

RESPONSE OF N<sub>2</sub>O TO NITROGEN MANAGEMENT AND  
BREEDING FOR SEED OIL IN BIODIESEL DEDICATED  
CANOLA

by

Labib El-Ali

Submitted in partial fulfilment of the requirements  
for the degree of Master of Science

at

Dalhousie University  
Halifax, Nova Scotia

in co-operation with

Nova Scotia Agricultural College  
Truro, Nova Scotia

May 2011

DALHOUSIE UNIVERSITY  
NOVA SCOTIA AGRICULTURAL COLLEGE

The undersigned hereby certify that they have read and recommend to the Faculty of Graduate Studies for acceptance a thesis entitled “RESPONSE OF N<sub>2</sub>O TO NITROGEN MANAGEMENT AND BREEDING FOR SEED OIL IN BIODIESEL DEDICATED CANOLA” by Labib El-Ali in partial fulfillment of the requirements for the degree of Master of Science.

Dated: May 30, 2011

Supervisor: \_\_\_\_\_

Readers: \_\_\_\_\_

\_\_\_\_\_

\_\_\_\_\_

DALHOUSIE UNIVERSITY

DATE: May 30, 2011

AUTHOR: Labib El-Ali

TITLE: RESPONSE OF N<sub>2</sub>O TO NITROGEN MANAGEMENT AND  
BREEDING FOR SEED OIL IN BIODIESEL DEDICATED CANOLA

DEPARTMENT OR SCHOOL: Department of Environmental Science

DEGREE: MSc CONVOCATION: May YEAR: 2012

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For my father.

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## Abstract

While breeding for increased oil yield has generated new lines of spring canola (*Brassica napus L.*) for biodiesel production, emissions of N<sub>2</sub>O from fertilized canola fields threaten to undermine the climate change mitigation benefits of canola as a biodiesel alternative to conventional diesel. This study determined the response of N<sub>2</sub>O emissions to canola line and N treatment in a maritime setting (Truro, Nova Scotia). Tissue N uptake was measured to determine whether differences in N uptake between the lines could explain any observed effect of canola line. Nitrate Exposure (the summation of daily soil NO<sub>3</sub><sup>-</sup> concentrations over a growing season, serving as an integrated measure of the exposure of soil biomass to nitrate over the growing season) was determined to investigate its potential as a predictor of N<sub>2</sub>O emissions. Four spring canola lines ('Topaz', 'Sentry', 'Polo', and 04C204, in order of increasing seed oil content) were paired with five N treatments (40, 60, 80, 100, and 120 kg N ha<sup>-1</sup>) in an incomplete two-factor factorial design over two growing seasons (2008 and 2009). N<sub>2</sub>O emissions were determined using a non-steady state vented chamber method. N<sub>2</sub>O emissions peaks closely followed increases in soil water content in both years, indicating that limited aerobicity was the trigger for N<sub>2</sub>O emissions events, and suggesting that denitrification was the predominant microbial process responsible for N<sub>2</sub>O emissions. The magnitude of average N<sub>2</sub>O emissions both years was considerably low when compared to other studies (0.55 and 0.56 kg N<sub>2</sub>O ha<sup>-1</sup> in 2008 and 2009 respectively). Increasing N treatment resulted in significantly increased N<sub>2</sub>O emissions in 2008. Though the same trend was observed in 2009, it was not found to be significant. Differences in weed cover, soil C, soil N supplying capacity, and elevation between the sites may have contributed to the inability to detect an N<sub>2</sub>O emissions response to N treatment in 2009. Canola line had no effect on N<sub>2</sub>O emissions in either study year, though heavy competition by weeds significantly affected canola plant health and survival in 2009. Tissue N uptake increased with increasing N treatment, but did not change with choice of line, which is consistent with the observation of no N<sub>2</sub>O emissions response to line. Nitrate Exposure was found to be strongly correlated with N<sub>2</sub>O emissions in a linear relationship, supporting the conclusion that Nitrate Exposure can be a promising indicator of N<sub>2</sub>O emissions when they are limited by soil N. Finally, FluxPerOil, the ratio of N<sub>2</sub>O emissions per unit oil yield (kg N<sub>2</sub>O kg<sup>-1</sup> oil) was found to decrease with decreased N treatment in 2008, though only very little, indicating a marginal abatement of N<sub>2</sub>O emissions at a significant cost of oil. FluxPerOil was unreliable in 2009 due to weeds compromising the line effect and therefore oil yield.

## List of Abbreviations and Symbols Used

ANOVA	Analysis of Variance
DEA	Denitrifier Enzyme Activity
ECD	Electron Capture Device
GHG	Greenhouse Gas
GWC	Gravimetric Water Content
GWP	Global Warming Potential
IPCC	Intergovernmental Panel on Climate Change
NDSU-ES	North Dakota State University Extension Service
TEA	Terminal Electron Acceptor
UNFCCC	United Nations Framework Convention of Climate Change
VWC	Volumetric Water Content
WFPS	Water Filled Pore Space

## Acknowledgements

This project would not have been possible without the guidance and faith of my supervisor, Dr. David Burton. I feel privileged and lucky to have had the opportunity to work and learn with him.

I would like to thank committee member Dr. Kevin Vessey for being available and instructive throughout the project, and also committee member Derek Lynch for his advice. Thanks go also to Dr. Peter McVetty, who supplied the germplasm for this work, contributed lab analysis, and took time to provide advice on the presentation and interpretation of the results.

I would like to also thank the Green Crop Network (GCN) for funding of this project, and their work in linking this research with other relevant other work happening within the network. Special thanks go to Dr. Donald Smith, Magali Merkx-Jacques, and Xiaomin Zhou at the GCN.

Thanks go to Drucie Janes and Laura Jollymore at the Greenhouse Gas laboratory of the Nova Scotia Agricultural College for their support in sample analysis and for guiding me in the necessary field and laboratory methods that were crucial to this work. A thank you as well to Briana Laybolt for her hard work during the 2008 field season.

Special thanks go also to Anne Lelecheur for her time and instruction on the LECO CNS combustion analyzer.

Finally, I wish to thank my colleague Adam Wile for his support and insight as we both worked through our masters projects.

## Chapter 1. Introduction

The capacity of a given biofuel to reduce GHG loading in the atmosphere depends on the greenhouse gas emissions savings gained throughout its life cycle when compared with that of its petroleum derived counterpart. In principle there are no net emissions from the combustion of a biofuel alone, since all carbon (C) contained in the fuel is fixed from the atmosphere. However, GHG emissions occur at other stages in the biofuel life cycle. In considering net GHG emissions from the lifecycle of biodiesel derived from oil seed crops, such as spring canola (*Brassica napus* L.), soil emissions of nitrous oxide (N<sub>2</sub>O) must be considered.

N<sub>2</sub>O is a greenhouse gas with a global warming potential (GWP) of 296 over a 100 year time frame (IPCC 2006). This means that a unit of N<sub>2</sub>O will trap 296 times the heat of a unit of carbon dioxide (CO<sub>2</sub>) over a 100 year period. N<sub>2</sub>O is emitted from soils primarily as a product of anaerobic respiration of soil denitrifiers and aerobic nitrification (Beauchamp 1997). When oxygen (O<sub>2</sub>) is limited, facultative denitrifying soil bacteria can respire by using soil nitrate (NO<sub>3</sub><sup>-</sup>) as a terminal electron acceptor in an electron transport phosphorylation chain (Firestone and Davidson 1989). The two major end products of this process are N<sub>2</sub>O and N<sub>2</sub>, and the ratio of their production is environmentally dependent (Weier et al. 1993). Nitrification, the aerobic oxidation of soil ammonium (NH<sub>4</sub><sup>+</sup>) to nitrite (NO<sub>2</sub><sup>-</sup>), and NO<sub>2</sub><sup>-</sup> to nitrate (NO<sub>3</sub><sup>-</sup>), involving different microfauna at each step (Stuart et al. 2007), has been shown to result in net N<sub>2</sub>O emissions (Bateman and Baggs 2005; Bremner and Blackmer 1978). However, most studies of N<sub>2</sub>O emissions from croplands have tended to focus on denitrification as the main contributor to soil N<sub>2</sub>O emissions (Beauchamp 1997). This could be due to the assumption that any available or applied NH<sub>4</sub><sup>+</sup> would be quickly and mostly converted to NO<sub>3</sub><sup>-</sup> through nitrification in an aerated soil (Myrold 1998).

Canola can be bred to increase seed oil content, as it is itself a product of breeding of older varieties of *B. napus* to achieve more desirable nutritional characteristics (Booth and Gunstone 2004). Breeding for seed oil would in principle increase oil yield per

hectare of crop. This may have consequences on plant N uptake from the soil and partitioning of N between seed and other plant tissue. Differences in plant N uptake between lines may affect soil  $\text{NO}_3^-$  levels, thereby indirectly influencing soil  $\text{N}_2\text{O}$  emissions when  $\text{NO}_3^-$  is limiting. In addition, it is unclear whether breeding for seed oil will influence nutrient flows in the rhizosphere (especially C and N) which may have an impact on biomass in the rhizosphere (Jones et al. 2009). Changes in chemical properties of the rhizosphere can have important impacts on the emission of greenhouse gasses from soil bacteria (Philippot et al. 2009).

Canola requires considerable N inputs, and has been observed to respond to N fertilization when soil  $\text{NO}_3\text{-N}$  is  $100 \text{ kg N ha}^{-1}$  and lower in Western Canada (Grant and Bailey 1993). Regardless of the form in which N is applied, an increase in soil  $\text{NO}_3^-$  can have implications on denitrification and  $\text{N}_2\text{O}$  emission, since  $\text{NO}_3^-$  can limit factor for denitrification (Beauchamp 1997). Also, the application of fertilizer containing  $\text{NH}_4^+$  (including ammonium nitrate, urea, and manure, among others) increases the quantity of substrate for nitrification. Numerous studies have observed an increase in  $\text{N}_2\text{O}$  emissions in response to added fertilizer (Snyder et al. 2009), and the IPCC (2006) suggests that 1% of added N is lost as gaseous  $\text{N}_2\text{O}$ . Nitrate Exposure, the summation of daily soil  $\text{NO}_3^-$  concentrations over a growing season, has been shown to be strongly correlated to soil  $\text{N}_2\text{O}$  emissions where soil mineral N limits denitrification (Zebarth et al. 2008b; Burton et al. 2008a). As an integrated measure of the exposure of soil denitrifiers to  $\text{NO}_3^-$ , it promises to be a good indicator (or even a predictor) of soil  $\text{N}_2\text{O}$  emissions.

Understanding the combined implications of breeding for seed oil content and managing N treatment for these new high oil yielding breeds of canola on soil  $\text{N}_2\text{O}$  emissions is an important task. It will aid in understanding the capacity of canola derived biodiesel to diminish the loading of GHG's in the atmosphere. As more agricultural land is being put under biodiesel production, farmers and policy makers will be interested in understanding the associated climate change abatement potential. Decreasing N applications can diminish  $\text{N}_2\text{O}$  emissions (Snyder et al. 2009), but this will most likely come at a yield cost. FluxPerOil (the units of  $\text{N}_2\text{O}$  emitted for every unit of oil yield gained,  $\text{kg N}_2\text{O kg}^{-1}$

oil) is a measure that can be useful in better understanding the yield costs associated with lowering N<sub>2</sub>O emissions through decreasing N treatment. It has the potential to aid both farmers and policy makers in quantifying the yield costs of GHG emissions reductions that are achieved through planting breeds selected for seed oil content and through more prudent N application.

### 1.1 Objectives

In this study our first and primary objective was to investigate the response of N<sub>2</sub>O emissions from canola to breeding for seed oil and N rate, and any interaction between the two. In so doing, we investigated the suitability of Nitrate Exposure as a predictor of soil N<sub>2</sub>O emissions in a conventional canola system. We also investigated tissue N uptake to understand its role in explaining any response of N<sub>2</sub>O emissions to line or N treatment. We also determined the response in FluxPerOil to increasing N treatment, with the aim of identifying an optimal N treatment rate where oil yields are maximized and N<sub>2</sub>O emissions reduced.

## Chapter 2. Literature Review

### 2.1 N<sub>2</sub>O from Agriculture in Canada

Lack of precision in data and the complexity of the associated biological and chemical processes create significant uncertainty in estimates of N<sub>2</sub>O emissions from agriculture (Beauchamp 1997). In 2007, Canada's contribution to global greenhouse gas emissions was estimated to be 747 Mt CO<sub>2</sub> equivalents, 60 Mt CO<sub>2</sub> equivalents (8%) of which were from the agricultural sector (Environment Canada 2007). Of those 60 Mt CO<sub>2</sub> equivalents from agriculture, 29 Mt CO<sub>2</sub> equivalents were in the form of N<sub>2</sub>O, and 7.1 Mt CO<sub>2</sub> equivalents of those were attributed to synthetic fertilizer application. In short, 0.95% of Canada's GHG emissions in 2007 were attributed to N<sub>2</sub>O release due to synthetic fertilizer application. To put this in perspective, GHG emissions from all Annex Parties to the United Nations Framework Convention on Climate Change (UNFCCC) in 2007 were an estimated 18,112 Mt CO<sub>2</sub> equivalents (UNFCCC 2007), of which Canada's contribution was 747 Mt CO<sub>2</sub> equivalents, or 4.1% (Environment Canada 2007).

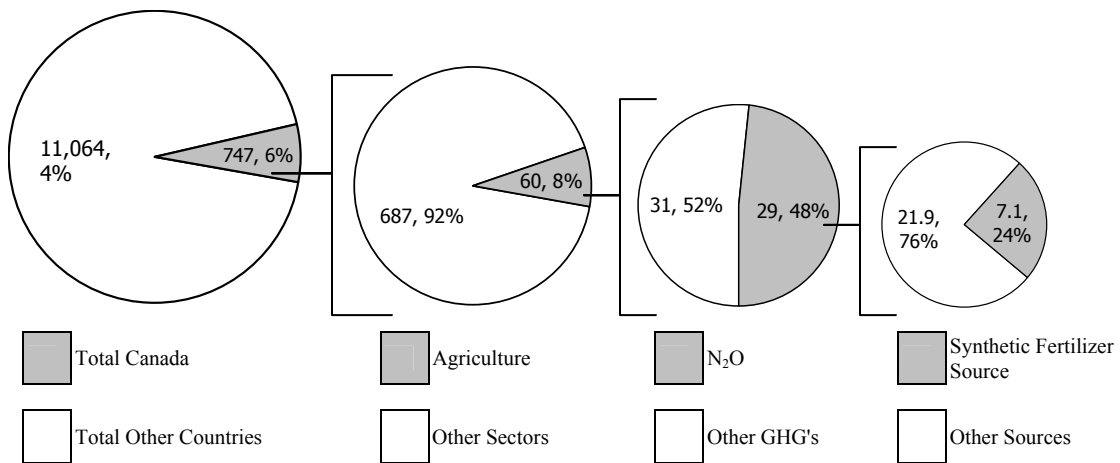


Figure 1. Breakdown of the share of N<sub>2</sub>O emissions from synthetic fertilizer application in Canadian Agriculture of Global GHG emissions in 2007 (CO<sub>2</sub> equivalents, %) (Environment Canada 2007)

From 1990 to 2007, Canada's agricultural sector contributions to GHG's grew by 11.2 Mt CO<sub>2</sub> equivalents (a 23.1% from 1990 levels), representing 7.2% of the 155 Mt CO<sub>2</sub> equivalents overall national increases in that same period (Environment Canada 2007). N<sub>2</sub>O emissions from agriculture increased from 26 to 29 Mt CO<sub>2</sub> equivalents in that

period, translating to 27% of the increase from agriculture, and 1.9% of the national increase in GHG emissions overall. The N<sub>2</sub>O emissions attributed to synthetic fertilizer application rose from 5.9 to 7.1 Mt CO<sub>2</sub> equivalents (a 17% increase), translating to 40% of the increase in N<sub>2</sub>O emissions from agriculture, 11% of the increase of all GHG's from agriculture, and 0.77% of the increase in national GHG emissions overall. With an estimated lifetime residency of 170 years in the atmosphere (Beauchamp 1997), emissions of N<sub>2</sub>O today will have global warming impacts for generations to come.

In addition to its global warming potential, N<sub>2</sub>O is an Ozone Depleting Substance (ODS). In a review on the impact of N<sub>2</sub>O on ozone (O<sub>3</sub>), Ravishankara et al. (2009) showed that it is the most important ozone depleting emission in the 21<sup>st</sup> century. N<sub>2</sub>O shares many similarities with Chlorofluorocarbons (CFCs), among the most widely known ODSs. Both are stable in the troposphere. When transported to the stratosphere, both transform into active chemicals that destroy O<sub>3</sub>. The authors also calculated the O<sub>3</sub> Depleting Potential (ODP) of N<sub>2</sub>O to be 0.017, making N<sub>2</sub>O comparable to CFCs in its capacity to destroy O<sub>3</sub>. ODP is the proportion of the amount of ozone destroyed by a unit mass of a given chemical at the earth's surface to that destroyed by a unit of CFC-11 (CFC<sub>11</sub>).

## 2.2 The Denitrification Pathway

Denitrification in the context of soil systems can be defined as “a form of anaerobic respiration in bacteria during which nitrogen oxide (NO<sub>3</sub><sup>-</sup>, NO<sub>2</sub><sup>-</sup>, NO, and N<sub>2</sub>O) reduction is coupled to electron transport phosphorylation” (Firestone et al. 1989). The microorganisms that carry out this process are found in cultivated and non-cultivated soils, and represent up to 5% of the total soil microbial community (Phillipot et al. 2007). An enzyme-catalyzed pathway reduces NO<sub>3</sub><sup>-</sup> into progressively reduced nitrogen oxides and eventually to N<sub>2</sub>. At each step reductases enable the denitrifying microorganisms to deposit electrons, completing electron transport as part of phosphorylative respiration (Firestone and Davidson 1989). In an environment of abundant NO<sub>3</sub><sup>-</sup>, denitrifying bacteria are likely to terminate the reduction sequence at N<sub>2</sub>O (Weier et al. 1993; Gillam et al. 2008), since NO<sub>3</sub><sup>-</sup> is a more energetically favorable compound to reduce than N<sub>2</sub>O



(Cho et al. 1997). This results in an accumulation of  $N_2O$  in the soil and its subsequent release into the atmosphere.

The conditions under which denitrification occurs are an anaerobic environment, the presence of nitrate, and an accessible source of reduced C (Philippot et al. 2007). Limited  $O_2$  availability prompts the utilization of  $NO_3^-$  as an alternate terminal electron acceptor (TEA). Reduced forms of C are electron donors for the denitrifying respiratory pathway. Finally,  $NO_3^-$  is the TEA upon which the whole denitrification sequence depends. Therefore the three most proximal factors influencing denitrification are aerobicity ( $O_2$  availability), availability of reduced C, and  $NO_3^-$  (Firestone and Davidson 1989). Note that when soil C is referenced in relation to denitrification in this paper, the reference is reduced forms of soil C, ones with the capacity to donate electrons to the denitrification process. The three proximal factors are discussed separately and in detail below. More distal factors (including climate, soil, and crop management) influence denitrification and associated  $N_2$  and  $N_2O$  emissions indirectly by influencing the three most proximal factors. One important additional factor is temperature, since it determines the rate of denitrification and nitrification (Snyder et al. 2009).

Spatial heterogeneity in denitrifier activity and end product ( $N_2$  and  $N_2O$ ) emissions is therefore expected and has been established (Beauchamp 1997). This spatial heterogeneity is expected, because denitrification is dependent on a complex set of distal conditions that vary spatially, and influence proximal factors. One possible way to identify spatial patterns is to examine soil aggregate size fractions as determiners of optimal conditions for denitrification, thereby explaining hot spots or pools of active denitrifiers (Seech and Beauchamp 1988). Looking at an arable cropping and a permanent grassland system, Miller et al (2009) found that aggregate size fraction did not influence denitrifier abundance in their arable cropping system. However they found that the smallest size fraction contained the greatest abundance of those same denitrifiers in the permanent grassland system. In that soil, they found that Denitrifier Enzyme Activity (DEA) did not differ among the size fractions in either system, and therefore concluded

that aggregate size fraction was uncoupled from the location of denitrification activity and denitrifier abundance in both systems.

### 2.3 Relevance of Nitrification

The relationship between  $N_2O$  emissions from nitrification and  $O_2$  availability is complex (Firestone and Davidson 1989). Whereas anaerobic soil microsites induce denitrification, nitrification is dependent on  $O_2$  availability for the oxidation of  $NH_3$  and as such diminishes with decreasing  $O_2$ . However, it is the reduction of accumulated  $NO_2^-$ , the product of the first step in the nitrification process, which generates  $N_2O$  under  $O_2$ -suppressed conditions. Predicting nitrification's share of  $N_2O$  emissions based on soil water content can therefore be difficult. Bateman et al. (2005) showed that autotrophic nitrification accounted for the majority of  $N_2O$  emissions between 35 to 60% WFPS, accounting for upwards of 81% at 60% WFPS in a fertilized silt loam.

### 2.4 Oxygen and Soil Water Content

Pathak (1999) summarizes the importance of soil water on  $N_2O$  flux: (a) it affects the growth and activity of soil microbes; (b) it affects the anaerobicity of soil micro sites; (c) it makes C and N substrates more accessible for nitrification/denitrification; and (d) it influences the diffusivity of substrates to and from soil bacteria. Of these, the influence of soil water on available  $O_2$  is of most interest in this study.

Denitrification occurs mainly in anaerobic environments, where denitrifiers reduce  $NO_3^-$  in the absence of  $O_2$  for respiration.  $O_2$  is the preferred electron receptor in phosphorylative respiration, meaning that denitrifiers favor the reduction of  $O_2$  over the reduction of  $NO_3^-$ , because  $NO_3^-$  has a smaller affinity coefficient for electrons than  $O_2$  (Cho et al. 1997). WFPS is a convenient measure of  $O_2$  availability in the soil, and several studies have found strong correlation between WFPS and  $N_2O$  emissions. Clayton et al. (1997) found 65% WFPS to be a critical threshold above which  $N_2O$  emissions increased dramatically. Similarly, Bateman and Baggs (2005) found tenfold increases in  $N_2O$  emissions when WFPS increased from 60% to 70% from a silt loam soil from an arable field, while Dobbie and Smith (2001) found up to 12 and 30 times increases of

N<sub>2</sub>O emissions from temperate grassland and aerable soils, respectively, when WFPS increased from 60 to 80%.

Wet field conditions and freeze-thaw events have been documented to cause dramatic responses in N<sub>2</sub>O flux (Burton and Beauchamp 1994; Wagner-Riddle and Thurtell 1998). In addition to N<sub>2</sub>O emissions due to the high WFPS associated from these scenarios, Burton and Beauchamp (1994) observed that N<sub>2</sub>O accumulated in subsurface regions following ice layer formations and was released to the atmosphere after thaw.

## 2.5 Carbon

The presence of plants has been observed to increase denitrification in soils (Scaglia et al. 1985). This increase is likely due to an increase in C availability from root exudates and the exudation of amino acids that increase the rate of certain steps in the enzyme dependent reduction process. Increased C availability affects N<sub>2</sub>O emissions in two ways. First, C limits denitrification as it is the source of electrons generating the demand for electron acceptors (Firestone and Davidson 1989). Second, increased soil C (possibly from root exudates or other sources) can stimulate microbial respiration and demand for O<sub>2</sub> as a TEA, thereby increasing the frequency of anaerobic soil micro sites necessary for denitrification (Garcia-Montiel et al. 2003; Gillam et al. 2008). Several studies have shown that movement of C to lower depths in the soil profile instigated greater denitrification in those layers than would be normally observed (Paul et al. 1997; McCarty and Bremner 1992; Yeomans et al. 1992).

Fazzolari et al. (1998) showed that under anaerobiosis and with limited C, denitrification can be more competitive at utilizing soil C than other processes, specifically dissimilatory nitrate reduction to ammonium (DNRA). On the other hand, C immobilization of nitrogen in the rhizosphere may limit inorganic NO<sub>3</sub><sup>-</sup> supply to denitrifier populations (Qian et al. 1997), thereby potentially reducing N<sub>2</sub>O emissions.

## 2.6 Canola N Fertility Management

As documented in a review of canola fertility management (Grant and Bailey 1993), a healthy and productive canola crop requires levels of soil N that match and at times exceeding the requirements of cereal crops. The authors have found that in Western Canada, canola generally responds to N inputs when soil  $\text{NO}_3\text{-N}$  concentrations are  $100 \text{ kg N ha}^{-1}$  or lower, and that maximum canola yields have been reported at N fertilization rates between  $100$  to  $200 \text{ kg N ha}^{-1}$ . The current recommendation by the Nova Scotia Department of Agriculture's Feed and Soil Testing Lab for canola is  $100 \text{ kg N ha}^{-1}$  (MacDonald 2008). Most fertilizer rate recommendations depend on the results of soil nitrate tests, and some take into account the soil's capacity for N mineralization. Of late, there has been increased interest in evaluating indices of potentially mineralizable N in soil (Sharifi et al. 2007), some of which could aid in providing more prudent fertilizer N recommendations. Crop farmers often look for the economically optimal N fertilization rate, which will vary by region due to soil and climate. The economically optimal N rate is also dependent on fertilizer N costs and the value of crop yield.

The effect of timing of N application on yield is influenced by climate and plant requirements (Grant and Bailey 1993). Where leaching, denitrification, or immobilization is thought to occur, early N application is encouraged, as shown in N-limited clay loam soils by Grant et al. (2002).

The Government of Saskatchewan reported that applying N at soil test recommended levels resulted in canola yields of  $1577 \text{ kg ha}^{-1}$ , and that application of 150% of soil test recommended level resulted in an additional  $50 \text{ kg ha}^{-1}$  only (Government of Saskatchewan 2010). NDSU-ES (2005) recommended about  $145 \text{ kg N ha}^{-1}$  to achieve yields of about  $900 \text{ kg ha}^{-1}$  in their region. This indicates that relatively high N fertilizer additions are required in North Dakota to achieve only moderate yields when compared to Saskatchewan. These 2 examples emphasize the high variability in N fertilizer rate recommendations and associated yields across canola growing regions.

To provide an example of a simplified regional guideline, the North Dakota State University Extension service recommends the use of the following rough formula to calculate fertilizer additions for canola (NDSU-ES 2005):

$$NR = (YG*0.05) - STN - PCC$$

where NR is the required supplemental N ( $\text{kg N ha}^{-1}$ ), YG is the desired yield ( $\text{kg ha}^{-1}$ ), STN is soil  $\text{NO}_3^-$  in the 0 to 24cm layer ( $\text{kg NO}_3\text{-N ha}^{-1}$ ), and PCC is previous crop credit from legumes.

Fertilizer N addition increases seed protein content but decreases seed oil content. Residual soil N will increase the pool of available N for canola growth, therefore cropping history should be taken into account in canola N management. There is likely to be little residual N however from porous soils subject to leaching, as often is the case with soils in Atlantic Canada. Hocking et al. (2002) has shown that at lower rates of N application, N removal in canola through seed harvest can exceed the N applied. N uptake in excess of added fertilizer would have come from the soil's residual N supply, or a significant soil capacity to supply N. The authors also demonstrated that high N uptake occurs around anthesis, which explains the benefit of split application at emergence and just before anthesis. Furthermore, they reaffirmed that concentration of N in shoots decreases from anthesis to maturity, which may in large part be due to migration of N to seeds and also to decreased N uptake after flowering.

## 2.7 Nitrate Exposure

Nitrate Exposure is the summation of daily  $\text{NO}_3^-$  concentration for a given depth of soil over a fixed period of time (Zebarth et al. 2008b). It serves as an integrated measure of the exposure of soil microbes to nitrate over a growing season. Few studies have looked specifically at the correlation of Nitrate Exposure<sup>1</sup> to  $\text{N}_2\text{O}$  flux (Burton et al. 2008a, Burton et al. 2008b; Zebarth et al. 2008a; Zebarth et al. 2008b). Nitrate Exposure has been found to be positively correlated with  $\text{N}_2\text{O}$  flux but not denitrification rate (Burton

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<sup>1</sup> In these early publications Nitrate Exposure was referred to as nitrate intensity.

et al. 2008a; Zebarth et al. 2008a). The lack of response in denitrification rate to Nitrate Exposure suggests that the affect of  $\text{NO}_3^-$  is on the ratio of denitrification end products ( $\text{N}_2$ :  $\text{N}_2+\text{N}_2\text{O}$ ) rather than the rate of the denitrification process itself. Similarly, Weier et al. (1993) and Gillam et al. (2008) found that greater  $\text{NO}_3^-$  availability through fertilization is associated with a greater proportion of  $\text{N}_2\text{O}$  released from denitrification, even though it may not affect denitrification rates. Some studies have however observed little response of  $\text{N}_2\text{O}$  flux to Nitrate Exposure (Burton et al. 2008b; Zebarth et al. 2008b). Soils with large N supplying capacity can diminish the impact of N addition on Nitrate Exposure and also minimize  $\text{N}_2\text{O}$  response to fertilization (Burton et al. 2008b). Also, limited soil C, especially later in the growing season, can limit denitrification and at times explain diminished  $\text{N}_2\text{O}$  response to Nitrate Exposure (Zebarth et al. 2008b).

Hynst et al. (2007) found that the splitting of nutrient (C and N) treatments did not significantly affect overall  $\text{N}_2\text{O}$  response in their experiment, while Burton et al. (2008) found split N application decreased  $\text{N}_2\text{O}$  emissions in years where there was significant rainfall between the periods of N application.

## 2.8 Canola for Biodiesel

Canola is a well-known Canadian success story in agriculture. The term itself is short for “Canadian oil-low acid,” and is reserved for rapeseed that has been bred to contain less than 2% erucic acid and less than  $30 \mu\text{M g}^{-1}$  glucosinolates in the meal (Booth and Gunstone 2004). Roughly 11.3 million acres of canola are harvested annually in Canada (Canola Council 2010), mostly for processing into cooking oil.

Diesel engines were designed more than a century ago for burning vegetable oil, according to Wang et al. (2000), whom cite the use of peanut oil in a diesel engine in the late 1800s. The authors indicate however that there were problems in using unaltered vegetable oils directly as fuel, problems mainly attributed to the oils’ high viscosity and low volatility. Today, a transesterification process is applied to vegetable oils to reduce their viscosity and make them more useful as a fuel (Chauhan et al 2008), where triacylglycerides are reacted with methanol using a base catalyst (e.g. NaOH) to generate

a mono-alkyl ester product (biodiesel) and glycerol (Rapier 2008). Other alcohols (e.g. ethanol and long chain alcohols) can also be used. A wide variety of vegetable oils (including canola oil) are suitable candidates for biodiesel production. The resulting biodiesel product can be used directly in conventional compression-ignition engines, or mixed with conventional diesel (Wang et al 2000).

Rapier (2008) describes how the combustion of conventional diesel fuel is by itself a more energy efficient process than the combustion of conventional gasoline and its substitutes. Diesel fuels (the category of fuels including diesel and home heating oil) are naturally more energy dense than gasoline. Also, a compression ignition engine (CIE), which is what is used to combust diesel oil, ignites the fuel through compression, whereas a spark ignited engine (SIE) ignites the fuel through a spark plug. Gasoline is not resistant to ignition as it is being compressed, which is why it is not used in CIE's and cannot be compressed to the extent that distillates can. This means that CIE's achieve a higher compression ratio when compared to an SIE, resulting in a more powerful combustion, and therefore realizing more useful energy output than an SIE.

Studies comparing the emissions of CO<sub>2</sub>, CO, NO<sub>x</sub>, and particulate matter from the combustion of biodiesel with those produced from the combustion of conventional diesel have shown mixed results (Rapier 2008). It is likely that emissions of the above toxins and greenhouse gasses are engine dependent. Never the less, since it is a biofuel – the C emitted from the combustion of biodiesel is offset by the C fixed from the atmosphere by the crop. Studies on net energy production associated with biodiesel crops also show mixed results (Rapier 2008). However, Smith et al. (2007) found the ratio of energy produced to energy input in canola to range from 2.08 to 2.36 under Canadian conditions, indicating that it is a promising candidate for biodiesel development in Canada. Samson et al. (2008) found that there was 45 GJ ha<sup>-1</sup> in solar energy collected by canola for 11.3 GJ ha<sup>-1</sup> fossil fuel energy used in its production. The same study found that canola-derived biodiesel could offset 58% of emissions from the combustion of conventional diesel when looking at diesel use in transportation.

Canola and soybean are among the top candidates for biodiesel feedstocks in Canada (Smith et al. 2007). Samson et al (2008) found that a slightly higher proportion of GHG's were offset when conventional diesel was switched for canola-based biodiesel instead of soybean-based biodiesel in transportation (58% and 50% for canola and soybean, respectively). Another reason Canola is considered a strong option for biodiesel development is due to the very favorable growing conditions for the crop in Canada.



## Chapter 3. Methods

### 3.1 Sites

Trials were established at the Plumdale Research Facility in 2008 (45°37'59"N, 63°23'99"W, 42.0 m above sea level) and Brookside Field Research Site in 2009 (45°39'12"N, 63°23'61"W, 30 m above sea level). The two sites are 2.40 km apart, and are located in the town of Bible Hill, Nova Scotia. Different sites were chosen between years to avoid continuous canola production and to test repeatability of results at different agricultural fields with similar soils and climatic conditions. Soil at the Plumdale site was Truro class and sandy loam in texture. Soil at the Brookside site was Pugwash class and coarse loamy in texture. The Plumdale (2008) site was fallowed in 2007. The Brookside (2009) site was planted to barley in 2008 and received 150 kg N ha<sup>-1</sup> in the form of ammonium nitrate. Climate data for the region was acquired from the National Climate Data and Information Archive (Environment Canada 2009). Seasonal precipitation was determined by summing daily precipitation for the duration of the growing season at the Debert, Nova Scotia weather station (45°25'00"N, 63°28'00"W, 37.5m above sea level). The weather station was the closest one to the two sites that was operation. It is 24.45 km from the Plumdale (2008) site, and 26.82 km from the Brookside (2009) site.

**Table 1.** Site characteristics

Site	Plumdale, 2008 45°38'N, 63°24'W	Brookside, 2009 45°39'N, 63°24'W
Elevation (m)	42	30
Annual precipitation (mm)	1202	1202
Growing season precipitation (mm)	455	455
Annual mean air temperature (°C)	5.8	5.8
Soil Type	Truro Sandy Loam	Pugwash Coarse Loamy

Gaining and understanding of the magnitude and pattern of precipitation at the study sites is important, as they will have an affect on soil water content and therefore O<sub>2</sub> availability in the soil. The area receives on average 1202 mm of rainfall annually, 455 mm during

the growing season (May to September, inclusive) (Environment Canada 2009). Mean annual air temperature is 5.8 °C. Highest temperatures are observed in June, July, and August. Despite significant rainfall, lengthy dry spells are not uncommon during the summer. Temperatures well below freezing and significant snow cover are typical of winter months (Dec to Feb). Soil bulk density was assumed to be 1.3 g cm<sup>-3</sup>.

### 3.2 Canola Lines

Seed for four lines of spring canola (*Brassica napus L.*) were sourced from the Canola/Rapeseed Breeding Program at the University of Manitoba in 2008. In order of increasing seed oil content, the lines and their respective seed oil percentages are: Topaz (low, 42 to 44%); Sentry (moderate, 45%); Polo (high, 47 to 48%), 04C204 (50% or higher) (McVetty 2008). Seed from the same source was used in the 2008 and 2009 trials.

### 3.3 Fertilizer Rates

Five N fertilizer rates (40, 60, 80, 100, and 120 kg N ha<sup>-1</sup>) were chosen. The current recommendation by the Nova Scotia Department of Agriculture's Feed and Soil Testing Lab for canola is 100 kg N ha<sup>-1</sup> (MacDonald 2008). In the case of a strong N treatment effect on N<sub>2</sub>O emissions, the 120 kg N ha<sup>-1</sup> treatment will provide useful information on the N<sub>2</sub>O emissions cost of fertilizer application that exceeds the optimum N rate for yield. Likewise 40 kg N ha<sup>-1</sup> was chosen as the lowest treatment to determine N<sub>2</sub>O emissions savings at a significant yield cost. Half the total amount of N was applied as ammonium sulfate shortly after germination and the other as ammonium nitrate at bolting. N application was split in order to minimize loss of N due to leaching in the spring. Since low soil S limits canola growth (Grant et al. 2003), fertilizing with ammonium sulfate avoided any effect of limited S on plant health. The application of S as ammonium sulfate took place shortly after seeding, which is preferable to application at bolting (Malhi and Gill 2002).

### 3.4 Experimental Design

An unbalanced two-factor factorial design was used to assess N<sub>2</sub>O response to both line and N treatment, and any interaction between the two. The unbalanced design allowed for

a greater number of replications. All four lines were fertilized with three different N rates (40, 80, and 120 kg N ha<sup>-1</sup>), and two of the lines (Topaz and Polo) were fertilized with two additional N rates (60 and 100 kg N ha<sup>-1</sup>). There were 16 treatment combinations in all, and each pairing was replicated 5 times, giving a total of 80 plots in a randomized complete block design.

This set-up allows for two separate full-factorial designs to be implemented: one better able to detect an effect of N treatment and one better able to detect an effect of line. In the first, two lines are treated with 5 different N treatments (2L5R), each combination replicated 5 times, providing 25 treatment combinations per line. In the second, all 4 lines are treated with 3 N treatments (4L3R), each combination replicated 5 times, providing 20 treatment combinations per N rate.

A planting error in 2009 resulted in the loss of two plots. One would have been seeded to Sentry and fertilized with 40 kg N ha<sup>-1</sup> and the other with Polo and fertilized with 120 kg N ha<sup>-1</sup>. The analyses were therefore conducted with one less rep (4 instead of 5) for both treatment combinations.

Two-factor Analyses of Variance (ANOVA) were conducted to determine the effect of line and N treatment on cumulative N<sub>2</sub>O emissions, Nitrate Exposure, and N uptake. Separate ANOVA's were conducted for the 2L5R and 4L3R designs for all responses (except for N uptake, since only 2 lines and three N treatments were included in studying N uptake, therefore requiring only one ANOVA for each N uptake sampling date). The coefficient of determination ( $r^2$ ) between cumulative N<sub>2</sub>O emissions and Nitrate Exposure will be determined using the method utilized by Zebarth et al. (2008b).

### 3.5 Crop Management

Crop management was conducted and overseen by the Crop Development Institute of the Nova Scotia Agricultural College. Planting took place on May 13 in both 2008 and 2009. Seed was drill planted. Each plot was seeded into 16 rows, with 15 cm spacing between rows and a seeding rate of 130 seeds m<sup>-2</sup>. Prior to planting, the soil was disked, then

treated with HeliXTRA (combination fungicide/insecticide), Treflan EC herbicide at 2.3 L ha<sup>-1</sup>, and 40 kg ha<sup>-1</sup> of P<sub>2</sub>O<sub>5</sub> and 40 kg ha<sup>-1</sup> of K<sub>2</sub>O. Shortly after emergence, the Brookside site was hand weeded using hoes due to significant weed presence.

Nitrogen fertilizer application was conducted by hand broadcasting fertilizer prills over each plot. Ammonium sulfate was applied on May 26 in 2008 and on May 25 in 2009, and ammonium nitrate on July 7 in 2008 and on July 10 in 2009.

During broadcasting the collar area was covered, prohibiting any prills from falling within the collars. The exact amount of fertilizer that should fall within the specific collar area was calculated and applied separately. This ensured that N<sub>2</sub>O gas emissions from the soil area contained in the collars was more representative of the fertilizer treatment for each plot, which could not be guaranteed by hand broadcasting over the entire plot area alone. This system was conceived mid season in 2008, and therefore was not used in the application of ammonium sulfate in 2008.

A 1.25m length of each plot was harvested with a Hege125C combine to acquire yield. Plots were harvested on September 12 in 2008 and September 11 in 2009. Seed was threshed, air dried, and weighed.

Plant survival to maturity was severely affected by weeds and birds in 2009 and treatment effects on yield for that year were deemed unreliable (see below). Weed cover was determined through visual approximation of the proportion of the area covered by weeds in each plot.

### 3.6 N<sub>2</sub>O Flux Measurement

A non-steady state, vented chamber method (Burton et al. 2008a) was used to determine N<sub>2</sub>O flux. Circular base collars (20.3 cm diameter and 5 cm high) made of roughly 1 cm thick PVC piping, were placed at least 0.5 m in from any side of the plot. Collars were inserted at least 10 cm into the soil. A collar-height measuring tool (a circular disc fitted with six sliding metal rods) was used to determine the volume between collar tops and

soil surface. On deployment, vented and circular insulated chamber tops (20.3 cm diameter and 15 cm high, 4.9 L in volume) were placed on top of collars. A closed cell foam gasket attached to the lower edge of the chamber top formed a seal with collar. Headspace gas samples (20 mL) were collected at 0, 10, 20, and 30 minutes from the time of chamber deployment. Samples were collected using syringes and injected into evacuated 12 mL Exetainers (Labco, UK). Exetainers were purged with N<sub>2</sub> gas and evacuated to 300 millitorrs prior to use. They also contained a desiccant (4 mg of magnesium perchlorate) to remove water from collected gas samples. Five evacuated exetainers were injected with a standard gas on sampling days and carried to the field to simulate the conditions to which field-collected samples were exposed. This was conducted to confirm the integrity of field-collected samples. Sampling took place weekly in the spring and then approximately biweekly in the summer and fall. The higher sampling frequency in the spring was due to expected higher N<sub>2</sub>O flux under elevated soil moisture conditions and following fertilizer application (Beauchamp 1997).

N<sub>2</sub>O gas concentrations were determined using gas chromatography, using a Varian CP-3800 GC (Varian, Mississauga, ON) fitted with an electron capture detector (ECD) with a Combi-Pal autosampler. The autosampler removes a 2.5 mL volume from the sample tube and injects this into a sample valve that delivers 0.1 mL to the ECD. The ECD was operated at 300 °C, 90%Ar, 10%CH<sub>4</sub> carrier gas at 10 mL min<sup>-1</sup>, Haysep N 80/100 pre-column (0.32 cm diameter x 50 cm length) and Haysep D 80/100 mesh analytical columns (0.32 cm diameter x 200 cm length) in a column oven operated at 70 °C. Pre-column was used in combination with a valve to remove water from the sample. Operational conditions and data handling was performed with Varian Star<sup>TM</sup> software. In each analytical run of 150 samples, five replicates of three concentrations of standard gas mixtures were run between each tray of 50 samples for quality assurance/quality control purposes.

N<sub>2</sub>O flux (kg N<sub>2</sub>O ha<sup>-1</sup> d<sup>-1</sup>) was determined using the following formula (Hutchinson and Livingston 1993):

$$F_{N_2O} = \frac{\partial C}{\partial t} \frac{V_c M_{mol}}{AV_{mol}}$$

where  $\partial C$  is the change in N<sub>2</sub>O gas concentration in the chamber (uL L<sup>-1</sup>),  $\partial t$  is the change in time (min),  $V_c$  is chamber volume after correction for temperature and relative humidity (L),  $M_{mol}$  is the molar mass of N<sub>2</sub>O (g mol<sup>-1</sup>),  $A$  is the collar area (m<sup>2</sup>), and  $V_{mol}$  is the volume of one mol of N<sub>2</sub>O gas after correction for air temperature using the ideal gas law. Linear approximations of the rate of change of N<sub>2</sub>O gas concentrations in the chamber were conducted using a simple linear-regression. Non-linear N<sub>2</sub>O accumulation patterns were typically associated with a mistake in sampling order (which was then fixed), or corrupted samples. At least three sound gas samples per plot were necessary to deduce flux; therefore plots with 2 or more corrupted samples were deemed lost on that particular sampling day (a rare occurrence). Cumulative N<sub>2</sub>O flux for the field season was calculated by summing the products of flux and the time period associated with that flux (half the number of days to the previous sampling date plus half the number of days to the next sampling date). Linear change in flux between sampling dates was assumed.

An air temperature and air humidity gauge (Cole Palmer) were used to determine both. A Hydrosense gauge (Campbell Scientific) was used to determine soil water content. Soil temperature was measured using a soil temperature probe (OAKTON Instruments). All were measured every flux sampling day.

### 3.7 Soil Nitrate

Three 2.5 cm diameter soil core samples from the 0 to 15 cm layer of each plot were taken biweekly or monthly and combined, then frozen. Samples were taken out to thaw at room temperature the day before analysis. Mineral NO<sub>3</sub>-N was extracted by mixing 25 g of soil in 50 mL of 0.5 M K<sub>2</sub>SO<sub>4</sub> in 125 mL French Square flasks, shaking for 1 hr, and then pouring the mixture through Whatman No. 42 filter paper into scintillation vials before freezing. Blank samples of 0.5 M K<sub>2</sub>SO<sub>4</sub> were carried for each analysis date. The

filtrate deposited in scintillation vials was analyzed colorimetrically for  $\text{NO}_3^-$  and  $\text{NO}_2^-$  on a Technicon Autoanalyzer II (Technicon AAI, Pulse Instruments, Saskatoon, SK). At the time of extraction, a separate subsample of soil (10g) was weighed, oven dried at  $60^\circ\text{C}$  for 24 hours, and weighed again to determine soil water content. Nitrate Exposure for the growing season was determined using the same linear interpolation method used to determine cumulative  $\text{N}_2\text{O}$  emissions. Nitrate Exposure was calculated for a period of 170 days in 2008 (May 12 to October 29) and 140 days in 2009 (May 13 to September 30).

### 3.8 N Uptake

Plant N uptake was determined for plots cropped to Topaz and Polo lines and treated with 40, 80, or 120 kg N  $\text{ha}^{-1}$ . Two lines and three N treatments were deemed sufficient to investigate how much N uptake can explain any effect of line or N treatment on  $\text{N}_2\text{O}$  flux. Ten plants from each of these plots were harvested at random at three times during the growing season (bolting, mid-season, and harvest) to determine plant N uptake. Plants were harvested 1" above the soil surface. The decision to include plant N uptake as a part of the project was not made until early in the season in 2008, and therefore no sample plants were taken at the bolting stage in that year. Harvested plants were dried at  $55^\circ\text{C}$  for 48 hours, ground using Wiley mill, then further ground using a ball mill. Ground tissue was analyzed for total N using a LECO CNS 1000 dry combustion analyzer (LECO, Michigan). Dried tissue weights of the ten plants harvested were also used to determine the above ground biomass in the entire plot. Plant N uptake sampling was conducted on the following dates: July 21, 2008 (mid-season); Sept 4, 2008 (harvest); July 3, 2009 (bolting); July 31, 2009 (mid-season); Aug 31, 2009 (harvest).

### 3.9 Weed Cover

Weed presence in 2009 was much larger than in 2008. The Brookside site is known to historically be more prone to weed infestations. The problem was severe enough in 2009 to affect the survival of germinated canola plants, and therefore seed and oil yield. A significant number of plots were effectively over grown with weeds, enough to diminish canola stands in those plots considerably, especially later in the season. In order to

determine approximate N uptake by canola plants and weeds, the last N uptake measurement in 2009 was conducted by harvesting the area enclosed by a 0.61 ft by 0.61 ft quadrat in all plots, and using the N uptake analysis procedure outlined in the methods section for canola tissue. Also, a rough estimate of the proportion (%) weed cover in each plot was conducted by eye estimation on August 31, 2009. This was done in order to investigate whether differences in weed cover among plots could explain any response from Line or N treatments in 2009.



## Chapter 4. Results

Growing season precipitation was higher in 2008 than 2009 by more than 30% (Table 2). Volumetric Water Content (VWC) and Gravimetric Water Content (GWC) were slightly higher in 2009 than in 2008 (Table 2). Growing season (May to September, inclusive) average daily temperature was nearly the same in both years (15.3 and 15.0 in 2008 and 2009, respectively). Average soil nitrate concentration was nearly equal in 2008 and 2009 (15.5 and 16.0 g NO<sub>3</sub>-N kg<sup>-1</sup> soil, respectively). Average growing season soil nitrate in the 0 to 15cm soil layer in the periods of highest N<sub>2</sub>O flux (May 13 to July 22, 2008 and May 13 to July 14, 2009, Figures 2 and 3, respectively) was 23.0 and 19.7 kg NO<sub>3</sub>-N kg<sup>-1</sup> soil for 2008 and 2009, respectively.

**Table 2.** Mean annual values for climate and soil descriptors

Site	Plumdale, 2008	Brookside, 2009
Nitrate (mg NO <sub>3</sub> -N kg <sup>-1</sup> soil)	15.5	16.0
Nitrate during highest N <sub>2</sub> O flux <sup>†</sup> (mg NO <sub>3</sub> -N kg <sup>-1</sup> soil)	23.0	19.7
Gravimetric Water Content (g g <sup>-1</sup> )	19.3	21.3
Volumetric Water Content (cm <sup>3</sup> cm <sup>-3</sup> )	24.3	27.6
Growing Season* Precipitation (mm)	552.2	402.8
Total precipitation during high N <sub>2</sub> O flux period <sup>x</sup> (mm)	194.8	182.6
Growing Season* Average Daily Temperature (°C)	15.3	15.0

<sup>†</sup>May 13 to July 22 in 2008 and May 13 to July 14 in 2009

\*May to September, inclusive

<sup>x</sup>May 15 to July 31 for both years

Average cumulative N<sub>2</sub>O emissions were nearly equal between the two years (0.56 and 0.55 kg N<sub>2</sub>O ha<sup>-1</sup> in 2008 and 2009) and varied within the same range (Table 3).

Cumulative emissions were calculated for a period of 191 days in 2008 (May 12 to November 19) and 171 days in 2009 (May 13 to October 31). Flux measurement ended 21 days earlier in 2009 due to the limited resources, however this did not affect cumulative emissions analysis since N<sub>2</sub>O flux was nearly negligible from the beginning of September and onwards in both years. Nitrate Exposure was less in 2008 than in 2009 (1.86 and 2.12 g NO<sub>3</sub>-N day kg<sup>-1</sup> soil, respectively) but varied within the same range as well (Table 3).

**Table 3.** Cumulative N<sub>2</sub>O emissions and Nitrate Exposure in 2008 and 2009<sup>+</sup>

	Cumulative N <sub>2</sub> O flux (kg N <sub>2</sub> O ha <sup>-1</sup> )		Nitrate Exposure (kg NO <sub>3</sub> <sup>-</sup> -N days ha <sup>-1</sup> )	
	2008	2009	2008	2009
Average	0.56	0.55	1.85	2.12
Maximum *	1.55	1.85	3.93	3.92
Minimum *	0.13	0.17	1.13	1.17

<sup>+</sup> Calculated for 191 days in 2008 and 170 days in 2009

\* Represent maximum and minimum observed in any one plot

The implications of weed competition in 2009 on yield, soil nitrate, and flux are outlined in the discussion section below. An analysis of variance was conducted on differences in weed cover within plots, in case by chance (or through some effect of treatment) certain plots with similar line and N treatments had similar weed cover rates (Table 4). No significant differences were found. It was also found that weed cover and cumulative flux in 2009 were poorly correlated ( $r^2 = 0.096$ ), and that weed cover and Nitrate Exposure were also poorly correlated did not explain differences in Nitrate Exposure ( $r^2 = 0.059$ ).

**Table 4.** Analysis of variance on differences among line and N treatments in plot weed cover for 2009 (%)

N treatment [N] (kg N ha <sup>-1</sup> )	2L5R	4L3R <sup>+</sup>
40	26.0	23.7
60	25.5	-
80	27.0	25.6
100	21.0	-
120	25.0	24.7
Line [L]		
Topaz	17.4	13.8
Sentry	-	17.1
Polo	32.7	30.6
04C204	-	18.8
ANOVA		
N	0.83	0.90
L	0.075	0.14
N*L	0.88	0.75

<sup>+</sup> means are the anti-log of those derived from an ANOVA conducted on log transformed data

The 2L5R analysis (designed to better detect effect of line) showed no significant effect in either year of line or N treatment on cumulative N<sub>2</sub>O emissions, plant N uptake, or FluxPerOil. The only significant response in the 2L5R design was that of Nitrate Exposure to N rate in 2008, but not in 2009. However, all four response variables (N<sub>2</sub>O emissions, Nitrate Exposure, plant N uptake, and FluxPerOil) responded to N rate in both years in the 4L3R analysis, which was designed to better analyze the effect of N rate. Therefore, only the 4L3R analysis is referenced in the discussion below in reference to the above mentioned four responses (Tables 5 and 10).

An analysis of variance on the effect of line and N treatment on cumulative N<sub>2</sub>O emissions over the growing season showed inconsistent results between the two years (Table 5). There was no effect of line in either year. N treatment did have a significant effect on cumulative N<sub>2</sub>O flux in 2008 ( $p < 0.001$ ). The increase in fertilization from 40 to 120 kg N ha<sup>-1</sup> resulted in an increase in cumulative N<sub>2</sub>O emissions from 0.37 to 0.64 kg N<sub>2</sub>O ha<sup>-1</sup>. The increase in cumulative N<sub>2</sub>O emissions in response to N treatment in 2009 was not significant.

**Table 5.** Analysis of Variance of N treatment and canola line effect on cumulative N<sub>2</sub>O emissions, Nitrate Exposure, and FluxPerOil (4L3R analysis only)

N treatment [N] (kg N ha <sup>-1</sup> )	Cumulative Flux (kg N <sub>2</sub> O ha <sup>-1</sup> )		Nitrate Exposure (g NO <sub>3</sub> -N day kg <sup>-1</sup> soil)		FluxPerOil (g N <sub>2</sub> O kg <sup>-1</sup> oil)	
	2008 <sup>+</sup>	2009 <sup>+</sup>	2008 <sup>+</sup>	2009	2008 <sup>+</sup>	2009 <sup>+</sup>
40	0.37 <sub>b</sub>	0.43	3.05 <sub>b</sub>	3.60 <sub>b</sub>	1.06	3.62
80	0.54 <sub>a</sub>	0.49	3.53 <sub>b</sub>	4.33 <sub>ab</sub>	1.45	2.83
120	0.64 <sub>a</sub>	0.58	4.39 <sub>a</sub>	4.60 <sub>a</sub>	1.56	2.53
Line [L]						
Sentry	0.44	0.46	3.76	4.21	1.02	2.68
Topaz	0.29	0.31	3.56	4.01	1.15	3.79
Polo	0.44	0.43	3.68	4.12	1.33	2.90
04C204	0.32	0.58	3.46	4.35	0.82	2.65
ANOVA						
N	1.651*	0.385	0.666*	4.930*	0.827 <sup>x</sup>	0.669
L	0.231	0.285	0.008	0.378	0.215	0.047
N*L	0.80	0.193	0.014	0.398	0.299	0.088
Error	0.194	0.285	0.042	1.296	0.276	0.443

<sup>+</sup> Means are geometric means (the anti-log of means derived from log transformed data). ANOVA values presented are Mean Sum of Squares (MSS). Where data was log transformed, MSS values are those associated with log transformed data.

\*P < 0.05

<sup>x</sup>P < 0.10

The average temporal fluctuations in daily N<sub>2</sub>O flux tended to closely follow trends in soil water content in both years (Figures 2 and 3). Daily N<sub>2</sub>O flux peaked shortly after the first N application (ammonium sulfate), which coincided with a peak in soil moisture. N<sub>2</sub>O flux decreased considerably shortly after that (mid-June), coinciding in both cases with a decrease in water content. It peaked again after an increase in soil moisture, a peak that also immediately followed the second N application (ammonium nitrate). The third and final N<sub>2</sub>O flux peak took place in mid July, and was more pronounced than in 2008 than in 2009, where it also followed fluctuations in soil moisture. Finally, in both years, N<sub>2</sub>O flux diminished quickly after that and remained low for the remainder of the season despite increases in soil moisture content.

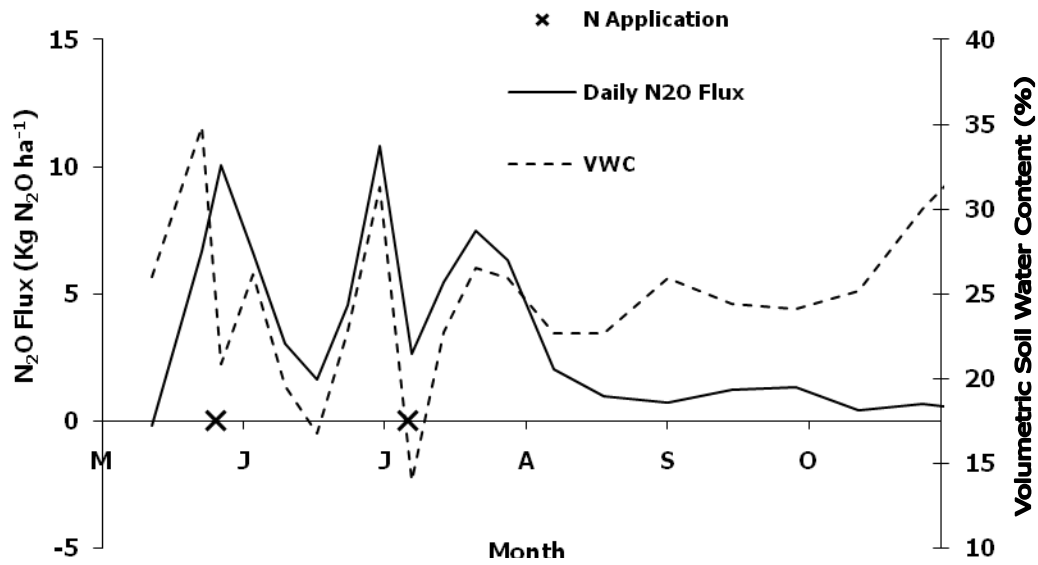


Figure 2. Average daily N<sub>2</sub>O flux and Volumetric Water Content (VWC) during the 2008 growing season (tick marks on the x-axis represent first day of each month).

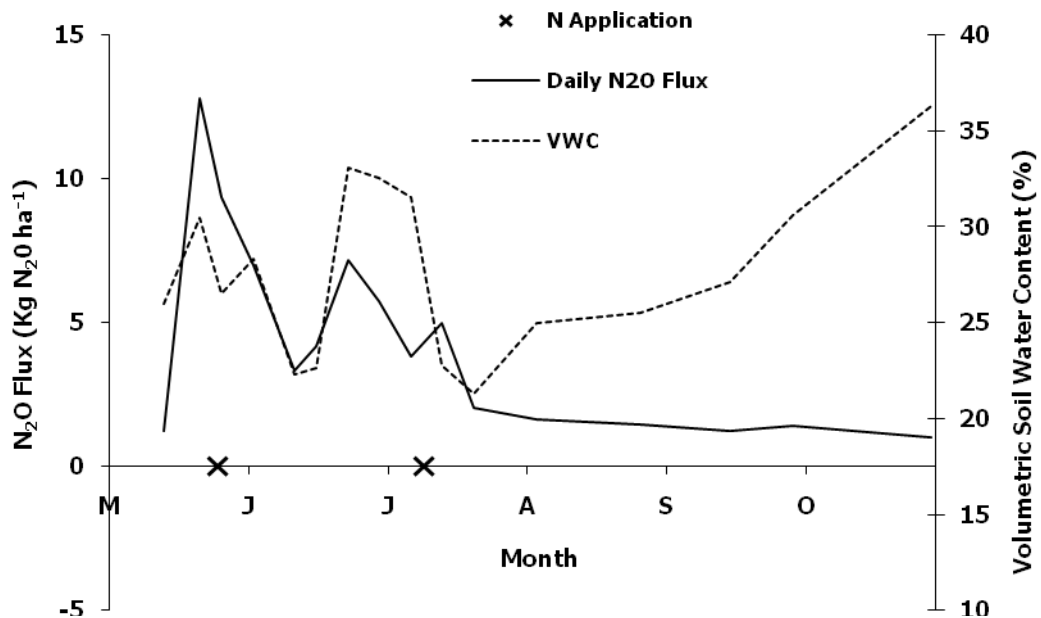


Figure 3. Average daily N<sub>2</sub>O flux and Volumetric Water Content (VWC) during the 2009 growing season (tick marks on the x-axis represent first day of each month).

Differences were observed between the two years in the temporal patterns of N<sub>2</sub>O flux as influenced by N fertility treatment during the period of high N<sub>2</sub>O flux, or late May to late

July (Figures 4 and 5). In 2008, from mid June onwards, flux tended to be higher with higher N treatments (Figure 4). The same cannot be said for 2009, where a consistent trend in N<sub>2</sub>O flux as a function of N treatment is not apparent at any time in the season. In fact earlier in the season, plots with lower N rate had the highest N<sub>2</sub>O flux of the entire season.

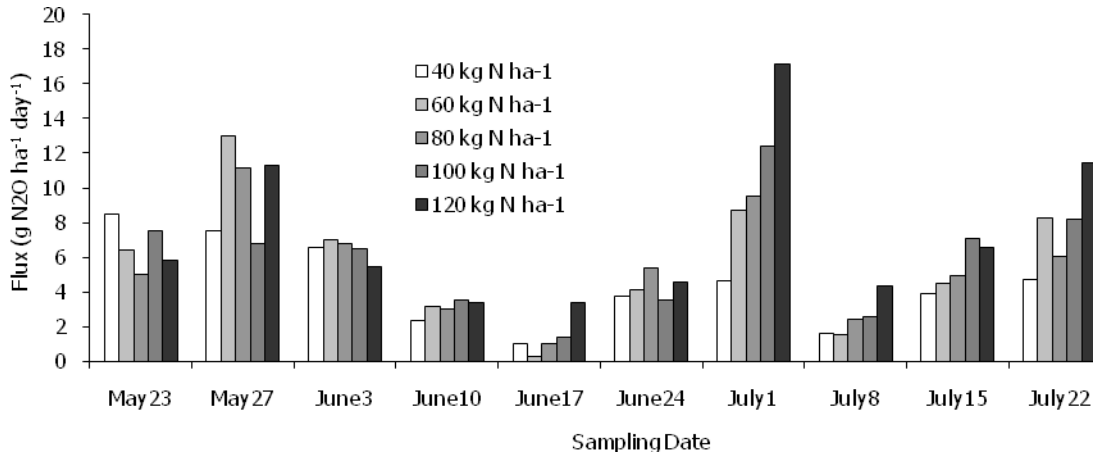


Figure 4. N<sub>2</sub>O flux as effected by N rate in 2008 (note that fertilizer was applied on May 26<sup>th</sup> and July 7<sup>th</sup> in 2008)

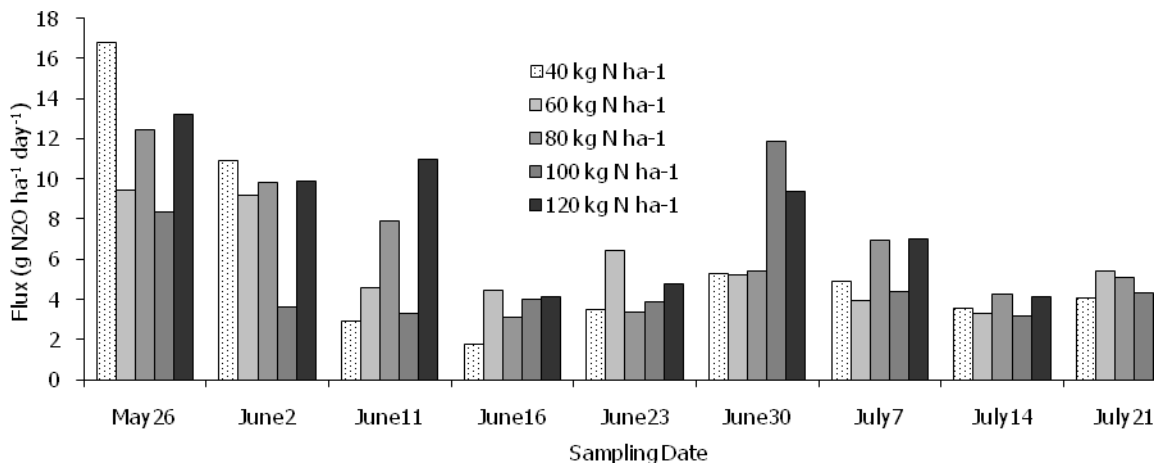


Figure 5. N<sub>2</sub>O flux as effected by N rate in 2009 (note that fertilizer was applied on May 25<sup>th</sup> and July 10<sup>th</sup> in 2009)

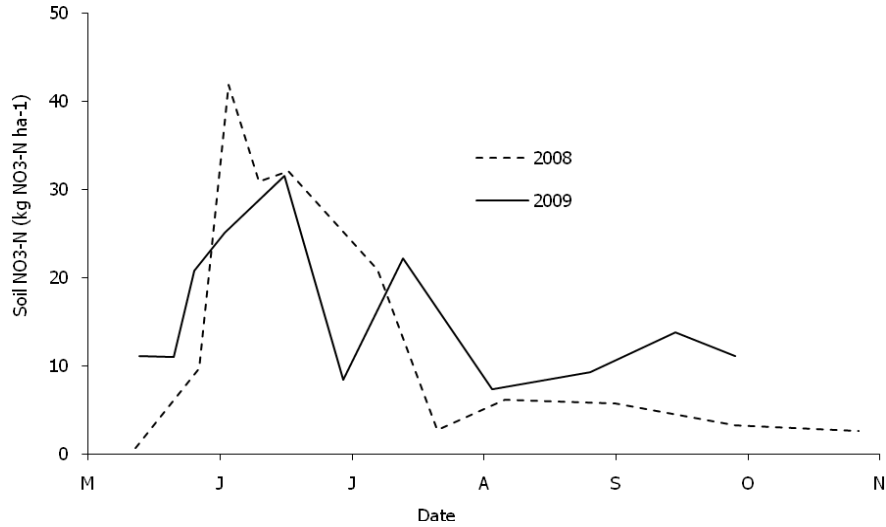


Figure 6. Temporal variation in soil NO<sub>3</sub>-N in 2008 and 2009 (averaged across line and N rate treatments; tick marks on the x-axis represent first day of each month).

Temporal variation in soil NO<sub>3</sub>-N concentrations were similar between the years (Figure 6), though in 2008 the maximum and minimum average soil NO<sub>3</sub>-N rates were more extreme than in 2009.

Nitrate Exposure responded to N treatment in both years, but not to canola line (Table 5). Nitrate Exposure increased from 1.2 to 1.8 g NO<sub>3</sub>-N days kg<sup>-1</sup> soil in 2008, and 1.8 to 2.3 g NO<sub>3</sub>-N days kg<sup>-1</sup> in 2009 with an increase in N treatment from 40 to 120 kg N ha<sup>-1</sup>.

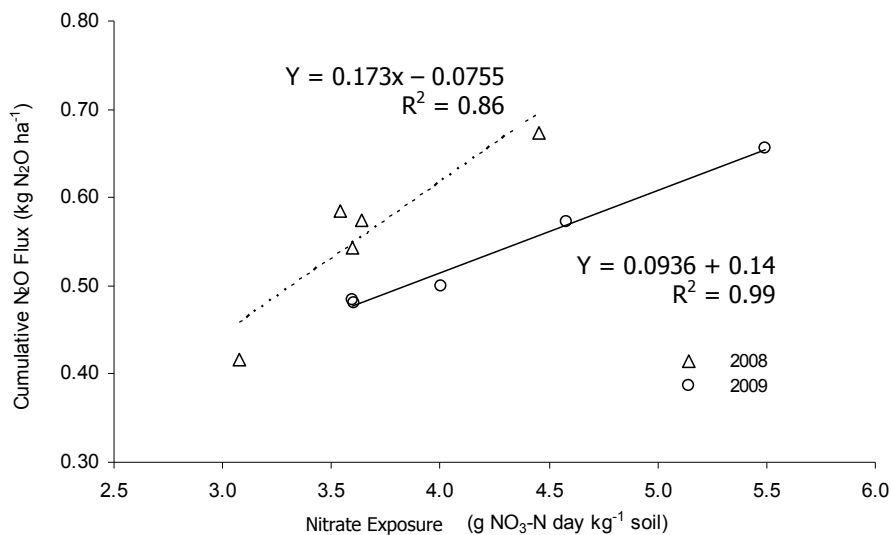


Figure 7. Cumulative N<sub>2</sub>O emissions as a function of Nitrate Exposure (each data point represents the average cumulative N<sub>2</sub>O flux and average Nitrate Exposure for each N treatment).

Cumulative N<sub>2</sub>O flux was found to be strongly correlated to Nitrate Exposure in 2008 and 2009 ( $r^2 = 0.99$  and  $0.86$ , and  $p = 0.023$  and  $<0.001$  respectively, Figure 7). Each data point in Figure 7 reflects mean Nitrate Exposure and mean cumulative N<sub>2</sub>O Flux at a given N rate.

Plant N uptake was included in this study in order to investigate the mechanisms by which either line or N treatment might have affected N<sub>2</sub>O emissions. There was no significant difference in plant N uptake among lines in any of the N uptake samplings in either year (Table 6). Increasing rate of N fertilizer application, however, did result in significantly increased N uptake at N uptake sampling date in both 2008 and 2009, though this was only significant at the  $\alpha = 0.10$  level in mid-season 2008 (Table 6). The increases in both years were comparable in both years at mid-season, where N uptake increased from 70 to 107 kg N ha<sup>-1</sup> in 2008 and from 60 to 130 kg N ha<sup>-1</sup> in 2009 with an increase in N treatment from 40 to 120 kg N ha<sup>-1</sup> (Table 6). N uptake determination at harvest however varied considerably between years, where it increased from 50 to 71 kg N ha<sup>-1</sup> in 2008 but from 113 to 194 kg N ha<sup>-1</sup> in 2009 when N rate was increased from 40 to 120 kg N ha<sup>-1</sup>. It is important to recall that due to significant weed presence in 2009, N uptake was that of canola plus that of weeds, while in 2008 it was that of only canola. Midseason N uptake did not include weeds in either year.



**Table 6.** Analysis of variance of N treatment and line effect on plant N uptake (kg N ha<sup>-1</sup>) at bolting, mid season, and harvest

N treatment [N] (kg N ha <sup>-1</sup> )	2008			2009		
	Bolting	Midseason	Harvest <sup>+</sup>	Bolting	Midseason	Harvest*
40	-	70.3	49.9 <i>b</i>	23.9 <i>b</i>	60.4 <i>b</i>	113.3 <i>b</i>
80	-	95.7	57.9 <i>ab</i>	30.7 <i>ab</i>	75.2 <i>b</i>	171.0 <i>ab</i>
120	-	107.0	71.2 <i>a</i>	40.7 <i>a</i>	130.4 <i>a</i>	194.1 <i>a</i>
Line [L]						
Topaz	-	82.1	65.5	25.5	90.7	146.1
Polo	-	100.0	52.6	22.4	86.6	172.9
ANOVA						
N	-	3545 <sup>x</sup>	0.368*	710*	13,595*	17,328*
L	-	2	0.107	25	1,538	101
N*L	-	1296	0.056	156	876	797
Error		197	0.052	175	1,244	2,983

<sup>+</sup> Means are the anti-log of those derived from an ANOVA conducted on log transformed data. ANOVA values presented are Mean Sum of Squares (MSS). Where data was log transformed, MSS values are those associated with log transformed data.

\*P < 0.05

<sup>x</sup>P < 0.10

Seed oil content (%) was examined in this study in part to determine FluxPerOil as a means of assessing net GHG impact of N fertilization strategies. Seed oil content responded in nearly identical fashion to line and N treatment in both years and under both 2L5R and 4L3R designs (Table 7). Both line and N treatment had a significant effect. Increasing N treatment from 40 to 120 kg N ha<sup>-1</sup> diminished seed oil content, from 48.0 to 44.7% in 2008 and from 48.2 to 45.2% in 2009. The highest seed oil content was observed in the Polo line (49.1 and 49.0% in 2008 and 2009 respectively), followed by 04C204 (46.0 and 47.0% in 2008 and 2009, respectively), Topaz (45.5 and 46.7% in 2008 and 2009, respectively), and finally Sentry (43.7 and 43.4%, in 2008 and 2009 respectively).

**Table 7.** Analysis of variance of N treatment and line effect on seed oil content (%)

N treatment [N] (kg N ha <sup>-1</sup> )	2008		2009	
	4L3R	2L5R	4L3R	2L5R
40	48.0 <i>a</i>	48.8 <i>a</i>	48.2 <i>a</i>	49.2 <i>a</i>
60	-	48.6 <i>a</i>	-	48.7 <i>a</i>
80	45.9 <i>b</i>	47.2 <i>b</i>	46.2 <i>b</i>	47.8 <i>ab</i>
100	-	46.7 <i>bc</i>	-	47.4 <i>ab</i>
120	44.7 <i>c</i>	45.8 <i>c</i>	45.2 <i>b</i>	46.2 <i>b</i>

Line [L]				
Topaz	45.5 <i>b</i>	45.5 <i>b</i>	46.7 <i>a</i>	46.8 <i>b</i>
Sentry	43.7 <i>c</i>	-	43.4 <i>b</i>	-
Polo	49.1 <i>a</i>	49.3 <i>a</i>	49.0 <i>a</i>	49.0 <i>a</i>
04C204	46.6 <i>ab</i>	-	47.0 <i>a</i>	-
ANOVA				
N	56.21*	16.18*	36.46*	11.41*
L	21.87*	21.08*	20.48*	10.57*
N*L	2.50	0.89	1.39	0.47
Error	1.12	0.98	2.25	0.67

\* P < 0.05

**Table 8.** Analysis of Variance of N treatment and line effect on oil yield (kg ha<sup>-1</sup>)

N treatment [N] (kg N ha <sup>-1</sup> )	2008		2009	
	4L3R <sup>x</sup>	2L5R	4L3R	2L5R
40	-	388 <i>b</i>	133 <i>b</i>	137 <i>b</i>
60	-	420 <i>ab</i>	-	182 <i>ab</i>
80	-	425 <i>ab</i>	195 <i>a</i>	181 <i>ab</i>
100	-	502 <i>ab</i>	-	224 <i>a</i>
120	-	437 <i>a</i>	244 <i>a</i>	264 <i>a</i>
Line [L]				
Topaz	-	500	187	198
Sentry	-	-	149	-
Polo	-	369	197	194
04C204	-	-	228	-
ANOVA				
N	34,231*	17,773*	65,086*	23,280*
L	29,522*	23,595 <sup>x</sup>	5,723	1,660
N*L	19,648*	11,070	4,100	5,488
Error	4,494	6,183	5,440	4,615

<sup>x</sup>See Table 9 for the means ranking for the interaction effect of N treatment and line in 2008 for the 4L3R design

\* P < 0.05

<sup>x</sup> P < 0.10

Oil yield (oil content ratio multiplied by seed yield in kg ha<sup>-1</sup>) responded to N treatment similarly in both years, but differently to line (Table 8). Increasing N treatment caused a significant increase in oil yield in both the both the 2L5R and 4L3R designs. Oil yields increased from 130 to 247 kg ha<sup>-1</sup> in 2009 with an increase in N application from 40 to 120 kg ha<sup>-1</sup> (Table 8). In 2008 there was an interaction effect in the 4L3R design, where yields from Topaz and 04C204 responded more to increased N treatment than yields from Sentry and Polo (Table 9). In 2009 there was no effect of line on oil yield. Variability associated with canola line in that year was comparable to that associated with error

(Table 8), a likely consequence of weeds compromising the oil yield response in that year.

**Table 9.** LS means letter groupings for the interaction effect of line and N treatment on oil yield in 2008 in the 4L3R design

Line	N treatment (kg N ha <sup>-1</sup> )	Oil Yield (kg ha <sup>-1</sup> )
04C204	120	556a
04C204	80	346bcd
04C204	40	390bcd
Polo	120	320cd
Polo	80	381bcd
Polo	40	339bcd
Sent	120	318cd
Sent	80	337bcd
Sent	40	257d
Topaz	120	554a
Topaz	80	469ab
Topaz	40	436abc

The ratio of cumulative N<sub>2</sub>O flux per unit of oil yield (FluxPerOil) was incorporated into this study to understand cost or benefit in oil yield from changing N treatments or lines to reduce cumulative N<sub>2</sub>O flux. A lower FluxPerOil ratio reflects a lower N<sub>2</sub>O emissions per unit of oil produced. In 2008, FluxPerOil responded to N treatment but only at the  $\alpha = 0.10$  level (Table 5), where reducing N treatment from 120 to 40 kg N ha<sup>-1</sup> brought about a reduction of FluxPerOil from 1.56 to 1.06 kg N<sub>2</sub>O-N kg oil<sup>-1</sup>. Line did not have an effect on FluxPerOil in 2008, and neither line nor N treatment had an effect on FluxPerOil in 2009.

## Chapter 5. Discussion

### 5.1 Magnitude of N<sub>2</sub>O emissions

Average cumulative N<sub>2</sub>O emissions over the growing season in 2008 and 2009 were very low (0.56 and 0.55 kg N<sub>2</sub>O ha<sup>-1</sup>, respectively), even when compared to low emissions observed in other studies of fertilized canola (Wagner-Riddle et al. 1997; Hao et al 2001; Malhi et al. 2006; Malhi and Lemke 2007) or N<sub>2</sub>O emissions measured from other crops within the region (Burton et al. 2008a; Zebarth et al. 2008a). It is important to note however that while spring thaw events were not included in annual N<sub>2</sub>O emissions in our study, they were in Wagner-Riddle et al. (1997), Hao et al (2001), Burton et al. (2008a), but not in Malhi et al. (2006), Malhi and Lemke (2007), nor Zebarth et al. (2008a).

Malhi et al. (2006) observed very low cumulative N<sub>2</sub>O emissions (0.75 kg N<sub>2</sub>O ha<sup>-1</sup>) from fertilized canola in Saskatchewan. Malhi and Lemke (2007) observed an average of 1.5 kg N<sub>2</sub>O ha<sup>-1</sup> from canola in Saskatchewan fertilized with 120 kg N ha<sup>-1</sup>. Hao et al (2001) observed roughly 3.1 and 6.3 kg N<sub>2</sub>O ha<sup>-1</sup> from canola with spring and fall fertilizer application (100 kg N ha<sup>-1</sup>), respectively. Wagner-Riddle and Thurtell (1998) observed 0.92 kg N<sub>2</sub>O ha<sup>-1</sup> from canola fertilized with 100 kg N ha<sup>-1</sup> in Ontario when considering only emissions occurring from January to April (spring thaw alone). Wagner-Riddle et al. (1997) observed 1.54 kg N<sub>2</sub>O ha<sup>-1</sup> from May to Sept (equal time period to this study) from canola fertilized with 100 kg N ha<sup>-1</sup> in Guelph Ontario. May to Sept cumulative precipitation in that study was roughly 350mm, slightly less than observed at our sites (Table 2). However, the soil at the Wagner-Riddle et al. (1997) site was a silt loam, presumably draining less than the sandy loam (2008) and coarse loamy (2009) soils in our study.

Cumulative N<sub>2</sub>O emissions observed in this study were also low in comparison with crops grown in a maritime climate and other than canola. Burton et al. (2008a) observed an average of 0.94 kg N<sub>2</sub>O ha<sup>-1</sup> in one of their study years and 3.14 kg N<sub>2</sub>O ha<sup>-1</sup> the next

year from fertilized potato in New Brunswick. Zebarth et al (2008a) observed average emissions as low as 1.6 kg N<sub>2</sub>O ha<sup>-1</sup> from commercial corn in New Brunswick.

## 5.2 N treatment effect on N<sub>2</sub>O emissions

Cumulative N<sub>2</sub>O flux responded to N treatment in 2008 but not in 2009. Differences between cumulative N<sub>2</sub>O emissions at the 40 and 120 kg N ha<sup>-1</sup> treatments were greater in 2008 than in 2009, and the variability attributed to error greater in 2009 than in 2008 (Table 5). Figure 4 shows that a clear pattern of increasing N<sub>2</sub>O flux with increasing N treatment in 2008 on sampling dates June 17<sup>th</sup> and onwards (with the exception of June 24<sup>th</sup> when the pattern is less discernible). No such pattern can be discerned at anytime in 2009 with the exception of one sampling date (June 30<sup>th</sup>).

N<sub>2</sub>O emissions increases in response to increased fertilization are well documented, but can vary widely among sites (Snyder et al. 2009) and years (Burton et al. 2008a). Some studies where no N response was detected attributed their findings to a high soil N supply, thereby diminishing the effect of added N (Burton et al. 2008b, Zebarth et al. 2008a). In our study, including a zero N treatment rate would have helped more clearly identify whether soil N supply played a significant part in N<sub>2</sub>O emissions response to N treatment. Cumulative N<sub>2</sub>O emissions at 120 kg N ha<sup>-1</sup> were equivalent to 0.48 and 0.53% of applied N in 2008 and 2009, respectively. This is less than the 1% IPCC N<sub>2</sub>O emission coefficient (IPCC 2006). Though significant, the 2008 increase was modest (a difference of only 0.27 kg N<sub>2</sub>O ha<sup>-1</sup>) and took place with an increase in fertilization from 40 to 120 kg N ha<sup>-1</sup>. A fertilizer increase from 40 to 80 kg N ha<sup>-1</sup> resulted in no significant increase in cumulative N<sub>2</sub>O flux. In 2009, though cumulative N<sub>2</sub>O flux tended to increase with an increase in N, the increase was small (0.15 kg N<sub>2</sub>O ha<sup>-1</sup> with an increase in N treatment from 40 to 120 kg N ha<sup>-1</sup>) and not significant.

Residual soil N at the Plumdale (2009) site is the most plausible explanation for the lack of response in N<sub>2</sub>O to N treatment in 2009. The site was planted to barley in 2008 and received 150 kg N ha<sup>-1</sup> in the form of ammonium nitrate. The expectation was that little or no residual N would remain in the soil, since the fertilizer was applied in June of 2008.

In hindsight, including a zero N treatment rate in the experimental design would have allowed us to determine whether there were in fact differences in residual soil N (or soil N supplying capacity) between the sites, and whether these differences could have accounted for an observed response (or lack thereof) of N<sub>2</sub>O emissions to N treatment.

It is possible that the difference between the two years in canola plant populations due to weed infestations contributed to the discrepancy in cumulative N<sub>2</sub>O flux response to N treatment. Increased weeds may have depleted available nitrate pools in the soil, leaving less available for denitrifier respiration. Indeed, plant N uptake (which includes weeds in 2009) was much higher in 2009 than in 2008, but only at harvest and not at mid-season. This logic however is contradicted by the higher Nitrate Exposure observed in 2009 across all N treatments when compared to 2008 (Figure 6), which would indicate that on average, the soil microbial community was exposed to higher levels of soil nitrate throughout the year in 2009.

The difference in landscape and hydrology between the two sites may have played a role in the difference in response of N<sub>2</sub>O emission to N treatment between the years. The Plumdale (2008) site was situated on a hill top and 12 m higher than the Brookside (2009) site, the latter being situated on relatively level terrain. Temporal variations in VWC during the growing season were similar between the two sites (Figures 2 and 3), with VWC reaching below 15% in 2008 but never below 20% in 2009. It is impossible however to determine whether this difference had an effect on N<sub>2</sub>O response to N treatment. WFPS was not determined in this study, and would have been a much more useful measure of the soil water content, as there are studies available which provide information on threshold WFPS levels which trigger dramatic increases in N<sub>2</sub>O emissions.

Differences in N<sub>2</sub>O emissions response to N treatment between the years may have also been due to differences in the availability of reduced carbon between the two sites. Soil carbon was not measured in this study, making its influence is impossible to assess. Since

denitrification is dependent on carbon as an electron donor, without which the step-wise reduction of  $\text{NO}_3^-$  to  $\text{N}_2$  and  $\text{N}_2\text{O}$  would not take place (Firestone and Davidson 1989).

### 5.3 Soil Water Content and $\text{N}_2\text{O}$ Emissions

Soil water content was found to be important in influencing the timing of  $\text{N}_2\text{O}$  emissions (Figures 3 and 4). The influence of soil water content in determining the temporal pattern of  $\text{N}_2\text{O}$  flux in both 2008 and 2009 was similar to that observed in other studies (Burton et al 2008a). Peaks in flux coincided with peaks in VWC (Figures 2 and 3), making high soil water content the likely trigger for peak  $\text{N}_2\text{O}$  emissions events. The sites did receive more precipitation in 2008 than in 2009 (Table 2), however this did not seem to translate into increased VWC or GWC during the growing season (May to September, inclusive) or during the period of high flux (mid-May to the end of July). It is during high magnitude peaks of flux in the early part of the growing season that the major portion of annual  $\text{N}_2\text{O}$  emissions was generated. Therefore the differences in flux response to N treatment at those peaks mattered most.

Zebarth et al. (2008b) observed peak  $\text{N}_2\text{O}$  emissions near crop harvest, and attributed that to increased N availability due to soil wetting at that time. This was not observed in our study, even though soil moisture was high at harvest. Unlike the rewetting event at harvest reported by Zebarth et al. (2008b), the high moisture content in the current study was consistent throughout the growing season, depleting the soil nitrate pool to less than 1.4 mg  $\text{NO}_3\text{-N kg}^{-1}$  soil in 2008 and less than 3.8 mg  $\text{NO}_3\text{-N kg}^{-1}$  soil in 2009. This indicates that  $\text{NO}_3^-$  availability limited  $\text{N}_2\text{O}$  emissions at our sites during the growing season.

### 5.4 Canola line effect on $\text{N}_2\text{O}$ emissions

Cumulative  $\text{N}_2\text{O}$  emissions did not respond to line in either year, under both the 2L5R and 4L3R designs. In the 2L5R design (Topaz and Polo, low and high seed oil content, respectively) were combined with 5 N treatments and replicated 5 times in order to provide a better ability to detect cumulative  $\text{N}_2\text{O}$  emissions response to line. In both years the 2L5R design was unable to detect a response in cumulative  $\text{N}_2\text{O}$  flux, Nitrate

Exposure, plant N uptake, or FluxPerOil to either N treatment or line (with the exception of a Nitrate Exposure response to N treatment in the 2L5R design for 2008). It must be recalled however that in 2009 the line main effect was seriously compromised by weeds (see section 5.5 below).

Likewise, plant N uptake did not respond to line. N uptake was measured in this study in order to help explain the response of N<sub>2</sub>O emissions to N fertilizer application. Different N uptake rates have the potential to indirectly impact N<sub>2</sub>O emissions by influencing the amount of nitrate that remains in the soil, that is when other factors (like C and aerobicity) are not limiting. That both N<sub>2</sub>O emissions and N uptake did not respond to line is therefore consistent with this logic.

The magnitude of N uptake observed in this study is consistent with other studies of fertilized canola (Malhi et al. 2006). At mid-season, more N was removed by plants than the sum of N applied in fertilizer and available in the soil at the beginning of the season. This confirms the role of mineralization in supplying N to the crop, and is consistent with findings of other studies (Hocking et al. 2002). The high N demand of the canola crop would reduce soil NO<sub>3</sub><sup>-</sup> accumulation, explaining the relatively low N<sub>2</sub>O emissions observed relative to other crops. Maximum nutrient uptake in canola occurs around the time of flowering (Malhi et al 2007), which (alongside tissue loss due to senescence) explains the decline in tissue N uptake from mid-season to harvest in 2008. The increase in N uptake in 2009 is due to weeds overtaking plots by harvest of that year.

### 5.5 Nitrate Exposure and N<sub>2</sub>O emissions

Nitrate Exposure is an integrated measure of the exposure of soil microbes to nitrate over a growing season. It is not a product of N treatment only, but is influenced by soil, climate, crop, and management. Some studies that have determined Nitrate Exposure have observed that it strongly correlates to cumulative N<sub>2</sub>O flux (Burton et al. 2008a; Zebarth et al. 2008b). In studies that have found little or weak correlation, it was determined that soil nitrate did not limit N<sub>2</sub>O emission because of the large N supplying capacity of the soils at the sites in question (Burton et al. 2008b, Zebarth et al. 2008a).



This study found a strong correlation in both 2008 and 2009 (Figure 8), which further supports the observation that  $\text{NO}_3^-$  was a factor limiting  $\text{N}_2\text{O}$  emissions in this study. This is consistent with  $\text{NO}_3^-$  being the limiting factor controlling  $\text{N}_2\text{O}$  emissions. The strong correlation of Nitrate Exposure to cumulative  $\text{N}_2\text{O}$  flux despite the differences in climate, soil, and crop, and management in all the studies that have reported Nitrate Exposure indicates that Nitrate Exposure is a promising indicator of cumulative  $\text{N}_2\text{O}$  flux when N is the main factor limiting  $\text{N}_2\text{O}$  emissions. Further research could focus on generating a database of cumulative  $\text{N}_2\text{O}$  flux and Nitrate Exposure relationships based on different soil, climate, crop, and management. This would aid in the development of more accurate  $\text{N}_2\text{O}$  emissions inventories, and assist producers in determining and managing their contribution to global  $\text{N}_2\text{O}$  emissions.

#### 5.6 Impact of Weeds in 2009

Significant weed cover in 2009 was problematic especially for investigating the effect of line on cumulative  $\text{N}_2\text{O}$  flux. On average 25% of all plots (and up to 50% in some plots) were significantly populated by weeds by mid season, enough to kill or stunt the crop. The line main effect was therefore compromised in 2009, and any measures directly associated with it are unreliable. This includes oil yield and FluxPerOil specifically. To maintain integrity in the N uptake measure, a different method was used in 2009 than in 2008, where the tissue N uptake for all plants (including weeds) inside a quadrat area was determined. The analysis of variance on the differences in weed cover shows that neither line nor N treatment induced greater weed cover, and also that weeds did not by chance occupy plots treated with a specific line or N treatment more than others (Table 4).

#### 5.7 Oil yield, seed oil content, and FluxPerOil

As expected, increasing N treatment resulted in decreased seed oil content in both years (Table 6 and Figure 8). This is consistent with well-established patterns of declining seed oil content with increased N application (Grant and Bailey 1993). Polo had the highest seed oil content, though it was not significantly different from 04C204, which had the second highest. This is in contrast with the expected seed oil content rankings, where

04C204 was to exceed all others. Since line did not have an effect on N<sub>2</sub>O flux, this discrepancy is of no consequence to the objectives of this study.

Oil yield (the product of seed yield and seed oil content) responded to line and N treatment in 2008 in the 4L3R design. In that year an interaction effect was observed, where oil yields from Topaz and 04C204 responded more to N treatment than the other two lines (Table 9). It was surprising to observe that oil yield of Polo, even with the highest N rate (120 kg N ha<sup>-1</sup>), was low relative to other lines (Table 9). Though seed oil content of Polo was highest among the four lines in both years (Table 7), it is clear that seed oil yield in Polo responded less to increasing N rate at our sites.

Oil yield was measured in this study to assess the ratio of cumulative N<sub>2</sub>O flux per unit of oil yield (FluxPerOil, g N<sub>2</sub>O kg<sup>-1</sup> oil). Minimizing the FluxPerOil ratio will maximize the atmospheric C offset potential of growing the crop as a biofuel in a life cycle analysis. FluxPerOil did not respond to line in either year, but did respond differently to N treatment in 2008 and 2009. FluxPerOil increased 1.06 to 1.56 g N<sub>2</sub>O kg<sup>-1</sup> oil with an increase in N treatment from 40 to 120 kg N ha<sup>-1</sup> in 2008, a significant difference at the  $\alpha = 0.10$  level (Table 5). It would be useful to calculate the ratio of N<sub>2</sub>O emissions per kg of oil yield lost due to decreasing N rate, however this is made impossible for 2009 due to unreliable yield data. In 2008 it is only possible for line 04C204, since it was the only line where a significant oil yield increase was observed due to N rate increase (Table 9). Only 1.63g of N<sub>2</sub>O (0.48 kg CO<sub>2</sub> eq) emissions reduction were gained for every kg of oil yield lost due to N rate reduction for 04C204 in 2008. This translates to a very small drop in N<sub>2</sub>O emissions. This, and the very small change in FluxPerOil with increased N treatment in 2008, indicates that we achieved only minor reductions in N<sub>2</sub>O emissions by reducing N treatment. Producers as such would incur a significant yield loss for minor reduction in greenhouse gas emissions from their fields. No FluxPerOil response to either line nor N treatment was detected in 2009, where FluxPerOil is unreliable, since the line effect was compromised due to weeds.

## Chapter 6. Conclusion

This study was undertaken in order to better understand the influence of breeding for seed oil and N treatment on N<sub>2</sub>O emissions from canola in Eastern Canada. Increased N treatment did result in increased N<sub>2</sub>O emissions, however the increase was found to be significant only in one of the study years (2008). This difference was found to be significant even though the average magnitude of emissions observed was very low (on average near 0.5 kg ha<sup>-1</sup> in both years of the study). Residual soil N at the 2009 site is the most plausible explanation for the lack of response in N<sub>2</sub>O to N treatment in that year. Differences in weed cover and elevation between the sites, and potential differences in soil carbon and soil N management may have contributed to the inability to detect an N<sub>2</sub>O emissions response to N treatment in the second study year. Increases in Nitrate Exposure explained the majority of the response of N<sub>2</sub>O to increased N treatment in both years, indicating that Nitrate Exposure is a useful predictor of N<sub>2</sub>O emissions when soil available N limits N<sub>2</sub>O emissions. A look into FluxPerOil (a measure of the cost in N<sub>2</sub>O emission per unit of increased oil yield gained by increasing N treatment) showed that relatively meager N<sub>2</sub>O emissions savings were gained from reducing yield through a reduction in N treatment. Unfortunately, this pattern was not verified in the second of year of the study due to weeds compromising the line effect. Breeding for seed oil had no impact on N<sub>2</sub>O emissions in this study, nor did it have a significant impact on plant N uptake at anytime during the growing season. Further research could focus on verifying the observed relationship between Nitrate Exposure and N<sub>2</sub>O emissions in this study and others ((Burton et al. 2008a; Zebarth et al. 2008b), and building a database of these for different regions and climates across Canada and in other countries. Also, investigating the ratio of N<sub>2</sub>O emissions per kg of oil yield in regions where the average magnitude of N<sub>2</sub>O emissions is higher may provide an optimal N treatment rate for minimizing flux and achieving adequate yields in those regions.

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