 to 1.7-fold higher in rapid growing plants that slow-growing species (Poorter et al., 1991; Scheurwater et al., 1998).

Currently several methods have been developed to separate rhizosphere respiration from soil respiration. These methods can be put into broad categories, namely, component integration, isotopic and the root exclusion methods (Hanson et al., 2000). Component integration involves the excluding various components of the soil, such as roots, sieved soil and litter, and then measuring the CO_2 efflux from each component (Anderson, 1973; Hendrickson and Robinson, 1984). Isotopic methods utilize the uptake of either radioactive ^{14}C (Cheng, 1996; Swinnen et al., 1994) or stable ^{13}C (Rochette and Flanagan, 1997; Rochette et al., 1999) by plant shoot to derive rhizosphere respiration from the respiration of photosynthetically-derived C. The root exclusion method (Anderson, 1973; Buchanann, 2000; Hanson et al. 2000) involves the estimation of rhizosphere respiration from the difference between the respiration from soils with roots and soils without roots.

Although $^{14}C/^{13}C$ tracers have been used to study the fate of photosynthetically-derived C in various components of the soil-plant system (i.e. shoot, root and soil), not much work has been done to study how this partitioning relates to the rhizosphere respiration, as well as variations of this rhizosphere respiration with plant phenology. This is because plant phenology can affect the quantity and quality of C-flow into the rhizosphere and hence the rhizosphere respiration (Hale et al., 1971). Fu et al. (2002) reported that maize roots delivered more C through rhizosphere-induced respiration to soil respiration before harvest when plants were fruiting than after harvest. In soybean, rhizosphere respiration increased significantly from the vegetative to the flowering stage, declining thereafter. Principal differences also exist in the utilization of C by C_3 plants (e.g. soybean) and C_4 plants (e.g. corn). It is known that C_3 plants fix C by the action of the enzyme ribulose biphosphate carboxylase whilst C_4 plants take up CO_2 through the carboxylation of phosphoenolpyruvate (O'leary, 1988). Also C_3 are isotopically distinct from C_4 plants with background $\delta^{13}C$ varying between C_3 plants and C_4 plants, averaging about -27‰ for C_3 plants (e.g. soybean) and averaging -12‰ for C_4 plants (e.g. corn) (Smith and Epstein, 1971).

The objective of this study was to determine rhizosphere respiration in corn and soybean, measured from the root exclusion method, and to investigate how ^{13}C use and partitioning varies with plant phenology, and contributes to this rhizosphere respiration. We hypothesize that corn will show a greater amount of assimilated ^{13}C partitioned to the roots and the rhizosphere-associated soil, leading to higher fluxes of CO_2 from the corn pots relative to the soybean pots.

3.3. MATERIALS AND METHODS

The greenhouse study was conducted with grain corn (*Zea mays L.*) and soybean (*Glycine max*) under natural lighting. Pots were prepared with soil (sandy loam, mixed typic Endoaquent, pH 6.1) taken from the top 10 cm of the Macdonald Research Farm, Ste. Anne de Bellevue, Quebec. The soil was a mixed, frigid Typic Endoaquent (Humic Gleysol) of the St. Damase series (Lajoie and Baril, 1953), containing 700 g kg^{-1} of sand, 140 g kg^{-1} of silt and 160 g kg^{-1} of clay with 15.4 g organic C kg^{-1} and pH 6.1 in the top 15 cm. The sandy-loam layer (28 cm mean thickness) was underlain by sand (6 cm mean thickness) and clay starting at depths below 34 cm, on average. Coarse fractions were removed by sieving through a 2mm sieve. The soil was then homogenized and air-dried (Cheng et al., 1993; Kuzyakov and Siniakina, 2001) after which we weighed 1500 g of soil into pots (PVC pipe, 25 cm height and 10 cm diameter) to a bulk density of 1.12 g cm^{-3}, leaving a headspace volume approximately 677 cm^3. The bottom of the pot was sealed to prevent nutrient loss via leaching. Phosphorous fertilizer (sodium phosphate tribasic) was added to the soil at a rate of 0.28 g P kg^{-1} of soil to the soils before seeding. Seeding with grain corn and soybean was done at a rate of 5 per pot and plants were thinned to a single plant per pot a week after seeding. The pots were covered with lids containing an opening for the plant shoot and a rubber septum for headspace gas sampling (Fig.1). Soil moisture content was maintained at 60% water-filled pore space (WFPS) throughout the experiment by monitoring the weight of the pots and adding distilled water when necessary.

3.3.1. Experimental design

The experimental design consisted of a completely randomized design consisting of three treatments (pots seeded with corn, pots seeded with soybean and control unseeded pots). There were five replicates for each treatment, for a total of fifteen pots for each sampling event on days 20, 60 and 80 following seeding. On day 20, corn was at the early vegetative growth stage (V1 stage) while soybeans were at the second node stage (V3 stage). By day 60, the corn was at the VT (tasseling) stage and soybeans were at the R2 (flowering) stage, while on day 80, corn was at the R3 (milk) stage and soybeans were at the R6 (pod-filling) stage (Ontario Ministry of Agriculture, Food and Rural Affairs, 2002).

3.3.2. ^{13}C labeling procedure

The ^{13}C labeling procedure was adapted from Bromand et al. (2001). A Plexiglas chamber with dimensions of 60 cm depth x 120 cm width x 104 cm height was used for ^{13}C labeling. Ice packs were placed in the bottom of the chamber to prevent overheating for the duration of the plant labeling. The pots were then placed in the chamber for 4 hours, and pulse-labeled with ^{13}C at 30 minute intervals. The ^{13}C-labeled CO_2 was generated by reacting $NaH^{13}CO_3$ with concentrated lactic acid. The $^{13}CO_2$ evolved was injected into the sealed chamber through the rubber septum (Fig. 1) whilst a Radioshack fan powered by 6V battery was used to circulate the $^{13}CO_2$ in the chamber to ensure a uniform distribution of the ^{13}C in the chamber headspace. The first step in the labeling process involved priming the system with ^{13}C-labeled CO_2 generated by injecting 30 ml of 99% $NaH^{13}CO_3$ through a septum into a flask containing concentrated lactic acid. The ^{13}C-labeled CO_2 generated in the flask was then extracted using a syringe and injected into the labeling chamber through the rubber septum (Fig. 1). After priming the system, we injected 8 applications of 35 ml of 50% $NaH^{13}CO_3$ (50% $NaH^{13}CO_3$ + 50% $NaH^{12}CO_3$) at 30-min intervals. We estimate that a total of 1700 mg of ^{13}C was injected into the labeling chamber during each 4 h labeling event. A LICOR LI-6400 infrared gas analyzer (LICOR Inc, Lincoln, NE) was placed in the chamber to monitor the CO_2 concentration to ensure that CO_2 concentration never fell below 100 ppm. Immediately following the ^{13}C-labeling, the holes in the lid were sealed with a layer of low melting point paraffin (Kuzyakov and Siniakina, 2001).

3.3.3. Gas sampling

Headspace gas concentrations were sampled using a 20-ml syringe immediately after sealing the lids with paraffin wax (initial headspace concentration at time $t = 0$) and then after 24 h ($t = 1$). The gas samples were stored in 12-ml previously-evacuated exetainers (LABCO, Wycombe, UK) and analyzed for CO_2 using a Varian Model 3800 gas chromatograph (Walnut Creek, CA, U.S.A.) equipped with automated valve injectors to simultaneously quantify CO_2, N_2O and O_2 concentrations (expressed in ppm v/v units). A Haysep A column followed by a molecular sieve and He carrier at 46 ml min^{-1} were used to separate CO_2 from O_2 whereas N_2O was quantified on a Porapak Q column with Ar/CH_4 (90:10) carrier gas at 20 mL min^{-1}.

3.3.4. Gas efflux calculations

Units of gas concentration (CO_2, N_2O or O_2) as expressed in ppm v/v is equivalent to 1cm^3 of gas per m^3 of air. From the ideal gas equation:

$$PV = nRT \tag{1}$$

where $P \approx$ atmospheric pressure (1 atm), V is the volume of gas (cm^3), n is the number of moles of gas, R is the ideal gas constant (82.06 atm cm^3/mol K) and T is the temperature under which pots were incubated expressed in kelvins (303 K).

Rearranging,

$$n = {}^{PV}\!/_{RT} \tag{2}$$

and since

$$d = {}^{g}\!/_{V} = M({}^{n}\!/_{V}) \tag{3}$$

where density d is mass of gas (g) per unit volume (V) and (M) is the molecular mass of gas.

Combining equations (2) and (3),

$$d = \frac{MP}{RT} \tag{4}$$

Multiplying the concentration of gas (cm^3 m^{-3}) with the density of the gas, d (g N_2O cm^{-3}) yields concentration of the gas in the headspace expressed as g m^{-3} of the headspace. We converted gas concentration to per C basis by multiplying by the ratio of C atoms per molecule of CO_2 (12/44). Conversion to mg C m^{-3} was done by multiplying by 1000 after which we multiplied by the volume of the headspace ($\approx 6.77 \times 10^{-4}$ m^3) in order to determine the gas concentration on a per pot basis (mg pot^{-1}). Gas efflux into the chamber headspace, f, (mg C pot^{-1} h^{-1}) was estimated as:

$$f = {(C_1 - C_0)}/{t} \qquad [5]$$

where C_1 and C_0 are the concentrations of gas (mg C pot^{-1}) at time $t = 1$ and $t = 0$ respectively and t is the duration between sampling events (24 h).

3.3.5. Separating rhizosphere respiration from soil respiration

The term soil respiration as used in this study refers to the sum of the rhizosphere respiration (root respiration and root-derived respiration) and soil microbial respiration (derived from mineralization of soil organic matter). Rhizosphere respiration was determined using the root exclusion technique (Anderson, 1973; Buchanann, 2000; Hanson et al. 2000). CO_2 efflux from the planted soil represented the sum of the rhizosphere respiration and the soil microbial respiration (soil respiration), whilst CO_2 efflux from the unplanted soil (controls) at any sampling event represented the soil microbial respiration. The rhizosphere respiration was calculated as the difference between the CO_2 efflux from soil with and without plants. Specific rhizosphere respiration (mg C g^{-1} root h^{-1}) was calculated by dividing the rhizosphere respiration (mg C pot^{-1} h^{-1}) by the mass of recovered roots per pots (g).

3.3.6. Shoot, root and soil sampling and analyses

After gas sampling, plants were cut off at the base, the root-soil column was pulled out and the roots was handpicked from the soil. Roots and shoots were then washed to remove soil particles, and shoots and roots were dried in an oven (65°C for 48 hours) and weighed. Soil samples were air-dried for about a week at 25°C. Plant tissue and soil samples were then finely ground (<0.5 mm mesh), weighed into tin capsules, and analysed for $^{13}C/^{12}C$ content. Delta (δ) ^{13}C and total C content in the samples were determined by flash combusting the samples at 1800°C in an elemental analyzer, EA 1110 (CE Instruments, Italy). Gases were carried by helium gas from the EA into an isotope ratio mass spectrometer, DeltaPlus Advantage IRMS (ThermoFinnigan, Germany) for isotope analysis. Data was normalized using internal standards. Analytical precision was +/- 0.2 permil (‰). The atom percentage of ^{13}C in soil and tissue samples, *at*% were determined from the relation:

$$at\% = \frac{100 * AR * \left(\frac{\delta^{13}C}{1000} + 1\right)}{1 + AR * \left(\frac{\delta^{13}C}{1000} + 1\right)} \qquad [6]$$

where AR is the Absolute Ratio, equivalent to 0.0111796, calculated after compilation of minimum and maximum isotope ratios of the element in naturally occurring terrestrial materials and reagents (U.S. Geological Survey, 2002).

Atom percentage of ^{13}C enrichment ($^{13}C_{en}$) in soil and tissue samples were determined from the difference between $at\%$ of soil and tissue samples in the experimental pots and the background $at\%$. Background $at\%$ in soil was calculated from the average δ ^{13}C value in the soils from the control treatment (-20.2‰) using equation [6]. Background δ^{13}C used for this study were -27‰ for soybean and -12‰ for corn (Smith and Epstein, 1971). Thus, we determined background $at\%$ for corn and soybean by inputting background δ^{13}C for corn and soybean into equation [6]. The mass of ^{13}C enrichment, m in soil and tissue samples, was obtained from the relation:

$$m = M * \frac{\%C}{100} * \frac{^{13}C_{en}}{100} \qquad [7]$$

where M is the dry mass of the total root or shoot recovered from each pot or soil used in each pot (1500 g) and $\%C$ is the percent of total C in the soil or tissue sample.

3.3.7. Statistical analyses

All statistical analyses were performed using the SAS statistical software (Version 8.02, SAS Institute Inc., Cary, NC). Gas efflux, as well as rhizosphere and specific rhizosphere respiration data were analyzed as two-way analyses of variance (ANOVA) test for the effect of growth stage and crop. Root, shoot and soil samples were analyzed separately to test the effect of growth stages and crop type on δ^{13}C levels and mass of ^{13}C enrichment in the roots and shoots. Growth stages were not considered to be repeated measures because they were represented by destructive sampling of random replicates (Cheng et al., 2003). Least square (LS) means were separated at a 95% confidence level using the least significant difference (LSD) method.

3.4. RESULTS AND DISCUSSION

3.4.1. Plant biomass

Plant growth appeared to be normal, with no symptoms of pests or pathogens. Generally, corn plants produced more biomass than soybean plants at all growth stages of the plants (Fig. 2). We observed a 3.2, 3.1 and 3.4-fold higher level of corn biomass than soybean biomass on days 20, 60 and 80 respectively. These results support our classification of corn as rapid-growing (Poorter et al., 1991), and soybean with a low RGR ranging between 97.8 and 138.2 (Barkosky and Einhellig, 2003), as slow-growing.

3.4.2. ^{13}C enrichment in soil under corn and soybean

Plant phenology (sampling date) did not affect the $\delta^{13}C$ levels, the mass of ^{13}C enrichment or the C content in the soil (Tables 1a, 1b and 1c). However, $\delta^{13}C$ levels and mass of ^{13}C enrichment varied significantly between corn and soybean-planted soils as shown by the significant treatment effect observed (Tables 1a and 1b). Enrichment of ^{13}C in the soil was highest in the corn pots, with $\delta^{13}C$ values averaging -18.85‰, compared to a $\delta^{13}C$ value of -21.2 ‰ and -20.18 ‰ in the in the soybean pots and control soils respectively. This was similar to results obtained by Qian et al. (1997), who reported that over time, soils planted under corn, had a greater (less negative) δ^{13}C value than the original soil. This observation is due to the fact that the photosynthetic pathway of C_3 plants such as soybean discriminate against the heavier ^{13}C isotope, and have a mean δ^{13}C level of -27‰ compared to C_4 plants like corn that have an average δ^{13}C value of -12‰ (Smith and Epstein, 1971). Over time therefore, under soybean, the δ^{13}C values of below-ground C assumes the δ^{13}C signature of the plant (Smith and Epstein, 1971). Soil total C did significantly change with sampling date or crop type (Table 1c, Fig. 3a).

3.4.3. ^{13}C partitioning in corn and soybean

Generally, carbon allocated to below-ground organs has various possible fates: carbon may be incorporated into root and nodule biomass; respired for synthesis and maintenance processes; or used to provide carbon skeletons for the amino compounds that are translocated to the shoots (Voisin et al., 2003). Our results in Table 1a show that sampling date significantly affected δ^{13}C levels in shoots and roots, but did not significantly affect the mass of ^{13}C enrichment (Table 1b). Generally, crop type significantly affected the δ^{13}C levels and mass of ^{13}C enrichment in the shoots and roots (Tables 1a), with corn shoots having an average mass of ^{13}C enrichment of 4.27 mg pot^{-1} compared to 0.99 mg pot^{-1} in the soybean shoots. Similarly, crop type significantly affected ^{13}C enrichment in roots (Table 1b), with a significantly higher mass of ^{13}C enrichment in corn roots (1.11 mg pot^{-1}) than soybean roots (0.13 mg pot^{-1}). On the average, corn roots contained an estimated

23.6% of the total ^{13}C per plant whilst soybean roots only contained 7.2% of the total ^{13}C per plant. These observations are consistent with findings that C_3 plants show more discrimination against ^{13}C than C_4 plants, during their respective photosynthetic pathways (Hobbie and Werner, 2004; O'Leary, 1988), which confounds any possible inferences into differences in the C utilization and partitioning between the roots and shoots of the corn and soybean plants. Thus, in contrast to our initial hypothesis, it was difficult to isolate differences in photosynthesis as well as translocation of newly assimilated C to the roots of corn and soybean, using the pulse labeling method.

3.4.4. Soil respiration

Generally, we observed very high significant positive correlations between CO_2 production and O_2 consumption (Pearson's coefficient $r = 0.97$). Soil respiration and O_2 consumption were not significantly affected by sampling date but varied significantly between the corn, soybean and control pots (Table 3). Except for day 80, when CO_2 efflux and O_2 consumption was not significantly different between the soybean and the unplanted control pots, the general trend we observed was higher CO_2 efflux and O_2 consumption in the planted soils than the unplanted control soils (Table 4). This observation is an indication of the significant contribution of rhizosphere respiration to soil respiration. At all growth stages however, we observed higher levels of soil respiration and O_2 consumption in the corn pots than the soybean pots (Table 4). The higher rate of soil respiration coupled with higher rates of C assimilation/fixation through photosynthesis adds to the growing body of literature that demonstrate a strong correlation between photosynthesis and root or total soil respiration (Irvine et al., 2005).

3.4.5. Rhizosphere respiration

Rhizosphere respiration was significantly affected ($P < 0.05$) by sampling date and treatment type (Table 5). We estimated an average of about 79% of the soil respiration in the corn pots came from rhizosphere respiration, whilst in the soybean pots, rhizosphere respiration constituted 58% of the soil respiration. This observation falls in the range of estimates for rhizosphere respiration of 30 to 80% of total below-ground efflux of CO_2, observed in various ecosystems (Rochette and Flanagan, 1997; Hanson et al., 2000). Rhizosphere respiration was much higher in corn than in soybean, especially on day 60 (Fig. 4a). This is likely due to the fact that corn produces a greater quantity of C-rich rhizodeposits, which have the capacity to stimulate a greater level of microbial respiration than soybean (Kogel-Knabner, 2002). In soybean, rhizosphere respiration peaked around the flowering stage and decreased significantly by the pod-filling stage, in a similar pattern to that reported by Fu et al. (2002), who observed rhizosphere respiration in soybean increasing up until the flowering stage, then declining thereafter. Thus, rhizosphere respiration varied with plant phenology being highest in the milk stage for corn and the flowering stage in the case of soybean. Our results suggest that any estimation of CO_2 production from corn and soybean agroecosystems must take into consideration the growth stages of the plants in order to provide more accurate estimates.

3.4.6. Specific rhizosphere respiration

Specific rhizosphere respiration (Fu et al., 2002) can serve as an effective index of root activity because rhizosphere respiration is normalized with root biomass. Treatment and sampling date significantly affected the specific rhizosphere respiration (Table 5), whilst in both corn and soybean, specific rhizosphere respiration declined significantly over time (Fig. 4b), indicating a decrease in the root activity with plant maturity, and is similar to the findings of various studies (Fu et al., 2002; Keith et al., 1986; Kuzyakov and Domanski, 2000; Swinnen et al., 1994). This is because generally, younger plants respire more strongly than older plants, and growing parts of the plant like the roots respire at a particularly high intensity (Larcher, 1995). Our results show that on average, specific rhizosphere respiration was significantly higher in soybean (0.29 mg C g^{-1} root h^{-1}) than corn (0.09 mg C g^{-1} root h^{-1}), and this difference was more pronounced in the early stages of growth (Fig. 4b). Thus on average, on a per unit mass of tissue basis, soybean rhizosphere was more active than the corn rhizosphere. This observations is contrast with findings that corn roots input about 3400 kg C ha^{-1} into the soil as rhizodeposits, while soybean roots contribute only a fraction of this amount at 1300 kg C m^{-2} (Kogel-Knabner, 2002), thereby suggesting that the differences in the specific rhizosphere respiration we observed could not have been due to differences in the root-induced respiration component of rhizosphere respiration, but most likely due to the differences in the root respiration component between the two crops.

Our results agree with those observed by Poorter et al. (1991), who reported that although slow-growing species (e.g. soybean) have lower rates of root respiration than rapid-growing species (e.g. maize), considering the low rate of plant growth and nutrient uptake, the rate of root respiration of slow-growing plants was relatively higher. Scheurwater et al., (1998) observed that on average rapid-growing species had a 1.4-fold higher rate of root respiration than the slow-growing ones, despite their 2-fold higher relative growth rate (RGR). Similarly, we observed a 1.84 and 1.89-fold higher rate of rhizosphere respiration in the soybean pots than the corn pots on days 20 and 60 respectively. Several factors may be responsible for this disparity.

The possibility of a greater degree of inhibition of root respiration in the faster-growing plants, as CO_2 concentration accumulate in the root enclosure/pots has been investigated and found to be mostly insignificant (Bouma et al., 1997a,b; Poorter et al., 1991; Scheurwater et al.,1998). Some past studies have reported a diurnal variation in root respiration (Hansen, 1980, Neals and Davis, 1965). Considering that our methodology estimated specific rhizosphere respiration over a 24-h period, spanning the light and dark phases, the effect of these diurnal variations on our estimates of specific rhizosphere respiration is not clear. However, to our knowledge, the only study that has investigated the diurnal variations in root respiration between slower-growing and faster-growing plant species was that by Scheurwater et al. (1998), who found no distinct differences between the light and dark periods of rapid-growing and slow growing plants. Differences in the activity of alternate pathways in respiration, relating to how efficiently rapid- and slow-growing species produce ATP, have also been investigated and found to be not significant in their contribution to the relatively higher levels of root respiration in slower-growing plants (Poorter et al., 1991; Scheurwater et al., 1998).

Poorter et al. (1991) discussed the possibility that the relatively higher turnover of carbohydrates in slower-growing species compared to more rapid-growing ones (Farrar, 1989) may enhance the respiration cost of root maintenance in the roots of rapid-growing plants, and estimated that root maintenance only accounts for about 4% of total root respiration. In addition, Poorter et al. (1991) observed that specific respiratory cost of growth was higher in rapid-growing plants and could not possibly explain the discrepancy. Similarly, Scheurwater et al. (1998) found slightly higher cost of root maintenance in rapid-growing grass species than the slower growing species.

By exclusion of the other possibilities, Poorter et al. (1991) speculated that the variation in the specific cost of nutrient acquisition was mostly responsible for the unexpected low rate of root respiration of rapid-growing species. In support of this theory, they estimated the O_2 consumption necessary for the uptake of one mol of anion and concluded that the cost of nutrient uptake was three times as low in rapid-growing species as for the slow-growing ones. Nutrient uptake accounted for 50-70% of root respiration compared to an estimated 20-45% of root respiration attributed to growth (Poorter et al., 1991). Thus, although plant growth contributes more to root respiration in rapid-growing plants compared to slow-growing plants, this is often overshadowed by the lower cost of nutrient uptake in the rapid-growing plants. Similarly, Scheurwater et al. (1998) observed that the specific cost of nutrient uptake in grasses (obtained by plotting the rate of root respiration against the rate of net nitrate uptake) was three times as high in the rapid-growing grass species than the slower-growing species. We speculate that nutrient acquisition by the slower-growing soybean roots was a relatively more energy-intensive process than the faster-growing corn.

3.4.7. Methodological limitations

The root exclusion technique employed in this study has the disadvantage of not being able to account for the interaction between the plant roots and the soil organic matter and vise versa. This is because the rhizosphere and soil organic matter may be linked through rhizosphere interactions (Andrews et al., 1999), and may exert a priming effect or a suppressive influence on the soil organic matter decomposition (Van Veen et al., 1991). Plant roots affect soil physical properties such as porosity and aeration, which all impact on soil organic matter decomposition. In addition, in a pot study like ours, there are certain inherent drawbacks including a restriction of the normal root, water and gas distribution and movement in the soil, and it is often difficult to determine the optimal volume of soil required under such conditions (Fu et al., 2002).

3.5. CONCLUSIONS

Generally, CO_2 efflux and O_2 consumption was higher in the planted soils than the unplanted control soils, an indication of the significant contribution of rhizosphere respiration to soil respiration. We estimate an average of about 78.9% of the soil respiration in the corn pots came from rhizosphere respiration whilst in the soybean pots rhizosphere respiration constituted 57.9% of the soil respiration. Although we observed a higher rate of rhizosphere respiration in corn than soybean, at all stages of growth, specific rhizosphere respiration was significantly higher in soybean (0.29 mg C g^{-1} root h^{-1}) than corn (0.09 mg C g^{-1} root h^{-1}), which supports previous observations made with regards to slower-growing plants (e.g. soybean) having relatively higher root respiration than faster growing plants (e.g. corn).

Table 1. Analysis of variance table for a) $\delta^{13}C$ (‰) b) mass of ^{13}C enrichment and (mg ^{13}C pot^{-1}) and c) total C (%), in soil, shoots and roots, as affected by sampling date(20, 60 and 80 days after seeding), crop type (corn and soybean) and their respective interactions.

a)

		Soil		**Shoots**		**Roots**	
Source	**df**	**F Value**	**Pr > F**	**F Value**	**Pr > F**	**F Value**	**Pr > F**
Sampling date (A)	2	0.06	0.9439	44.03	<.0001	12.73	0.0003
Crop type (B)	1	21.52	0.0002	13.1	0.0017	3.87	0.0633
A*B	2	0.44	0.6505	4.77	0.0203	0.13	0.8768

b)

		Soil		**Shoots**		**Roots**	
Source	**df**	**F Value**	**Pr > F**	**F Value**	**Pr > F**	**F Value**	**Pr > F**
Sampling date (A)	2	0.03	0.9711	1.69	0.2184	2.43	0.1152
Crop type (B)	1	19.12	0.0003	4.66	0.0475	16.7	0.0006
A*B	2	0.41	0.6705	2.78	0.094	2.44	0.114

c)

		Soil		**Shoots**		**Roots**	
Source	**df**	**F Value**	**Pr > F**	**F Value**	**Pr > F**	**F Value**	**Pr > F**
Sampling date (A)	2	2.30	0.1263	55.06	<.0001	33.28	<.0001
Treatment (B)	1	1.33	0.2623	85.33	<.0001	16.39	0.0007
A*B	2	0.50	0.6146	7.23	0.0063	3.67	0.0449

Table 2. Means of $\delta^{13}C$, ^{13}C content in soil, shoots and roots of corn and soybean (soy) treatments. The % ^{13}C recovery represents the ratio of the total recovered ^{13}C to the total ^{13}C used in the labeling process (1700 mg). Means with similar letters are not significantly different at $p<0.05$. $\Delta^{13}C$ represents the mass of ^{13}C enrichment.

Crop	Sampling date	Soil ^{13}C		Shoot ^{13}C		Root ^{13}C		% ^{13}C recovery (% per pot)
		$\delta^{13}C$ (‰)	$\Delta^{13}C$ (mg/pot)	$\delta^{13}C$ (‰)	$\Delta^{13}C$ (mg/pot)	$\delta^{13}C$ (‰)	$\Delta^{13}C$ (mg/pot)	
Corn	20	-19.02^{b}	0.34^{a}	260.25^{c}	2.39^{a}	360.21^{c}	1.03^{ab}	0.21
Soy	20	-20.80^{a}	-0.17^{a}	274.42^{c}	1.50^{a}	220.01^{b}	0.28^{a}	0.10
Corn	60	-18.84^{b}	0.42^{a}	127.43^{b}	1.61^{a}	66.23^{a}	0.52^{a}	0.15
Soy	60	-21.16^{a}	-0.31^{a}	41.30^{ab}	0.99^{a}	-16.31^{a}	0.02^{a}	0.04
Corn	80	-18.63^{b}	0.47^{a}	118.57^{b}	9.93^{b}	70.07^{a}	1.93^{b}	0.73
Soy	80	-21.64^{a}	-0.39^{a}	-19.93^{a}	0.24^{a}	-24.60^{a}	0.01^{a}	-0.01

Table 3. Analysis of variance table for the CO_2 efflux (mg C pot^{-1} h^{-1}) and O_2 (mg O_2 pot^{-1} h^{-1}) consumption as affected by sampling date (20, 60 and 80 days after seeding) and treatment (corn, soybean and control) and their respective interactions.

		CO_2 efflux		**O_2 efflux**	
Source	**df**	**F Value**	**Pr > F**	**F Value**	**Pr > F**
Sampling date (A)	2	1.55	0.2285	3.18	0.0551
Treatment (B)	2	85.14	<0.0001	73.59	<0.0001
A*B	4	2.26	0.0850	2.77	0.0441

Table 4. Mean CO_2 efflux (mg C pot^{-1} h^{-1}) and O_2 consumption (mg O_2 pot^{-1} h^{-1}) in corn, soybean and control pots at different sampling dates and overall means for the 3 sampling dates (20, 60 and 80 days after seeding). Means with similar letters are not significantly different at $p<0.05$.

Treatment	**Sampling date**	**CO_2 efflux**	**O_2 consumption**
Corn	20	0.23^{d}	0.68^{d}
Soy	20	0.16^{c}	0.50^{c}
Control	20	0.08^{ab}	0.28^{ab}
Corn	60	0.25^{d}	0.76^{d}
Soy	60	0.13^{bc}	0.43^{bc}
Control	60	0.03^{a}	0.13^{a}
Corn	80	0.26^{d}	0.73^{d}
Soy	80	0.08^{ab}	0.19^{a}
Control	80	0.05^{a}	0.17^{a}

Averages

Treatment	**CO_2 efflux**	**O_2 consumption**
Corn	0.25^{c}	0.73^{c}
Soy	0.12^{b}	0.38^{b}
Control	0.05^{a}	0.19^{a}

Table 5. Analysis of variance table for rhizosphere respiration (mg C pot^{-1} h^{-1}) and specific rhizosphere respiration (mg C g^{-1} soil h^{-1}) as affected by sampling date (20, 60 and 80 days after seeding) and treatment (corn, soybean and control) and their respective interactions.

		Rhizosphere respiration		**Specific rhizosphere respiration**	
Source	**df**	**F Value**	**Pr > F**	**F Value**	**Pr > F**
Sampling date (A)	2	5.14	0.0158	7.91	0.0029
Treatment (B)	1	36.29	<0.0001	6.31	0.0207
A*B	2	2.46	0.1110	1.76	0.1982

Figure 1. Experimental design for ^{13}C pulse-labeling grain corn (*Zea mays L.*) and soybean (*Glycine max*) sampling gases from soil, adapted from Bromand et al., 2001.

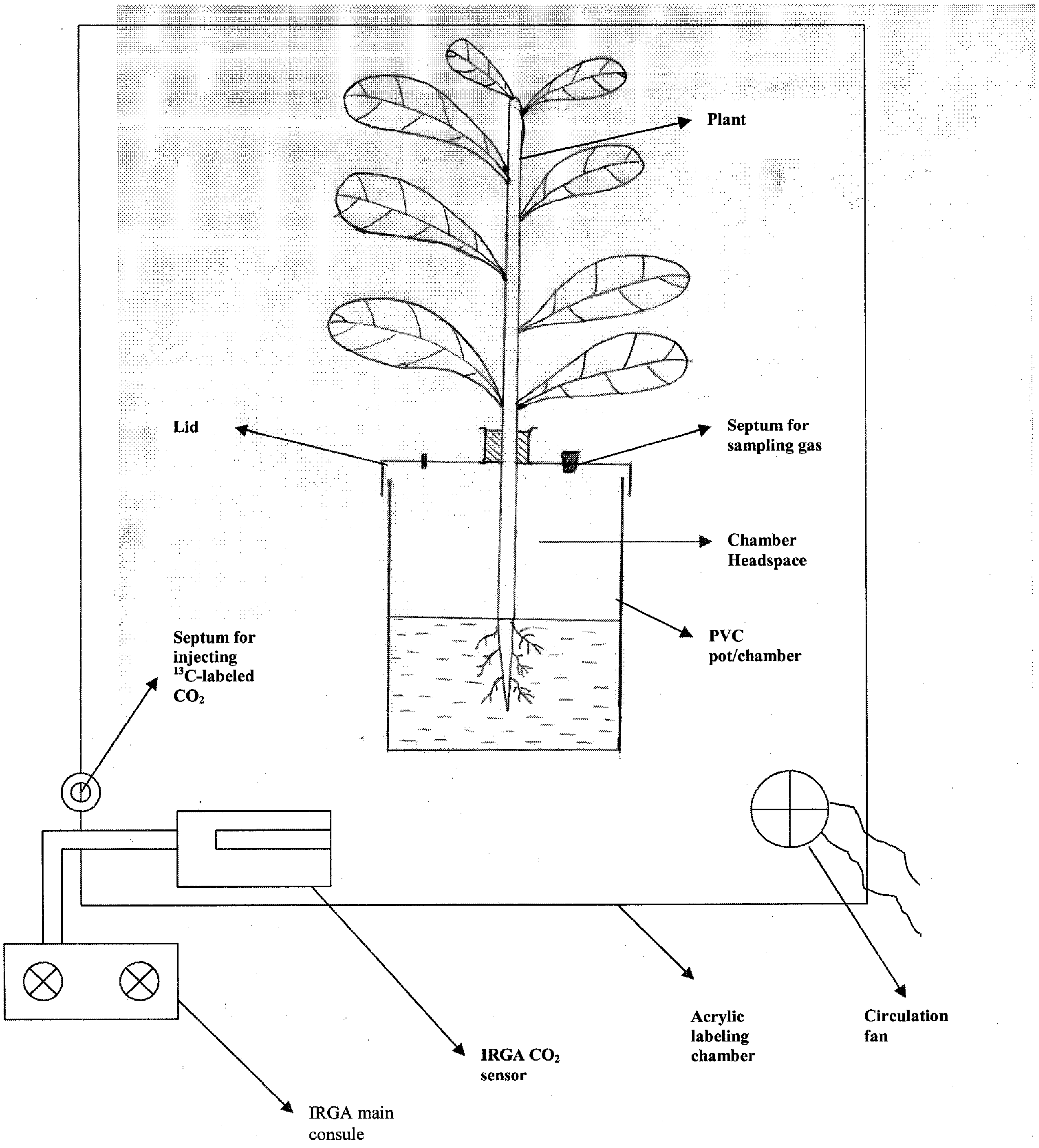

Plant
Lid
Septum for sampling gas
Chamber Headspace
PVC pot/chamber
Septum for injecting ^{13}C-labeled CO$_2$
Acrylic labeling chamber
Circulation fan
IRGA CO$_2$ sensor
IRGA main consule

Figure 2. Mass of shoots and roots of corn and soybean (soy) recovered from pots after each sampling event at 20, 60 and 80 days after seeding. Vertical bars represent the standard errors.

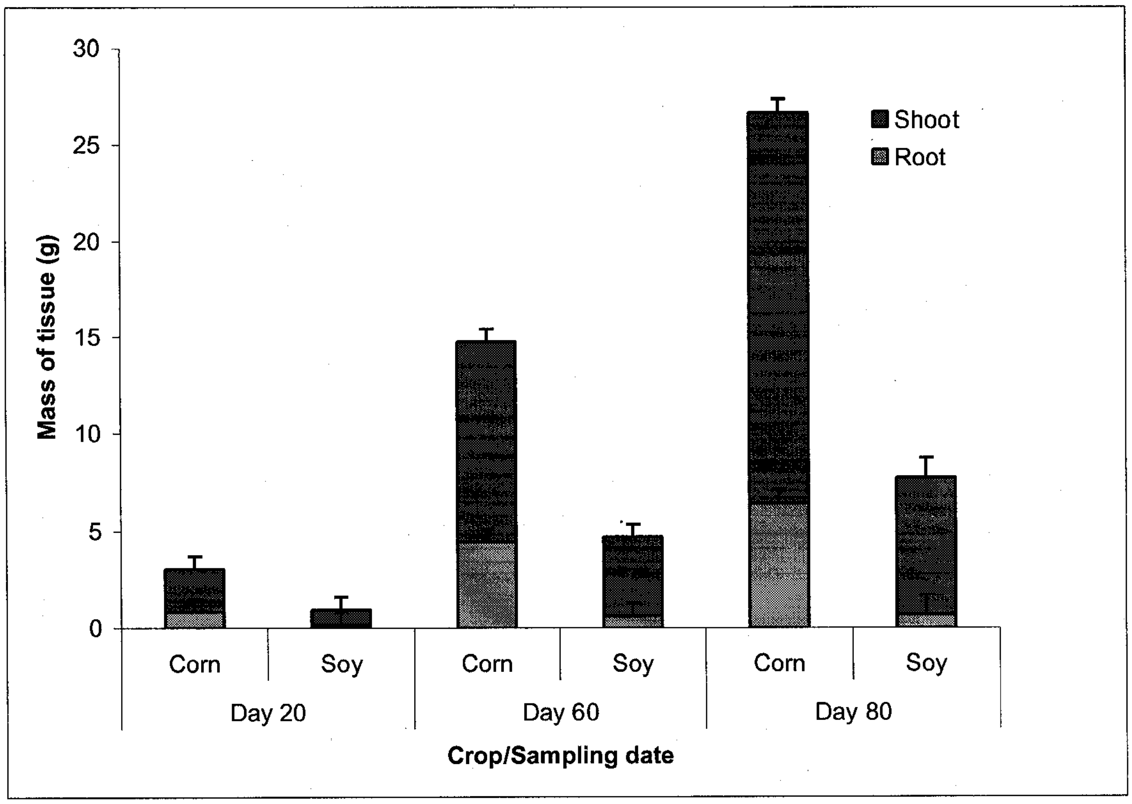
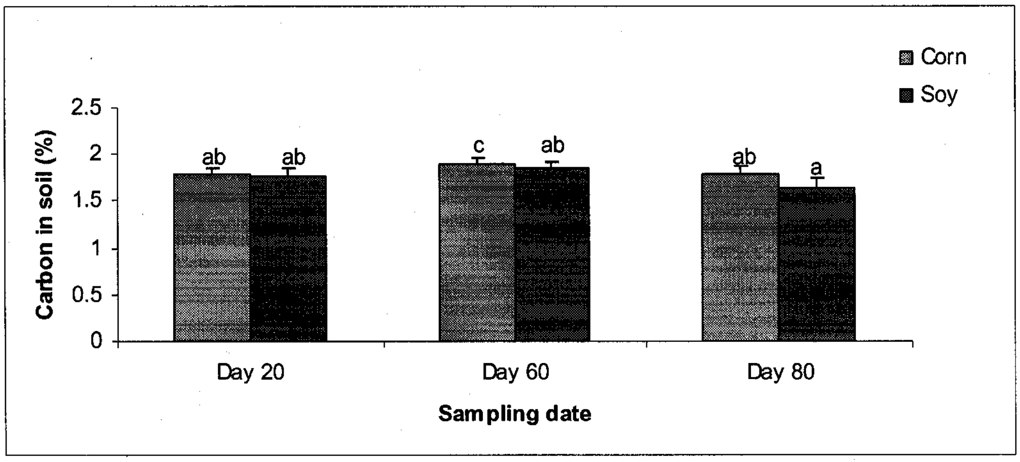
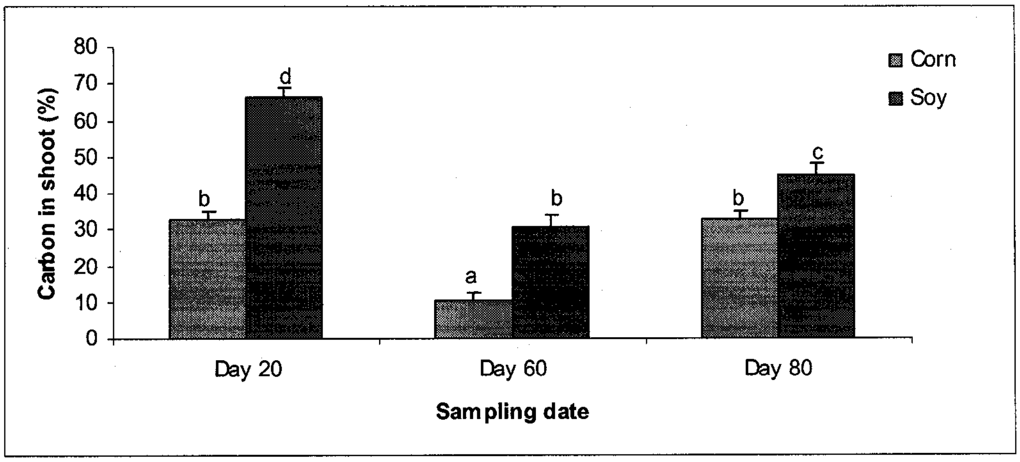

30
25
20
15
10
5
0
Mass of tissue (g)
Shoot
Root
Corn
Soy
Corn
Soy
Corn
Soy
Day 20
Day 60
Day 80
Crop/Sampling date

Figure 3. Percentage C content in a) soil, b) shoots and c) roots after each sampling event at 20, 60 and 80 days after seeding. Vertical bars represent the standard errors. Bars with identical letters are not significantly different at $P<0.05$.

a)


Carbon in soil (%)
2.5
2
1.5
1
0.5
0
ab
ab
c
ab
ab
a
Day 20
Day 60
Day 80
Sampling date
▨ Corn
■ Soy

b)


Carbon in shoot (%)
80
70
60
50
40
30
20
10
0
b
d
a
b
b
c
Day 20
Day 60
Day 80
Sampling date
▨ Corn
■ Soy

c)

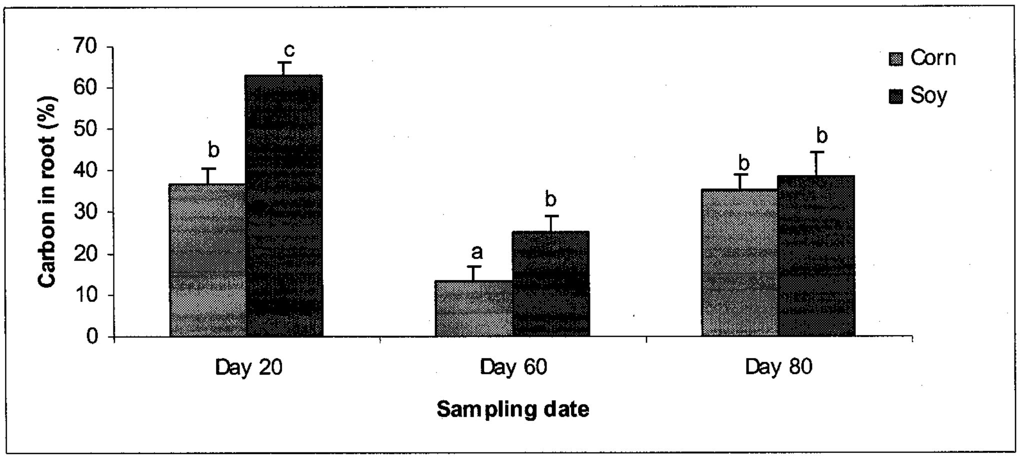
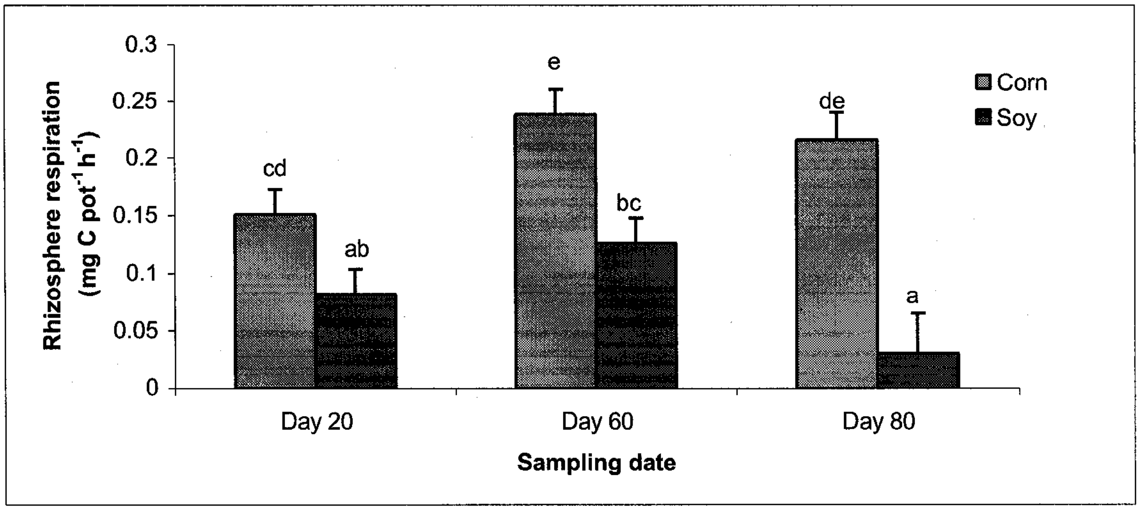
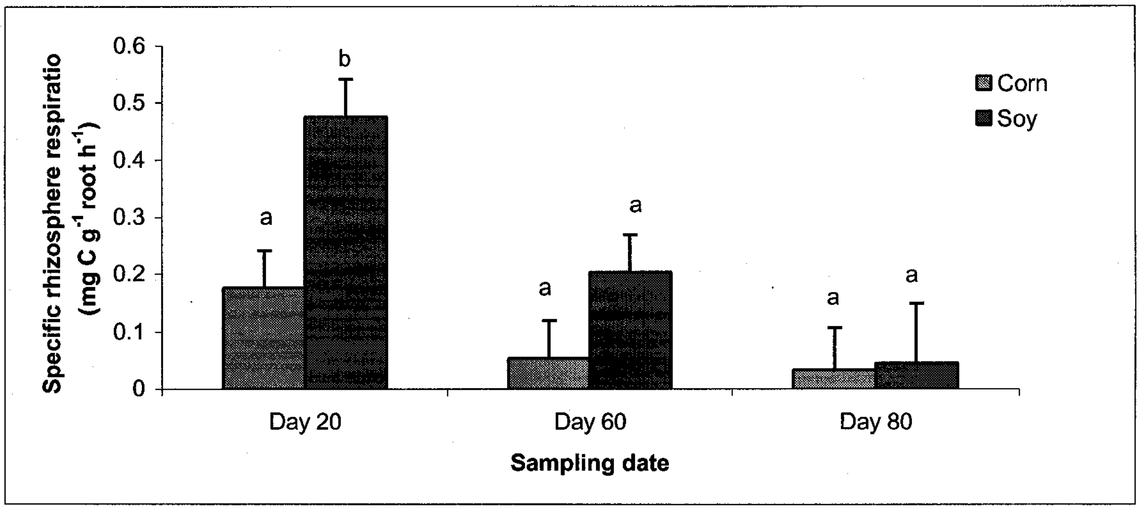

Carbon in root (%)
70
60
50
40
30
20
10
0
b
c
a
b
b
b
Day 20
Day 60
Day 80
Sampling date
▨ Corn
■ Soy

Figure 4. a) Rhizosphere respiration and b) Specific rhizosphere respiration in pots seeded with corn (*Zea mays L.*) and soybean (*Glycine max*) at 20, 60 and 80 days after seeding. Vertical bars signify the standard errors. Bars with identical letters are not significantly different at *P*<0.05.

a)


Rhizosphere respiration
(mg C pot^{-1} h^{-1})
0.3
0.25
0.2
0.15
0.1
0.05
0
cd
ab
e
bc
de
a
▨ Corn
■ Soy
Day 20
Day 60
Day 80
Sampling date

b)


Specific rhizosphere respiratio
(mg C g^{-1} root h^{-1})
0.6
0.5
0.4
0.3
0.2
0.1
0
a
b
a
a
a
a
▨ Corn
■ Soy
Day 20
Day 60
Day 80
Sampling date

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CONNECTING PARAGRAPH

In the Chapter 3, the first of our greenhouse studies, we investigated the differences in the rhizosphere and specific rhizosphere respiration in corn and soybean systems, and discussed how they related to total soil respiration. The root exclusion method served a valuable tool for estimating the differences in soil and rhizosphere respiration between corn and soybean. We discovered that although corn rhizosphere produced more CO_2 than soybean rhizosphere, on a per unit mass of root biomass basis, soybean rhizosphere produced more CO_2 than corn rhizosphere. In our second greenhouse study, represented in the next chapter, we will investigate the dynamics of fertilizer N uptake and utilization in the corn and soybean plants and how this relates to the efflux of N_2O, as well as N loss from the root zone.

CHAPTER 4: NITROUS OXIDE EFFLUX FROM N-FERTILIZED CORN AND SOYBEAN

4.1. ABSTRACT

Direct and indirect emissions of nitrous oxide (N_2O) from agricultural systems contribute an estimated 6.2 Tg N_2O-N yr^{-1} out of the 17.7 Tg N_2O-N yr^{-1} produced globally, and most of it is associated with the use of N fertilizer. The objective of this study was to investigate how partitioning of newly incorporated N between the roots and shoots affects the N_2O efflux from corn and soybean systems at different growth stages. The experiment was conducted in a greenhouse under natural lighting at an air temperature of approximately 30°C. A solution containing 10% ^{15}N-labelled KNO_3 fertilizer was applied to pots seeded with corn or soybean at a rate of 100 mg N per pot to those with the N fertilizer treatment after which the opening in the lid for the plant shoot was sealed with low melting point paraffin wax. Headspace gas concentrations were then sampled after three hours later using a 20-ml syringe. Soil ^{15}N concentration was lowest in corn pots during the early vegetative stage and tasseling stage and highest during the pod-filling stage of soybean pots. Shoot uptake of ^{15}N was not significantly different between corn and soybean except for day 80, when corn plants were in the milk stage and soybean were in the pod-filling stage. On the average however, corn shoots and roots contained significantly higher levels of ^{15}N than soybean shoots and roots. Our results show that N_2O efflux did not correspond well with ^{15}N losses observed suggesting however this disparity was related to the difficulty involved in maintaining uniform soil water content in this pot study.

4.2. INTRODUCTION

Direct and indirect emissions of nitrous oxide (N_2O) from agricultural systems are now thought to contribute 6.2 Tg N_2O-N yr^{-1} out of the 17.7 Tg N_2O-N yr^{-1} produced globally, and most of it is associated with the use of N fertilizer (Kroeze et al., 1999; Baggs et al., 2003). In Canada alone, N_2O gas emissions account for about two-thirds of the greenhouse gas emissions, expressed on a carbon dioxide (CO_2) equivalent basis, with agricultural sources contributing about 60% of the total anthropogenic emissions of N_2O (Janzen et al., 1999). The N_2O emitted from these soils results from microbial activity in the nitrification and denitrification pathways (Firestone 1982; Payne, 1973).

Generally, emissions of N_2O from agricultural soils have been associated with the use of N fertilizers (Nielsen et al., 1996; Paul et al., 1994). For example, N fertilizer loss as N_2O from fertilized wheat, barley, beet, and rape fields in Germany have been estimated as between 1-8% of fertilizer N (Kaiser et al., 1998). In a agricultural field setting, measured N_2O and is also affected by agricultural management factors such as tillage (Grevers and de Jong, 1982; Aulakh et al., 1984), and crop type (O'Hara and Daniel, 1985), however, although generally, researchers have a good understanding of many of the individual factors that affect N_2O production from soils, a true understanding of how these factors interact in agricultural soils have been more elusive (Mosier, 1994).

It is also known that soil associated with the rhizosphere, close to plant roots, is known to support a diverse microbial community and high microbial activity

(Barber and Lynch, 1977; Foster, 1988; Bazin et al., 1990), which may stimulate the production of N_2O. The presence of plant roots has a significant effect on the soil microbial population and activity, because conditions for microbial growth are especially favorable in the rhizosphere due to the process of rhizodeposition. Rhizodeposits are easily decomposable substrates translocated from the aboveground parts of the plant to the roots and subsequently transferred into the surrounding soil as root exudates, mucilage, and sloughed cells and tissues (Qian et al., 1997). It has been estimated that two-third of the O_2 consumption in the rhizosphere is attributed to plant roots, and the remaining one-third results from root-associated microbes (Woldendorp, 1962). Plant root respiration as well as root-induced respiration from the decomposition of root rhizodeposits by root-associated bacteria may deplete the level of O_2, leading to an increased N_2O production in the rhizosphere. Legumes and non-legumes differ in their C composition of rhizodeposits. For example, although corn roots are estimated to input about 3400 kg C ha^{-1} into the soil as rhizodeposits, soybean roots contribute only a fraction of this amount at 1300 kg C m^{-2} (Kogel-Knabner, 2002), the significance of this difference in N_2O production from corn and soybean Agroecosystems is not clear. Thus, Smith and Tiedje (1979) reported that the potential for denitrification was much higher near corn roots (*Zea mays* L.) and decreased rapidly in the first few millimeters of soil away from the roots. However, this rhizosphere-induced denitrification may be limited by NO_3-N availability as soil moisture increases above field capacity. This is because the low O_2 availability limits the nitrification process, which produces the NO_3-N needed for N_2O production. In some cases, there is no increase in denitrification when plants are present, probably due to competition between roots and denitrifying bacteria for NO_3-N (Guenzi et al., 1978). The complexity in studying differences in loss of fertilizer N in the form of N_2O is also related to the fact that differences exist in the fertilizer N use of legumes and non-legumes. Leguminous crops such as soybean (*Glycine max* (L.) Merr.), that fix N_2 from the atmosphere are known to increase the soil mineral N pool, thereby providing additional substrate for N_2O production through nitrification and denitrification (Rochette et al., 2004).

Although legumes and non-legumes differ in the composition of rhizodeposits and fertilizer N use efficiencies (Singh, 2004), which may ultimately affect N_2O production from such Agroecosystems, there has been very little work done to investigate how N_2O production varies in the rhizosphere-associated soils of legumes and non-legumes.

The objectives of this study were 1) to investigate the N_2O production from rhizosphere-associated soil (corn and soybean) and bulk soil receiving N fertilizer, and 2) to investigate the partitioning of incorporated N between the roots and shoots and determine how N partitioning affects the production of N_2O in rhizosphere-associated soil of corn and soybean at different growth stages. We hypothesize that N fertilization will lead to a greater N_2O production in the soybean as compared to corn as a result of a lesser demand on soil NO_3-N pool due to N fixation in the soybean.

4.3. MATERIALS AND METHODS

4.3.1. Experimental setup

The experiment was conducted in a greenhouse under natural lighting at an air temperature of approximately 30°C. The soil, a Humic Gleysol (fine silty, mixed Typic Endoaquent, pH 6.1) was taken from the top 10-cm of the Macdonald Research Farm, Ste. Anne de Bellevue, Quebec. The soil was a mixed, frigid Typic Endoaquent of the St. Damase Series (Lajoie and Baril, 1953), containing 700 g kg^{-1} of sand, 140 g kg^{-1} of silt and 160 g kg^{-1} of clay with 15.4 g organic C kg^{-1} and pH 6.1 in the top 15 cm. The sandy-loam layer (28 cm mean thickness) was underlain by sand (6 cm mean thickness) and clay starting at depths below 34-cm, on average. The soil was sieved through 2-mm sieve to remove the coarse fractions, homogenized and air-dried (Cheng et al., 1993; Kuzyakov and Siniakina, 2001). We added Na_3PO_4 to the soil as a P fertilizer at a rate of 0.28 g P kg^{-1} and then packed 1.5 kg of soil into pots (PVC pipe, 30-cm height and 10-cm diameter) to a bulk density of 1.12 g cm^{-3}. The bottom of the pot was sealed to prevent N loss via leaching. The pots were covered with lids containing an opening for the plant shoot (Fig.1). Each lid was fitted with a rubber septa for gas sampling from the headspace of the closed chamber. Soil moisture content was maintained at 60% water-filled pore space (WFPS) throughout the experiment by monitoring the weight of the pots and adding distilled water when necessary.

The experiment consisted of three treatments (five replicates per treatment) arranged in completely randomized design: corn + N fertilizer (Corn + N), soybean + N fertilizer (Soy + N) and bulk soil + N fertilizer (Soil + N). Five corn seeds (*Zea* *mays L.*) and five soybean seeds (*Glycine max* (L.) Merr.), were planted in the Corn + N and Soy + N treatments, and then thinned to one seedling per pot after a week. The pots were sampled at three growth stages (on day 20, day 60 and day 80) for headspace gas concentration (described below). On day 20, corn was at the early vegetative growth stage (V1 stage) while soybeans were at the second node stage (V3 stage). By day 60, the corn was at the VT (tasseling) stage and soybeans were at the R2 (flowering) stage, while on day 80, corn was at the R3 (milk) stage and soybeans were at the R6 (pod-filling) stage (Ontario Ministry of Agriculture, Food and Rural Affairs, 2002). Soil and plant tissues were destructively sampled after each gas-sampling event in order to determine ^{15}N content. In addition to the experimental pots, three unfertilized control treatments (five replicates each of unseeded soil, pots seeded with corn and pots seeded with soybean) were destructively sampled on day 80 to determine the background levels ^{15}N in soil, corn tissue and soybean tissue.

4.3.2. ^{15}N-labelled fertilizer application and gas sampling

At each gas sampling event, a solution containing 10% ^{15}N-labelled KNO_3 fertilizer was applied at a rate of 100 mg N per pot to those with the N fertilizer treatment after which the opening in the lid for the plant shoot was sealed with low melting point paraffin wax. Headspace gas concentrations were then sampled after three hours later using a 20-ml syringe. Gas samples were taken 1 h ($t = 1$) and 24 h ($t = 2$) after sealing the headspace with paraffin wax (Fig. 1). The gas samples were transferred to 12-ml previously-evacuated exetainers (LABCO, Wycombe, UK) and analyzed for N_2O content (ppm v/v) using a Varian Model 3800 gas chromatograph

(Walnut Creek, CA, U.S.A.) equipped with automated valve injectors to simultaneously quantify CO_2 and N_2O concentrations. A Haysep A column followed by a molecular sieve and He carrier at 46 ml min^{-1} were used to separate CO_2 from O_2 whereas N_2O was quantified on a Porapak Q column with Ar/CH_4 (90:10) carrier gas at 20 ml min^{-1}.

4.3.3. Gas efflux calculations

Units of gas (CO_2, or N_2O) as expressed in ppm v/v is equivalent to 1cm^3 of gas per m^3 of air. From the ideal gas equation:

$$PV = nRT \qquad [1]$$

where $P \approx$ atmospheric pressure (1 atm), V is the volume of gas (cm^3), n is the number of moles of gas, R is the ideal gas constant (82.06 atm cm^3/mol K) and T is the temperature under which pots were incubated expressed in Kelvins (303 K).

Rearranging,

$$n = {}^{PV}\!/_{RT} \qquad [2]$$

Since density d is mass of gas (g) per unit volume (V) and the mass of gas is moles of gas times molecular mass of gas (M)

$$d = {}^{g}\!/_{V} = M({}^{n}\!/_{V}) \qquad [3]$$

Combining equations (2) and (3),

$$d = \frac{MP}{RT} \qquad [4]$$

Multiplying the concentration of gas (cm^3 m^{-3}) with the density of the gas, d (g cm^{-3}) yields concentration of the gas in the headspace expressed as g m^{-3} of the headspace.

Conversion to mg m^{-3} or ug m^{-3} was done by multiplying by 1000 or 1 x 10^6 respectively, after which we multiplied by the volume of the headspace ($\approx 6.77 \times 10^{-4}$ m^3) in order to determine the gas concentration on a per pot basis (μg pot^{-1}). Gas efflux into the chamber headspace, f (μg pot^{-1} h^{-1}) was estimated as:

$$f = {(C_2 - C_1)}/{t} \qquad [5]$$

where C_2 and C_1 are the concentrations of gas (μg pot^{-1}) at time $t = 2$ and $t = 1$ respectively and t is the duration between sampling events (23 h). Gas efflux was converted to per C or N basis by multiplying by the mass ratio of carbon atoms per molecule of CO_2 (12/44) or the mass ratio of nitrogen atoms per molecule of N_2O (28/44).

4.3.4. Shoot, root and soil analyses

After gas sampling, plants were cut at the base, the root-soil column was pulled out and the roots were handpicked from the soil. Roots and shoots were then washed with deionized water to remove soil particles, dried in an oven (65°C for 48 hours) and weighed. Soil samples were air-dried for about a week at 25°C. Plant tissue and soil samples were then finely ground (<0.5 mm mesh) and weighed into tin capsules for analyses of $^{15}N/N$ content. Delta ^{15}N (δ ^{15}N) and total N content in the samples were determined by flash combusting the samples at 1800°C in an EA 1110 elemental analyzer (CE Instruments, Italy). The resulting gases were carried by helium through the EA for purification and separation into N_2 and CO_2, and then to a DeltaPlus Advantage isotope ratio mass spectrometer (ThermoFinnigan, Germany) for isotopic analysis. Data was normalized using internal standards. Analytical precision was +/- 0.2 permil (‰). However, we could not analyze soil samples from the soybean pots taken after the first sampling event (day 20) due to accidental contamination of the samples at the greenhouse. We derived the atom percentage, ($at\%$) of ^{15}N in the samples:

$$at\% = \frac{100 * AR * \left(\frac{\delta^{15}\mathrm{N}}{1000}+1\right)}{1 + AR * \left(\frac{\delta^{15}\mathrm{N}}{1000}+1\right)} \qquad [6]$$

where AR is the Absolute Ratio, equivalent to 0.0036764, calculated after compilation of minimum and maximum isotope ratios of the element in naturally occurring terrestrial materials and reagents (U.S. Geological Survey, 2002). Atom percentage of ^{15}N enrichment ($^{15}N_{en}$) in soil and tissue samples were determined from the difference between $at\%$ of soil and tissue samples in the experimental pots and the background $at\%$. Background $at\%$ in soil and tissue samples was calculated from the background δ ^{15}N values in soil (28.6‰) , corn tissue (6.3‰) and soybean tissue (0.8‰) obtained from the control treatments, using equation [6].

The mass of ^{15}N enrichment, m in soil and tissue samples, was obtained from the relation:

$$m = M * \frac{\%N}{100} * \frac{{}^{15}N_{en}}{100} \qquad [7]$$

where M is the dry mass of the total root or shoot recovered from each pot or soil used in each pot (1500 g) and $\%N$ is the percentage composition of total N in the soil or tissue sample.

The percent ^{15}N recovery for each pot, R_{pot} in the soil, shoot and root were calculated as:

$$R_{pot} = \frac{(s+r+t)}{M_a} * 100 \qquad [8]$$

where s, r, and t represent the mass enrichment of ^{15}N in the shoot, root and soil respectively per pot (mg) at any given sampling event, M_a represents the mass of ^{15}N amendment per pot (10 mg). The average recovery, R_{treat} for each treatment was then calculated from the mean recovery from all pots within each treatment.

$$R_{treat} = \sum R_{pot} \qquad [9]$$

4.3.5. Statistical analysis

Statistical analyses were performed using the SAS statistical software (SAS Institute Inc., 1999). Gas efflux data were analyzed as two-way analyses of variance (ANOVA) test for the effect of growth stage (day 20, 60 and 80) and treatment (Corn+N, Soy+N and Soil+N). Root, shoot and soil samples were analyzed separately to test the effect of growth stages and crop type on $\delta^{15}N$ levels and mass of ^{15}N enrichment in the roots and shoots. Growth stages were not considered to be repeated measures because they were represented by destructive sampling of random replicates (Cheng et al., 2003). Least square (LS) means were separated at a 95% confidence level using the least significance difference (LSD) test.

4.4. RESULTS AND DISCUSSION

4.4.1. ^{15}N enrichment in soil

Sampling date significantly affected ($P<0.05$) δ ^{15}N values in the planted soils; however, it did not affect the mass of ^{15}N enrichment in those soils (Table 1a). Crop type, on the other hand, had a significant effect on both the δ ^{15}N values and the mass of ^{15}N enrichment in the soils (Table 1a), with a higher average mass of ^{15}N of 7.04 mg ^{15}N pot^{-1} observed in the soybean pots compared to an average value of 2.36 mg of ^{15}N pot^{-1} in the corn pots. Although we did not observe a significant interaction between sampling date and crop type on the δ ^{15}N values or the mass of ^{15}N enrichment in the corn or soybean-planted soils, we observed that soil ^{15}N concentration was least in the corn pots during the early vegetative stage (1.36 mg ^{15}N pot^{-1}) and tasseling stage (2.03 mg ^{15}N pot^{-1}), and highest in the soybean pots during the pod-filling stage (7.74 mg ^{15}N pot^{-1}) (Table 2). However, ^{15}N concentration in the soil needs to be examined in the context of plant root and shoot uptake in order to provide a fuller picture of N losses in the plant-soil system.

4.4.2. ^{15}N partitioning between root and shoot

Our results show that δ ^{15}N values and the mass of ^{15}N enrichment in shoots and root were mostly significantly affected by sampling date (Tables 1b and 1c). Shoot uptake of ^{15}N was not significantly different between corn and soybean except for day 80, when corn plants were in the milk stage and soybean were in the pod-filling stage (Table 2). However, corn roots contained significantly higher levels of ^{15}N, averaging 1.66 mg ^{15}N pot^{-1} compared to 0.55 mg ^{15}N pot^{-1} contained in the soybean shoots (approximately 3 times more ^{15}N in corn shoots than soybean shoots). Similarly, assimilation of ^{15}N was greater in the corn roots (0.91 mg ^{15}N pot^{-1}) than soybean roots (0.11 mg ^{15}N pot^{-1}). The general trend we observed was that whilst ^{15}N content in soybean shoots and roots remained statistically constant with plant phenology, ^{15}N content in corn shoots and roots stayed constant between the early vegetative growth stage and the tasseling stage but increased significantly between the tasseling and milk stages (Table 2). This is similar to findings by, Karlen et al. (1998) who reported sharp increases in the N uptake of corn during the onset of tasseling (VT stage) and milk (R3 stage).

Although dinitrogen (N_2) fixation in soybean has been reported to increase rapidly in the R2 reproductive phase and decline rapidly in the R6 stage (Fabre and Planchon, 2000) we did not observe any decline in the root and shoot uptake of ^{15}N between the different growth stages, suggesting that the increase in N_2 fixation that may have occurred during soybean development did not prevent the plant from assimilating the ^{15}N-labelled fertilizer. This may be possibly due to the higher N demand in the soybean plants as they reached the flowering and pod-filling stages. Although the ^{15}N levels in soybean shoots did not differ at the growth stages studied, the pattern observed are consistent with patterns of N uptake in soybean shoots found by Thibodeau and Jaworski (1975), who reported increasing shoot N over the season and a maximum level of N fixation reached at mid-pod fill.

In general, total N composition of shoots and roots, on a percentage basis, was significantly affected by sampling date as well as crop type (Table 3). The percentage total N content of roots and shoots was mostly significantly higher in soybean compared to corn, declined significantly in both crops between day 20 and day 60, and did not significantly change between day 60 and day 80 (Fig. 2). These results suggest therefore that the increases in the mass of ^{15}N uptake by corn roots or shoots we observed between the tasseling (day 20) and the milk stages (day 80) resulted from the increase in root and shoot biomass of corn between the two sampling periods (Fig. 3).

4.4.3. N_2O efflux and N loss from the soil-plant system

Sampling date did not significantly affect CO_2 efflux, but affected N_2O efflux (Table 4). Our results show a significant difference between the CO_2 and N_2O efflux from the corn, soybean and bulk soil treatments (Table 4). Generally, CO_2 efflux in the corn pots was significantly higher at 0.75 mg C pot^{-1} h^{-1} compared to soybean pots at 0.1 mg C pot^{-1} h^{-1}. Similarly, N_2O efflux was higher in the corn pots (2.84 ug N pot^{-1} h^{-1}) than the soybean pots (0.06 ug N pot^{-1} h^{-1}), however most of this difference was only observed on day 80 (Fig. 4b). Generally, CO_2 and N_2O efflux from soybean-planted soil did not significantly differ from the efflux from bulk soil amended with N fertilizer treatment (Fig. 4). Our observation of non-significant difference between N_2O efflux in the soybean-planted soil and unplanted bulk soil was in contrast to other studies that have reported that atmospheric nitrogen fixed by the legumes could stimulate more N_2O production from the soil by increasing the N pool through N_2 fixation and also through direct denitrification by the symbiotically living *Rhizobia* in root nodules (Freney, 1997). This is because N fixed by legumes can be nitrified and denitrified in the same way as fertilizer nitrogen (Freney, 1997). Delucchi (2003) cites Robertson et al. (2000), who suggests that it is “high soil nitrogen availability” in general, rather than synthetic fertilizer N, that causes high N_2O emissions. To support this proposition, they noted that their own field experiments found that an N-fixing crop system (alfalfa), that received no synthetic fertilizer, produced as much N_2O as did crop systems fertilized with synthetic N. However, in agreement with the results of this study, Rochette et al. (2004) observed no increase in the N_2O production on one field site under legume cultivation despite high mineral N accumulation and high rainfall. Jarvis et al. (1996) concluded that variations in the soil N pools are imperfect indicators of the rates at which N transformations, including denitrification, occurs in soil.

Interaction between sampling date and treatment had a significant effect on CO_2 and N_2O efflux (Table 4). CO_2 efflux in the corn pots significantly increased between the vegetative stage and the tasseling stage; however, there was no significant change between the tasseling and the milk stages. Nitrous oxide efflux in corn pots followed a similar trend, but, unlike CO_2 efflux that peaked during the tasseling stage, N_2O efflux peaked during the milk stage. This pattern observed for N_2O efflux is similar to that reported by Qian et al. (1997), showing an increase in N_2O production from the root zone of corn between 4 and 12 weeks after plant emergence.

The relationship between CO_2 efflux and N_2O efflux highlights the significantly positive ($P<0.001$) correlations of 0.32 we observed between CO_2 efflux and N_2O efflux, and supports observations made by other workers, who have reported that N_2O production may indeed be coupled with soil respiration even in aerobic soils, due to the occurrences of anaerobic hotspots in zones of high microbial respiration and O_2 depletion (Abbasi and Adams, 1998; Firestone, 1982). Differences observed between CO_2 and N_2O efflux in corn and soybean suggest that a rhizosphere process may actually be responsible for this disparity. That is because corn roots are estimated to input about 3400 kg C ha^{-1} into the soil as rhizodeposits, whilst soybean roots contribute only a fraction of this amount at 1300 kg C m^{-2} (Kogel-Knabner, 2002). Pothoff et al. (2005) reported that C availability was the main prerequisite to establish the anoxic conditions necessary for N_2O production. Additionally, differences exist in the N compositions of the rhizodeposits between leguminous and non-legminous plants. Ta et al. (1986) reported that alfafa (*Medicago sativa* L.) nodules excreted N compounds into the rhizosphere whilst Mayer et al. (2003) reported that faba beans (*Vicia faba* L.) could release as much as 13% of their fixed N into the rhizosphere as rhizodeposits. In corn however, the estimates are much higher, with corn roots estimated to release 24% of total N uptake into the soil as rhizodeposits (Molina et al., 2005), thus making more N available for increased N_2O production from higher denitrifier and nitrifier activity. Therefore, decomposer and denitrifier activity in corn rhizosphere is expected to be higher than in the soybean rhizosphere, resulting from these differences in the rhizosphere C and N pools. Also, corn root respiration and root-derived respiration is generally higher than in soybean root due to a greater flow of photosynthates to corn roots compared to soybean roots (Cardon et al., 2002).

Nitrous oxide reduction to N_2 has been reported in soybean nodules and may have contributed significantly to suppress the N_2O production in the soybean pots. Hoch et al. (1960) observed the evolution of $^{15}N_2$ from $^{15}N_2O$ with detached soybean nodules whilst Coyne and Focht (1987) observed similarly that $^{15}N_2O$ consumption could directly be attributed to $^{15}N_2$ production in detached cowpea nodules. Thus, although N produced from biofixation in soybean should be made available to the

N_2O producing pathway in much the same way as applied N fertilizer in the soil (Delucchi, 2003), theoretically providing a larger pool of NO_3-N for denitrification and nitrification after N fertilizer amendment, the higher N_2O efflux in the corn pots suggest the more C input from rhizodeposition and higher root respiration in corn, relative to soybean, may have overshadowed this effect of N fixation in the soybean pots.

In conclusion, although corn plants in general uptake more added N, and may indeed release more of this assimilated N back into the rhizosphere (Molina et al., 2005), this N does not persist in the soil and is quickly lost, as represented by the lower N recovery in the corn pots compared to the soybean pots (Table 2). However, our results show that N_2O efflux did not correspond well with ^{15}N losses observed (Table 2). For example, although the general trend was increasing levels of N_2O efflux from corn pots between the early vegetative stage and the milk stage (Table 2), the trend in ^{15}N recovery in the corn pots suggests decreasing N loss as plants matured. This disparity emphasizes the inaccuracies and complexities involved in using N_2O emissions as a criterion for estimating fertilizer N loss in soils.

4.5. CONCLUSIONS

Soil ^{15}N concentration was lowest in corn pots during the early vegetative stage and tasseling stage and highest during the pod-filling stage of soybean pots. Shoot uptake of ^{15}N was not significantly different between corn and soybean except for day 80, when corn plants were in the milk stage and soybean were in the pod-filling stage. On the average however, corn shoots and roots contained significantly higher levels of ^{15}N than soybean shoots and roots. Our results show that N_2O efflux did not correspond well with ^{15}N losses observed suggesting however this disparity may be related to the difficulty involved in maintaining uniform soil moisture content in this pot study. We recommend further investigation into the dynamics of the interactions between C and N pools, and soil microbes in soil-plant systems.

Table 1. Analyses of variance tables showing the significance of sampling date (20, 60 and 80 days after seeding), crop type [^{15}N-amended corn (corn + N) and ^{15}N-amended soybean (Soy + N)] and their interactions on $\delta\,^{15}$N and mass of ^{15}N in a) soil b) shoots and c) roots.

a)

		$\delta\,^{15}$N		**Mass of ^{15}N**	
Source	**df**	**F Value**	**Pr > F**	**F Value**	**Pr > F**
Sampling date (A)	2	8.24	0.0032	2.19	0.1586
Crop type (B)	1	6.56	0.0202	19.08	0.0011
A*B	2	0.11	0.7471	0.01	0.9081

b)

		$\delta\,^{15}$N		**Mass of ^{15}N**	
Source	**df**	**F Value**	**Pr > F**	**F Value**	**Pr > F**
Sampling date (A)	2	7.35	0.0034	22.11	<0.0001
Crop type (B)	1	22.93	<0.0001	13.51	0.0013
A*B	2	9.62	0.0009	14.26	<0.0001

c)

		$\delta\,^{15}$N		**Mass of ^{15}N**	
Source	**df**	**F Value**	**Pr > F**	**F Value**	**Pr > F**
Sampling date (A)	2	1.65	0.2192	11.76	0.0005
Crop type (B)	1	13.08	0.0018	39.63	<0.0001
A*B	2	1.17	0.3331	10.99	0.0007

Table 2. Means of $\delta^{15}N$, ^{15}N content in soil, shoots and roots of corn and soybean (soy) treatments as well as the % recovery of ^{15}N in corn and soybean pots at 3 sampling events (20, 60 and 80 days after seeding). The % ^{15}N recovery represents the ratio of the average total recovered ^{15}N per pot to the total ^{15}N applied per pot (10 mg). Means with similar letters are not significantly different at *P*<0.05.

Crop	Sampling date	Soil ^{15}N		Shoot ^{15}N		Root ^{15}N		%^{15}N recovery per pot
		$\delta^{15}N$ (‰)	$\Delta^{15}N$ (mg)	$\delta^{15}N$ (‰)	$\Delta^{15}N$ (mg)	$\delta^{15}N$ (‰)	$\Delta^{15}N$ (mg)	
Corn	[1]20	1002.32^{c}	1.36^{a}	1735.93^{a}	0.47^{a}	3563.65^{abc}	0.30^{ab}	21.3
Soy	[1]20	[4]ND	ND	907.64^{a}	0.20^{a}	2044.72^{a}	0.07^{a}	ND
Corn	[2]60	276.18^{a}	2.03^{a}	2020.10^{a}	0.50^{a}	4730.14^{bc}	0.66^{b}	31.9
Soy	[2]60	679.66^{bc}	6.16^{bc}	1388.57^{a}	0.61^{a}	2425.18^{a}	0.12^{a}	68.9
Corn	[3]80	430.95^{ab}	3.84^{ab}	4871.72^{b}	3.53^{b}	6586.13^{c}	1.75^{c}	91.2
Soy	[3]80	742.89^{bc}	7.74^{c}	994.61^{a}	0.80^{a}	2569.94^{ab}	0.12^{a}	86.6

[1] V1 (Early vegetative growth) stage in corn; V3 (second node) stage in soybean

[2] VT (tasseling) stage in corn; R2 (flowering) stage in soybean

[3] R3 (milk) stage in corn; R6 (pod-filling) stage in soybean

[4] ND = not determined due to sample contamination

Table 3. Analysis of variance table for effect of sampling date (20, 60 and 80 days after seeding), crop type [^{15}N-amended corn (corn + N) and ^{15}N-amended soybean (Soy + N)] and their interactions on % total N content in shoots and roots.

		Shoot N		**Root N**	
Source	**df**	**F Value**	**Pr > F**	**F Value**	**Pr > F**
Sampling date (A)	2	89.61	<0.0001	80.57	<0.0001
Crop type (B)	1	46.68	<0.0001	64.22	<0.0001
A*B	2	3.09	0.0646	8.74	0.0020

Table 4. Analysis of variance table for the CO_2 efflux and N_2O as affected by sampling date (20, 60 and 80 days after seeding), treatment [^{15}N-amended corn (corn + N), ^{15}N-amended soybean (Soy + N) and ^{15}N-amended bulk soil (Soil + N)] and their interactions.

		CO_2		**N_2O**	
Source	**df**	**F Value**	**Pr > F**	**F Value**	**Pr > F**
Sampling date (A)	2	2.13	0.1326	5.37	0.0083
Treatment (B)	2	24.93	<0.0001	11.72	<0.0001
A*B	4	3.29	0.0210	6.48	0.0004

Figure 1. Experimental setup for sampling headspace gas from soil-plant systems using an adaptation of the closed chamber method.

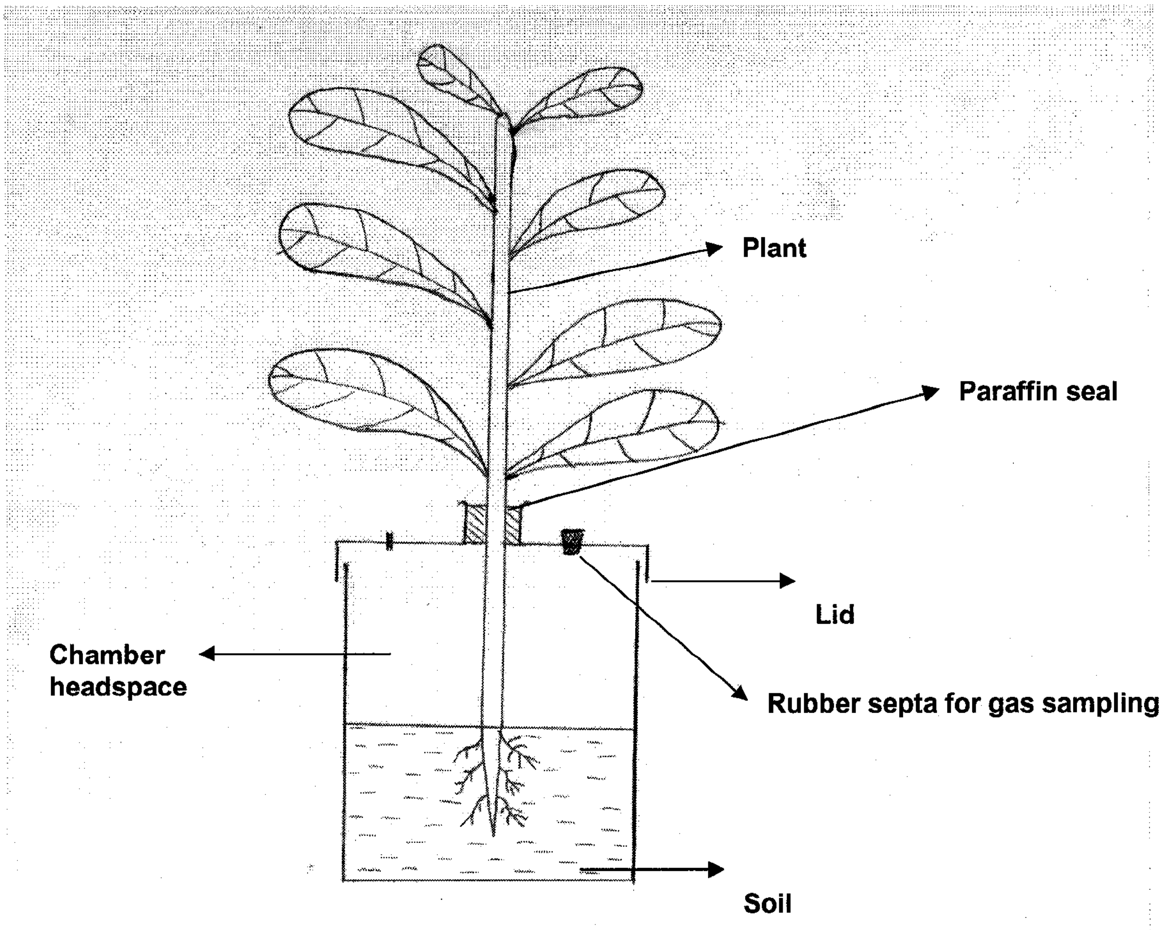
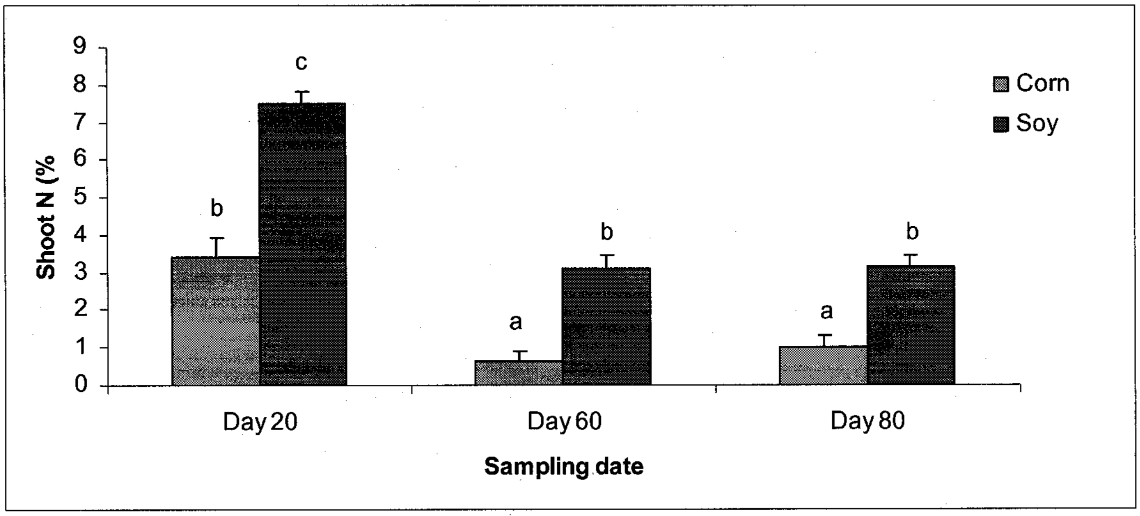

Plant
Paraffin seal
Lid
Chamber headspace
Rubber septa for gas sampling
Soil

Figure 2. Percentage total ^{15}N in a) shoots and b) roots of corn and soybean at 20, 60 and 80 days after seeding. Bars with different letters were significantly different (*P*<0.05, LSD test).

a)


Shoot N (%
9
8
7
6
5
4
3
2
1
0
c
b
b
b
a
a
Corn
Soy
Day 20
Day 60
Day 80
Sampling date

b)

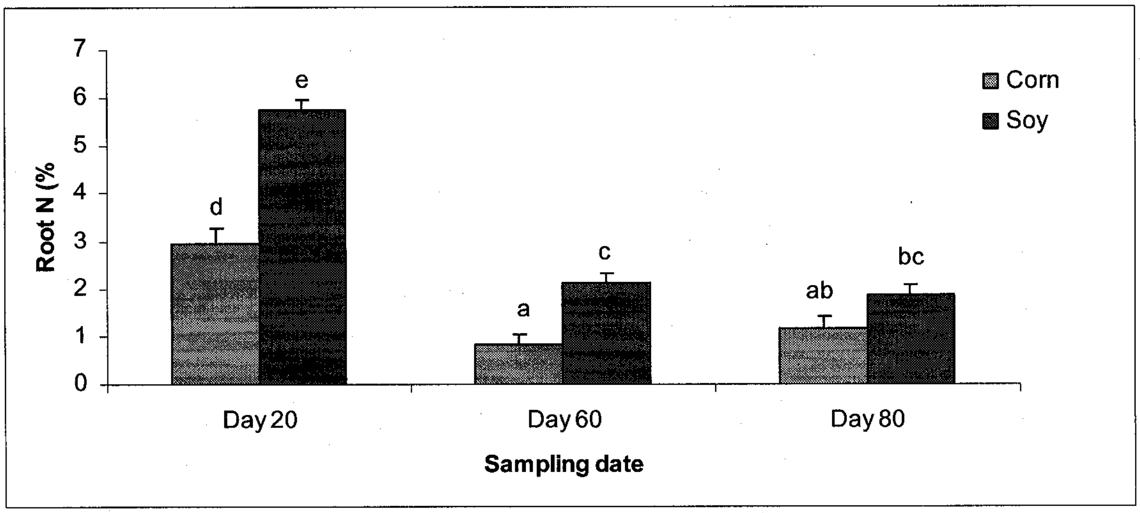

Root N (%
7
6
5
4
3
2
1
0
e
d
c
a
ab
bc
Corn
Soy
Day 20
Day 60
Day 80
Sampling date

Figure 3. Mass of shoots and roots of corn and soybean (soy) recovered from pots after each sampling event at 20, 60 and 80 days after seeding. Vertical bars signify standard errors.

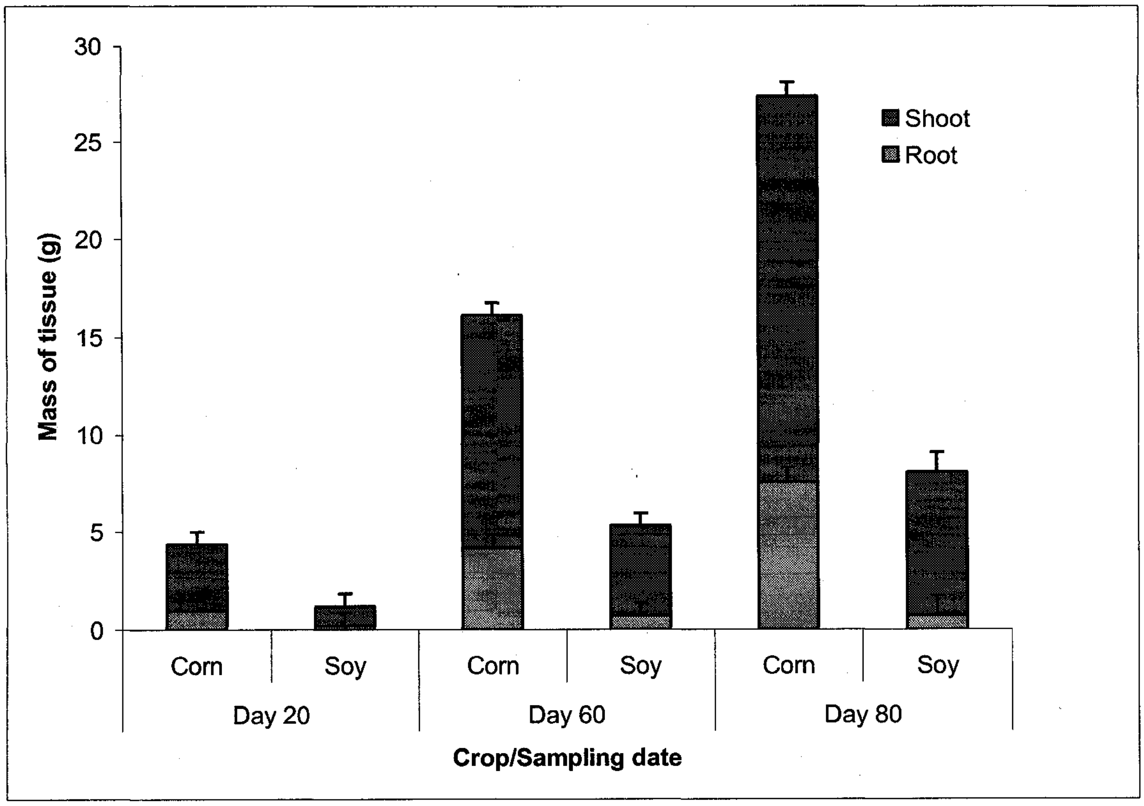
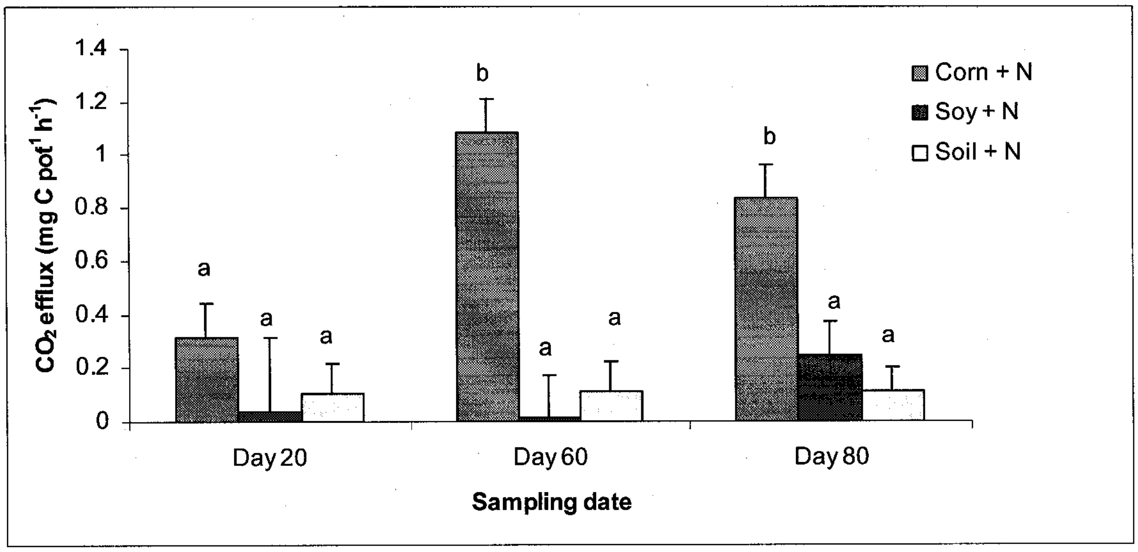

30
25
20
15
10
5
0
Mass of tissue (g)
Shoot
Root
Corn
Soy
Corn
Soy
Corn
Soy
Day 20
Day 60
Day 80
Crop/Sampling date

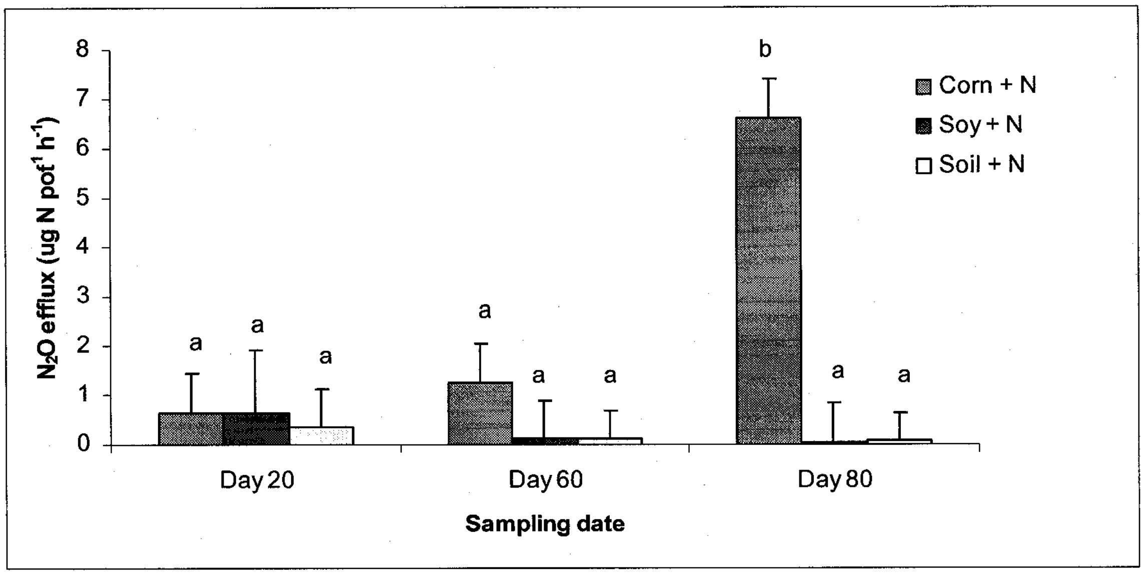
Figure 4. Efflux of a) CO_2 and b) N_2O into the headspace of ^{15}N-amended corn (corn + N), ^{15}N-amended soybean (Soy + N) and ^{15}N-amended bulk soil (Soil + N)at 20, 60 and 80 days after seeding. Bars with different letters were significantly different ($P<0.05$, LSD test).

a)



CO$_2$ efflux (mg C pot^{-1} h^{-1})
1.4
1.2
1
0.8
0.6
0.4
0.2
0
a
a
a
b
a
a
b
a
a
Day 20
Day 60
Day 80
Sampling date
▨ Corn + N
■ Soy + N
☐ Soil + N

b)



N$_2$O efflux (ug N pot^{-1} h^{-1})
8
7
6
5
4
3
2
1
0
a
a
a
a
a
a
b
a
a
Day 20
Day 60
Day 80
Sampling date
▨ Corn + N
■ Soy + N
☐ Soil + N

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CONNECTING PARAGRAPH

In chapter 4, we investigated the dynamics of N uptake in corn and soybean and how this related to the production of N_2O. We discovered that corn-planted pots produced more N_2O than the soybean contrary to our initial hypothesis that N fixation in soybean could lead to higher levels of denitrification. Also N_2O efflux did not follow a similar trend as N loss in the plant-soil system, which highlights the complexities involved in understanding N_2O production from soils. The next chapter investigates CO_2 and N_2O efflux from various soil aggregate classes at different soil moisture levels, and discusses how this may relate to certain management practices such as tillage and use of organic fertilizers.

CHAPTER 5: EFFECT OF SOIL MOISTURE ON CO_2 AND N_2O EFFLUX FROM SOIL AGGREGATE FRACTIONS

5.1. ABSTRACT

Soil aggregates are the habitat for soil microflora and fauna and are likely to be hotspots for decomposition and denitrification due to reduced predation from protozoa. Soil moisture affects the availability of substrate such as organic C, ammonium and nitrate that are essential for microbial functions. The objective of this study was to determine the effect of soil moisture on CO_2 and N_2O efflux from bulk soil and soil aggregate fractions. This study consisted of two separate 4 x 4 factorial experiments consisting of four different soil fractions (bulk sieved soil, large macroaggregates, small macroaggregates and microaggregates) incubated at four levels of soil moisture (20%, 40%, 60% and 80% of WFPS). The first batch of soil fractions were incubated in the presence of 10 kPa (10% v/v) of acetylene whilst the second were incubated in the absence of acetylene. On the average, efflux of CO_2 and N_2O were 52.5% and 55.5 % respectively lower in the acetylene treated fractions than the non-acetylene treated fractions. Our results indicated that the acetylene treatment may have reduced the differences in the N_2O production from nitrification in the various aggregate factions. Denitrification in the microaggregates proceeded at about 4.4 to 39.6 times higher rate compared to the other fractions, and showed a greatest response to high moisture levels.

5.2. INTRODUCTION

About 25% of CO2 emissions and 90% of the N2O emissions worldwide are believed to come from agroecosystems (Duxbury, 1994). Soil aggregates are the habitat for soil microflora and fauna and are likely to be hotspots for decomposition and denitrification due to reduced predation from protozoa (Griffiths 1994, Gupta and Germida, 1988). Several studies have shown that soil microorganisms and their activities are heterogeneously distributed across aggregate-size classes (Gupta and Germida, 1988). Whilst some studies show greater microbial biomass and higher activities in macroaggregates (>0.25 mm) compared with microaggregates (<0.25 mm) (Gupta and Germida, 1988, Franzluebbers and Arshad, 1997), others report similar levels of activities between macroaggregates and microaggregates (Seech and Beauchamp, 1988; Miller and Dick, 1995).

Agricultural practices such as tillage and organic fertilizer applications influence the distribution and activity of decomposer and denitrifier organisms in soils by directly impacting on soil aggregation. Soil disturbance through tillage influences CO_2 production through (i) reduced aggregation and (ii) increased decomposition through aggregate disruption (Beare et al., 1994; Adu and Oades, 1978). Tillage increases the rate of organic-matter decomposition and mineralization by aerating the soil, burying surface residues, breaking soil aggregates, and thereby increasing the exposure of soil organic matter to microbial activity (Phillips and Phillips, 1984). Over time, studies indicate that tillage practices tend to increase soil respiration by increasing the availability of the previously-protected soil organic matter in aggregates to decomposers (Wander and Bollero, 1999). This promotes O_2

consumption by heterotrophic microbes, thereby maintenaning the anoxic conditions suitable for denitrification to proceed (Abassi and Adams, 1998; Firestone 1982). Aoyama et al. (1999) observed that long-term application (18-yr) of cattle manure favored the formation of macro-aggregates resistant to shaking, and that, although manure application increased the concentration of organic matter both in macro- and microaggregates, manure-derived organic matter accumulated preferentially in macroaggregates. They hypothesized that manure-derived organic matter first enters the soil primarily as particulate material, and during decomposition is transformed within the macroaggregate structure into mineral-associated material of microbial origin, thereby contributing to the stabilization of macroaggregates (Aoyama et al., 1999a).

Water is essential for microbial survival and activity. Moisture level and its effect on CO_2 production was described by Skopp et al. (1990) as a delicate balance between having sufficient water for substrate diffusion and microbial requirements and adequate oxygen for respiration. Soil moisture affects the availability of substrate such as organic C, ammonium and nitrate that are essential for microbial functions (Weitz et al., 2001). Microbial respiration is maximized when water-filled pore spaces (WFPS) is from 50 to 75% (Olness et al., 2002) (Fig. 4). The potential for denitrification in soils on the other hand, is a complex interaction between aeration, NO_3-N availability, C substrate availability and other intrinsic "soil factors" (Firestone, 1982). Although high levels of N_2O production have been reported in some soils after a heavy downpour, in flooded organic soils, the N_2O production in most soils is limited by the NO_3-N availability (Firestone 1982). It is well established that denitrification enzymes are synthesized and active only when O_2 availability is low or O_2 is absent. That is because under low oxygen tension, nitrate (NO_3-N) replaces O_2 as the terminal electron acceptor for energy production used by denitrifying bacteria, forming dinitrogen gas and nitrous oxide (N_2 and N_2O) which are lost to the atmosphere (Watts and Seitzinger, 2000). Saturated soils incubated anaerobically in the presence of NO_3-N amendments increased denitrifier populations (Doner et al., 1975; Voltz et al., 1975). However, when soil solution contained more than 0.020 mg NO_3-N g^{-1} soil, denitrification is rarely limited by NO_3-N levels (Myrold and Tiedje, 1985a; Myrold and Tiedje, 1985b). While water content, matric potential and water-holding capacity all serve as relative predictors of microbial activity in soil, the expression of water content as WFPS encompasses the role of water as a barrier to O_2 diffusion and as such is a better predictor of aeration-dependent microbial processes such as denitrification than any other factor (Sommers et al., 1981). As the water filled pore space (WFPS) approaches and exceeds 30%, nitrification increases and NO is produced, however as the soil gets wetter beyond 30% WFPS, nitrification continues while denitrification begins, with N_2O as the predominant product of both processes (Sylvia et al., 1998). Beyond 60% WFPS, nitrification slows and at 80% WFPS, the denitrification rate plateaus, with N_2 as the main product (Sylvia et al., 1998).

Changes in the WFPS are strongly influenced by soil aggregation, which affects the distribution of macro- and micro-pores and hence the water holding capacity of soils. In well-aggregated surface soils, large macro-pores contribute to a major portion of the inter-aggregate pore space and drain rapidly after a precipitation event (Tomasella and Hodnett, 1996). Deeper in the soil profile (4-7 cm depth), more micro-pores are found; these small pores drain slowly and have a greater water holding capacity than the overlying soil (Weitz et al., 2001). Weitz et al. (2001) also reported that surface aggregated soils supported mainly nitrification whilst the micro-pores supported mainly denitrification. However, nitrification and denitrification may occur simultaneously in soil aggregates, with denitrification occurring predominately within aggregates (anaerobic microsites) and nitrification occurring mostly in the aerobic microsites of aggregate surfaces and inside the small aggregates (Abbasi and Adams, 1998).

Although soil moisture and soil aggregates play a major role in the production and emission of CO_2 and N_2O, our knowledge of how soil moisture interacts with soil aggregates to affect this production is limited. The objective of this study was to determine the effect of soil moisture on CO_2 and N_2O efflux from soil aggregate fractions.

5.3. MATERIALS AND METHODS

This study employed the acetylene (C_2H_2) inhibition method (Klemedtsson et al., 1977) to quantify total denitrification, by estimating the efflux of N_2O in the absence of the N_2O reduction to dinitrogen (N_2) gas. This is because at levels of acetylene between 1 and 20 kPa, the reduction of N_2O to N_2 by the enzyme N_2O reductase is blocked, so that accumulated N_2O can be measured instead of N_2 (Yoshinari et al., 1977). The acetylene treatment also inhibits ammonium oxidase, which is responsible for the oxidation of ammonia to hydroxylamine, a precursor of

N_2O produced through nitrification, thereby effectively blocking N_2O production through nitrification (McCarty and Bremner, 1986). Thus, we assumed that the 10 kPa acetylene treatment was sufficient to block N_2O production from nitrification as well as the enzymatic reduction of N_2O to N_2, thereby ensuring that all the N_2O measured was from denitrification alone (Garrido et al. 2000; Klemedtsson et al., 1977).

5.3.1. The soil

The soil was a Typic Endoaquent containing, on average, 700 g sand kg^{-1}, 140 g silt kg^{-1} and 16 g clay kg^{-1} with15.4 g total C kg^{-1}, 1.24 g total N kg^{-1}, and pH 6.1 in the 0- to 15-cm sandy loam layer. It was collected adjacent to a field located on the Macdonald Research Farm, Ste. Anne de Bellevue, Quebec, Canada (45° 28' N, 73° 45' W, elevation 35.7 m). For this study, soil was collected from the 0- to 10-cm depth, homogenized by passing through a 6-mm mesh sieve and air-dried in the laboratory. Then, soil was spread carefully on top of sieves with openings of 2 mm and 0.25 mm, placed in a rotary sieve and shaken for 30 min to collect three aggregate size fractions: large macroaggregates (>2 mm), small macroaggregates (0.25-2 mm) and microaggregates (<0.25 mm). The bulk soil used in this study was also sieved (<2 mm mesh), and included small macroaggregates and microaggregates. We weighed 40 g of the aggregates fractions into ninety-six 160-mL serum bottles (twenty-four bottles per each fraction) and packed them to a bulk density of 1.11 g cm^{-3}. Soil moisture was adjusted to 60% WFPS by adding distilled water to each bottle, based on the relationship between gravimetric soil moisture content and WFPS:

$$WFPS = 100\left(\frac{w\rho_b/\rho_w}{1-\rho_b/\rho_s}\right) \qquad [1]$$

where w is the gravimetric water content (%), ρ_b is the soil bulk density (g cm^{-3}), ρ_w is the density of water (1 g cm^{-3}) and ρ_s is the soil particle density (2.65 g cm^{-3}).

The serum bottles were capped and then pre-incubated at 25°C for 48 h. The pre-incubation period was meant to re-establish soil nitrifying activity of the soil and to avoid the large initial pulse of N_2O released as a result of rewetting the dry soils (Godde and Conrad, 1999).

5.3.2. Experimental design

This study consisted of two separate factorial experiments (or batches of aggregate fractions) run concurrently; the first batch were incubated in the presence of 10 kPa (10% v/v) of acetylene (Klemedtsson et al., 1977) whilst the second consisted of soil fractions incubated in the absence of acetylene. Each experiment was laid out in a 4 x 4 factorial design consisting of four different soil fractions (bulk sieved soil, large macroaggregates, small macroaggregates and microaggregates) incubated at four levels of soil moisture (20%, 40%, 60% and 80% of WFPS), for a total of 16 treatment combinations. Each treatment combination was replicated three times to yield 48 experimental units. Five empty bottles, incubated under the same conditions but without acetylene were included to serve as blanks for the experiment.

5.3.3. Experimental procedure

After the pre-incubation stage, the soil moisture content in the fractions were gradually adjusted within a period of a week to the treatment levels by air-drying or adding distilled water, based on the following equation:

$$M_w = \Delta WFPS * M_s * \left(\frac{\rho_s - \rho_b}{\rho_s \rho_b} \right) \qquad [2]$$

where M_w is the mass of water added or lost through drying, $\Delta WFPS$ is the difference between the target WFPS and the actual WFPS, M_s is the mass of soil solids (40 g).

After adjusting the soil moisture content of the fractions to treatment levels, the serum bottles were sealed with a septa, secured by a metal cap. In the acetylene treatments, we extracted 10% v/v of headspace air using a gas-tight syringe and injected acetylene to replace the volume of air extracted-plus-pore space volume. Soils were then incubated at 25°C for 48 h.

5.3.4. Gas sampling

Gas samples were taken immediately following the 48-h incubation period after acetylene injection. Gas samples (20 mL) were taken using a gas-tight syringe and injected into previously-evacuated 12 mL exetainers (LABCO, Wycombe, U.K.). Gas samples were analyzed with a Varian Model 3800 gas chromatograph (Walnut Creek, CA, U.S.A.) equipped with automated valve injectors to simultaneously quantify CO_2, N_2O and O_2 concentrations. A Haysep A column followed by a molecular sieve and He carrier at 46 ml min^{-1} were used to separate CO_2 from O_2

whereas N_2O was quantified on a Porapak Q column with Ar/CH_4 (90:10) carrier gas at 20ml min^{-1}.

Units of gas concentration (CO_2 or O_2) as expressed in ppm v/v is equivalent to $1 cm^3$ of gas per m^3 of air. From the ideal gas equation:

$$PV = nRT \qquad [3]$$

where $P \approx$ atmospheric pressure (1 atm), V is the volume of gas (cm^3), n is the number of moles of gas, R is the ideal gas constant (82.06 atm cm^3/mol K) and T is the temperature under which serum bottles were incubated expressed in Kelvins (298 K). Rearranging,

$$n = {}^{PV}\!/_{RT} \qquad [4]$$

Since density d is mass of gas (g) per unit volume (V) and the mass of gas is moles of gas times molecular mass of gas (M)

$$d = {}^{g}\!/_{V} = M({}^{n}\!/_{V}) \qquad [5]$$

Combining equations (2) and (3),

$$d = \frac{MP}{RT} \qquad [6]$$

Multiplying the concentration of gas ($cm^3\ m^{-3}$) with the density of the gas, d ($g\ cm^{-3}$) yields concentration of the gas in the headspace expressed as $g\ m^{-3}$ of the headspace. We converted gas concentration to per C or per N basis by multiplying by the mass ratio of carbon atoms per molecule of CO_2 (12/44) or the mass ratio of nitrogen atoms per molecule of N_2O (28/44). Conversion to mg C m^{-3} was done by multiplying by 1000 after which we multiplied by the volume of the headspace ($\approx 1.24 \times 10^{-4}\ m^3$) in order to determine the gas concentration on a per bottle basis (mg bottle^{-1}). Gas efflux into the headspace, f (mg C kg^{-1} soil h^{-1}) was estimated as:

$$f = {}^{(C_1 - C_0)}\!\big/_{(m*t)} \qquad [7]$$

where C_1 is the concentrations of gas (mg C bottle^{-1}) after 48 h, C_0 is the concentration of gas in the blanks, m is the mass of dry soil in each bottle (0.04 kg), and t is the duration of the incubation (48 h).

5.3.5. Soil analyses

Soil samples were taken from a separate set of twelve bottles (three replicates per fraction) maintained at 60% WFPS and pre-incubated at 25 °C for 48h, after which samples were taken and kept in plastic bags at 4°C until chemical analyses. The mineral N (NH_4-N and NO_3-N) concentration in 0.5 M K_2SO_4 soil extracts was measured calorimetrically using the salicylate and cadmium reduction-diazotization methods with a Lachat Quik-Chem AE flow-injection autoanalyzer (Lachat Instruments, Milwaukee, WI). The dissolved organic N (DON) concentration was the difference between the NO_3-N concentration in an alkaline persulfate digest of the soil extract and the mineral N concentration in the soil extract (Cabrera and Beare, 1993). Microbial biomass N (MBN) was determined using the chloroform fumigation-direct extraction method followed by persulfate digestion (Brookes et al., 1985a; Brookes et al., 1985b) and calculated as: (total extractable N after fumigation – total extractable N before fumigation)/ K_{EN}, where $K_{EN} = 0.54$ and is used to correct for the efficiency of the extraction process. The extractable C concentration in 0.5M K_2SO_4 extracts of fumigated and unfumigated soil samples was measured by wet combustion with a Shimadzu TOC-V C analyzer (Shimadzu Corporation, Kyoto, Japan). The extractable C concentration in unfumigated soil samples was assumed to represent a pool of dissolved organic C (DOC). Microbial biomass C (MBC) was calculated as: (total extractable C concentration after fumigation – total extractable C before fumigation)/K_{EC}. A K_{EC} value of 0.43 was used to correct for the efficiency of the extraction process (Vance et al., 1987).

5.3.6. Statistical analysis

Statistical analyses were performed using the SAS statistical software (SAS Institute Inc., 1999). Analyses of variance (ANOVA) was initially performed separately for the acetylene and non-acetylene treatment experiments, to test the effect of soil fractions and soil moisture and their interactions on CO_2, N_2O efflux and O_2 consumption. Means comparison was performed using the least significance difference (LSD) test using the SAS statistical software (SAS Institute Inc., 1990) at the 95% confidence level. Pearson Correlation was performed on the gas data in order to explore the relationships between CO_2, N_2O efflux and O_2 consumption.

5.4. RESULTS AND DISCUSSIONS

5.4.1. Extractable soil C and N pools in aggregate fractions

Our results show that after the pre-incubation phase, with the exception of microbial biomass carbon (MBC), extractable C and N between the aggregate fractions pools were significantly different ($P< 0.05$) (Table 1). This difference was particularly most significant in the case of ammonium (NH_4-N), nitrate (NO_3-N) and mineral nitrogen (Min N). Table 2 shows that the NH_4-N concentration was highest in the microaggregates and lowest in the macroaggregates. This result could have been due to higher rates of nitrification in the macroaggregates, leading to higher NH_4-N depletion in the macroaggregates. This is because nitrification tends to decrease with aggregate size, which is related to the decrease in the ease of O_2 diffusion into the inter-aggregate space with decrease in soil aggregate size (Kremen et al., 2005). However, the levels of NH_4-N we observed were relatively higher than the benchmark level of 0.5 mg N kg^{-1} soil reported to be the levels at which the concentration becomes limiting to nitrification (Garrido et al., 2000). Aggregate NO_3-N concentration also appeared to be related to aggregate size, with the highest levels observed in the large macroaggregates (52.87 mg N g^{-1} soil) and the lowest levels observed in the microaggregates (30.36 mg N g^{-1} soil). This observation further suggested that the rate of nitrification increased with aggregate size, leading to depletion in NH_4-N and an accumulation of NO_3-N in the larger aggregates relative to the smaller aggregates. Generally, NO_3-N concentration in aggregate fractions ranged between 30.8 to 52.9 ug N kg^{-1} soil (Table 2), higher than the NO_3-N levels reported by Myrold and Tiedje (1985a, 1985b) to limit nitrification (>20 mg N kg^{-1} soil). In addition, denitrifying enzyme induction will most likely not be hindered at the NO_3-N levels observed. This is because under low NO_3-N concentration (< 0.001 mg N kg^{-1} soil) denitrification might be restricted since 2-8 μg of NO_3-N is required to produce 10^6 denitrifiers in the soil (Jacobson and Alexander, 1980).

Dissolved organic C levels were lowest in the large macroaggregates (Table 2), suggesting higher activity of decomposers and denitrifiers in the large macroaggregates than the smaller aggregate fractions. This is because there has been a strong link established in past studies between DOC and CO_2 production (Seto and Yanagiya 1983; Jandl and Sollins 1997) as well as with denitrification activity (Burford and Bremner, 1975). Dissolved organic nitrogen levels decreased with aggregate size, being were lowest in the large macroaggregates, and highest in the micoaggregates (Table 2). This result further indicates an increase in C and N mineralization with aggregate size.

Microorganisms and their activities are heterogeneously distributed across aggregate-size classes (Gupta and Germida, 1988). Although microbial biomass carbon (MBC) was not significantly different between the different fractions (Table 1), we observed significant differences in the microbial biomass nitrogen (MBN), with the highest levels of MBC in the bulk soil fraction (Table 2), an implication of more microbial biomass in the bulk soil and macro aggregates, compared to the other aggregate fractions. Microaggregates, on the other hand, had the least amount of MBN and MBC (Table 2). The heterogeneous distributions of microbial biomass and the associated activities among the different aggregate size classes may be conceptually explained in two ways. Firstly, it may be a reflection of their structural properties that selectively or generally restrict the transfer of nutrients and O_2 for respiration (Mendes et al., 1999). Alternately, variations in microbial biomass might imply that the structural variation among the aggregates restricts predatorial grazing to certain aggregate-size classes but not to others (Mendes et al., 1999). Thus, our results may be an indication that microbial population was more restricted in the microaggregates compared to the larger aggregates, due to greater restrictions to the flow of nutrients and O_2, as well as greater access to soil microbes from by grazers.

5.4.2. Correlations between CO_2, N_2O efflux and O_2 consumption

Table 3 shows very high significant positive correlation ($p < 0.05$) observed between CO_2 efflux and O_2 consumption in both the acetylene-treated and non-acetylene treated soils. In the non-acetylene treatment, there was significant positive correlation between N_2O efflux and CO_2 efflux ($r = -0.40$) and between N_2O efflux and O_2 ($r = -0.41$) consumption (Table 3), either due to the process of microbial respiration enhancing denitrification by increasing the occurrences of anaerobic microsites in the fractions (Petersen et al., 1996), or directly produced from the process of nitrification (Denmead et al., 1979; Kuenen and Robertson, 1994; Payne 1973). However, In the 10 kPa acetylene-treated fractions, we observed a non-significant negative correlation between N_2O efflux and CO_2 efflux ($r = -0.07$) and between N_2O efflux and O_2 consumption ($r = -0.08$), indicating that the latter scenario was most likely the case, and that a significant portion of the correlation observed in the non-acetylene treated soil could be attributed to the nitrification process which is inhibited by the 10 kPa acetylene treatment. Our observation supports our initial assumption that although generally, the acetylene method is used as an estimate of the total denitrification, levels of acetylene of around10 kPa have the potential to block nitrification as well as the reduction of N_2O to N_2. This conforms to observations made by Garrido et al. (2000), who reported a 100% reduction of nitrification in the presence of 10 kPa of acetylene, thereby implying that at that level, acetylene.

Similarly, Malone et al. (1998) observed no enrichment in the NO_3-N pool in soils incubated under 10 kPa of acetylene as compared to enrichment in soils incubated with acetylene, an indication that nitrification was blocked at that level of acetylene treatment.

5.4.3. CO_2 and N_2O efflux from aggregate fractions

Our results in Table 4 shows that CO_2 efflux and O_2 consumption were significantly affected ($p < 0.05$) by soil aggregate fraction, soil moisture and the interaction between soil aggregate and soil moisture in both the acetylene and non-acetylene treated fractions. On the average, efflux of CO_2 and N_2O were 52.5% and 55.5 % respectively lower in the acetylene treated fractions than the non-acetylene treated fractions. Our result suggests that at 10 kPa, acetylene may also inhibit the activity of decomposers by inhibiting the nitrification process, thereby reducing the mineral N available for organic matter mineralization in the soil fractions. However, to our best knowledge, literature on this possible link between the acetylene inhibition process and the activity of decomposers is very limited. A more direct inhibition of the activity of decomposers by exposure to10 kPa acetylene, may also have been possible and requires further investigation in future studies. The lower efflux of N_2O in the acetylene treated soil indicates that although acetylene blocks the reduction of N_2O to N_2 (Yoshinari et al., 1977), which should lead to more accumulation of N_2O in the acetylene-treated fractions, the treatment also blocks the significant input of nitrification to the total N_2O efflux (Garrido et al., 2000; Malone et al., 1998), and causes a reduction in the microbial respiration, thereby reducing the occurrence of anaerobic microsites that favour N_2O production (Maag and Vinther, 1999).

Table 4 shows that CO_2 efflux was significantly different between the various soil fractions. Although we did not observe a clear trend between the fraction in both the acetylene and non-acetylene treatments, CO_2 efflux was highest in the bulk soil (Tables 5 and 6), which could have been related to the higher levels of microbial biomass observed in the bulk soil fraction (Table 2). Although aggregate size, together with soil moisture are perhaps the two major parameters determining the existence and extent of anaerobic zones in aggregates (Kremen et al., 2005), our results show that soil aggregate size did not have a significant effect on the efflux of N_2O in the acetylene-treated soil (Table 4b). Thus, statistically the acetylene treatment eliminated differences in the N_2O production between the fractions.

There are two possible explanations for this observation. Firstly, by blocking the nitrification process, the acetylene treatment may have reduced the differences in the N_2O production from nitrification resulting from differences in O_2 diffusivity in the various soil aggregate fractions. Secondly, by blocking the reduction N_2O to N_2, the acetylene treatment possibly eliminated differences in the rate at which this process occurs in the soil fractions. However, the degree to which either process may have contributed to our observation is impossible to isolate with our current methodology.

Our results indicate that N_2O efflux from microaggregates in the acetylene treatment was between 4.4 to 39.6 times higher than that observed in the other fractions, suggesting that N_2O production from denitrification did indeed proceed at a faster rate in the microaggregates than in the other fraction. Diffusivity of O_2 in microaggregates is higher due the greater proportion of micropores, thereby providing a more suitable anaerobic environment for denitrification to proceed (Weitz et al., 2000). In the non acetylene treatment on the other hand, the highest N_2O efflux was observed from the bulk soil whilst that from the other aggregate fraction were not significantly different (Table 5).

There are two possible scenarios that may have led to our observation. In the first instance, our observation could have been due to increased denitrifier activity from increased occurrences of anaerobic zones in the bulk soil as a result of higher microbial activity in the bulk soil compared to the other aggregate fractions. This is due to the fact that compared to the other aggregate fractions, bulk soil had the highest microbial population, as indicated by the higher microbial N content (Table 2), and had up to 2.7 times more respiration rates than the other factions. The second scenario is that nitrification may have proceeded at a much higher rate in the bulk soil than the other fractions in the absence of acetylene, hence increasing N_2O production from the nitrification process.

Generally, CO_2 production increased with increasing soil moisture in both the acetylene and non-acetylene experiments (Tables 5 and 6), suggesting that decomposers were still active even at 80% WFPS. Efflux of CO_2 and N_2O was lowest between 20 and 40% WFPS and increased between 60% and 80% WFPS (Tables and 7). These results conform to that observed by Olness et al. (2002) and Skopp et al. (1990), who reported that microbial respiration is maximized when water-filled pore spaces is around 50 to 75%. Similarly, Maljanen et al. (2003), who observed the highest levels of denitrification between a WFPS of 70-90%. Oxygen consumption was highest at 60% and 80% WFPS, corresponding to the high efflux of CO_2 and N_2O in the soil at that moisture level (Tables 5 and 6). This observation suggests that as the soils got wetter, decomposer activity increased with increased microbial mobility and substrate availability, thereby depleting the O_2 levels in the soil and creating anaerobic conditions in the soil conducive for high denitrifier activity. Given that the rate of diffusion of O_2 in water is about 0.0001 of its diffusion in the atmosphere, the presence of water in the soil also creates the right anaerobic conditions to favor denitrification even in aerobic soils (Hassett and Banwart, 1992).

The interaction effect between soil fractions and soil moisture on CO_2 and N_2O was significant in both the acetylene and non-acetylene (Table 2). However, we did not observe a clear trend in the interaction effect on CO_2 efflux in the non-acetylene treatments. Contrary, to other observation made in previous studies of a close relationship between CO_2 and N_2O efflux (Blackmer et al., 1980; Goreau et al., 1980; Firestone, 1982, Garrido et al., 2002), trends in the CO_2 efflux differed from N_2O efflux in the various fractions and at different moisture levels (Tables 7 and 8). Generally, N_2O efflux increased exponentially in all fractions between WFPS 60% and 80% (Tables 7 and 8). In the acetylene treatments, although not following a clear trend, N_2O efflux was highest in the microaggregates at 80% WFPS, likely because microaggregates have the least inter-aggregate space and was more saturated at 80% WFPS, providing the most conducive environment for denitrifiers. In the non-acetylene treatment, in a sharp contrast to the results obtained from the acetylene treatment, microaggregates had the least N_2O efflux (Table 7). According to Sylvia et al. (1998), at 60% WFPS, nitrification slows and at 80% WFPS, the denitrification rate plateaus, with N_2 as the main product. Our observation therefore suggests that in the non-acetylene treatment at 80% WFPS, air content was least in the microaggregates, thereby limiting the nitrification process to a greater extent than the other fractions. In the microaggregates therefore, nitrification likely contributes the least amount of N_2O at 80% WFPS compared to the other fractions. Also, at that moisture level, the process of reduction of N_2O to N_2 likely occurred at a faster rate in the microaggregates than the other fractions, contributing to our observation.

5.5. CONCLUSIONS

During the pre-incubation stage, nitrification appeared to proceed at a faster rate in the larger aggregate size fraction and much slower in the smaller aggregate fractions. For example, NH_4-N concentration was highest in the microaggregates and lowest in the macroaggregates, whilst NO_3-N concentrations accumulated in the large macroaggregates and was depleted in the microaggregates. We observed CO_2 efflux and O_2 consumption were significantly by soil aggregate fraction, soil moisture and the interaction between soil aggregate and soil moisture in both the acetylene and non-acetylene treated fractions. On the average, efflux of CO_2 and N_2O were 52.5% and 55.5 % respectively lower in the acetylene treated fractions than the non-acetylene treated fractions. Our result suggests that at 10 kPa, acetylene may inhibit the activity of decomposers by inhibiting the nitrification process, thereby reducing the mineral N available for organic matter mineralization in the soil fractions. Although we did not observe a clear trend between the fraction in both the acetylene and non-acetylene treatments, CO_2 efflux was highest in the bulk soil. Our results indicated that the acetylene treatment may have reduced the differences in the N_2O production from nitrification in the various aggregate factions that results from differences in O_2 diffusivity. Denitrification in the microaggregates proceeded at about 4.4 to 39.6 times higher rate compared to the other fractions, and showed a greatest response to high moisture levels (80% WFPS).

The results from this study suggest that agricultural management practices such as tillage, and organic fertilizer usage, which affect soil aggregation and soil moisture status, may indeed alter the C and N cycles in soils by altering the rate of nitrification, denitrification and N and C mineralization in the soils.

Table 1. Analysis of variance table for the effect of soil fractions on the composition of ammonium (NH_4-N), nitrate (NO_3-N), mineral nitrogen (MinN), microbial biomass nitrogen (MBN), microbial biomass carbon (MBC), dissolved organic nitrogen (DON),and dissolved organic carbon (DOC) in soil.

PARAMETER	**DF**	**MS**	***P* value**
NH_4-N	3	110.17	<0.0001
NO_3-N	3	4.13	<0.0001
Min N	3	23.31	0.0005
MBN	3	5.68	0.0456
MBC	3	6.19	0.1422
DON	3	5.20	0.0335
DOC	3	5.34	0.0395

Table 2. Concentrations of ammonium (NH_4-N; mg N kg^{-1} soil), nitrate (NO_3-N; mg N kg^{-1} soil), mineral nitrogen (MinN; mg N kg^{-1} soil), microbial biomass nitrogen (MBN; mg N kg^{-1} soil), microbial biomass carbon (MBC; mg C kg^{-1} soil), dissolved organic nitrogen (DON; mg N kg^{-1} soil), and dissolved organic carbon (DOC; mg C kg^{-1} soil) in bulk soil (BS), large macroaggregates (LM), small macroaggregates (SM) and microaggregates after pre-incubation at 60% WFPS for 48 h. Values within a column followed by the same letter are not significantly different at $P < 0.05$.

FRACTION	NH_4-N	NO_3-N	Min N	MBN	MBC	DON	DOC
LM	2.72^{a}	52.88^{c}	55.60^{c}	8.27^{ab}	11.34^{a}	6.79^{a}	14.67^{a}
B	3.93^{b}	38.95^{b}	42.88^{b}	13.86^{b}	8.58^{a}	9.74^{a}	21.77^{b}
SM	6.17^{c}	35.14^{ab}	41.31^{ab}	7.19^{a}	22.68^{a}	12.88^{ab}	26.85^{b}
M	6.87^{d}	30.88^{a}	37.24^{a}	4.78^{a}	5.10^{a}	16.40^{b}	21.92^{b}

Table 3. Pearson correlation coefficients (*r*) between CO_2, O_2 and N_2O efflux from acetylene-treated soil fractions and non-acetylene treated soil fractions (bulk sieved soil, large macroaggregates, small macroaggregates and microaggregates) (n = 48). Significance levels were *P*<0.05 (*), *P*<0.01 (**) and *P*<0.001 (***).

With 10 kPa treatment			
	CO_2	N_2O	O_2
CO_2	-	-0.07	0.97^{***}
N_2O	-0.07	-	-0.08
O_2	0.97^{***}	-0.08	-
No acetylene treatment			
	CO_2	N_2O	O_2
CO_2	-	0.40^{**}	0.99^{***}
N_2O	0.40^{**}	-	0.41^{**}
O_2	0.99^{***}	0.41^{**}	-

Table 4. Analysis of variance table for the effect of soil fraction (bulk sieved soil, large macroaggregates, small macroaggregates and microaggregates), soil water-filled pore space (20%, 40%, 60% and 80% WFPS) and interactions on the efflux of CO_2, N_2O and O_2 consumption in the non-acetylene treatment and 10 kPa acetylene-treatment.

Non-acetylene treatment

		CO_2		**N_2O^***		**O_2**	
Source	**df**	**F Value**	**Pr > F**	**F Value**	**Pr > F**	**F Value**	**Pr > F**
Fraction (A)	3	53.99	<0.0001	8.48	0.0003	37.93	<0.0001
WFPS (B)	3	120.94	<0.0001	120.80	<0.0001	81.73	<0.0001
A*B	9	6.29	<0.0001	2.76	0.0171	3.97	0.0018

10 kPa acetylene treatment

		CO_2		**N_2O ***		**O_2**	
Source	**df**	**F Value**	**Pr > F**	**F Value**	**Pr > F**	**F Value**	**Pr > F**
Fraction (A)	3	50.97	<0.0001	1.09	0.3693	34.40	<0.0001
WFPS (B)	3	105.62	<0.0001	44.57	<0.0001	78.91	<0.0001
A*B	9	3.75	0.0026	9.51	<0.0001	2.41	0.0327

* Log-transformed data

Table 5. Efflux of CO_2 (mg C kg^{-1} h^{-1}), N_2O (ng N kg^{-1} h^{-1}) and consumption of O_2 (mg O_2 kg^{-1} h^{-1}) in the non-acetylene experiment by a) soil fraction [bulk sieved soil (B), large macroaggregates (LM), small macroaggregates (SM) and microaggregates (M)] and b) soil water-filled pore space (20%, 40%, 60% and 80% WFPS). Means in columns with similar letters are not significantly different at *P*<0.05.

a)

FRACTION	**CO_2**	**N_2O**	**O_2**
LM	0.78^{d}	14.18^{b}	2.42^{d}
B	2.08^{a}	33.13^{a}	6.20^{a}
SM	1.38^{c}	14.46^{ab}	4.20^{c}
M	1.77^{b}	2.27^{b}	3.10^{b}

b)

WFPS	**CO_2**	**N_2O**	**O_2**
20	0.80^{c}	0.25^{b}	2.36^{c}
40	2.02^{b}	0.18^{b}	3.88^{b}
60	0.77^{c}	0.16^{b}	2.66^{c}
80	2.43^{a}	63.46^{a}	7.04^{a}

Table 6. Efflux of CO_2 (mg C kg^{-1} h^{-1}), N_2O (ng N kg^{-1} h^{-1}) and consumption of O_2 (mg O_2 kg^{-1} h^{-1}) in the acetylene experiment by a) soil fraction [bulk sieved soil (B), large macroaggregates (LM), small macroaggregates (SM) and microaggregates (M)] and b) soil water-filled pore space (20%, 40%, 60% and 80% WFPS). Means in columns with similar letters are not significantly different at $P<0.05$.

a)

FRACTION	**CO_2**	**N_2O**	**O_2**
LM	0.70^{b}	5.04^{a}	3.58^{b}
B	1.02^{a}	0.56^{a}	4.64^{a}
SM	0.62^{b}	0.68^{a}	3.48^{bc}
M	0.51^{c}	22.2^{a}	3.20^{c}

b)

WFPS	**CO_2**	**N_2O**	**O_2**
20	0.272^{c}	0.03^{c}	2.44^{c}
40	0.70^{b}	0.04^{c}	3.54^{b}
60	0.91^{a}	5.09^{b}	4.40^{a}
80	0.96^{a}	23.32^{a}	4.54^{a}

Table 7. Effect of interaction of soil fraction [bulk sieved soil (B), large macroaggregates (LM), small macroaggregates (SM) and microaggregates (M)] and soil water-filled pore space (20%, 40%, 60% and 80% WFPS) on the efflux of CO_2 (mg C kg^{-1} h^{-1}), N_2O (ng N kg^{-1} h^{-1}) and consumption of O_2 (mg O_2 kg^{-1} h^{-1}) in the non-acetylene experiment. Means in columns with similar letters are not significantly different at *P*<0.05.

FRACTION	WFPS	CO_2	N_2O	O_2
LM	20	0.25^{a}	0.01^{a}	0.79^{a}
	40	1.06^{cd}	0.05^{a}	3.23^{cde}
	60	0.57^{ab}	0.32^{ab}	1.95^{abc}
	80	1.21^{de}	56.34^{cd}	3.80^{def}
B	20	1.60^{ef}	0.23^{ab}	4.61^{ef}
	40	2.79^{gh}	0.38^{b}	8.10^{gh}
	60	0.92^{bcd}	0.15^{ab}	3.25^{cde}
	80	3.04^{h}	131.77^{d}	8.85^{h}
SM	20	0.42^{a}	0.45^{ab}	1.25^{ab}
	40	1.69^{f}	0.10^{ab}	4.90^{f}
	60	0.90^{bcd}	0.13^{ab}	3.06^{cd}
	80	2.53^{g}	57.17^{d}	7.56^{gh}
M	20	0.95^{bcd}	0.31^{ab}	2.80^{cd}
	40	2.52^{g}	0.17^{ab}	7.30^{g}
	60	0.68^{abc}	0.04^{a}	2.34^{bcd}
	80	2.92^{gh}	8.57^{c}	7.96^{gh}

Table 8. Effect of interaction of soil fraction [bulk sieved soil (B), large macroaggregates (LM), small macroaggregates (SM) and microaggregates (M)] and soil water-filled pore space (20%, 40%, 60% and 80% WFPS) on the efflux of CO_2 (mg C kg^{-1} h^{-1}), N_2O (ng N kg^{-1} h^{-1}) and consumption of O_2 (mg O_2 kg^{-1} h^{-1}) in the acetylene experiment. Means in columns with similar letters are not significantly different at $P<0.05$.

FRACTION	**WFPS**	**CO_2**	**N_2O**	**O_2**
LM	20	0.26^{a}	0.024^{ab}	2.25^{a}
	40	0.73^{de}	0.035^{abc}	3.19^{b}
	60	0.99^{g}	19.41^{gh}	4.69^{fg}
	80	0.81^{ef}	0.67^{def}	4.16^{def}
B	20	0.46^{b}	0.03^{abc}	3.20^{b}
	40	0.95^{fg}	0.07^{abc}	4.33^{ef}
	60	1.24^{h}	0.08^{bcd}	5.25^{gh}
	80	1.43^{i}	2.06^{fg}	5.83^{h}
SM	20	0.18^{a}	0.05^{abc}	2.09^{a}
	40	0.62^{bcd}	0.05^{abc}	3.48^{bc}
	60	0.71^{de}	0.83^{cde}	3.75^{bcde}
	80	0.98^{fg}	1.81^{ef}	4.59^{f}
M	20	0.19^{a}	0.02^{a}	2.18^{a}
	40	0.52^{bc}	0.02^{ab}	3.13^{b}
	60	0.68^{cde}	0.02^{abc}	3.88^{cde}
	80	0.64^{cde}	88.74^{h}	3.58^{bcd}

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GENERAL CONCLUSIONS & CONTRIBUTIONS TO KNOWLEDGE

At the start of our field study, we hypothesized that below-ground content of CO_2 and N_2O would differ between the rhizosphere-associated soil of corn and soybean, however, we observed no such differences in the corn and soybean systems with regards to below-ground CO_2 or N_2O content. Our greenhouse experiment using potted corn and soybean plants enabled us to study CO_2 and N_2O efflux from the rhizosphere-associated soils, in a controlled environment. Thus, we observed rhizosphere respiration in corn from our greenhouse experiment averaged significantly higher than soybean. Also, corn rhizosphere contributed a greater proportion to the total soil respiration, averaging 79% of the soil respiration, whilst in the soybean pots, rhizosphere respiration constituted a lesser amount, averaging 58% of the soil respiration. Our results show that although rhizosphere respiration in corn exceeded that in soybean, on a per unit mass of root basis (specific) rhizosphere respiration was significantly higher in soybean (0.29 mg C g^{-1} root h^{-1}) than corn (0.09 mg C g^{-1} root h^{-1}), and this difference was more pronounced in the early stages of growth. We speculate that this difference was as a result of the higher respiratory cost of nutrient acquisition in soybean compared to corn, however, there is a need for more studies into this phenomenon.

Nitrogen assimilation in the roots and shoots appeared to be much higher in corn than in soybean and this difference grew more significant as the plants matured. Similarly, N loss from the plant-soil system was consistently higher in corn pots than in soybean pots with the highest N loss occurring in the early stages of growth for the corn pots, which may be an indication of higher denitrifier activity in the soil at that stage of growth.

Contrary to our initial hypothesis that tillage activity would improve aeration and oxygen content in the soil, thereby reducing below-ground content of CO_2 and N_2O, we observed significantly higher ($p<0.05$) CO_2 contents in conventionally-tilled soils (6.36 g C m^{-3}) than in the no-till soils (4.43 g C m^{-3}), whilst the effect on N_2O efflux was mostly insignificant. This was likely because tillage improved accessibility of organic matter and nutrients to soil microbes, increased mineralization of organic matter and plant residue, thereby creating more anaerobic microsites in the soil which favoured denitrification. Tillage effect on CO_2 was most dominant in the first month of the growing seasons, suggesting that the greatest tillage-induced losses of soil C may occur shortly after tillage, and that this is the period was important for C mineralization processes. Tillage also had a varied effect on the decomposition of corn and soybean tissue, significantly increasing CO_2 content in the soybean plots but not in the corn plots. This relates to the fact that soybean tissue is more labile and more easily incorporated into the soil through the tillage action than corn tissue. Although the trend was not consistent in both growing seasons, fertilizer amendment significantly affected CO_2 content in 2002, with higher content of CO_2 in the compost-treated soil (6.48 g C m^{-3} soil) than the inorganic fertilizer-treated soil (4.3 g C m^{-3}).

Temporal variations in the below-ground concentrations of CO_2 and N_2O were reflected in the disproportionately high CO_2 and N_2O content we observed during the first month of the growing seasons; 45 and 91% of total content of CO_2

and N_2O, respectively, measured in 2002, and 17% and 38% of total CO_2 and N_2O content, respectively, in 2003. These temporal variations may have been linked to differences in soil moisture levels within seasons. Accordingly, CO_2 and N_2O content declined as WFPS fell below 50% in both 2002 and 2003. On the average we observed that increases in soil temperature positively affected below-ground CO_2 and N_2O content only when soil moisture was not limiting.

Efflux of CO_2 and N_2O were significantly affected by the 10 kPa acetylene treatment, being 52.5% and 55.5 % respectively lower in the acetylene treated fractions than the non-acetylene treated fractions. Our result suggests that at 10 kPa, acetylene may inhibit the activity of decomposers by inhibiting the nitrification process, thereby reducing the mineral N available for organic matter mineralization in the soil fractions. However, a more direct inhibition of the activity of decomposers by exposure to10 kPa acetylene, may also have been possible and requires further investigation in future studies. Denitrification in the microaggregates proceeded at about 4.4 to 39.6 times higher rate compared to the other fractions, and showed a greatest response to high moisture levels (80% WFPS).

In our field study, we observed high autocorrelation of 0.64, 0.47 and 0.16 for successive measures of CO_2, N_2O and O_2 respectively, whilst in 2003, the autocorrelation was 0.91, 0.90 and 0.78 for successive measures of CO_2, N_2O and O_2 respectively indicating that CO_2 and N_2O content observed in our soils were not erratic or random, but were dependent on site specific factors. This strongly suggests that for field studies like ours involving replicate measurements of CO_2 and N_2O in time; it may be possible to reduce the number of replicates in time without compromising excessively on the reliability of the results.