NITROGEN FERTILIZATION AND NUTRITION OF CANOLA IN EASTERN CANADA

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November, 2014

A thesis submitted to McGill University in partial fulfillment of the requirements for the degree of Master of Science

ABSTRACT

Canola (*B. napus*) is an ideal feedstock for biodiesel production due to its high oil and low saturated fatty acid concentration. In recent years, there is a growing interest to expand canola production in Eastern Canada. Canola producers in this region lack fertilization guidelines and need appropriate N fertilizer recommendations to achieve high yields and N fertilizer use efficiency. Nitrogen (N) is a limiting nutrient in canola and plays a determinant role in improving oilseed yield and quality. The objective of this study was to evaluate the status of canola N in relation to soil N mineralization and nitrification and microbial biomass N (MBN) at three sites in Eastern Canada. Experimental sites were located in Ste. Anne-de-Bellevue (Quebec), St. Augustin-de-Desmaures (Quebec) and Ottawa (Ontario). During 2012, the experiment was designed as a randomized complete block with four pre-plant N fertilizer treatments (0, 50, 100 and 150 kg N ha^{-1} from urea), replicated four times. Canola biomass and N concentration were assessed at four growth stages namely rosette, 20% flowering, 80% pod formation and 90% maturity. Soil N pools (NH_4, NO_3, MBN, net N mineralization rate and net nitrification rates) were also determined at stages. Canola N concentration was greater with 100 kg N ha^{-1} than at 0 kg N ha^{-1} at the rosette stage

(P<0.001) in Ste. Anne-de-Bellevue and flowering stage (P<0.005) in St. Augustin-de-Desmaures, but by maturity there was no difference among N fertilizer treatments. Net N mineralization and nitrification rates as well as MBN concentration varied significantly (P<0.001) with canola growth stage, but this was not affected by N fertilization, suggesting that the soil N supply was derived from decomposition of organic residues by the activity of a relatively stable microbial population. At the end of the growing season, the NO_3 concentration was elevated in plots that received 150 kg N ha^{-1} indicating that canola did not utilize all of the N in the soil and so did not benefit from fertilization. This residual soil NO_3 represents economic inefficiency and pose environmental risk. Future research on seed yield and harvest index (HI) under the climatic conditions of Eastern Canada will contribute to the development of a precise N fertilization guideline.

RÉSUMÉ

Le canola (*B. napus*) est une matière première idéale pour la production de biodiesel en raison de sa teneur élevée en huile et sa concentration faible en acides gras saturés. Au cours des dernières années, il y a un intérêt croissant pour accroître sa production dans l'Est du Canada. Cependant, les producteurs de canola dans cette région n'ont pas encore de formulations d'application d'engrais azotés et il est nécessaire d'avoir des recommandations appropriées pour atteindre des rendements élevés et l'efficience de l'utilisation de l'azote. L'azote (N) constitue un facteur limitant pour le canola et l'élément nutritif déterminant pour améliorer les rendements et la qualité des oléagineux. L'objectif de cette étude est d'évaluer le statut de N dans le canola en relation avec celui fourni par le sol par la minéralisation, la nitrification et la biomasse microbienne (MBN) sur trois sites dans l'Est du Canada. Les sites expérimentaux ont été situés à Ste. Anne-de-Bellevue (Québec), Saint-Augustin-de-Desmaures (Québec) et Ottawa (Ontario). En 2012, L'expérience était structurée en blocs complets aléatoires dans lequel quatre niveaux de fertilisant azotés ont été appliqués, avant plantation (0, 50, 100 et 150 kg N ha^{-1} de l'urée), répétés quatre fois. La biomasse du canola et sa concentration en N ont été évalués à quatre stades de croissance, à savoir le stade rosette: 20%

floraison, 80% formation des gousses et 90% maturité. Les sources de N du sol (NH_4, NO_3, MBN, le taux de minéralisation nette de N et les taux de nitrification nette) ont également été déterminés à ces stades. La concentration en N du canola était plus grande avec 100 kg N ha^{-1} qu'avec 0 kg N ha^{-1} au stade de la rosette ($P < 0,001$) à Ste. Anne-de-Bellevue et au stade de la floraison ($P < 0,005$) à Saint-Augustin-de-Desmaures, mais en maturité aucune différence n'a été constaté entre les différents traitements. Les taux de minéralisation et de nitrification nettes de N, ainsi que la concentration MBN ont varié significativement ($P < 0,001$) avec le stade de croissance du canola sans être affectée par la fertilisation azotée; ce qui suggère que l'apport en N du sol provient de la décomposition des résidus organiques par l'activité d'une population microbienne relativement stable. A la fin de la saison de croissance, la concentration en NO_3 a été élevée dans les parcelles qui ont reçues 150 kg N ha^{-1} indiquant que le canola n'a pas utilisé la totalité de l'azote dans le sol et n'a donc pas bénéficié de la fertilisation appliquée. La concentration résiduelle de NO_3 dans le sol représente l'inefficacité économique et constitue un risque environnemental. Les recherches futures sur le rendement en grain et l'indice de récolte (IR) dans les

conditions climatiques de l'Est du Canada contribueront à l'élaboration des références pertinentes pour la fertilisation azotée du canola.

CONTRIBUTION OF AUTHORS

This thesis consists of a literature review and two manuscripts. Literature review, preceded by a general introduction, summarizes the results of other studies, establishes the research context, and presents the objectives and hypotheses of the study. Experiments and results are presented in chapter two and three, which were written in a manuscript format according to the guidelines of the Graduate and Postdoctoral Studies Office. A connecting paragraph between these chapters is used to clarify the linkage between manuscripts. Finally, the fourth chapter, Conclusions, provides a summary of the key findings of this thesis and areas for further research. The two manuscripts are co-authored by the candidate and her supervisors Dr. Joann Whalen and Dr. Philippe Seguin. The research work of the candidate was part of a larger project initiated by Eastern Canada Oilseed Development Alliance (ECODA). Overall direction for the thesis research, guidance in soil and plant sampling, analysis, data interpretation and editorial assistance came from Drs. Joann Whalen and Philippe Seguin. The candidate was responsible for data collection, laboratory analysis, statistics, data interpretation and manuscripts writing.

ACKNOWLEDGEMENTS

I would like to express my deepest gratitude to my supervisor Dr. Joann Whalen for her invaluable guidance, encouragement and quick and professional advice on scientific questions during the course of this study. Her full length editorial correction during the preparation of this thesis was particularly helpful to me, a non-native English speaker. Along with her knowledge, she passed on very useful life and working philosophies that will be beneficial to my future life goals.

I would also like to thank Dr. Philippe Seguin, who served as my supervisor and provided me insightful comments and helpful advice throughout the program. Thank you for always having time to talk over things that needed to be discussed.

I would like to thank the FQRNT for funding this project. I am thankful to everybody at the Emile A. Lods Agronomy Research Centre of Macdonald Campus of McGill University in Ste. Anne-de-Bellevue, Laval University Experimental Farm near Saint-Augustin-de-Desmaures, and the Central Experimental Farm of Agriculture and Agri-Food Canada, Ottawa (45°38′ N, 74°58′ W) where my field trials were conducted.

My deepest thanks to Hicham Benslim, Helene Lalande and Amelie Roy for the help they offered me in favour of the completion of the work.

I would like to extend my appreciation to the soil ecology group for their help during field and lab work. I would particularly like to thank Kiara, Habib, Ben, Jinghan and Zhor for their continuous encouragement, company and support.

I would like to acknowledge and thank my friends Jamuna and Kaberi for their endless support and encouragement which helped me to remain enthusiastic about my work and finish my M.Sc. studies at McGill.

I am grateful to my family for their unconditional love and support. I will forever remain thankful to my father, who is now up in heaven, for watching my every move and standing by me when I decided to pursue my higher education in Canada. I thank you for all the love you conveyed to me.

There are many others that in one way or another contributed to this work, I thank you all.

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LIST OF ABBREVIATIONS

ABBREVIATION	MEANING
ANOVA	Analysis of variance
B	Boron
Ca	Calcium
CCC	Canola council of Canada
Cd	Cadmium
Cl	Chlorine
CND	Compositional nutrient diagnosis
CO_2	Carbon dioxide
CRAAQ	Centre de référence en agriculture et agroalimentaire du Québec
Cu	Copper
CVA	Critical value approach
d	Day
DRIS	Diagnosis and recommendation integrated system
Fe	Iron
g	Grams
GHG	Green house gas
h	Hectares

ha	Hour
HI	Harvest Index
K	Kilograms
K_2SO_4	Potassium sulphate
L	Litre
LAD	Leaf area duration
LFOMN	Light fraction organic matter
M	Molar
MBN	Microbial biomass N
Mg	Magnesium
mg	Milligrams
mL	Milliliters
Mn	Manganese
Mo	Molybdenum
N	Nitrogen
NH_4-N	Ammonium
Ni	Nickel
NO	Nitric oxide
NO_2	Nitrite
NO_3	Nitrate

NUE	Nitrogen use efficiency
O_2	Oxygen
OCGA	Ontario canola growers association
OMAFRA	Ontario Ministry of Agriculture, Food and Rural Affairs
P	Phosphorus
POMN	Particulate organic matter nitrogen
S	Sulphur
SAS	Statistical Analysis System
SOM	Soil organic matter
SON	Soil organic nitrogen
StatsCan	Statistics Canada
USDA	United states department of agriculture
UV	Ultraviolet
WEON	Water extractable organic nitrogen
WFPS	Water filled pore space
Zn	Zinc

GENERAL INTRODUCTION

Biofuel is an alternative renewable energy source that would reduce our reliance on non-renewable fossil fuels and mitigate greenhouse gas (GHG) emissions, since biofuel crops are carbon-neutral with regards to atmospheric CO_2 concentrations. Due to its low level of saturated fatty acid, canola (*Brassica napus L.* and *B. rapa L.*) is an ideal biofuel in Canada where cold winter conditions require fuel to perform well under freezing conditions (CCC, 2014). Consequently, the demand for canola increased after the enforcement of the B2 biodiesel mandate (a minimum of 2% biodiesel content in diesel is required) in July 2011 by the federal government of Canada. Production of 15 million metric tonnes of canola by the end of 2015 is anticipated to meet the demand of canola for the food and biodiesel market in Canada (CCC, 2008). A total of 17,960,100 metric tonnes of canola was produced in 2013 (StatsCan, 2014). However, much of this production is largely attributed to the western provinces of Canada. In 2013, Eastern provinces of Ontario and Quebec, contributed only 0.43% (77,700 metric tonnes) of the total canola produced in Canada (StatsCan, 2014).

Canola production in Eastern Canada is low compared to that in western provinces of the country (StatsCan, 2012) mainly because canola was originally bred in the Prairie provinces and therefore not many cultivars are adapted to the humid growing conditions of Eastern Canada. As a result, canola is grown as an alternative crop in Eastern Canada where corn, soybean and wheat are the major crops. However, the

rising demand for canola as food and biofuel led farmers to consider canola as a profitable cash crop option. Another feature of canola that makes it a good choice is its shorter growing season than soybean, which fits into the crop rotation prior to winter wheat in the cool weather conditions of northern Ontario and Quebec (OCGA, 2009). Canola production is also encouraged by the new markets in Eastern Canada developing from the establishment of a crushing facility in Quebec in 2010, in addition to the two canola crushing facilities in Ontario. As a result, the production of canola in Eastern Canada increased significantly from 57,200 metric tonnes in 2002 to 77,700 metric tonnes in 2013, with a record production of 103,900 metric tonnes in 2011 (StatsCan, 2014).

Agronomic practices for canola production need to be optimized for profitable yields. At present, canola fertilization guidelines in Eastern Canada are based on recommendations for the Prairies. It is important to note that arable land in the west tends to be in the semiarid regions with a cool dry climate while Eastern Canada has a cool moist climate (Halpern et al., 2010). Climatic factors like temperature and precipitation not only affect canola production (Kutcher et al., 2010) but also affect soil nitrogen (N) supply, which in humid environment is controlled by N mineralization and nitrification (Zebarth et al., 2005), microbially mediated processes depending on temperature and moisture (Goncalves and Caryle, 1994). Therefore, region-specific

nutrient management strategies need to be adopted to achieve canola yield and quality goals (Zebarth et al., 2009b).

As with other oilseed crops, inadequate N fertilization will limit canola growth, which has a higher N requirement than cereal crops (Grant and Bailey, 1993). Insufficient N inputs reduce yield and quality while excess N increases chances of N loss to the environment (Zebarth and Rosen, 2007). In particular, excess N in agricultural soils contributes to the production of nitrous oxide, a potent GHG that represents 5% of Quebec's emission (MDDEP, 2008) and thus negates the environmental benefits of using canola as biofuel. Economically, excess N application is increasingly expensive because of a long term increase in the price of N fertilizer (USDA, 2013). Therefore, region specific guidelines for N fertilizer need to be developed to optimize canola productivity in an economically and environmentally sustainable manner.

For optimum growth and development, the exact amount of N needed by the crop should be supplied by the soil system. To do this, the soil N supply should match the plant nutrient uptake for optimal growth (Ingestad et al., 1981). However, a review of the literature shows that native soil N supply is rarely sufficient for optimal growth. In Saskatchewan, canola requires up to 120 kg N ha^{-1} for maximum yield (CCC, 2014). However, it is not clear whether this rate is appropriate for canola production in Eastern Canada. The accuracy of N fertilizer recommendations in the humid temperate region of

Eastern Canada is improved by estimating the soil N supply (from soil organic matter), which is regulated by microbial activities. Soil organic nitrogen, which comes from soil organic matter (SOM), is the main source of plant available N during the growing season in the humid temperate regions (Zebarth et al., 2009b). Therefore, understanding the microbial processes of mineralization and nitrification under field conditions is key to predicting the soil N supply in agricultural soils.

The objective of this thesis is to compare soil N supply from microbial activities with crop N uptake by canola. The outcomes of this work contribute to the general research effort (OCGA, 2012) to formulate more precise N fertilizer recommendations for canola in Eastern Canada that will help agricultural producers to save money on fertilizer costs, increase revenues from crop production and protect environmental quality.

CHAPTER ONE: LITERATURE REVIEW

1.1 Canola

1.1.1 History and classification

Canola belongs to one of the most widely cultivated families, the Brassicaceae or Cruciferae. The major canola (and rapeseed) species include *Brassica napus, B. rapa,* and more recently *B. juncea*. *B. napus and B. juncea* are both hybrids developed through natural hybridization between *B. rapa x B. oleracea,* and *B. rapa x B. nigra,* respectively. The genetic relationship between these, and other Brassica species, is shown in Fig 1. The origin of these different species is not clear and multiple areas of origin have been speculated by different authors. These crops are currently grown commercially as oilseed crops in regions of China, Canada, USA, Europe, India, Pakistan, and Australia.

Canola was bred naturally from rapeseed by Keith Downey and Baldur R. Stefansson at the University of Manitoba in the 1970s. The name "canola" stands for "Canadian oil, low acid" and was coined in 1978 by the Western Canadian Oilseed Crushers Association (now the Canadian Oilseed Processors Association) (CCC, 2014). It describe "double-low" varieties of rapeseed that have both low-erucic acid and low-glucosinolate levels, undesirable attributes because erucic acid is a fatty acid linked to heart disease and glucosinolate breakdown products are toxic to animals (Duan et al., 2011). Double low indicates that the processed oil contains less than 2% erucic acid

and the meal contains less than 3 mg/g of glucosinolates. These features distinguish canola hybrids from their rapeseed parents, as the hybrids and have different nutritional profiles.

1.1.2 Plant characteristics

Canola is an annual plant and is relatively tall, ranging from 120-180 cm with a long and slender taproot (Brown et al., 2008). Typically, an individual plant produces 3-20 branches, with each branch terminating in an elongated spike. The leaves of the plant are dark green, pinnate on the lower and lanceolate, sessile and clasping the stem. The inflorescence consists of bright yellow flowers that have four distinct sepals and petals, six stamens and one carpel. The petals are arranged diagonally opposite each other, which is a distinguishing characteristic of the Brassicaceae family. The number of flowers on one plant can range from 12-25 depending on variety, climate and farming techniques. About 65-70 % of these flowers become pods.

Although cross-fertilization between canola plants through insect pollination is reported to exceed 30 percent of seed set, the majority of canola (*B. napus* and *B. juncea*) seeds are set through self-pollination because the self-incompatibility from their diploid ancestors is overcome as a result of polyploidy (containing more than two sets of chromosomes). Therefore, canola is primarily self-pollinating and can produce high yields without insect pollination. *B. rapa* canola, however, is a diploid species with a

strong self-incompatibility that results in more than 95 percent cross-pollination (Brown et al., 2008).

The fruit consists of a pod that is long and narrow, approximately 5-10 cm in length. The pod consists of two carpels divided by a false septum which, when mature, shatters. These pods may contain fifteen to forty small round seeds that are about 1-2.5 mm long. Often the seed coat is rough and pitted. These seeds are dark brown to black in color. The protein content of these seeds ranges from 10-45% and the oil content from 30-50%. Some varieties are reported to have 60% oil content (Brown et al., 2008).

1.1.3 Growth stages

Canola requires 90 to 120 growing days to reach maturity (CCC, 2014). Harper and Berkenkamp (1975) have divided the life cycle of the canola plant into five principle growth stages (Table 1.1, Fig. 1.2). These overlapping growth stages can be determined by examining the main flowering (terminal) stem. The early flowering stage (i.e., 20 % flowering, about 6-8 weeks after seeding) is the most critical period for pod and seed development. This is the stage when canola is most susceptible to pests, disease, heat stress (>28 ^{o}C) and drought. Another notable phenomenon at the early flowering stage is substantial leaf abscission as N is remobilised and translocated from leaves to pods and seeds. The timing and length of each growth stage are greatly influenced by temperature, moisture, light (day length), nutrition and variety. Researchers at the University of Manitoba showed that temperature is the most

important environmental factor regulating growth and development of canola in western Canada (CCC, 2012). Therefore, understanding the canola life cycle helps producers make critical management decisions at the right developmental stage to enhance oilseed quality and yield.

1.1.4 Environmental conditions affecting growth and development

Growth and development in canola is affected by several environmental factors. Producers can optimize canola production and reduce the risk of yield losses by implementing management practices that allow plant to compensate for environmental stresses.

1.1.4.1 Climate

Temperature and precipitation are important climatic factors affecting canola growth and development. Canola performs well in many areas under variable temperatures. However, it grows best when temperatures are between 12 ºC and 30 ºC, with the optimal temperature being 20 ºC (Brown et al., 2008). In the early plant stage, temperature below 10 ºC results in poor germination and delayed emergence (CCC, 2014). Protein production is impaired at low temperatures (< 4 ºC), reducing metabolic processes that affect germination, and emergence. Another reason that cold soil temperature limits growth is that it reduces water and nutrient absorption by seedling roots. Delayed emergence increases the seedlings' susceptibility to diseases. After emergence, canola seedlings prefer relatively cool temperatures (for leaf area

development in the rosette stage) up to flowering, between 13 and 22°C (17°C mean temperature). Higher temperature causes faster growth, resulting in shorter leaf area duration (LAD) and hence lower photosynthetic potential during the early growth stage (Dewey and Lu, 1959).

Plant development is adversely affected by both low and high temperatures immediately before and during flowering (Kutcher et al., 2010). Development rate as well as flowering is delayed at low (but not freezing) temperatures prior to flowering. The amount of pollen shed is also reduced. However, yield is not affected by low temperature except in the case of frost. On the other hand, high temperature, particularly during later stages results in reduced yield and oil content by affecting formation of pods, seeds, seed size and oil content.

Precipitation and soil moisture also affects canola growth and development. Germination rate in canola depends on soil moisture, as well as temperature. Insufficient moisture delays germination and emergence whereas waterlogging affects nutrient uptake and therefore is not favourable for plant growth and development. It is important to note that soil moisture is controlled partially by soil texture. Coarse soils drain more easily and therefore have lower water retention capacity. Researchers at Agriculture and Agri-Food Canada Melfort working in northern Saskatchewan reported that clay soils with higher moisture storage capacity had quicker canola emergence than sandy loam soils with lower moisture storage capacity at 100% field capacity (CCC,

2014). In the flowering stage, excess rain or sprinkler irrigation may cause flower damage, reduce pollination and yield. Similarly, water stress during the flowering period may result in reduced dry matter production, fewer pods, early leaf loss and reduced yield. Therefore, it is necessary to maintain an optimum level of water in the field by using proper soil water management strategies (for both irrigation and drainage), depending on soil texture.

1.1.4.2 Soil

Canola growth and development is affected by various physical, chemical and biological properties of soil. Soil texture is an important physical property, which refers to the size and proportion of sand, silt and clay minerals present in the soil. Soil texture affects crop growth and development by primarily affecting water holding capacity of the soil and therefore indirectly affecting available water in the soil. However, Brennan et al. (2000) found that soil texture does not affect the oil and protein content in canola when soil moisture was not limiting. This suggests that canola can be grown on all types of mineral soil, as long as moisture is controlled.

1.2 Soil fertility and canola nutrition

Soil is the major source of nutrients for canola. Canola receives both macronutrients (N, P, K, S, Mg, Ca), and micronutrients (Fe, Mn, Zn, Cu, B, Mo, Cl and Ni) from the soil. Each of these minerals has an important role in plant growth and development. However, as in other oilseed crops, N limits canola growth because it

generally has higher N requirements than cereal crops (Grant and Bailey, 1993). The role of N in canola, crop response to N and the N supplied by soil and fertilizers in the canola production system will be discussed in the following section.

1.2.1 Role of nitrogen in canola

Nitrogen is an integral part of essential plant compounds like amino acids, genetic materials, enzymes, vitamins and hormones, which help to regulate important biological processes. Besides, N also regulates and stimulates vegetative and reproductive development in plant by enhancing the uptake of other essential nutrients (Barker and Bryson, 2006).

In canola, Allen and Morgan (1972) reported that N increases yield by influencing yield components such as branches per plant, buds per plant, and flowers per plant. They reported that N affects growth and development by increasing stem length, number of flowering branches, total plant weight, leaf area index, and weight and number of pods and seeds per plant. Wright et al. (1988) reported that N prolongs the life length of leaves, improves leaf area duration (LAD) after flowering and increases overall crop assimilation, thus contributing to seed yield. However, excess N leads to lodging (Bailey, 1990; Scott et al., 1973; Sheppard and Bates, 1980; Wright et al., 1988) and thereby reduces seed yield and quality. Scott et al. (1973) reported that excess N supply in later growth stages causes a delay in maturity by extending the pod development phase. Delayed maturity increases the number of green kernels and the

amount of chlorophyll in the oil (Grant and Bailey, 1993). This produces undesirable oil colour and off-flavour that affects marketability.

1.2.2 Nitrogen assimilation and distribution in canola

According to Masclaux-Daubress et al. (2010), about 70 % of the N required by canola is taken up before flowering and then translocated from the leaves and stems to pods and seeds. Schjoerring et al. (1995) reported that until the rosette stage, leaves are the main N reservoir (75%) regardless of the amount of N fertilizer applied. At the early flowering stage N is mostly stored in the stem (50%) and at the end of flowering stage, pod wall is the main N reservoir (40%). At maturity, about 80% N is stored in the seed, 10 % N in the stem and 10% N in the pod wall.

N fertilization generally increases the protein content of canola seed and meal but at the expense of oil concentration (Brennan et al., 2000; Malhi 2001 and Gan et al., 2007). The total seed protein content is approximately 60 to 65% and is inversely related to oil content (Brennan et al., 2000; CCC, 2014). Seed oil content was reported to decrease 0.6 to 1.2% per additional 100 kg N ha^{-1} applied (Mendham and Roberson, 2004). Although oil content decreased at high N rates, the gain in yield is greater than the decline in oil content decline, resulting in a total oil yield increase per unit area (CCC, 2014).

1.2.3 Canola response to nitrogen fertilizer

Crops receive N from the soil in the form of nitrate (NO_3^-) and ammonium (NH_4^+) ions arising from the mineralization of soil organic nitrogen (SON), crop residues or fertilizer residue from previous years, and residual N from previous growing season (Sharifi et al., 2007). In deficient soil, N demand of the crop can be fulfilled by adding inorganic fertilizers, which is the standard agronomic practice to achieve top yields in grain and oilseed since most soils do not have the inherent ability to release enough NO_3^- and NH_4^+ at the critical growth stages for grain and oilseed formation.

Canola is highly responsive to N fertilizer in deficient soil, thus N fertilizer is an essential input (Hocking et al., 1997; Jackson, 2000; Karamanos et al., 2007; Malhi and Gill, 2004). Canola responds quickly to applied N fertilizer when soil NO_3-N concentration is less than 45 mg kg^{-1} (Soper, 1971). Research on the Prairies found that profitable dryland canola yield response to fertilizer N is unlikely when the soil contains more than 34 to 45 kg NO_3-N/ha in the top 60 cm (CCC, 2014). Besides soil NO_3-N concentration, canola response to N fertilization also varies with climate, soil type and management practice (Qaderi et al., 2006; Jackson, 2000).

Soil moisture influences yield response to N fertilizer. In dry conditions, root growth and activity is limited, resulting in poor N uptake. Microbial activity is also limited in dry soil, which results in low production of plant available N. Another important factor is the crop rotation, which determines how much available N was removed from the field

in the past year as well as the N supplied by previous crop residue. Greater N uptake was reported in canola following legume crops like pea (*Pisum sativum* L.), faba bean (*Vicia faba* L.) than non legume crops like canola and wheat (*Triticum aestivum* L.) (St. Luce et al., 2013). In addition, the disease break with different rotational crops is beneficial for canola, improving the overall crop health, yield potential and the economic response to fertilizer. Thus, we can conclude that canola's response to added N fertilizer varies with climate, soil and management practice. Therefore, it is important to understand soil N dynamics at a particular region in order to estimate the optimum N fertilizer recommendation for canola.

1.3 Nitrogen cycle and nitrogen supply to canola

1.3.1 Soil nitrogen cycle

Nitrogen is present in the atmosphere in an inert state and hence it cannot be utilised directly by plants unless it is transformed into simple nitrate and ammonium forms that are readily available to plants (Tinsdale et al., 1995). Nitrogen undergoes various transformations in the N cycle through a series of complex biochemical reactions that include N_2 fixation, mineralization, nitrification, denitrification and immobilisation (Tinsdale et al., 1995). **N mineralization** or ammonification is a biochemical process where soil microorganisms transform the complex organic nitrogen present in the soil, originating from animal, plant and microbial residues, into a simple

inorganic nitrogen form (NH_4^+) that is available for plant uptake (Myrold and Bottomley, 2008).

Nitrification is the biologically mediated conversion of reduced N in the form of ammonia (NH_3) or ammonium ion (NH_4^+) to oxidised N in the form of nitrite (NO_2^-) or nitrate (NO_3^-) (Sylvia et al., 1998).

Denitrification is the microbial process that reduces nitrite (NO_2^-) or nitrate (NO_3^-) in the soil to inert N_2 or reactive N_2O gases, releasing them back to the atmosphere and representing a loss of plant-available N from the soil-plant system. Under anaerobic condition, the nitrate in the soil is used by denitrifying bacteria as an electron acceptor in place of oxygen during respiration.

Immobilization is a process in which the N in the soil organic N is tied up temporarily by soil bacteria for using NO_3^- and NH_4^+ for their own growth. This process reduces the plant available N supply.

1.3.2 Transformations supplying plant available nitrogen

In recent years, many strategies have been developed to increase the efficiency of N use in agricultural production systems. These strategies can be classified into two groups: strategies to improve delivery of N to the soil; and strategies to minimise N loss from the cropping systems (Zebarth et al., 2009a). Among the different strategies to improve N delivery to the plant, matching the supply of N with crop N demand in both

space and time is an important one (Zebarth and Rosen, 2007) that requires a thorough understanding of microbial transformations supplying plant available N.

1.3.2.1 Nitrogen mineralization

As mentioned earlier, N mineralization involves microbial degradation of soil organic nitrogen into soluble NH_4^+ ions (Sylvia et al., 1998). Aerobic and anaerobic bacteria, fungi, and actinomycetes are the major microbial population involved in N mineralization (Tisdale et al., 1995). Extracellular and intracellular enzymes produced by these heterotrophic soil microorganisms help to metabolize organic N polymer in the soil into simple ammonium ions that are available for plant uptake, or subject to other microbially-mediated reactions (e.g., nitrification, denitrification) or chemical transformations like NH_4^+ fixation (Sylvia et al., 1998; Tinsdale et al., 1995). Microbial biomass and its activity is an important biotic component of soil environment that regulates N mineralization in the soil (Whalen and Sampredo, 2010).

N mineralization is a biologically mediated process, and therefore temperature and soil moisture affecting microbial activity are the two most important factors affecting N mineralization (Goncalves and Caryle, 1994). Soil moisture affects N mineralization by affecting O_2 diffusion within the soil as well as water availability, both of which are important for microbial respiration and activity (Sierra, 1997). Ma et al. (1999) reported varying rates of N mineralization due to change in annual precipitation rates. Increasing temperature increases N mineralization in soil (Tisdale et al., 1995). Nitrogen

mineralization is also affected by soil texture (Nyiraneza et al., 2012). In coarse textured soil, N mineralization is quicker due to less physical protection of organic N compounds, which increases microbial accessibility to decomposable residues and other forms of SON, and greater aeration compared to fine textured soil (Griffin, 2008).

Net N mineralization is the amount of NH_4^+ left in the soil after the loss of NH_4^+ through these consumptive processes. Gross N mineralization is the actual amount of NH_4^+ accumulated in the soil after mineralization regardless of consumption. Thus, the relationship between the net and gross N mineralization can be shown as:

Net mineralization= Gross mineralization - Gross NH_4^+ consumption

1.3.2.2 Nitrification

The process whereby ammonium (NH_4^+) is oxidised to nitrite (NO_2^-) is called ammonia oxidation and subsequently NO_2^- is transformed to nitrate (NO_3^-) through nitrification (Sylvia et al., 1998). Ammonia oxidation which is the first step is carried out mainly by autotrophic ammonia oxidising bacteria and archaea present in the soil. Nitrification results in further oxidation of NO_2^- to NO_3^- by nitrite oxidising bacteria. Besides these bacteria, fungi also help to oxides nitrite into nitrate. Most of the bacteria involved in this process belong to the *Nitro*-genera and are chemotrophs, using N oxidation as an energy producing reaction, and using CO_2 as carbon source. Only few heterotrophs are involved in nitrification (Sylvia et al., 1998). The conversion of NH_4^+ into NO_2^- or NO_3^- determines the mobility of N through the usually negatively charged

soil and therefore has strong influence on N cycle in the terrestrial systems. Nitrate moves more rapidly than NH_4^+ and therefore it is easily taken up by plant roots, or is leached out from the root zone or lost from the soil by denitrification (Norton and Stark, 2011). Therefore, it is desirable to manage agricultural soils to carefully match the nitrification rate with plant N uptake to increase the efficiency of N fertilizer use.

Since soil microorganisms are the major actors responsible, nitrification is affected by different soil environmental conditions such as temperature, moisture, pH and soil aeration that affect microbial activity in the soil. Temperature rise tend to increase nitrification (Cookson et al., 2002) whereas low temperature (<10°C) limits the growth and activity of autotrophic nitrifiers and thus decreases the nitrification rate (Cookson et al., 2002). Tisdale et al. (1995) reported that nitrification is favoured in well aerated soils and under conditions where there is optimum water content suitable for most aerobic bacteria. They also found that a pH of 8.5 is most favourable while pH as low as 4.5 could result in a negligible amount of nitrification.

Net nitrification rate helps to estimate the pool of soil NO_3-N available in the soil solution. It is the difference between gross nitrate production and consumptive processes like immobilisation, plant uptake, denitrification, gaseous loss and leaching in the soil ecosystem (Cookson et al., 2002). It can be differentiated from gross nitrification by the following relationship:

Net nitrification= Gross nitrification - Gross NO_3^- consumption

1.4 Soil nitrogen supply

Soil N supply is the sum of soil mineral N (NH_4^+ and NO_3^-) at the time of sampling, crop N uptake and mineralizable soil N in the rooting depth of the crop (Whalen et al. 2013). Crop N uptake is the concentration of N in the crop at the time of soil sampling. Mineralizable soil N is the N that becomes available during the growing season from the soil organic N through the process of N mineralization and nitrification. Soil mineral N and crop N uptake can be estimated by routine soil and plant nutrient analysis. Mineralizable soil N that is available to plant during the entire growing season (in the field) or long term incubation (in the lab) is often defined as potentially mineralizable nitrogen (Sharifi et al., 2007).

1.5 Indicators of soil nitrogen supply

1.5.1 Soil mineral nitrogen test

The soil mineral N in the soil provides farmers a reasonable guide to adjust N fertilizer inputs to achieve optimum yield. NO_3-N and NH_4-N concentration in the soil can be quantified by different methods like colorimetry, ion-selective potentiometry, steam distillation, microdiffusion, ion chromatography, and ultraviolet (UV) spectrophotometry (Mulvaney, 1996). Colorimetric methods and steam distillation are the most widely used. Colorimetric methods have advantage over steam distillation because 1) there are less environment impacts when copperised Cd is replaced by Devarda's alloy, and 2) there are fewer carryover contaminations between colorimetric analyses compared to

continuous flow analysis (Sims et al., 1995). Mineral N in the soil can be tested before planting or before sidedressing and consequently allow producers to split their N fertilizer application, which consistently boosts N fertilizer use efficiency by synchronizing N inputs with plant N demands (International Plant Nutrition Institute, 2012) .

Pre-plant mineral N test estimates N credits from the previous growing seasons and N mineralization in the early flowering season (Ma and Wu, 2008). However, it cannot consider spatial and temporal heterogeneity in soil NO_3-N and NH_4-N or predict N available to canola from in-season mineralization, especially in humid climatic region. Unpredicted rainfall in April or early May can increase N loss by leaching and runoff (Ma et al., 2005; Zebarth et al., 2005). Currently there are no pre-plant soil N test calculated from crops grown in Quebec, and research is ongoing in this area (St. Luce et al., 2011).

Estimating pre-sidedress soil NO_3-N and NH_4-N has been proven to be successful in maize cultivation in New Jersey, USA (Salardini et al., 1992) and southern Ontario, Canada (Vyn et al., 1999). In canola, split application of N fertilizer did not increase oilseed yield and other components of yield in Australia (Taylor et al., 1991) or Pakistan (Cheema et al., 2001). Low water availability (30 mm at the early flowering growth stage which is six to eight weeks after seeding) and high temperature (daytime > 25^{o} C and nighttime > 17 ^{o}C) were the primary reasons for inefficient N uptake and N

translocation from leaves to pods for N split application (Taylor et al., 1991; Thomas, 2003).

1.5.2 Plant tissue analysis as an indicator of crop nitrogen uptake and soil nitrogen supply

Plant tissue analysis is a good indicator of crop N uptake at any given point of time, based on the total N in root and shoot components. The Dumas technique, which converts all forms of N to molecular N_2 is the most extensively used method to determine total N in plant tissue (Jones and Case, 1990).

Hocking et al. (2001) studied the effect of N fertilizer (0-150 kg N ha^{-1}) on N uptake by canola during 1991 to 1992. They reported that N concentration averaged 26.0 mg N g^{-1} at the early flowering stage and varied between 20.6 and 34.8 mg N g^{-1} in a late sowing year (1991). In contrast, when sowing occurred at the recommended time (1992), plants at the early flowering stage contained 23.8 mg N g^{-1}, with values between 16.8 and 29.9 mg N g^{-1}. At harvest, shoot N concentration varied between 3.7 mg N g^{-1} and 7.2 mg N g^{-1} in two growing seasons and the mean N concentration in 1991 was 0.6 mg N g^{-1} higher than that of 1992. Plant biomass (kg ha^{-1}) at the early flowering stage and harvest were negatively correlated to straw N concentration. The highest biomass (14040 kg ha^{-1}) (shoots and oilseeds) was achieved in 1992 when the straw N concentration was 22.8 mg N g^{-1} at the early flowering stage and 5.6 mg N g^{-1} after harvest. Hocking et al. (2001) explained that the dry condition in 1991 stimulated the

premature ripening of canola and 2 ^{o}C lower temperatures at pod and seed filling stage in 1992 increased the oilseed potential.

One of the disadvantages of plant tissue test is that it may be too late to correct deficiencies for in-season crops due to the time needed for sampling and analysis (Scherer, 2001; Malhi and Gill, 2007). Therefore, agronomists suggest testing young tissue at early vegetative growth stages, between stem elongation and floral initiation, when N assimilation rate is highest (Thomas, 2003).

1.5.3 Labile organic N and potentially mineralizable nitrogen

In humid temperate soils, soil organic nitrogen (SON) is the primary source of nitrogen in the soil (Zebarth et al., 2005, 2009) because most of the plant available N from previous growing season is lost through the rooting zone during fall and winter due to high soil moisture. Therefore, soil N supply in such humid conditions depends on the microbial decomposition of SON during the growing season. Knowledge of the quantity of N that will be potentially available for microbial degradation to release plant available nitrogen during the growing season is therefore important for predicting soil nitrogen supply (Curtin and Campbell, 2008).

Soil labile organic N is the most active fraction of SON and is the major source of mineralizable N in agricultural soils (Wander, 2004). The soil N supply depends on the turnover of labile organic N, which undergoes mineralization-immobilization reactions and releases mineral N (Duxbury et al., 1991; Gregorich et al., 1994; Haynes 2005).

Labile organic N fractions accounts for over 20% of total soil N and include soil microbial biomass N (MBN), particulate organic matter N (POMN), light fraction organic matter N (LFOMN), water extractable organic N (WEON), and hot water extractable N (Haynes, 2005). Quantification of labile organic N in the soil gives an idea of the mineralizable soil N pool, which is a component of the soil N supply.

The mineralizable soil N pool is not easily quantified because test methods rely on lengthy laboratory incubations to determine the quantity of labile organic matter that can decompose and mineralize to release NH_4^+ and NO_3^- (Whalen et al. 2013). Potentially mineralizable N can be determined by long-term (>20-week) aerobic incubation method (Stanford and Smith, 1972). This method is generally regarded as the standard measure of N mineralization potential, but is time consuming and not practical for routine use. Research effort is ongoing to investigate N flow through several labile SON fractions in order to elucidate potential soil N availability (St. Luce et al., 2014). The pattern and magnitude of N flow through the labile SON can be studied by relating initial ^{15}N concentration in the labile SON fractions to the final ^{15}N concentration in the soil mineral N pool at the end of the incubation.

1.5.4 Soil microbial biomass nitrogen (MBN)

Soil microbial biomass nitrogen (MBN) is a relatively small component of SOM (about 5 % of total soil N) but is the most biologically active labile N pool (Deng et al., 2000). This is due to the rapid generation time of soil microbial biomass. MBN

represents both a source (substrate) and sink (assimilation) of mineral N (Brookes, 2001; Nicolardot et al., 2001) therefore can be used as a predictor of soil N supply in humid temperate regions (Sharifi et al., 2007). Madison et al., (1998) have also suggested MBN as a predictor of potentially mineralizable nitrogen. Microbial biomass in humid temperate regions is subjected to frequent variations in soil water potential, temperature and substrate availability during the growing season, which implies that it will vary in size and therefore alter the conversion of SON to inorganic NH_4^+ and NO_3^- forms. However, Holmes and Zak (1994) found that there was marked variability in net N mineralization during the growing season while MBN remained relatively constant. They concluded that N availability is therefore not controlled by large seasonal fluctuations in soil microbial biomass, but rather by changes in the activity of microbial biomass such that a relatively constant pool is maintained over time.

1.6 Summary, research objectives and hypotheses

Nitrogen is a limiting nutrient in canola (Grant and Bailey, 1993). When applied at the required amount (100 to 150 kg N ha^{-1}) and at the early growth stage, N fertilizer increases yield and seed quality (Allen and Morgan, 1972). Quantifying the plant-available N supplied by the soil during the growing season can help to estimate the amount of exogenous N to be applied as inorganic N to meet the N demand of canola in deficient soils. Soil N supply is in turn influenced by soil physical and chemical properties as well as various abiotic (rainfall temperature) and biotic factors

(microorganisms). Therefore, canola fertilization guidelines devised for the Prairie provinces are probably not suitable for canola cultivation in Eastern Canada. Site specific fertilization guidelines that integrate weather and soil nutrition status need to be formulated to improve canola production in eastern Canada.

Research to improve canola production in Eastern Canada needs to be directed towards 1) establishing the critical soil and plant concentrations of mineral N in (NO_3-N and NH_4-N) during important growth stages of canola (rosette, flowering, pod filling and maturity); 2) investigating SON's contribution to supply plant available N by the process of N mineralization and nitrification; 3) investigating MBN dynamics to predict potentially mineralizable N during the growing season; 4) correlating canola's N uptake with different soil parameters; and 5) identifying the optimal N fertilizer rate for maximum yield and yield quality.

For this research project, my objectives are: 1) to determine NO_3-N, NH_4-N and Microbial Biomass N (MBN) concentration in the soil during critical growth stages (rosette, 20% flowering, 80% pod formation and 90% physiological maturity) in canola; 2) to quantify net N mineralization and nitrification rates in the soil during different growth stages in canola; and 3) to determine plant N concentration at different growth stages and relate it to soil N supply.

The following hypotheses will be tested in this research project:

1) Soil NO_3-N and NH_4-N concentrations decrease during the growing season,

2) MBN concentration in the soil remains stable during the growing season,
3) Net N mineralization and nitrification rates increase with nitrogen fertilization rate,
4) N concentration in canola is responsive to N fertilizer input at the early vegetative (rosette) stage in N deficient soil.

Table 1.1: Key growth stages in canola (Harper and Berkenkamp, 1975).

Stage	Description of main Raceme
0 Pre-emergence	
1 Seedling	
2 Rosette	2.1 First true leaf expanded
	2.2 Second true leaf expanded
3 Bud	3.1 Inflorescence visible at center of rosette
	3.2 Inflorescence raised above the level of rosette
	3.3 Lower buds yellowing
4 Flower	4.1 First flower opens
	4.2 Many flowers opened, lower pods elongating
	4.3 Lower pods starting to fill
	4.4 Flowering complete, seed enlarging in lower pods
5 Ripening	5.1 Seeds in lower pods full size, translucent
	5.2 Seeds in lower pods green
	5.3 Seeds in lower pods green-brown or green- yellow mottled
	5.4 Seeds in lower pods yellow or brown
	5.5 Seeds in all pods brown, plant dead

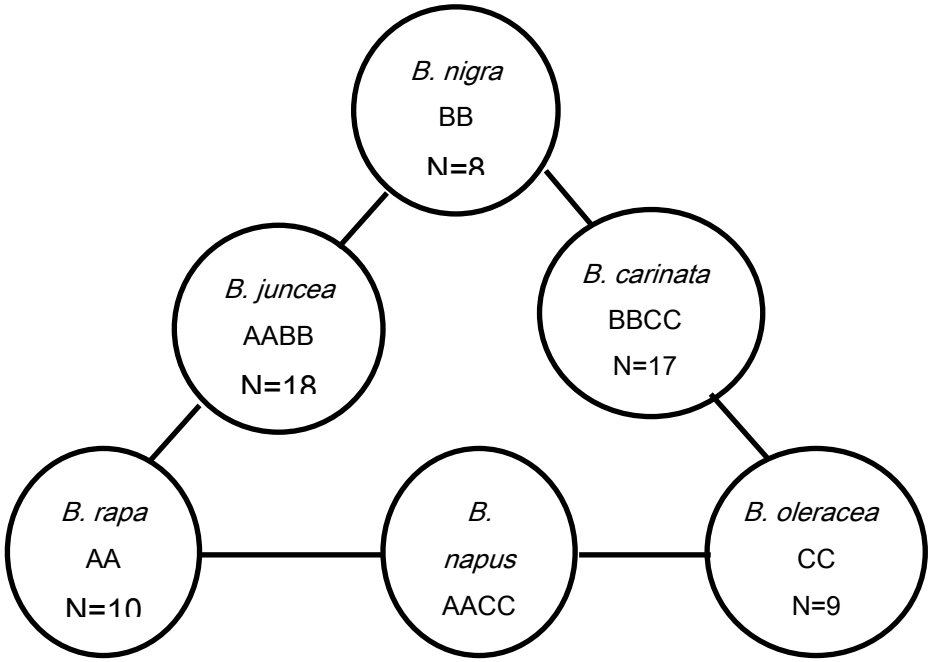
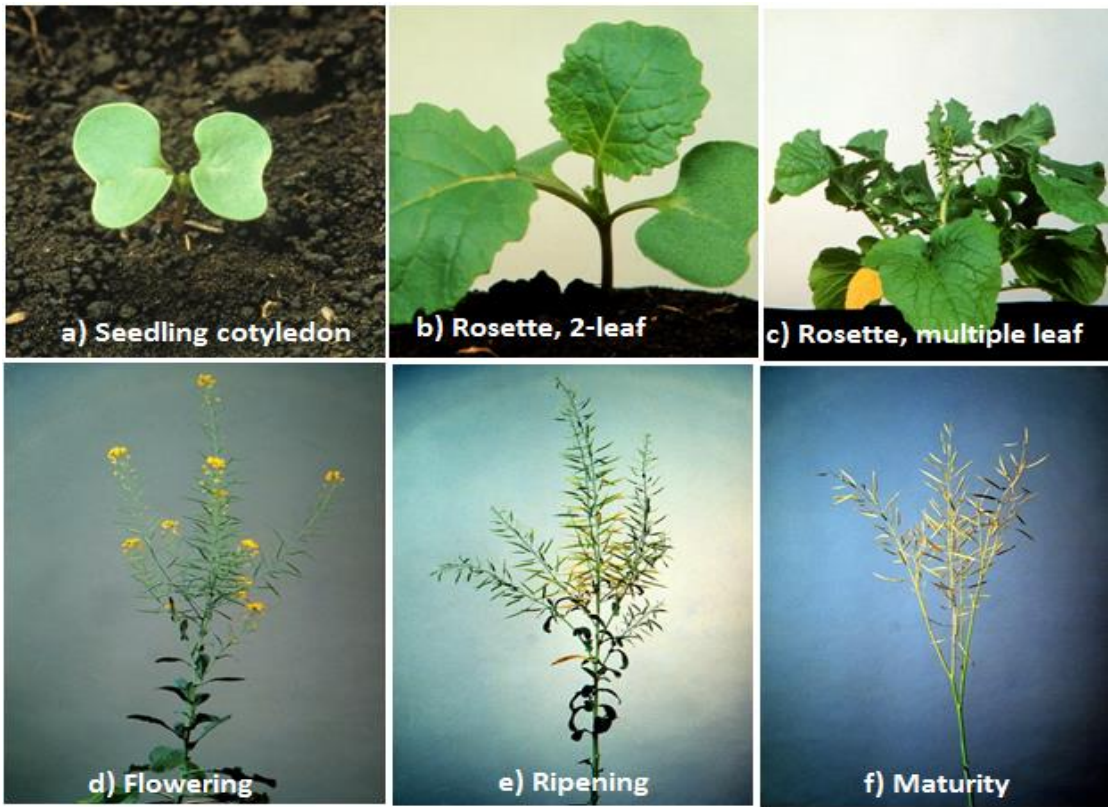


Fig. 1.1: Cross-pollination relationships between Brassica species commonly referred to as the Triangle of U (U, 1935).



Source: www.canolacouncil.org

Fig. 1.2**:** Key growth stages in canola (based on Harper and Berkenkamp, 1975).

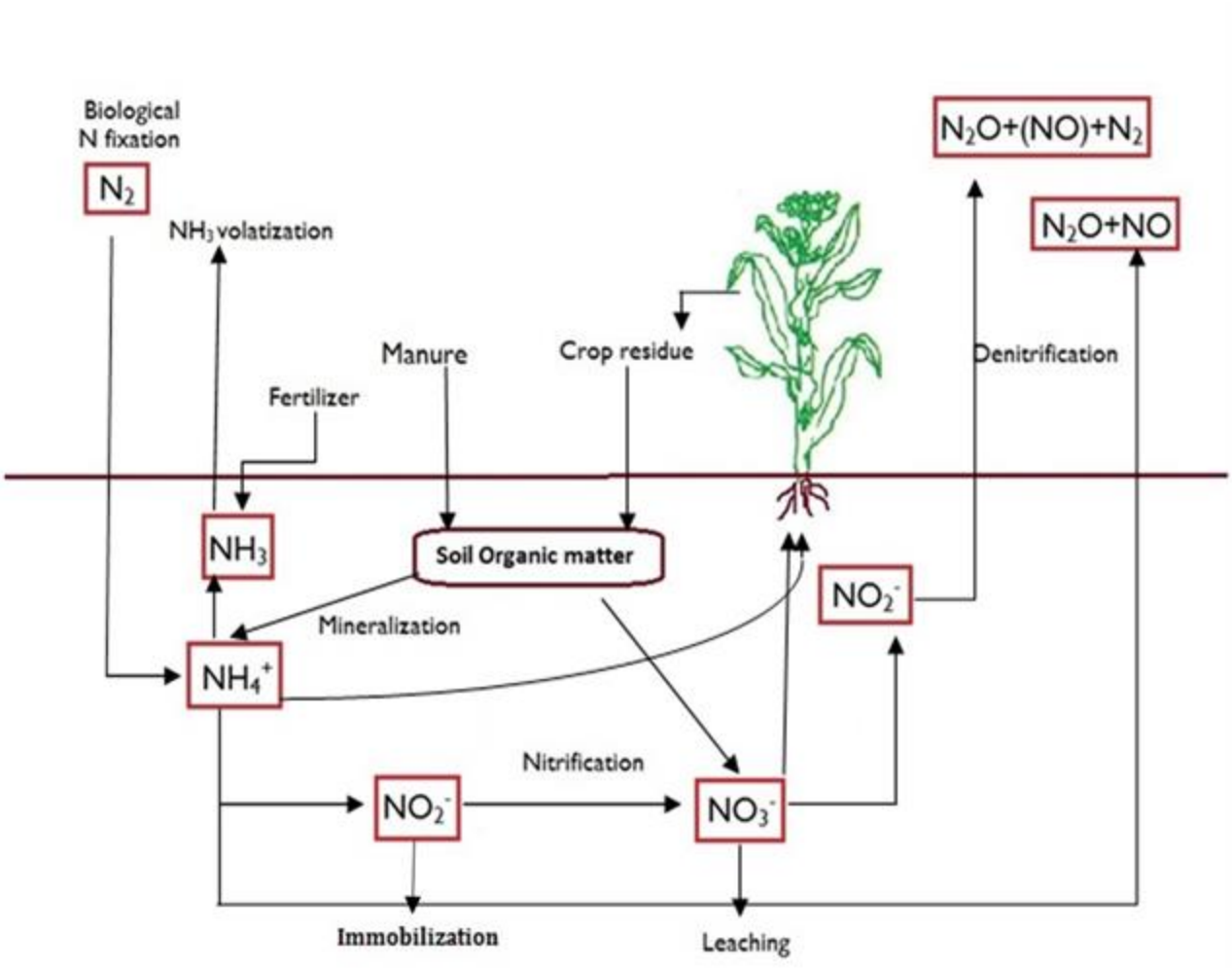


Fig. 1.3: Soil nitrogen cycle.

CHAPTER TWO: SOIL MINERAL NITROGEN AND MICROBIAL BIOMASS NITROGEN RESPONSE TO NITROGEN FERTILIZATION AT VARIOUS CANOLA GROWTH STAGES

2.1 Abstract

Canola (*B. napus*) requires high N supply for optimum yield. Since soils supply N, it should be possible to adjust the N fertilizer inputs for canola according to the soil mineral N concentration, but there are no guidelines for the humid temperate region of Eastern Canada. The purpose of this study was to evaluate the dynamics in soil mineral N and microbial biomass nitrogen (MBN) concentrations at various canola growth stages, as related to N fertilization. In 2012, experimental sites were located in Ste. Anne-de-Bellevue (Quebec), St. Augustin-de-Desmaures (Quebec) and Ottawa (Ontario). The experiment was designed as a randomized complete block with four pre-plant N fertilizer treatments (0, 50, 100 and 150 kg N ha^{-1} from urea), replicated four times. Soil N pools (NH_4, NO_3, MBN, net N mineralization rate and net nitrification rates) were measured at four growth stages namely rosette, 20% flowering, 80% pod formation and 90% maturity. Soil mineral N (NH_4^+ + NO_3^-) decreased during the growing season. Concentration of NH_4^+ was negligible indicating NH_4-N was rapidly nitrified. Soil mineral N concentration increased with increasing N fertilizer rates. However, the elevated mineral N concentration in plots receiving 150 kg N ha^{-1} at the end of the growing season suggested canola plants did not benefit from excess fertilization.

Findings indicate that net N mineralization and nitrification rates, as well as MBN concentration, were not affected by N fertilization, suggesting that the soil N supply was derived from decomposition of organic residues by the activity of a relatively stable microbial population.

2.2 Introduction

Canola production in Eastern Canada is low compared to that in Prairie provinces of the country (StatsCan, 2013) mainly because canola was originally bred in the Prairie provinces and therefore not many cultivars are adapted to the humid growing conditions of Eastern Canada. However, with increasing canola demand due to the growing interest in using it as a biofuel, the production of canola in Eastern Canada has increased significantly from 57,200 metric tonnes in 2002 to 77,700 metric tonnes in 2013, with a record production of 109,700 metric tonnes in 2011 (StatsCan, 2014). Profitable canola yields depend on adequate fertilization, among other good agronomic practices, but canola fertilization guidelines in the humid temperate region of Eastern Canada are currently based on recommendations for the semi-arid Canadian Prairies. Climatic factors like temperature and precipitation not only affect canola production (Kutcher *et al.*, 2010) but also affect soil nitrogen (N) supply, which in humid environments is controlled by the microbially-mediated processes of N mineralization and nitrification (Zebarth *et al.*, 2005). Therefore, region specific N management

strategies need to be adopted for achieving canola yield and quality goals (Zebarth *et al.*, 2009b).

In Quebec, 100 to 150 kg ha^{-1} N is currently recommended for canola cultivation (CRAAQ, 2010), which is similar to the recommendation for Ontario and other canola-producing areas in Eastern Canada However achieving synchrony between plant N demand and soil N supply is difficult because leaching contributes to greater N loss in the humid temperate region of Eastern Canada than in the semi-arid Prairies (St. Luce *et al.*, 2011). Fertilizer recommendations in Eastern Canada also need to consider the soil N supply from the decomposition of soil organic nitrogen (SON), a major source of plant available N during the growing season in humid temperate regions (Zebarth *et al.*, 2009b). Conversion of the organic nitrogen contained in SON into soluble ammonium ions (NH_4^+) (Sylvia *et al.*, 1998) by microbial action is known as N mineralization. The NH_4^+ produced by this reaction is rapidly oxidized to nitrite (NO_2^-) and nitrate (NO_3^-) through microbial action (Sylvia *et al.*, 1998) by the processes of ammonia oxidation and nitrification. These mineral N forms may eventually be used by plants or microbes, or removed from the soil solution by NH_4^+ fixation, NO_3^- leaching or denitrification (Power and Prasad, 2010). Thus, soil microbes can act as both source (by driving decomposition and mineralization process) as well as sink of N (by consuming and immobilizing N from the soil solution) (Brookes, 2001). Therefore, understanding of mineral N dynamics in the soil is essential to predict soil N supply, which is unknown for

canola production systems at present, as far as we are aware. The objectives of this study were to 1) measure soil NO_3-N, NH_4-N and microbial biomass N (MBN) concentration in the soil at key growth stages in canola field plots across Eastern Canada and 2) quantify net N mineralization and nitrification rates in soil, in response to N fertilizer.

2.3 Materials and methods

2.3.1 Site description

The experiment was conducted at three sites of Eastern Canada in 2012. The study sites were located at Emile A. Lods Agronomy Research Centre of Macdonald Campus of McGill University in Ste. Anne-de-Bellevue, Quebec (45°03′ N, 74°11′ W), Laval University Experimental Farm near Saint-Augustin-de-Desmaures, Quebec (46°44′ N, 71°31′ W), and the Central Experimental Farm of Agriculture and Agri-Food Canada, Ottawa (45°38′ N, 74°58′ W). For convenience, the three sites have been referred to as Montreal, Quebec and Ottawa throughout this chapter and chapter 3. The soil at the Montreal site was a clay loam, mixed frigid typic Endoaquent of the Chicot series (Humic Gleysol). In Quebec, the soil was a sandy loam, mixed frigid Typic Dystrudept of the Orthic series (Dystric Brunisol). While in Ottawa site, the soil was a Grenville Sandy Loam of the Orthic series (Melanic Brunisols).

2.3.2 Experimental design

The field experiment was laid out in a randomized complete block design at all three sites. There were four blocks each having 28 treatments that gave a total number of 112 plots per site. The plot dimensions were 2.6 x 4 m with 14 rows spaced at 30 cm. There was 50 cm spacing between two plots and 1.3 m wide buffer zone between blocks. In all sites, border plots were established at both ends of the field. Border plots received the same treatment as the neighboring plots.

From the larger field experiment, 4 treatments were selected for this study, each receiving different rates of N (0, 50, 100, 150 kg ha^{-1}) with zero application of S and B. Urea was broadcast on the soil surface and incorporated to a depth of 10 cm between 24 and 48 h before planting canola. This gave a total of 16 experimental plots at each site (48 plots in total, across the three sites) to be followed during the canola growing season (May to August 2012).

2.3.3 Soil sampling

Soil samples were collected from the four replicates of each treatment (N0, N50, N100, N150) at four sampling times: (1) rosette stage- May (2) 20% flowering stage- June (3) 80 % pod filling stage- July (4) 90 % physiological maturity stage-August. Composite soil samples were taken from two soil depths: 0-5 cm and 5-20 cm from random positions in each plot with a soil probe (2.5 cm diameter). The soils were passed through a size 4 mesh Tyler sieve to remove plant residues, and packed in

ziplock bags. The samples were then transported to the laboratory on ice to minimize microbial activity and stored at 4°C until analysis.

2.3.4 Soil Analysis

2.3.4.1 Mineral N concentration

Soils were extracted with 0.5M K_2SO_4 (1:2 soil:extractant) for determining mineral N (NH_4-N and NO_3-N) concentration. For this, 40 mL of 0.5M K_2SO_4 was added to 10 g of field moist soil in a 250 mL Erlenmeyer flasks, shaken for an hour and filtered into acid washed Nalgene bottles using Fisher Q5 filter paper (Fisher Scientific). The mineral N concentration was then determined using modified indophenol blue technique (Sims *et al.*, 1995). Spectrophotometric measurements of samples were done in triplicate using an EL312 Model microplate reader (Bio-Tek Instrument, Winooski, VT, USA). Simultaneously, gravimetric soil moisture content was determined by oven drying 10 g soil at 105°C for 48 hours.

2.3.4.2 Net N mineralization and nitrification

About 25 g of field moist soil was placed in a 120 mL polythelene cup. Water was added to the soil to adjust the moisture content to 60% water-filled pore space, assuming a particle density of 2.65 g cm^{-3} (Elliott *et al.*, 1999). Each cup with soil was then placed in an individual 1 L glass mason jars (= experimental unit) that contained 10 mL water in the bottom to maintain soil humidity. The mason jars were sealed to create an air tight condition and were incubated at 21°C in the dark. Mason jars were aerated

for 15-20 min every 7 days ito maintain aerobic condition. After 28 days, the incubated samples were extracted with 0.5M K_2SO_4 (1:2 soil:extractant) and mineral N (NH_4-N and NO_3-N) concentrations were determined using the modified indophenol blue technique (Sims *et al.*, 1995). The net N mineralization rate (mg NH_4-N kg^{-1} d^{-1}) was the difference in NH_4-N concentration in incubated and unincubated soils divided by the incubation time (28 d). Likewise, the nitrification rate (mg NO_3-N Kg^{-1} d^{-1}) was calculated as the difference between NO_3-N concentration in incubated and unincubated soils divided by incubation time.

2.3.4.3 Microbial biomass N

Microbial biomass nitrogen (MBN) was determined using the chloroform fumigation-direct extraction method (Brookes *et al.*, 1985; Vance *et al.*, 1987). Fumigated and unfumigated field moist soils were extracted with 0.5 M K_2SO_4 (1:4 soil:extractant) and then subjected to persulphate digestion (Cabrera and Beare, 1993). The MBN was calculated as [(total N in digests of fumigated soils- total N in digests of unfumigated soils)/K_{EN}] where K_{EN} is the extraction coefficient 0.54 (Brookes *et al.*, 1985).

2.3.5 Statistical Analysis

The data were tested for normality prior to analyses, using the Kolmogorov-Smirnov test (PROC UNIVARIATE procedure of SAS, SAS System 9.1, SAS Institute Inc., Cary, NC). For each site, the effect of fertilizer treatments on net nitrogen mineralization and nitrification rates as well as on soil microbial biomass N were

evaluated using PROC MIXED and PROC GLIMMIX procedure Repeated Measures ANOVA of SAS statistical software, version 9.2. We also looked at the effect of soil depth and canola growth stages on net nitrogen mineralization and nitrification rates, and microbial biomass N, separately for each site. When fertilizer treatment was significant ($P<0.05$), data from each fertilizer rate were compared using a Scheffe's test at 95% confidence level. Values in tables and graphs are untransformed means ± standard errors (n=4 unless mentioned otherwise).

2.4 Results

2.4.1 Mineral N concentration

Compared to the initial soil concentrations at rosette stage, mineral N decreased during the growing season, and NO_3^{-1} was at least ten times greater than NH_4^+ in the mineral N pool during early growth stages, indicating rapid nitrification in these fields (Tables 2.2, 2.3, 2.4). Towards the end of the growing season both NO_3^- and NH_4^+ were observed in equal amounts ranging from 0.12 to 7.76 mg N kg^{-1}. There was a sharp effect of growth stage in all three sites ($P<0.05$) (Table 2.1). Mineral N decreased towards the later stages. There was no difference in NH_4-N and NO_3-N concentration in the two soil depths in Montreal and Quebec soil, but in the Ottawa soil, the mineral N concentration was significantly ($P<0.05$) greater at the 0-5 cm depth than at 5-20 cm depth in rosette, flowering and pod filling stage. The NO_3-N concentration in the soil increased with increasing N fertilizer application. Increasing the N fertilizer rate

significantly ($P<0.05$) increased the NO_3-N concentration at the Montreal and Ottawa sites, but not at the Quebec site.

2.4.2 Net N mineralization and nitrification rates

There was difference ($P<0.05$) in net mineralization and nitrification rates between stages of development but the effect of depth was not significant at all three sites (Table 2.1). Net N mineralization and nitrification rates fluctuated throughout the growing season (Fig. 2.1). Highest rate was observed at the pod filling stage and the lowest rate was observed at the flowering stage in the sandy loam soils of Ottawa and Quebec. In Montreal where the soil type was a clay loam, highest rate of N mineralization and nitrification was observed in the rosette stage while lowest rate was measured at maturity. Within each stage, there was no effect of fertilizer treatment.

2.4.3 Microbial biomass nitrogen

There was difference ($P<0.05$) in microbial biomass nitrogen (MBN) between crop development stages but the effect of depth was not significant (Table 2.1). Soil MBN concentration was stable at the beginning and end of the growing season with fluctuations between stages in all 3 sites. There was no difference due to N fertilizer treatment within stages ($P>0.05$).

2.5 Discussion

2.5.1 Mineral N concentration

2.5.1.1 Soil Mineral N in Montreal

There was no effect of soil depth in soil mineral N concentration throughout the growing season. This could be because the soil was cultivated using a harrow to a depth of 10 cm immediately after fertilizer application leaving the N input well mixed among the two soil depths. In addition, some of the mineral N came from “soil N supply” and this was due to conversion of SON to NH_4 and NO_3. Since there was no effect of depth on MBN concentration, microbial activity generating mineral N must have been similar among the two soil depths resulting in no difference in mineral N concentration in the two soil depths.

Mineral N concentration decreased during the growing season. It was highest at the rosette stage, as high as 49.86 mg N kg^{-1}(average of two soil depths) in plots receiving 150 kg N ha^{-1}. It decreased in subsequent stages. The lowest concentration was found when plants were at the maturity stage in plots receiving 150 kg N ha^{-1}. This decrease in mineral N concentration could be due to plant uptake. It could also be due to loss of N through leaching and runoff during the growing season (Ma *et al.*, 2005; Zebarth *et al.*, 2005).

NH_4-N concentration in the soil was low, from 0.11 to 5.84 mg N kg^{-1}, on average. Whalen and Sampedro (2010) explained the low concentration of NH_4^+ in

humid temperate soils as a result of rapid conversion into NO_3^- through nitrification by chemoautotrophic microorganisms. There was no effect of fertilizer rate on soil NH_4-N concentration but 150N plots showed relatively higher level of NH_4-N at the rosette (16.16 mg N kg^{-1}) and flowering stages (11.6 mg N kg^{-1}). One possible reason could be the incomplete conversion of NH_4^+ to NO_3^- as these plots received higher dose of urea, which is an ammonia-based fertilizer.

There was an effect of fertilizer treatment on NO_3-N concentration ($P<0.05$). NO_3-N concentration in the soil increased with increasing N fertilizer application. NO_3-N concentration was highest in 150N plots and lowest in 0N plots at all growth stages. Ma and Wu (2008) explained that critical NO_3-N concentration can be lower for fields that have not been fertilized for a long time, which is the case in this study as the trial was conducted in a field that was fallowed in the previous year. A fallow season meant there was not only absence of exogenous nitrogen input but also no biomass returned to the soil to be decomposed, mineralized and nitrified.

At the maturity stage, there was a significantly higher level of NO_3-N in 150N plots when compared with N0 plots. This indicates that canola did not utilize all the mineral N in the soil. When soil N supply exceeds plant N demand, N mineralized during the growing season is susceptible to loss (Paul and Zebarth, 1997). Excess NO_3-N may remain in the soil as residual N but this is highly unlikely in humid temperate condition and so may be lost to the environment posing environmental risk. This residual soil

NO_3-N also indicates economic inefficiency due to incomplete use of costly urea fertilizer.

2.5.1.2 Soil Mineral N in Quebec

Like in the Montreal soil, there was no effect of soil depth in mineral N concentration because of the uniform distribution of N fertilizer in the two soil depths due to harrowing (Table 2.1). The concentration of mineral N decreased throughout the growing season (Table 2.3). This decrease was most likely due to plant uptake. Another reason could be loss of N by leaching and runoff (Ma *et al.*, 2005; Zebarth *et al.*, 2005).

NH_4-N concentration was very low ranging from 0.18 to 3.19 mg kg^{-1} indicating rapid conversion into NO_3-N. Concentration of NO_3-N ranged from 10.08 to 36.88 mg kg^{-1} at the early stage and subsequently decreased at the maturity stage (0.89 to 3.53 mg kg^{-1}).

There was no effect of fertilizer rate on NO_3-N concentration indicating that initial NO_3-N level was high and further N input did not yield much difference. Higher level of NO3 concentrations in the early part of the growing could be attributed to decomposition, N mineralization and nitrification of the residues from the previous barley crop (*Hordeum vulgare* L.) from previous season (Ma and Wu, 2008). Also, if fertilizer rate did not affect NO_3-N concentration, this meant that extra N released from the fertilizer was either taken up by the crop, or underwent transformation into stable SON and/or was lost to the environment.

2.5.1.3 Soil Mineral N in Ottawa

There was effect of soil depth in mineral N concentration (Table 2.1). Mineral N concentration was greater at 0-5 cm depth than at 5-20 cm depth at the rosette, flowering and pod filling stages in Ottawa (Table 2.4). Like in Montreal and Quebec, the soil in Ottawa was cultivated using a harrow to a depth of 10 cm before planting canola ensuring that N fertilizer was uniformly spread among the two soil depths. However, significant difference in mineral N concentration at the two depths was observed and this could be attributed to more microbial activity (leading to more N mineralization and nitrification) in the 0-5 cm depth than the 5-20 cm depth.

NH_4-N concentration in the soil was very low, ranging from 0.12 to 6.33 mg N kg^{-1}. This could be due to a rapid conversion into NO_3^- through nitrification by chemoautotrophic microorganisms (Whalen and Sampedro, 2010). There was no effect of fertilizer rate on soil NH_4-N concentration but 150N plots showed relatively higher level of NH_4-N at rosette (16.16 mg N kg^{-1}) and pod filling stages (10.86 mg N kg^{-1}). This could be due to incomplete conversion of NH_4^+ to NO_3^- as these plots received higher dose of urea, which is an ammonia-based fertilizer.

There was an effect of fertilizer treatment on NO_3-N concentration ($P<0.05$). NO_3-N concentration in the soil increased with increasing N fertilizer application. NO_3-N concentration was highest in 150N plots and lowest in 0N plots at all growth stages. The previous crop in the field was soybean, which provides an N credit of 30 kg N ha^{-1}

(OMAFRA, 2013) to the canola crops due to release of mineral N during decomposition. This contributed to the soil NO_3-N concentration, which was further boosted by urea fertilizer, and NO_3-N was either used by the crop or retained in the 0-20 cm depth of this sandy loam soil. The significantly higher level of NO_3-N in 150N plots when compared with N0 plots towards the end of the season indicated that canola did not benefit from fertilization. This inefficient use of fertilizer N applications poses environmental risk due to the fact that residual soil NO_3-N remaining in the soil following crop harvest is susceptible to environmental loss, leading to contamination of ground water and increased nitrous oxide emissions to the atmosphere. This residual soil NO_3-N also indicates economic inefficiency.

2.5.2 Net N mineralization and nitrification rates

Soils in all three sites, exhibited either positive rates of small magnitude or negative rates of net mineralization. This negative rate of mineralization indicates rapid nitrification which is common in humid region. When both net N mineralization rate and nitrification rate were combined there was significant variation in all three sites (Fig. 2.1). The combined net rate fluctuated throughout the season which could be due to fluctuation in microbial activity as a result of continuous drying and rewetting of the soil (Desserault-Rompre *et al.*, 2010).

Effect of soil depth on N mineralization and nitrification was absent in Montreal and Quebec soils but was seen in the Ottawa soil. There was no difference in net N

mineralization and nitrification rates in the two soil depths in Montreal and Quebec because the soil was cultivated using a harrow to a depth of 10 cm before planting canola creating a homogenous soil layer. However, in case of Ottawa, depth effect was seen with higher rates observed at 0-5 cm depth than at 5-20 cm, and was attributed to greater heterogeneity in microbial processes and nutrient retention capacity of soil at the Ottawa site.

There was no difference in net N mineralization and nitrification rates among treatments at any of the sites during any growth stage. This suggests that fertilizer N input did not change the rate at which soil organic N was converted into NH_4-N and NO_3-N. This finding is in accordance with Nissen *et al*. (2003) who reported that the soils under different management practices were affected chiefly from indigenous N contents instead of added urea as N. We can conclude that net N mineralization and nitrification rates are inherent soil properties depending on organic N in the soil and is not affected by external N application.

2.5.3 Microbial biomass nitrogen (MBN)

Soil microbial biomass N (MBN) concentration appeared to be stable at the beginning and end of the growing season with fluctuations between stages in all 3 sites (Tables 2.2, 2.3, 2.4). This is a result of continuous drying and rewetting of the soil (Desserault-Rompre *et al*., 2010). The effect of fertilizer treatment was also absent (Table 2.1). This is consistent with other studies reporting that the microbial biomass

remains relatively constant throughout the year in spite of changing soil conditions and nutrient availability (Patra *et al.*, 1990; Puri and Ashman, 1998). Griffin (2008) reported that soil MBN concentration was greater in clay soil compared to sandy soil. However, in this study MBN concentration was found to be higher in the sandy loam soils of Quebec and Ottawa, compared to the clay loam soils in Montreal. This could be due to extraction inefficiency with the MBN chloroform extraction procedure. Another possibility is that MBN relies on decomposing residues for their energy source and there were fewer residues/resources in the former fallow plot in Montreal than the other systems.

2.6 Conclusion

Elevated level of mineral N at the end of the growing season in plots receiving 150 kg N ha^{-1} suggested canola plants did not benefit from excess fertilization. Excess fertilizer N application may pose environmental risk in the form of NO_3-N contamination of ground water and increased nitrous oxide emissions to the atmosphere. It also indicates economic inefficiency. In order to avoid unnecessary loss of N and find out optimum level of N fertlizer, repeated measurement of canola N and monitoring soil mineral N status is warranted. Plant tissue analysis at critical growth stages and correlating it to soil mineral N, net N mineralization and nitrification rates, and microbial biomass N is recommended develop a soil and crop-based N indicator of the soil N supply to canola.

Table 2.1: Effect of nitrogen fertilizer application rate on soil characteristics in canola field in 2012 at three sites.

Parameter	Source of variation						
	trt	depth	trt*depth	stage	trt*stage	depth*stage	trt*depth*stage
Montreal							
NO_3-N (mg kg^{-1})	<.0001	NS	NS	<.0001	<.0001	<.0001	0.0132
NH_4-N (mg kg^{-1})	<.0001	NS	NS	<.0001	<.0002	NS	NS
MBN (mg kg^{-1})	NS	NS	NS	<.0001	NS	NS	NS
Net N mineralization and nitrification rates (mg kg^{-1} d^{-1})	NS	NS	0.0001	<.0001	NS	NS	NS
Quebec							
NO_3-N (mg kg^{-1})	NS	NS	NS	<.0001	<.0001	NS	0.0132
NH_4-N (mg kg^{-1})	NS	NS	NS	<.0001	NS	NS	NS
MBN (mg kg^{-1})	NS	0.001	NS	<.0001	NS	0.0415	NS
Net N mineralization and nitrification rates (mg kg^{-1} d^{-1})	NS	NS	NS	<.0001	NS	NS	NS
Ottawa							
NO_3-N (mg kg^{-1})	<.0001	0.0136	NS	<.0001	NS	0.0004	NS
NH_4-N (mg kg^{-1})	NS	0.0006	NS	<.0001	NS	0.0069	NS
MBN (mg kg^{-1})	NS	0.0139	NS	<.0001	0.0466	NS	NS
Net N mineralization and nitrification rates (mg kg^{-1} d^{-1})	NS	0.0094	NS	<.0001	NS	0.003	NS

Table 2.2: Mineral nitrogen and microbial biomass concentrations in soils at 0-5 cm and 5-20 cm depth from a canola field in 2012 in Montreal. Soils were collected at different growth stages. The different treatments 0N, 50N, 100N, 150N were 0 Kg N ha^{-1}, 50 Kg N ha^{-1}, 100 Kg N ha^{-1} and 150 Kg N ha^{-1}.

Treatment	NO_3-N		NH_4-N		MBN	
	(mg kg^{-1})					
	0-5cm	5-20cm	0-5cm	5-20cm	0-5cm	5-20cm
Rosette						
0N	14.89 ±3.00b	15.35 ±1.03b	0.78 ±0.28	4.62 ±2.28	64.46 ±17.24	*76.74 ±7.44
50N	*34.23 ±4.78a	22.93 ±3.21a	*4.98 ±2.63	5.84 ±4.20	*74.17 ±8.49	70.31 ±7.69
100N	56.59 ±1.48a	27.01 ±5.78a	4.21 ±2.65	0.52 ±0.11	*69.35 ±5.74	123.01 ±55.04
150N	78.78 ±14.74a	22.16 ±5.52a	43.1 ±17.72	1.01 ±0.46	73.8 ±8.59	*45.73 ±26.34
Flowering						
0N	18.27 ±9.13bc	8.711 ±4.35bc	0.39 ±0.19	0.3 ±0.15	31.43 ±15.71	37.94 ±18.97
50N	8.94 ±4.47c	9.45 ±4.72c	0.22 ±0.11	0.2 ±0.10	45.46 ±22.73	38.56 ±19.28
100N	30.05 ±15.02ab	8.87 ±0.69ab	2.89 ±1.44	0.28 ±0.11	53.97 ±26.98	37.22 ±2.42
150N	36.52 ±9.25a	21.15 ±10.57a	11.6 ±3.76	11.36 ±5.68	60.64 ±7.83	42.01 ±21.00
Pod filling						
0N	1.54 ±0.37b	*0.66 ±0.18b	0.49 ±0.06	1.29 ±0.20	*99.55 ±6.24	86.38 ±3.25
50N	1.17 ±0.28b	0.62 ±0.14b	1.16 ±0.11	1.56 ±0.11	98.86 ±4.38	75.45 ±2.98
100N	7.87 ±1.67a	8.65 ±2.07a	3.11 ±1.90	1.85 ±0.32	94.35 ±5.85	96.96 ±2.36
150N	11.18 ±2.51a	19.78 ±3.76a	2.14 ±0.30	2.48 ±0.91	116.0 ±7.21	78.92 ±8.15
Maturity						
0N	*0.12 ±0.06c	1.73 ±0.32c	2.24 ±0.19	2.26 ±0.16	57.77 ±10.34	*42.29 ±8.96
50N	1.01 ±0.38bc	1.78 ±0.29bc	1.7 ±0.21	1.64 ±0.23	55.46 ±12.87	57.49 ±7.13
100N	1.62 ±0.30b	*2.27 ±0.20b	2.24 ±0.26	2.12 ±0.06	52.21 ±12.56	65.85 ±6.01
150N	6.95 ±1.62a	7.76 ±2.20a	3.61 ±1.43	2.88 ±0.45	55.29 ±8.29	62.74 ±2.64

Note: Values are the mean ± standard error (n=4 except * indicates n=3). Within each column and at each sampling date, means followed lowercase letters are significantly different ($P<0.05$, Scheffe's test).

Table 2.3: Mineral nitrogen and microbial biomass concentrations in soils at 0-5 cm and 5-20 cm depth from a canola field in 2012 in Quebec. Soils were collected at different growth stages. The different treatments 0N, 50N, 100N, 150N were 0 Kg N ha^{-1}, 50 Kg N ha^{-1}, 100 Kg N ha^{-1} and 150 Kg N ha^{-1}.

Treatment	NO_3-N		NH_4-N		MBN	
	(mg kg-1)					
	0-5cm	5-20cm	0-5cm	5-20cm	0-5cm	5-20cm
Rosette						
0N	12.04 ±3.40	13.84 ±4.08	0.18 ±0.18	1.75 ±0.72	*65.07 ±3.76	99.98 ±7.86
50N	20.72 ±2.97	16.19 ±3.20	1.19 ±0.73	1.1 ±0.78	**64.74 ±5.52	89.22 ±12.78
100N	36.88 ±11.10	10.08 ±2.97	2.58 ±1.00	1.49 ±0.74	*49.85 ±6.51	82.59 ±14.39
150N	35.62 ±3.49	19.43 ±5.15	1.13 ±0.58	0.58 ±0.43	**52.7 ±0.06	99.61 ±4.42
Flowering						
0N	6.91 ±1.04	9.44 ±1.01	0.6 ±0.32	0.52 ±0.29	*71.36 ±30.17	67.89 ±8.62
50N	9.77 ±1.41	8.25 ±1.87	0.59 ±0.32	0.51 ±0.22	40.9 ±11.7	58.58 ±13.58
100N	8.65 ±1.63	6.91 ±1.49	0.47 ±0.23	0.72 ±044	51.83 ±11.51	70.31 ±8.9
150N	10.8 ±2.09	13.25 ±1.82	0.52 ±0.3	0.37 ±0.34	36.38 ±6.35	46.58 ±5.79
Pod filling						
0N	5.2 ±1.42	3.58 ±0.74	1.22 ±0.42	1.42 ±0.7	78.99 ±8.86	94.41 ±7.86
50N	4.73 ±0.42	3.5 ±0.47	0.86 ±0.23	0.9 ±0.2	74.45 ±11.85	78.52 ±8.5
100N	3.06 ±0.34	2.09 ±0.38	0.93 ±0.27	0.86 ±0.24	*88.61 ±3.57	*85.43 ±7.27
150N	1.84 ±0.65	3.34 ±1.14	0.9 ±0.18	0.9 ±0.38	*76.32 ±15.12	*86.94 ±6.56
Maturity						
0N	0.89 ±0.34	2.03 ±0.33	3.18 ±0.62	2.53 ±0.44	94.21 ±17.63	89.71 ±10.88
50N	1.8 ±0.34	3.53 ±0.74	2.55 ±0.25	2.09 ±0.42	79.78 ±9.21	84.03 ±13.37
100N	3.03 ±0.85	3.31 ±0.79	3.4 ±1.07	3.37 ±0.84	70.74 ±15.22	96.45 ±10.27
150N	2.07 ±0.76	0.97 ±0.47	3.38 ±0.69	3.19 ±0.47	84.39 ±14.26	82.85 ±16.24

Note: Values are the mean ± standard error (n=4 except * indicates n=3 and ** indicated n=2). Within each column and at each sampling date, means followed lowercase letters are significantly different ($P<0.05$, Scheffe's test).

Table 2.4: Mineral nitrogen and microbial biomass concentrations in soils at 0-5 cm and 5-20 cm depth from a canola field in 2012 in Ottawa. Soils were collected at different growth stages. The different treatments 0N, 50N, 100N, 150N were 0 Kg N ha^{-1}, 50 Kg N ha^{-1}, 100 Kg N ha^{-1} and 150 Kg N ha^{-1}.

Treatment	NO_3-N		NH_4-N		MBN	
	(mg kg^{-1})					
	0-5cm	5-20cm	0-5cm	5-20cm	0-5cm	5-20cm
Rosette						
0N	10.42 ±3.87b	13.11 ±1.32b	0.27 ±0.1	0.12 ±0.08	67.12 ±10.03	69.07 ±13.05
50N	18.48 ±2.65ab	21.56 ±2.6ab	0.28 ±0.11	0.25 ±0.06	54.91 ±18.06	51.72 ±15.46
100N	32.9 ±9.15a	30.36 ±0.91a	0.22 ±0.12	0.28 ±0.17	71.42 ±23.58	66.78 ±8.62
150N	56.08 ±8.98a	27.32 ±2.75a	16.2 ±12.91	0.16 ±0.05	76.33 ±28.67	37.88 ±10.56
Flowering						
0N	6.57 ±1.01a	4.31 ±0.99a	1.39 ±0.15	1.25 ±0.31	48.9 ±4.16	44.89 ±5.84
50N	4.97 ±0.55a	5.03 ±0.58a	1.05 ±0.09	1.19 ±0.18	60.81 ±5.67	54.35 ±4.8
100N	12.96 ±3.04a	6.57 ±1.45a	1.31 ±0.21	0.98 ±0.12	69.52 ±14.58	56.41 ±6.81
150N	14.2 ±3.45a	7.65 ±3.06a	1.13 ±0.13	1.05 ±0.18	52.18 ±5.72	34.17 ±5.64
Pod filling						
0N	4.09 ±1.22b	3.4 ±1.46b	4.55 ±0.44	2.76 ±0.28	*50.48 ±14.46	67.72 ±14.13
50N	8.57 ±2.46ab	3.41 ±0.51ab	5.13 ±0.41	2.62 ±0.29	92.85 ±7.81	76.47 ±12.56
100N	12.65 ±3.35ab	3.87 ±0.81ab	6.33 ±1.01	4.32 ±2.02	99.33 ±16.90	85.56 ±12.04
150N	16.53 ±3.79a	7.45 ±1.26a	10.9 ±3.87	3.29 ±0.32	*128.52 ±21.99	85.11 ±6.21
Maturity						
0N	3.87 ±0.21c	4.67 ±0.51c	2.98 ±0.43	2.43 ±0.16	122.7 ±2.92	68.52 ±8.22
50N	6.58 ±1.02b	8.03 ±1.08b	3.32 ±0.23	3 ±0.21	96.66 ±10.79	74.43 ±3.88
100N	8.1 ±1.15b	8.97 ±1.17b	3.15 ±0.24	3.56 ±0.73	109.1 ±12.73	*113.54 ±14.6
150N	14.58 ±1.66a	17.47 ±2.00a	3.69 ±0.70	2.08 ±0.33	112.1 ±19.78	78.79 ±14.09

Note: Values are the mean ± standard error (n=4 except * indicates n=3). Within each column and at each sampling date, means followed lowercase letters are significantly different ($P<0.05$, Scheffe's test).

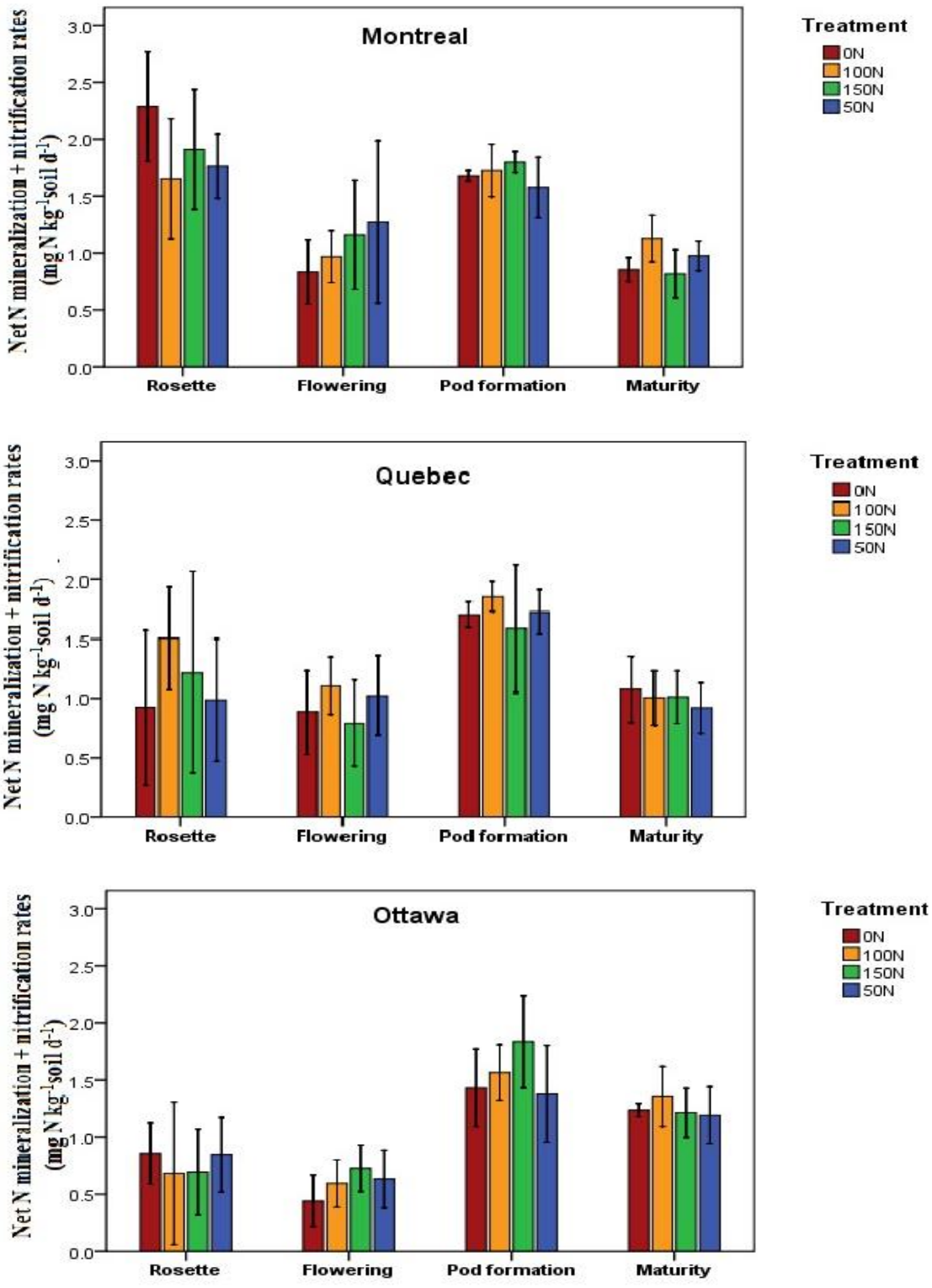


Fig. 2.1: Net N mineralization and nitrification in incubated soils (0-20 cm) from canola field in three sites. Soils were collected during four critical growth stages in canola.

CONNECTING PARAGRAPH

In the previous chapter, I determined soil mineral N content and microbial biomass nitrogen in canola fields at three sites during one growing season in 2012. I also monitored net N mineralization and nitrification rates in the soils at different times in the growing season by means of aerobic laboratory incubation that allowed practical treatment comparisons. Results indicated residual soil mineral N at the end of the growing season in plots receiving 150 kg N ha^{-1}, but not with 100 kg N ha^{-1}. Considering economic efficiency and environmental quality, I propose that N fertilizer should be applied at the rate of 100 kg N ha^{-1} for canola, which is in accordance with the recommendations of the Ontario Canola Growers' Association (Hall, 2012). However, sufficient mineral N concentration in the soil does not necessarily mean all of it is taken up by the plant. Mineral N is prone to loss due to leaching, runoffs and immobilization. To optimize N fertilizer for canola production in Eastern Canada, I studied crop N response to N fertilizer application.

CHAPTER THREE: CANOLA BIOMASS AND NUTRITION IN RESPONSE TO NITROGEN FERTILIZATION

3.1 Abstract

Field experiments were conducted in Ste. Anne-de-Bellevue (Quebec), St. Augustin-de-Desmaures (Quebec) and Ottawa (Ontario) in 2012 to investigate the effect of nitrogen fertilizer on canola biomass and crop nutrition. The experiment was designed as a randomized complete block with four pre-plant N fertilizer treatments (0, 50, 100 and 150 kg N ha^{-1} from urea), replicated four times. Soil N pools (NH_4, NO_3, MBN, net N mineralization rate and net nitrification rates) were measured at four growth stages namely rosette, 20% flowering, 80% pod formation and 90% maturity. Simultaneously canola biomass and plant N concentration were determined at these crop growth stages. Canola was most responsive to N fertilizer input at the rate of 100 kg N ha^{-1}during the rosette stage ($P<0.001$) in N deficient soils. Seed protein content was not affected by fertilizer application rate. Total seed protein content ranged from 19.57 to 21.69% in Ste. Anne-de-Bellevue, 20.46 to 20.82% in St. Augustin-de-Desmaures, and 26.75 to 27.96% in Ottawa. Correlations between plant N concentration and mineral N differed among soils: there was a negative relationship between these values for soils from Ste. Anne-de-Bellevue ($r= -0.551$, $P<0.001$) and St. Augustin-de-Desmaures soils ($r= -0.272$, $P<0.05$), and a positive relationship for Ottawa soils ($r= 0.319$, $P<0.05$). A strong negative correlation ($r= -0.701$, $P<0.001$) was observed between plant N

concentration and net N mineralization and nitrification rates in Ste. Anne-de-Bellevue while there was no relationship between plant N concentration and microbial biomass nitrogen in any of the three sites. Further study on seed yield and harvest index is warranted to identify optimum N fertilizer application rate for canola in Eastern Canada.

3.2 Introduction

In order to optimize Nitrogen (N) fertilizer application in Eastern Canada, it is important to understand how canola responds to N fertilizer, since N is often the most limiting nutrient in canola (Grant and Bailey, 1993). It affects canola growth and development by affecting yield attributing characters. Leaf area, number of leaves per plant, plant height, number of flowering branches, number of pods and oilseed yield have been found to increase with increasing N fertilizer application (Ogunlela et al., 1989; Ozer, 2003). Insufficient N slows plant growth and development resulting in shorter stems, fewer branches, and smaller canopy (Ogunlela et al., 1989). On the other hand, excess N causes lodging, reduction in canola seed oil content, increased seed chlorophyll content (Brennan et al., 2000; Karamanos et al., 2003 and 2007).

Although N fertilization boosts biomass accumulation in canola, oilseed concentration declines with increasing level of N application because there is an increasing protein concentration at the expense of the oil concentration (Mason and Brennan, 1998; Brennan et al., 2000, Malhi 2001; Gan, 2007). Mendham and Roberson (2004) reported that the oil concentration decreases by 0.6% to 1.2% per additional 100

kg ha^{-1} N applied. Since canola is primarily grown for oil extraction, N fertilizer recommendation needs to be calibrated to achieve optimal plant nutrition and maximize oil concentration in the harvested seed.

Canola is characterized by low nitrogen use efficiency (NUE) which ranges from 12% to 40% depending on the cultivar and management regime (Gan et al., 2007). It is categorized as low NUE crop in comparison to other typical grain oil seed production systems having NUE between 30% and 50% (Raun and Johnson, 1999). In canola, NUE is defined as the ratio of seed yield (kg N ha^{-1}) to N fertilizer input (kg N ha^{-1}) (Gan et al., 2008). The NUE can be quite low at high N fertilizer rates, since so little of the N is retained for oilseed production, or when the soil mineral N supply exceeds crop N requirement (Chamorro et al., 2002). Therefore, judicious amount of N needs to be applied to achieve yield targets in an economic manner while minimizing N losses to the environment.

Ontario recommends N application at 100-110 kg N ha^{-1} for canola. This rate of N application resulted in an average seed yield of 2863 kg ha^{-1} in 2011 (Earl, 2011). However, canola's ability to uptake N fertilizer depends on plant available N in the soil, which in turn is affected by various climatic and edaphic factors. Canola’s response to N fertilizer also depends on its ability to grown an extensive root system that can access N ions (plant-available N) in the soil solution. Therefore, an understanding of soil N supply

and crop N uptake at various growth stages is essential to achieve synchrony between the two in order to maximize crop productivity while ensuring economic viability.

The objectives of this study were to (1) Compare canola N concentration in response to N fertilizer (2) Correlate and identify soil parameters that affect crop N uptake in canola.

3.3 Materials and Methods

Detailed description of experiment sites, experimental design, and soil sampling and analysis methods have been discussed in section 2.3 of chapter two.

3.3.1 Plant sampling and analysis

Plant samples were collected from the four replicates of each treatment (N0, N50, N100, N150) at four sampling times: (1) rosette stage- May (2) 20% flowering stage- June (3) 80 % pod filling stage- July (4) 90 % physiological maturity stage-August (2012). While sampling, five representative plants were randomly uprooted from the first seven rows of each plot. The roots were separated from the shoots and were washed to remove soil. The plants were then dried to a constant weight at 60°C, and the biomass per plant was determined. The dried plants were ground to pass through a 2mm mesh sieve and stored in paper envelopes until further analysis. For determining the total N content, 12 mg of plant sample was weighed into tin capsules and N concentration was determined with Leco CN analyzer.

3.3.2 Statistical analysis

The data were tested for normality prior to analyses, using Kolmogorov-Smirnov test (PROC UNIVARIATE procedure of SAS, SAS System 9.1, SAS Institute Inc., Cary, NC). The effect of fertilizer treatments on plant N concentration and plant biomass were evaluated for each crop growth stage using PROC MIXED and PROC GLIMMIX statement in SAS. Significant effects were analyzed using Scheffe's multiple comparisons test at 95% confidence level. Correlation analyses with Spearman's correlation coefficient were performed to determine the relationship of plant N concentration with soil parameters like mineral N concentration, net N mineralization and nitrification rates, and MBN. Values in tables and graphs are untransformed means ± standard errors (n=4 unless mentioned otherwise).

3.4 Results

3.4.1 Dry biomass

The effect of treatment on dry biomass of plant was significant ((P<0.005) in Montreal but not in Quebec and Ottawa (Table 3.1). Dry biomass of plant increased with response to N fertilizer in all three sites at all growth stages, but the magnitude of biomass gain was very small (Table 3.2). There was a strong effect of stage in all three sites (P<0.0001). Dry biomass ranged from 2.5 to 7.42 g plant^{-1} at the rosette stage and was highest at the maturity stage, with 11.18 to 25.06 g plant^{-1}.

3.4.2 Plant nitrogen concentration

Plant N concentration increased with increasing N application in all three sites at all growth stages (Table 3.3). Despite the wide range of N inputs, there was small increment in the plant N concentration. Significant effect of fertilizer treatment was observed in Montreal ($P<0.001$) and Quebec ($P<0.005$) (Table 3.1). Stages of crop development strongly affected plant N concentration in Montreal ($P<0.0001$). It was lowest at the rosette stage (10.1 to 40.07 mg g^{-1}) and highest at the maturity stage (26.85 to50.44 mg g^{-1}).

3.4.1 Correlation between plant N concentration and soil parameters

Total plant N concentration reflects the demand of N over the season. Our data suggest that there was negative correlation between plant N content and mineral NO_3-N content in Montreal ($r= -0.551$, $P<0.001$) and Quebec ($r =-0.272$, $P<0.05$) (Table 3.4, Fig. 3.1). A positive correlation was seen in Ottawa ($r =0.319$, $P<0.05$).

Significant correlation between plant N concentration and net N mineralization and nitrification rates were not detected in the sandy soil of Ottawa and Quebec (Table 3.4). However, a strong negative correlation ($r =-0.701$, $P<0.001$) (Fig. 3.2) between plant N and net N mineralization and nitrification rates was detected in the clay loam soil of Montreal.

3.5 Discussion

3.5.1 Canola biomass in response to N fertilizer input

Dry biomass in canola increased with increase in N application rate. The highest biomass at all growth stages was recorded in plants receiving 150 kg N ha^{-1} in all three sites. However, the difference due to N application rate was not significant in Quebec and Ottawa. Similar results were reported by Asare and Scarisbrick (1995) and Allen and Morgan (1972), who reported that dry matter in plant increased with N application but not always to a statistically significant level. In Montreal, significant effect of N application rate on plant biomass was observed in the rosette stage ($P<0.005$) where the dry biomass increased from 2.56 to 4.79 g $plant^{-1}$ in response to N fertilizer. This could be due to low mineral N level in the soil since N is a limiting element and is necessary for stimulating vegetative growth in canola (Grant and Bailey, 1993). This finding was supported by the lower N concentration in plants receiving no N application than plants that received N fertilizer.

3.5.2 Plant N concentration in response to N fertilizer input

Plant N concentration increased with increasing N application rate at all growth stages in all three sites. Plants grown in the sandy loam soil of Montreal had greater N concentration compared to plants grown in the clay loam soil. In Montreal, different rate of N application affected plant N concentration in the rosette stage ($P<0.001$) (Table 3.1) where plant N concentration increased from 10.1 to 23.15 mg g^{-1}. This explained

the low biomass in plants receiving no N fertilizer compared to those receiving N fertilizer. One possible reason for this difference in plant N concentration could be poor N uptake due to low level of mineral N in the soil. In the previous chapter, concentration of mineral N (NO_3-N and NH_4-N) in top 5 cm of soil in the Montreal site at rosette stage was reported to be 17.91mg kg^{-1}. This low level of mineral N in the soil could be attributed to a previous fallow season as explained by Ma and Wu (2008). Another reason for poor crop N uptake could be the soil texture. Ammonium fixation by clay particles in plant unavailable form may have reduced the accessibility to plants of mineral N in the clay loam soil (Chantigny et al., 2004).

In Quebec, plant N concentration increased from 11.39 to 25.84 mg g^{-1} during the flowering season in response to N fertilizer. This could be attributed to low mineral N level in the soil which was recorded as 8.73 mg g^{-1} for plots receiving no N fertilizer. This low amount of N in the soil could be because of loss of N from the soil due to leaching, runoff and denitrification (Ma et al., 2005; Zebarth et al., 2005). In Ottawa, the increase in plant N concentration with N application rate was not at a statistically significant level. Towards the end of the growing season, there was no effect of fertilizer application in plant N concentration in all sites, suggesting that N mineralization and nitrification contribute to plant available N at later stages.

Plant N concentration in all three sites increased throughout the growing season and was recorded highest at the maturity stage. N concentration at maturity stage

included total straw N concentration and seed N content. Effect of crop growth stage on plant N concentration was significant ($P < 0.0001$) only in Montreal. One possible reason could be poor plant nourishment in the rosette stage in Montreal due to low mineral N in the soil. In Quebec and Ottawa, stage effect was not significant and plant N concentration in rosette stage did not differ much from that at maturity. This finding was consistent with Schjoerring et al.'s finding (1995) that more than 75 to 80 % of canola N is assimilated before the flowering stage and is translocated to floral structures including pod and seed for reproductive development.

Total seed protein content ranged from 19.57 to 21.69% in Montreal, 20.46 to 20.82% in Quebec and 26.75 to 27.96% in Ottawa. Data on the same variety from across Western Canada (Manitoba, Alberta and Saskatchewan), produced an average protein level of 21.1% (Canadian Grain Commission, 2012). This suggested that seed protein content was not affected by fertilizer application rate. Despite the high plant biomass in the Montreal site, the seed protein content was lower than that in the Ottawa site, which had a relatively lower plant biomass at maturity stage. Hocking et al., (1997, 2002) showed a negative relationships between plant biomass and N concentration in plants, which explains higher protein concentration in plants with low dry biomass in Ottawa site. According to the protein content of the oilseed, the crop in Ottawa allocated more N to seed production and less to biomass (whole plant) production. In contrast, the Montreal and Quebec sites had bigger plants (which need a bit more N to support

the plant body) and allocated a bit less N to the seed protein synthesis. Since it is the same hybrid, this is evidence of phenotypic responses to soil and climatic conditions at each site.

3.5.3 Correlation between plant N concentration and soil parameters

The negative correlation between plant N and soil mineral N content indicated that N in the soil was taken up by the plant. It also suggested that addition of N to the soil in this case will benefit the crop. The weak positive correlation between plant N and soil mineral N in Ottawa suggested that addition of N fertilizer in the soil did not benefit the crop and will remain in the soil, often prone to loss by leaching, runoff or denitrification. Lack of strong correlation between plant N and mineral N in the soil suggested that N-cycling processes and plant N uptake may be controlled by different set of factors (Turner et al., 1997).

Increase in plant N accumulation despite decrease in net N and nitrification rates in the clay loam soil of Montreal indicated that N assimilated by the plant in the soil did not come from mineralization of soil organic N, but from the exogenous application of N fertilizer. This is because N mineralization and nitrification rates are low in clay soil due to reduced microbial activity (Colman and Schimel, 2013; Roychand and Marschner, 2013). Therefore, application of N fertilizer in clay loam soil is recommended in order to fulfill plant N demand.

No significant correlation was observed between plant N and soil MBN at all three sites. However, there was a negative correlation in Montreal. The reason the MBN could be declining in Montreal with increase in N uptake could be because the Montreal soil does not have substrates from previous fallow season due to limited crop residues for decomposition.

3.6 Conclusion

Canola grown in the clay loam soils of Montreal was most responsive to N fertilizer input at the rate of 100 kg N ha^{-1} during the rosette stage. Soil texture and previous fallow season appeared to limit canola nutrition by affecting the amount of plant available N in the soil. In the sandy loam soils of Quebec and Ottawa, N input did not affect canola response in the early growth stage probably because they had lower NO_3-N retention capacity, making mineral N readily available for plant uptake. With time, the soil N supply was depleted in sandy loam soil for the same reason. Reduced level of NO_3-N in the soil affected canola nutrition in the flowering stage in Quebec and plant responded to fertilizer input at the rate of 100 kg N ha^{-1}. Therefore, fertilizer application at the rate of 100 kg N ha^{-1} is recommended in the early stage for better canola nourishment. Although no measurements were available for canola yield and harvest index because this study strictly focused on N content in canola, it is strongly suggested that these factors be taken into account while recommending optimum fertilizer

application rate. Further research on the effect of soil texture and preceding crop on canola production in Eastern Canada is warranted.

Table 3.1: Effect of nitrogen fertilizer application rate on plant biomass and N in canola field in different sites in 2012.

Parameter	Source of variation		
	trt	stage	trt*stage
Montreal			
Per plant biomass (g)	0.0029	<0.0001	NS
Total N per plant (mg g^{-1})	0.0006	<0.0001	NS
Quebec			
Per plant biomass (g)	NS	<0.0001	NS
Total N per plant (mg g^{-1})	0.0015	0.0563	NS
Ottawa			
Per plant biomass (g)	NS	<0.0001	NS
Total N per plant (mg g^{-1})	NS	0.0542	NS

Table 3.2: Plant biomass (g plant^{-1}) in canola fields across three sites in 2012. Soils were collected at different growth stages. The different treatments 0N, 50N, 100N, 150N were 0 Kg N ha^{-1}, 50 Kg N ha^{-1}, 100 Kg N ha^{-1} and 150 Kg N ha^{-1}.

Treatment	Rosette	Flowering	Pod filling	Maturity
		Montreal		
0N	2.56 ±0.14b	ND	ND	20.17 ±1.60
50N	4.17 ±0.52ab	ND	ND	21.17 ±2.20
100N	4.79 ±0.64a	ND	ND	22.05 ±1.30
150N	4.93 ±0.51a	ND	ND	25.06 ±2.67
		Quebec		
0N	4.1 ±0.14	6.7 ±0.55	10.61 ±2.52	11.18 ±1.9
50N	4.07 ±0.27	7.6 ±0.52	13.86 ±1.8	18.26 ±4.34
100N	3.65 ±0.46	9.44 ±1.5	16.62 ±1.39	22.17 ±2.6
150N	4.9 ±0.68	7.61 ±0.44	19.32 ±1.29	18.31 ±1.6
		Ottawa		
0N	5.41 ±0.39	11.6 ±0.66	14.72 ±1.55	14.52 ±2.56
50N	6.53 ±0.49	14.42 ±2.16	15.66 ±1.52	16.22 ±1.49
100N	5.68 ±1.01	14.77 ±1.12	18.87 ±2.14	17.18 ±3.1
150N	7.42 ±0.78	16.50 ±1.32	15.02 ±2.15	22.10 ±6.88

Note: Values are the mean ± standard error (n=4). Within each column and at each sampling date, means followed by lowercase letters are significantly different ($P<0.05$, Scheffe's test). ND= Not determined.

Table 3.3. Plant nitrogen concentrations (mg g^{-1}) in canola fields across three sites in 2012. Soils were collected at different growth stages. The different treatments 0N, 50N, 100N, 150N were 0 Kg N ha^{-1}, 50 Kg N ha^{-1}, 100 Kg N ha^{-1} and 150 Kg N ha^{-1}.

Treatment	Rosette	Flowering	Pod filling	Maturity
		Montreal		
0N	10.1 ±1.9b	ND	ND	30.94 ±3.29
50N	17.76 ±2.56ab	ND	ND	33.4 ±3.13
100N	23.15 ±2.56a	ND	ND	40.96 ±1.78
150N	24.91 ±2.05a	ND	ND	44.17 ±6.26
		Quebec		
0N	13 ±1.22	11.39 ±1.86b	12.84 ±2.52	18.58 ±3.41
50N	16.62 ±1.24	16.41 ±1.22ab	16.75 ±1.80	26.85 ±5.72
100N	17.58 ±2.55	25.84 ±5.20a	22.01 ±1.75	41.07 ±7.17
150N	20.99 ±2.01	17.27 ±1.77ab	23.75 ±1.84	28.11 ±4.13
		Ottawa		
0N	25.61 ±1.75	24.92 ±2.9	23.44 ±3.54	30.55 ±5.81
50N	34.61 ±2.07	39.41 ±8.09	28.48 ±3.31	33.03 ±2.33
100N	30.14 ±5.39	43.41 ±6.31	31.65 ±3.39	41.42 ±9.71
150N	40.07 ±4.3	50.19 ±7.8	30.33 ±4.33	50.44 ±7.52

Note: Values are the mean ± standard error (n=4). Within each column and at each sampling date, means followed by lowercase letters are significantly different ($P<0.05$, Scheffe's test). ND= Not determined.

Table 3.4: Spearman's correlation coefficients (r) between plant N concentration and NO_3-N, NH_4-N, Net N mineralization and nitrification rates (Net rates), and Microbial biomass nitrogen (MBN) in soils from canola field in different sites. Data were from plant and soils collected at different growth stages.

	NO_3-N	NH_4-N	Net N mineralization and nitrification rates	MBN
		Montreal		
Plant N	-0.551***	-0.098	-0.701***	-0.224
	(31)	(32)	(32)	(28)
		Quebec		
Plant N	-0.272*	0.413***	-0.028	0.159
	(64)	(64)	(64)	(62)
		Ottawa		
Plant N	0.319*	-0.038	0.015	0.017
	(64)	(64)	(64)	(62)

Correlation coefficients were significant at P<0.05*, P<0.01**, P<0.001*** or not significant. Numbers in the parenthesis are the total number of observations.

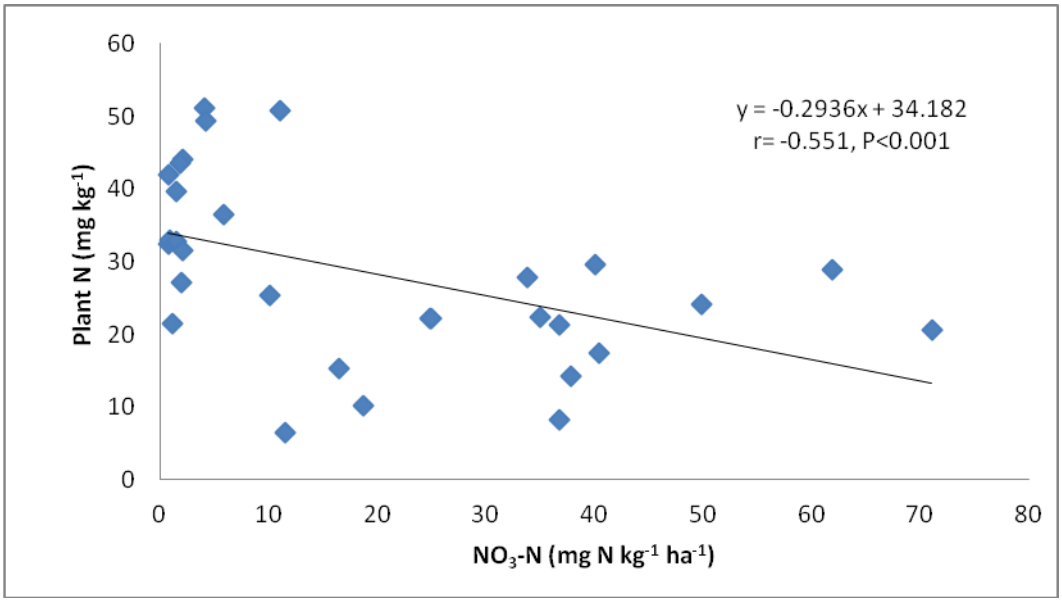


Fig. 3.1: Correlation between plant N concentration and NO_3-N concentration in soils from Montreal. The strength of the association between these two variables is calculated as Spearman's correlation coefficient (r value).

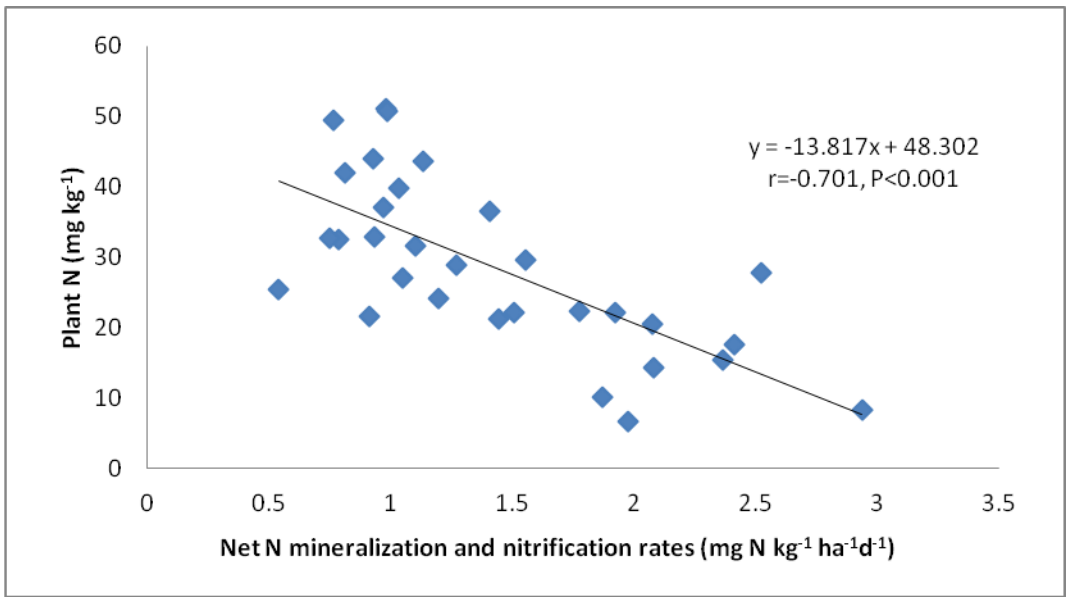


Fig. 3.2: Correlation between plant N concentration and net N mineralization and nitrification rates in soils from Montreal. The strength of the association between these two variables is calculated as Spearman's correlation coefficient (r value).

CHAPTER FOUR: CONCLUSIONS

4.1 General conclusion

In order to reduce fossil fuel consumption and GHG emission, the Canadian government has committed to increase the use of biofuel blend (diesel and ethanol) among fossil fuel. Canola based biodiesel is a good alternative to lower GHG emissions in Canada, but requires that canola cultivation be expanded in eastern Canada to meet regional demands, since these cannot be met feasibly with biodiesel from western Canada. Establishment of local oilseed crushing facility operated by TRT-ETGO is helping to expand canola production in this region, but producers lack necessary agronomic information and fertilizer recommendations to produce canola profitably. Nitrogen fertilizer inputs must be carefully managed to optimize canola yields, with high N fertilizer use efficiency being desirable from agronomic, economic and environmental perspectives. This can be accomplished by synchronizing N availability with plant N demands.

In the present study, total Mineral N ($NH_4^+ + N0_3^-$) was found to decrease during the growing season. Concentration of NH_4^+ was negligible indicating NH_4-N was rapidly nitrified. Soil mineral N concentration increased with increase in fertilizer N treatment. However, elevated level of mineral N at the end of the growing season in plots receiving150 kg N ha^{-1} suggested canola plants did not utilize the entire N supplied. Excess fertilizer N application may pose

environmental risk in the form of NO_3-N contamination of ground water and increased nitrous oxide emissions to the atmosphere. It also indicates economic inefficiency.

Our findings indicate that net N mineralization and nitrification rates, as well as MBN concentration, were not affected by N fertilization, suggesting that the soil N supply was derived from decomposition of organic residues by the activity of a relatively stable microbial population. Since the dynamics of the soil N supply are controlled by native soil microorganisms, further research should be conducted to identify abiotic (temperature, moisture content and substrate) factors that favor the microbial activities that produce mineral N from SON.

Plant tissue analysis revealed that canola was most responsive to N fertilizer input at the rate of 100 kg N ha^{-1}during the rosette stage in N deficient soils. Soil texture and previous fallow season appeared to limit canola nutrition by affecting the amount of plant available N in the soil. Further research on the effect of soil texture on canola production in Eastern Canada is warranted. It is also worthy to evaluate the influence of N fertilizer on residue decomposition and nutrient release from the common crops that are grown in rotation with canola. Although no measurements were available for canola yield and harvest index because this study strictly focused on N content in canola, it is strongly

suggested that these factors and other yield contributing parameters be taken into account while formulating optimum fertilizer.

In the long run, interpretative criteria such as critical range for soil N at various crop development stages and plant tissue analysis of elements that contribute to optimum canola nutrition should be formulated. These tests will identify any nutrients that may limit the yield, giving farmers additional opportunities to correct N and other nutrient deficiencies. Split applications, involving side-dressing of N fertilizer at the rosette stage, or foliar fertilization to supply N and other essential nutrients at key growth stages, must be identified. Multiple approaches such as critical value approach (CVA), compositional nutrient diagnosis (CND) and diagnosis and recommendation integrated system (DRIS) have been formulated for many crops. Development of such diagnostic tools for canola in eastern Canada will help to capture spatio-temporal heterogeneity in order to ensure validity and accuracy of canola fertilization guidelines.

4.2 Acceptance or rejection of hypotheses

Hypothesis 1. Soil NO_3-N and NH_4-N concentrations decrease during the growing season.

Our results showed that the concentration of soil NO_3-N and NH_4-N was initially high but decreased gradually with time. Thus, we accept hypothesis 1.

Hypothesis 2. MBN concentration in the soil remains stable during the growing season.

Soil MBN concentration was stable at the beginning and end of the growing season with fluctuations between stages in all three sites. So, we accept hypothesis 2.

Hypothesis 3. Net N mineralization and nitrification rates increase with nitrogen fertilization rate.

There was no difference in net N mineralization and nitrification rates among different fertilizer rate treatments at any of the sites during any growth stage. Therefore, we reject hypothesis 3.

Hypothesis 4. N concentration in canola is responsive to N fertilizer input at the early vegetative (rosette) stage in N deficient soil.

Canola grown in the clay loam soil of Montreal was most responsive to N fertilizer input at the rate of 100 kg N ha^{-1} during the rosette stage. Our results showed soil in Montreal site was deficient in NO_3-N content at the rosette stage compared to the soils in Quebec and Ottawa at the same stage. Thus, we accept hypothesis 4.

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