THESIS

SOIL ORGANIC MATTER AS A NITROGEN SOURCE FOR *BRASSICA NAPUS*

Submitted by

Candace Carter

Department of Soil and Crop Sciences

In partial fulfillment of the requirements

For the Degree of Master of Science

Colorado State University

Fort Collins, Colorado

Spring 2020

Master's Committee:

       Advisor: Meagan Schipanski

       Richard Fletcher
       Jorge Vivanco
       Matthew Wallenstein

ABSTRACT

SOIL ORGANIC MATTER AS A NITROGEN SOURCE FOR *BRASSICA NAPUS*

Decreasing nitrogen (N) fertilizer losses from agricultural systems is a major focus in sustainable agriculture research. Most research to date has focused on reducing and managing N fertilizer additions in time and space. However, approximately half of the N taken up by most field crops is not from that season's fertilizer but is derived from the mineralization of soil organic matter (SOM). Despite its importance, intentionally managing crop utilization of background SOM as a source of N has received little attention. Our study explored N uptake patterns of rapeseed or canola (*Brassica napus*) in a greenhouse pot study and in a field setting. In the greenhouse pot study, we explored the effects of rapeseed genotypic diversity on N uptake from organic and inorganic N sources. We used dual ^{15}N labeled ammonium-nitrate fertilizer to examine N uptake patterns of rapeseed in different N environments. Using a full factorial experiment, 10 varieties were grown under four treatments that included combinations of high and low N fertilizer and SOM. While we found limited varietal differences in N uptake dynamics, SOM was an important N source across all varieties even as N fertilizer availability increased. Our High SOM/High Fertilizer treatment obtained 64% of N from SOM, while the High SOM/Low Fertilizer obtained 89% of total N from SOM. Nitrogen source uptake was dependent on the treatment level N availability. We found evidence of enhanced SOM mineralization in higher N treatments, where high N fertilizer additions increased overall plant N uptake from SOM by 42% relative to low N fertilizer treatments. Although overall plant N uptake from SOM increased in high fertilizer treatments, microbial enzyme activity related to nutrient mineralization processes was suppressed in the high N fertilizer treatments relative to low fertilizer treatments in similar SOM environments by 16-58%. This result suggests high N fertilizer additions change microbial nutrient cycling dynamics. Based on

the general results from our greenhouse study that N from SOM had an additive effect to fertilizer additions on rapeseed biomass production, we estimated the potential yield contributions of SOM increases with the adoption of conservation tillage practices in Canada. We used yield data provided by a literature search and the Canola Council of Canada to examine how the adoption of conservation tillage practices over the last 25 years has contributed to crop yield improvements in the Canadian prairies. We found that on average canola yields increase by 54.9 kg/ha per year, with 13% of annual yields attributed to agronomic practices. We estimated that the adoption of conservation tillage has increased soil N by 320 kg N/ha per year. Although N mineralization is highly variable and dependent on many factors, we estimated that 2% of total soil N is available annually for plant uptake. This translated to an additional 6.4 kg N/ha per year available for plant nutrition. We estimated that 91 to 164 kg/ha of the annual canola yield increases could be contributed to an increase in soil N availability. It is important to acknowledge the complex nature of N mineralization and plant N uptake patterns. This complexity likely leads to an underestimation of the contribution of SOM as an N source in cropping systems. Because of the dynamic and complex nature of agricultural systems, an integrated approach to N management where both N fertilizer and SOM are considered in crop breeding and system management is an important step in improving agricultural sustainability.

ACKNOWLEDGEMENTS

TABLE OF CONTENTS

Chapter 1: Nitrogen uptake by rapeseed varieties from organic matter and inorganic fertilizer sources

Introduction

Over the last half century, the application of mineral nitrogen (N) fertilizer has been at the foundation of our agricultural system. Although N fertilizer is effective for increasing crop production, more than 50% of the world's N fertilizer is escaping agricultural systems (Tilman et al. 2002; Raun and Johnson 1999; Lassaletta et al. 2014). Because of the major environmental impacts of lost N to waterways and greenhouse gas emissions, management of N fertilizer has been a focus of investment and research for decades. Reducing mineral N inputs by improving crop nitrogen use efficiency (NUE), typically defined as the total plant biomass or seed yield divided by the N applied, is an essential part of sustainable agriculture. However, NUE is a complex trait that integrates the efficiency of plant N uptake from the soil environment as well as internal N utilization patterns within the plant (Kant, Bi, and Rothstein 2011; Stahl, Friedt, et al. 2016; Rathke, Behrens, and Diepenbrock 2006; Perchlik and Tegeder 2017). Improved NUE in crops by shifting internal plant N translocation has been one solution used to improve NUE. Despite decades of effort, crop NUE remains low, on average 30-50% of the total N applied is harvested in that season's grain crop (Raun and Johnson 1999; Garnett, Conn, and Kaiser 2009; Conant, Berdanier, and Grace 2013; Bodirsky et al. 2012).

Regardless of fertilizer management, soil organic matter (SOM) is often an equally important source of N for most crops (Kundu and Ladha 1995; Gardner and Drinkwater 2009). However, there has been limited research investment in utilizing SOM as a source of N through crop management or crop breeding. As a result, we have a limited understanding of belowground activity that affects crop utilization of N derived from the mineralization of SOM. Most modern crops have been primarily bred for high grain yield and field agronomic performance in systems where N is not a limiting factor. While roots are known to

mediate N uptake through physiological and morphological mechanisms (direct effect) and microbial activity (indirect effect) these belowground traits have mostly been ignored in traditional breeding programs (Stahl, Friedt, et al. 2016; Dawson, Huggins, and Jones 2008; Jilling et al. 2018).

Root biomass and structure affect soil carbon (C) inputs and root exudates, which can have profound influences on the crop's ability obtain nitrogen from diverse sources (Stahl, Friedt, et al. 2016). Root morphology is also important for nutrient uptake and has been shown to have a significant effect on aboveground growth and vigor (Garnett, Conn, and Kaiser 2009). A number of studies support that root traits, such as root-shoot ratios, root length density, and root N transport and metabolism could contribute to higher NUE in crops (Ju et al. 2015; Bingham et al. 2012; Bowles, Raab, and Jackson 2015; Garnett, Conn, and Kaiser 2009). Belowground traits are recognized as important for N uptake and may be particularly important for improving utilization of N from SOM. However, we have a limited understanding of root traits that increase mineralization of N from SOM.

Indirectly, roots can enhance soil N cycling via their effects on soil microbial communities and activity (Jilling et al. 2018). Microbial communities in agricultural soils tend to be C limited so plant belowground C inputs can shift microbial communities in the rhizosphere. Plants use complex chemical communication through the secretion of root exudates, which can enhance microbial mineralization of C, N, and other essential nutrients (Fontaine, Mariotti, and Abbadie 2003; Li et al. 2018; Faucon, Houben, and Lambers 2017). Microorganisms are essential to soil N cycling, transforming biologically unavailable N into bioavailable forms. Plants can also influence the rate of SOM decomposition by stimulating or inhibiting microbial activity resulting in a positive or negative priming effect (Huo, Luo, and Cheng 2017). Most soil N is bound in proteins and can only be used by plants and microorganisms after depolymerization of SOM to monomers by microbially-derived extracellular enzymes (Schimel and Bennett 2004).

2

Rapeseed (*Brassica napus*), also known as canola and oilseed rape, is the second most important oilseed crop in the world. Rapeseed is highly valued for its widely used, high quality vegetable oil as well as high protein meal used in livestock feed (Stahl, Friedt, et al. 2016). Generally, rapeseed requires high amounts of N inputs but is considered to have low NUE compared to other crops (Kessel, Schierholt, and Becker 2012; Rathke, Behrens, and Diepenbrock 2006). As N inputs continue to increase yield and productivity, large amounts of N are being lost from the production system. These losses are not necessarily due to over-fertilization from the farmers, but instead by narrowed acquisition efficiency of the crops (Rathke, Behrens, and Diepenbrock 2006; Stahl, Friedt, et al. 2016). The focus on improving N management has led to more interest in breeding rapeseed varieties with increased NUE capacity (Stahl, Friedt, et al. 2016; Kessel, Schierholt, and Becker 2012). These breeding efforts are primarily focused on one component of NUE, N fertilizer uptake efficiency (RE_N), or the percentage of N fertilizer recovered in the aboveground plant biomass during the growing season (Cassman, Dobermann, and Walters 2002). Little attention has been paid to the N supplied by SOM mineralization. Understanding the belowground dynamics of N recovery from SOM, rather than applied N fertilizer, could be an important new direction for improving the sustainability of this globally important crop.

The primary focus of rapeseed NUE studies have been on improving synthetic RE_N and N translocation within the plant, with yield as the primary response indicator (Stahl, Bissuel-Belaygue, et al. 2016; Stahl, Friedt, et al. 2016). In contrast, this study evaluated the effects of rapeseed genotypic diversity on N uptake from organic and inorganic N sources. We used ^{15}N enriched ammonium-nitrate (NH_4NO_3) fertilizer and soil enzyme activity to determine differences in N acquisition and microbially-mediated N cycling from organic and inorganic N pools as influenced by rapeseed varieties. We hypothesized that different rapeseed varieties would differ in N source uptake patterns and soil enzyme activity.

Materials and Methods

Experimental Design

To investigate N acquisition in rapeseed (*Brassica napus*), a greenhouse pot study was conducted at the

Colorado State University Plant Growth Facility in Fort Collins, CO (40.5717° N, 105.0812° W) from June

to September 2016. Ten rapeseed varieties were selected from the 51 founder lines of the Parkin, Vail,

and Robinson 2017 project, that were selected for development of a germplasm resource to dissect

complex traits in *Brassica napus*. This project collected diverse rapeseed lines from around the world to

make a nested association mapping (NAM) population that could be used to introduce new diversity

into breeding germplasm (Parkin, Vail, and Robinson 2017). The ten varieties for this study were

selected using 12,612 single nucleotide polymorphisms (SNP) markers to capture the widest possible

general genetic diversity based on genetic distance, and geographic locations of the markers while

controlling for common flowering time (Table 1.1, Figure 1.1).

The rapeseed plants were grown in 3.8 Liter pots with a water catch tray. A non-soil mixture of 2/8 sand,

3/8 calcined clay, and 3/8 vermiculite by volume was homogenized. A high organic matter field soil was

added to the non-soil mixture to create up to a 6-fold difference in soil organic matter levels across the

four treatments. The field soil was a fine loamy Aridic Argiustoll with 6.7% organic matter (3.7% C, 0.38%

N) collected from a farm near Fort Collins, Colorado, with a history of organic vegetable production. The

field topsoil (0-10cm) was collected and sieved to 8-mm. Soil mixtures were homogenized using a clean

cement mixer. A ^{15}N enriched N fertilizer solution using 98% ^{15}N enriched dual labeled NH_4NO_3, diluted

down to 8% ^{15}N enrichment, was applied weekly to obtain the specified total N additions for the high

and low fertilizer rates as outlined in Table 1.2 (Damon, Osborne, and Rengel 2007; Balint and Rengel

2008). Fertilizer rates of 50 mg N/pot for low N treatments and 150 mg N/pot for high N treatments

were chosen to provide sufficient N through vegetative growth based on the Balint and Rengel 2008

study. The SOM levels were selected by assuming less than 1-2% of total N in SOM would be mineralized during the short time period of the study, resulting in mineralization of approximately 50-100 mg N in high SOM and 10-20 mg N in low SOM treatments.

The plants were planted in randomized complete blocks with restricted randomization design with 5 blocks of each of the 4 treatments with each of the 10 varieties for a total of 200 pots. Each of the blocks were divided to have 1 plant of each variety and treatment in a randomly assigned block design in the greenhouse. Each block was planted 1 week apart for 5 weeks.

Four seeds were planted into each pot and at one week they were thinned to one plant per pot. Fertilizer treatments were initiated at 2 weeks after planting when the first true leaves were beginning to emerge. Once per week, 50ml of N-free Hoagland's nutrient solution was applied to each pot to ensure that nutrients other than N were not limiting across all treatments. Based on the treatment, supplemental ^{15}N enriched fertilizer was applied weekly to achieve desired N rates. The first week 100 ml of N-fertilizer solution was applied, and 50 ml was applied in all subsequent weeks for a total of 5 fertilizer applications. Any liquid that ran through the pot was caught in the trays below the pot and added back into the pot to eliminate N loss by leaching.

The pots were watered with a drip emitter irrigation system starting at week three. The irrigation system watered for two minutes each day fertilizer treatments were not applied. A moisture probe was used in the pots twice a week to measure pot moisture and the irrigation amounts were adjusted to equalize moisture levels between treatments and blocks.

Each block was destructively harvested when about 75% of the plants in a block were at the rosette stage, around 6 weeks. Each individual pot in the block was photographed and weighed. The plant was clipped at the base of the stem. The clipped plant shoot was put in a paper bag and dried at 55℃ and weighed for dry shoot biomass.

The pot of soil was turned upside down in a clean tub. The loose soil was gently brushed off leaving the root ball and the rhizosphere soil surrounding the roots. The rhizosphere soil and root ball and the bulk soil were placed in separate zip lock bags and placed in a cooler with ice until they were put in cold storage for further processing. A subsample of the bulk soil from each pot was weighed and dried at 105℃ to determine soil gravimetric water content.

Enzyme activity

The activity of four soil enzymes involved in SOM decomposition and soil nutrient cycling were measured using fluorescence-based enzyme activity assay (Table 1.3). Rhizosphere soil samples from each pot were analyzed using the microplate enzyme assay using fluorescence-based MUB (4-methylumbelliferone) and MUC (7-amino-4-methylcoumarin) substrate protocol (Bell et al. 2013). Briefly, the day after the plants were harvested, 1.1-1.3 g of soil was weighed from the rhizosphere soil sample. The soil was blended to homogenize sample with a 50mM sodium acetate buffer solution, that had been adjusted to the average soil pH of 7.5 to make a soil slurry. Soil slurry was pipetted into black, 96-well microplates with compound-specific fluorescing substrates. Samples were analyzed using a Tecan Infinite M200 plate reader (Tecan Austria GmbH, Salzburg, Austria).

Inorganic nitrogen

A sample of the rhizosphere soil from each pot was extracted with 100 mL of a 2 M Potassium chloride (KCl) solution to analyze levels of extractable ammonium (NH_4^+) and nitrate (NO_3) in the soil at the time of harvest using the microplate colorimetric method (Sims, Ellsworth, and Mulvaney 1995).

The Vanadium (III) Chloride (VCl_3) protocol was used to determine soil NO_3, where 30μL of the KCl extracted sample was pipetted into microplates with VCl_3 solution (Doane and Horwáth 2003). The salicylate-hypochlorite method was used to determine soil NH_4^+, where 70μL of KCl extracted sample was used in each of the microplate wells. Both assay reactions were read on a microplate reader (BioTek Instruments, Winooski, VT). Inorganic N values are not dependent on dry plant biomass so, all 5 blocks of data are used in analysis

Nitrogen Source Analysis

Isotopic values of the dried plant shoots were analyzed to determine the relative contributions of the fertilizer and SOM to plant N uptake. Dried plant samples were ground to 2 mm in a Wiley Mill and then roller ground until the sample was homogenized. All samples were analyzed for total C, total N and ^{15}N at EcoCore Analytical Services Lab, at Colorado State University, Fort Collins, CO, using an Elemental-Analyzer – Isotope Ratio Mass Spectrometry (Costech, Valencia, CA).

The contributions of the N from the labeled inorganic ^{15}N and the organic N acquired from the SOM were calculated by applying the isotopic mixing model (Hauck and Bremner 1976). The fraction of fertilizer-derived N ($f_{fertilizer}$) was calculated using the equation:

$$f_{fertilizer} = (\delta_{sample} - \delta_{soil}) / (\delta_{fertilizer} - \delta_{soil})$$

Where δ_{sample}, δ_{soil}, and $\delta_{fertilizer}$ represent the atom % ^{15}N of the total sample, natural abundance of the soil mixture, and fertilizer (8 atom% ^{15}N) respectively. The value for δ_{sample} was the sample value output

from EA-IRMS analysis (Figure 1.2). The δ_{Soil} was the natural abundance of the soil mixture, 0.3681 atom% ^{15}N for low SOM and 0.3699 atom% ^{15}N for High SOM treatments. The contribution of soil derived N was calculated using the equation $f_{SOM} = 1 - f_{fertilizer}$. The RE_N was calculated using the equation: Total Plant N/Applied N Fertilizer.

Root Biomass

The root biomass was obtained by washing the growth media away from the bulk and rhizosphere samples of blocks 1, 2 and 3. The washed roots were dried at 55℃ and weighed for dry biomass. The samples were a mixture of roots, vermiculite and particulate organic matter. The root samples were homogenized in a ball grinder. A subsample of each root sample was analyzed for organic content using the ash correction protocol to obtain an estimated root biomass for each sample (Sparks et al. 1996). Briefly, a subsample of the homogenized sample was weighed in tin weigh boats and placed into a 105℃ oven for at 24 hours. The sample was weighed again and then placed into a cold muffle furnace and baked at 450℃ for 4 hours. Once samples were cooled to at least 200℃, they were weighed again. The difference in the sample weights were used to correct root weights for inorganic compounds and get an ash corrected estimated root biomass for each sample (Harmon, Nadelhoffer, and Blair 1999). The root-shoot ratio was calculated as the ash corrected root biomass estimate divided by the dry shoot weight. Only blocks 1 and 3 were included in the root-shoot ratio estimate.

Data Analysis

The data were analyzed in R, using a mixed model approach (R Core Team 2017). Due to a data loss of block 2 aboveground dry plant weights, only four of the five blocks were used in analyses that relied on plant biomass. Block was included as a random variable, while the fixed predictor variables were rapeseed variety and treatment. The response variables were the four different enzyme activities, N

uptake, SOM and Fertilizer N uptake, percent N from SOM and fertilizer, total soil inorganic N, soil NH_4^+, NO_3^-, dry root and shoot biomass, and root-shoot ratio. The data were not normally distributed, so the lme() function in the nlme package to allow for unequal variances (Pinheiro et al. 2018). The exceptions are RE_N was analyzed with the lemr() function, and for NH_4^+ and NO_3^-, the data were transformed by taking the square root and then analyzed using lemr() function of the Lme4 package (Bates et al. 2015). Due to near zero nitrate levels in some samples, some samples had negative values after subtracting sample blanks. In this case, a constant was added to make all values positive and then were square root transformed. These values were then analyzed with the lme() function from the nlme package (Pinheiro et al. 2018). A type three analysis of variance Anova() from the with Kenward-Roger approximation for degrees of freedom was used from the car package (Fox et al. 2018). The emmeans function, from the emmeans package, was used to make pairwise comparisons of significant predictors (Russell Lenth 2019).

Results

Plant Growth

The SOM and fertilizer N treatments had a significant effect on dry shoot biomass (p<0.001) and resulted in a range of N available for plant uptake and biomass production. There was almost a 3-fold difference in biomass ranging from High SOM/High Fertilizer with the highest dry shoot biomass with 4.46 g/plant, followed by High SOM/Low Fertilizer, then Low SOM/High Fertilizer, and Low SOM/Low Fertilizer had the lowest biomass of 1.62 g/plant (Table 1.4). All treatments produced significantly different quantities of plant biomass except between the High SOM/Low Fertilizer and Low SOM/High Fertilizer treatments. Genotype had no effect on plant biomass and there were no interactions between variety and treatment.

Root Biomass

Root biomass also differed by soil treatment (p<0.0001). There was no difference between root biomass between the High SOM/High Fertilizer and the High SOM/Low Fertilizer treatments, but there were significant differences between all other treatments (Table 1.4). The biomass of the High SOM treatments had root biomass that was 2-4-fold higher than the root biomass of the Low SOM treatments (Table 1.4). Variety did not have a significant effect (p=.4197). The root-shoot ratio differed by treatment (p=.00091). The highest average root-shoot ratio was the High SOM/Low Fertilizer treatment, followed by the High SOM/High Fertilizer treatment.

Plant N Uptake

There was a significant treatment effect for total plant N uptake (p<0.001), with significant differences between all four treatments. The High SOM/High Fertilizer treatment had the highest total N uptake (195 mg N), and the highest amount of N from SOM (125 mg N) (Figure 3). The High SOM/Low Fertilizer treatment had the second highest total plant N, it had 35% less total plant N than the High SOM/High Fertilizer treatment with 128 mg N and 114 mg N derived from SOM (Figure 3). The High SOM/High Fertilizer and High SOM/Low Fertilizer treatments had a significant difference between total N uptake, but the amount of N derived from SOM (p=.066) was not different. Conversely, we saw a difference in N uptake from SOM between the Low SOM/High Fertilizer (36 mg N) and Low SOM/Low Fertilizer (25 mg N) treatments (p<0.001) (Table 1.4).

We found differences in the proportion of N acquisition from fertilizer and SOM sources between all four treatments (p<0.001) (Figure 1.4). Source acquisition indicated the importance of SOM in all four treatments. When comparing the extreme treatments High SOM/High Fertilizer and Low SOM/Low Fertilizer, both treatments obtained 64% of their total N uptake from SOM (Figure 1.4), suggesting a

similar balance between N sources at high and low treatment levels. In contrast, High SOM/Low

Fertilizer obtained 89% of the total N from SOM, and only 11% from fertilizer, which was more than

double the Low SOM/High Fertilizer treatment that obtained 33% of N from SOM (Figure 1.4).

The RE_N was affected by treatment (p=.012). The treatments had RE_N values that ranged from 44% to

49% (Table 1.4). The Low SOM/High Fertilizer treatment had the highest fertilizer RE_N. Although there

was not a large spread of RE_N between treatments, pairwise comparisons between treatments show

that only the Low SOM/High Fertilizer and the Low SOM/Low Fertilizer are significantly different each

other, while no other treatments were significantly different from each other. Variety did not have a

significant effect on RE_N (Table 1.4).

Variety had a significant effect on the percentage of total plant N that came from fertilizer and SOM

(p=.0022). Although variety is significant in the percentage of total plant N, the interaction between

variety and total plant N is not significant (p=.34). Looking at pairwise comparisons, no single variety was

significantly different from another likely due to the large number of varieties compared (Table 1.5).

Variety did not influence any other aspects of N uptake (Table 1.4).

Inorganic nitrogen

The SOM and fertilizer treatments influenced extractable soil inorganic N (NO_3^--N + NH_4^+-N mg/kg soil).

The highest to lowest average soil inorganic N by treatment was as follows: High SOM/High Fertilizer,

High SOM/Low Fertilizer, Low SOM/High Fertilizer, Low SOM/Low Fertilizer (Table 1.4). Pairwise

comparisons of total inorganic N show significant differences between all treatments except High

SOM/Low Fertilizer and Low SOM/High Fertilizer and between Low SOM/High Fertilizer and Low

SOM/Low Fertilizer treatments. Total extractable soil inorganic N (NO_3^-+ NH_4^+), NO_3^-, or soil NH_4^+ did

not differ by rapeseed variety. While total inorganic N differed by treatment, there was no treatment effect on the individual amounts of NH_4^+ or NO_3^- in the soil (Table 1.4).

Enzyme Activity

In all four enzymes measured we found a decrease in enzyme activity in High Fertilizer treatments relative to Low Fertilizer treatments. In High SOM treatments, the Low Fertilizer treatments had activity that was 1.2-1.3 times higher than the High Fertilizer treatments for all four enzymes. We saw a similar trend in the Low SOM treatments where activity was 2.2-2.4 times higher in the Low Fertilizer treatments than in the High Fertilizer Treatments (Figure 1.5). Although High SOM/High Fertilizer treatments had lower enzyme activity than the High SOM/Low Fertilizer treatments it was not to a significant degree for any of the measured enzymes (Table 1.4). Variety was not a significant predictor for any of the measured enzymes (Table 1.4).

Discussion

Our results highlight the potential importance of organic N sources in rapeseed and support that an ecosystem-based management system is likely to be an effective approach for improving N uptake and reducing N losses in rapeseed cropping systems. This approach actively manages organic and inorganic N pools and strategically uses all available nutrient sources, instead of focusing solely on inorganic N management (Drinkwater and Snapp 2007). Across all treatments, rapeseed plants effectively accessed N from SOM for crop growth, the effect was additive and not a substitute for fertilizer. In the two extreme treatments, High SOM/High Fertilizer and Low SOM/Low Fertilizer, plants obtained about two thirds of their total N from SOM. While rapeseed plants in the High SOM/Low Fertilizer treatment obtained the majority (89%) of their total N from SOM, even in the Low SOM/High Fertilizer treatment, plants still acquired approximately one third of total plant N from SOM (Figure 1.3).

Our study found that only 44%-49% of the total applied N fertilizer was taken up into the plant shoot (Table 1.4). This low rate of RE_N is within the range of RE_N of other grain crops worldwide, contributing to the growing problem of N pollution. As N fertilizer losses from agricultural systems continue to have major environmental impacts there has been an increased interest in integrated nutrient management (INM). The goal of INM is to integrate the use of synthetic and biological plant nutrient sources so that crops can be raised in a productive and sustainable way (Gruhn, Goletti, and Yudelman 2000). Most INM studies have focused on developing countries with degraded soils and on rice, maize and wheat crops (Fan, Zhang, and Jiang 2009). In INM systems with higher SOM, crops show improved yield and field performance, while reducing the need for inorganic N additions (Zhou et al. 2019). While oilseed rape is a major worldwide crop, fewer INM studies to this point have focused on rapeseed. Studies looking at INM in rapeseed systems have focused on organic fertilizer additions, and agronomic practices and have not focused on the importance of SOM as an N source. Rapeseed studies have primarily focused on enhancing RE_N and improving inorganic N fertilizer management in conventional managements systems (Stahl, Bissuel-Belaygue, et al. 2016; Gan et al. 2008; Ma and Herath 2016; Chamorro et al. 2002) and have widely ignored the important role of SOM as an N source.

There was indirect evidence of a positive priming effect on SOM decomposition and N mineralization in the Low SOM treatments. The rhizosphere priming effect for N is often dependent on the mineral N content of the soil and the amount of added fertilizer introduced to the system (Kuzyakov 2002). The Low SOM/Low Fertilizer treatment had significantly less N uptake from SOM than the Low SOM/High Fertilizer treatment (Table 1.4). This suggests that the Low SOM/Low Fertilizer treatment was N limited. The root biomass was significantly lower in the Low SOM/Low Fertilizer treatment than the other treatments (Table 1.4), the smaller root system can't explore as much of the soil and stimulate

mineralization of the SOM (Kuzyakov 2002). Root and microbial growth are both limited by N

availability. Plant roots compete with microbial communities for available N in the rhizosphere. Root

uptake of N decreases the amount of N available for microbial growth and therefore can reduce SOM

decomposition and N mineralization (Jingguo and Bakken 1997; Bottner, Pansu, and Sallih 1999;

Kuzyakov 2002). Studies suggest that the addition of N fertilizer to an N limited system can stimulate

plant and root growth, and therefore increase available C for exchange, this stimulates microbial growth

and activity that can increase N mineralization from SOM (Jingguo and Bakken 1997; Kuzyakov 2002;

Zhu, Vivanco, and Manter 2016; Geisseler and Scow 2014). The Low SOM/High Fertilizer treatment had

an average plant biomass that was twice of the Low SOM/Low Fertilizer treatment and a root biomass

that was about 70% greater (Table 1.4). Larger root systems contribute more C and stimulate more N

mineralization (Kuzyakov 2002). This larger root system likely contributed to the increased RE_N of 49% in

the Low SOM/High Fertilizer treatment relative to 44% in the Low SOM/Low Fertilizer treatment (Table

1.4). However, plants increased the relative allocation of resources to support belowground biomass

under greater N limitation. We found greater root-shoot ratio under the Low SOM/Low Fertilizer relative

to the Low SOM/High Fertilizer (Table 1.4). Within the high SOM treatments, the High SOM/High

Fertilizer treatment also obtained more N from SOM than the High SOM/Low Fertilizer treatment and

had slightly lower root-shoot ratio, but the differences were not significant (Table 1.4). This suggests

that plants in the High SOM treatments were not as N limited and the added N fertilizer has less of an

effect on belowground C dynamics.

We measured the activity of four enzymes that are known to be sensitive indicators in the

decomposition of SOM. We found that High SOM treatments had higher enzyme activity, in all four

measured enzymes, than the Low SOM treatments (Figure 1.5). The High SOM treatments have 6 times

the amount of field soil in their growth media than Low SOM treatments (Table 1.2). Microbial biomass

is an essential component of SOM parent material. SOM provides both habitat and a food source that can support larger and more active microbial communities. To access C and other nutrients in SOM, soil microorganisms produce exoenzymes to catalyze SOM decomposition and nutrient mineralization. Thus, it is not surprising then that enzyme activity was higher in high SOM treatments (Kögel-Knabner 2002).

While increasing SOM corresponded with increasing soil enzyme activity, increasing N fertilizer had the opposite effect. Our results showed that between High and Low SOM treatments, Low Fertilizer treatments had higher enzyme activity than High Fertilizer treatments (Figure 1.3). This could seem contradictory to see lower enzyme activity but an increase in plant productivity and N uptake from SOM in High Fertilizer treatments. Although traditional nutrient limitation theories are not able to explain the suppression of enzyme activity with N applications, there are several theories that could explain these observations. The *enzyme inhibition* hypothesis proposes that N additions directly inhibit enzymes that degrade complex-C compounds resulting in an overall reduction in microbial activity (Fog 1988; Gallo et al. 2004). Similarly, the *N-mining* hypothesis states that N additions reduce microbial N requirements so they no longer have to 'mine' the soil for recalcitrant C, and instead rely on labile C resulting in an overall reduction in enzyme activity (Craine, Morrow, and Fierer 2007; Moorhead and Sinsabaugh 2006; Mahal et al. 2019). We find limited support for these hypotheses as all 4 enzymes evaluated were similarly suppressed under high fertilizer, irrespective of the process that they catalyzed. Another explanation of the reduction in enzyme activity we observed is that the High Fertilizer treatments may have lowered the pH of the soil thus reducing microbial activity (Geisseler and Scow 2014). We did not find differences in soil pH by treatment (data not shown). Another possible explanation is the *copiotrophic* hypothesis, that suggests N additions shift the microbial community composition away from taxa that decompose recalcitrant C and towards those that rely upon more labile C pools (Ramirez,

Craine, and Fierer 2012; Fierer et al. 2012). This potential mechanism is more likely; however, our study

cannot draw any conclusions as we did not examine microbial community composition.

We did not find a significant effect of variety in our study. All varieties in this study are open pollinated

varities that were chosen based on common spring flowering time and overall genomic diversity. Some

studies suggest that high yielding herbicide tolerant hybrid rapeseed varieties have different N

requirements and uptake patterns than open pollinated varities. Hybrid varieties could have been

included in this study to examine differences in uptake patterns (Karamanos, Goh, and Flaten 2005; K. N.

Harker et al. 2012; E G Smith et al. 2010; Brandt et al. 2007). An approach more targeted on diversity of

belowground traits could show significance differences in varieties. We have a limited understanding of

the role of root traits on NUE because of the challenges of studying root function and architecture in a

field setting. Studies support genomic links between belowground traits and N uptake, suggesting that it

is possible to incorporate NUE root systems into a breeding program (Coque et al. 2008). We suspect

that studies without significant results, which may be more common with studies investigating

belowground traits, are not always published due to the bias toward reporting significant differences.

This makes belowground traits even harder to study and integrate into belowground targeted breeding

approaches because there is not enough literature to help guide what has or has not been examined

already. Although this study did not find significant effects of variety on N uptake patterns, this study

provides a new understanding of plant response to different N environments and the importance of

SOM as a N source and could be valuable for future studies focused on belowground trait genetics.

Our study examined N uptake in the shoots at the plant bolting stage. One study examined the

consistency of NUE between vegetative and mature stages in rapeseed. They found that NUE of varieties

were not consistent across maturity stages and for breeding purposes may require evaluation at plant

maturity (Balint and Rengel 2008). This suggests that further studies should measure N uptake at

multiple stages and include assessment at grain maturity. As a greenhouse pot study, our study was limited in scope. Further examination of N source uptake in a field setting will be needed to further guide integrated nutrient management recommendations.

Conclusion

Much of sustainable agriculture research has focused on how to decrease the need for surplus N fertilizer additions by increasing fertilizer use efficiency (RE_N) of crops. While many studies have focused on N fertilizer uptake and use, we have focused on the belowground mechanisms in relation to N uptake from SOM. Our results indicate the importance of SOM as an N source to support crop growth, even when an abundance of mineral N is available. Our results also suggest that the interaction between plants and soils mediates the dynamic nature of N uptake as demonstrated by the priming effect in N limited environments. The suppression of enzyme activity in high N environments suggests that N fertilizer has a direct effect on soil N cycling dynamics, which warrants further investigation. These findings support an ecosystem-based management system that manages both synthetic and organic N sources for effective nutrient management in rapeseed cropping systems.

Table 1.1: *Brassica napus* varieties selected for this study chosen from the founder lines of NAM project (Parkin, Vail, and Robinson 2017) and their country of origin.

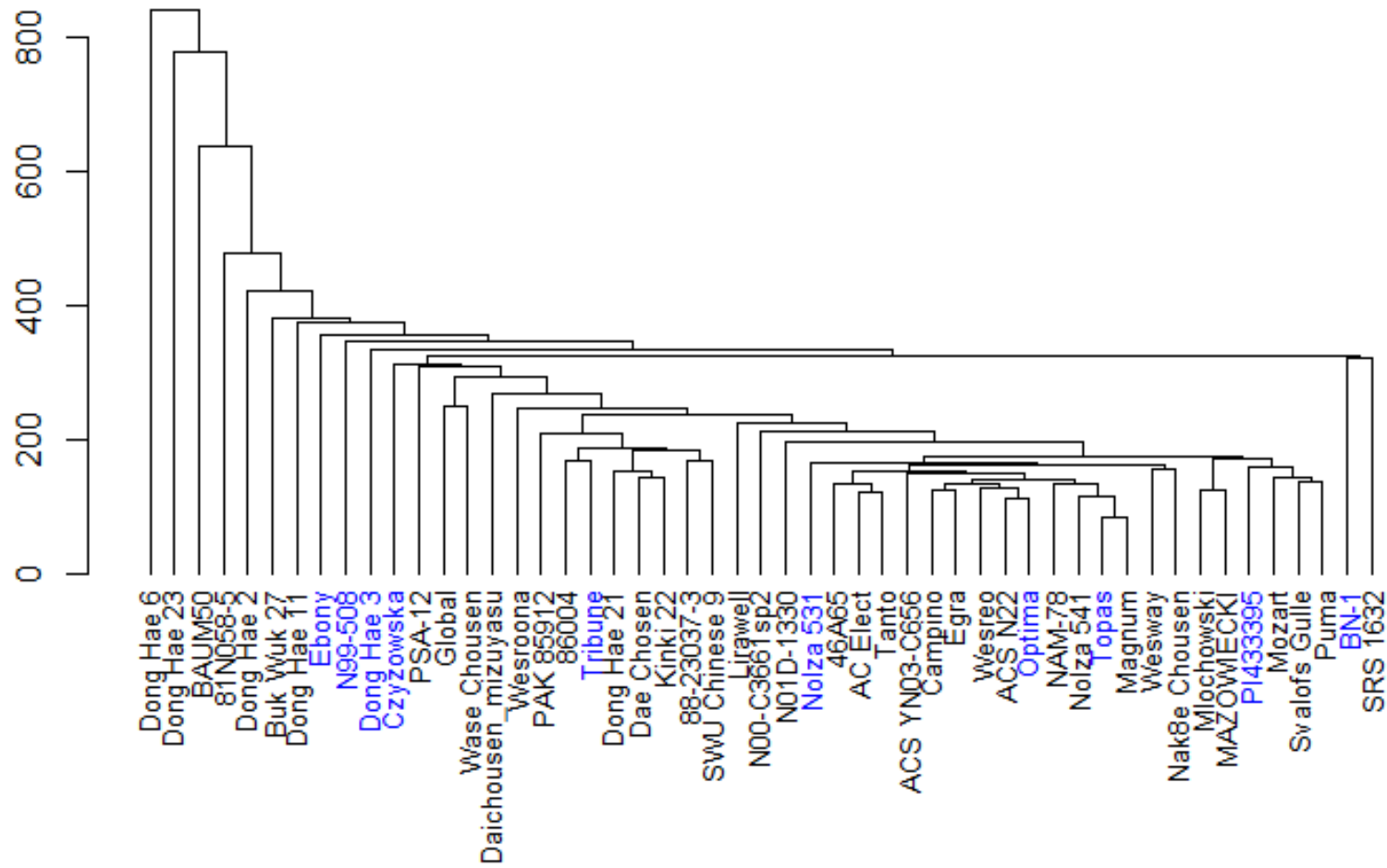| NAM Founder Line ID	Variety Name	Origin
NAM-0	N99-508	Common line
NAM-1	Czyzowska	Poland
NAM-5	BN-1	India
NAM-26	Noiza 531	Argentina
NAM-28	Topas	Sweden
NAM-33	Dong Hae 3	S. Korea
NAM-43	PI433395	Unknown
NAM-73	Optima	Denmark
NAM-76	Ebony	Canada
NAM-82	Tribune	Australia

Figure 1.1: Dendrogram of genetic relationships and distances of *Brassica napus* founder lines of Nested Association Mapping (NAM) project (Parkin, Vail, and Robinson 2017). Higlighted lines are varieties selected for this study.

Table 1.2: The four treatments evaluated in this study included two levels of organic nitrogen (N) from soil organic matter (SOM) from field soil and two levels of mineral nitrogen fertilizer. Total milligrams (mg) of N from fertilizer and grams (g) of N in SOM per pot by treatment. The percentage (%) of SOM and % by volume of field soil and non-soil mix in each pot for each treatment.

Treatments	Fertilizer mg N/ pot	SOM g N/ pot	SOM level (%)	Field Soil %	Non-Soil Mix %
High SOM/ High Fertilizer	150	5.36	3	60	40
High SOM/ Low Fertilizer	30	5.36	3	60	40
Low SOM/ High Fertilizer	150	0.89	0.5	10	90
Low SOM/ Low Fertilizer	30	0.89	0.5	10	90

Table 1.3: Enzyme name, abbreviation, function in the soil (nutrient cycle indicator), and final product for the four soil enzymes assayed.

Enzyme Name	Abbreviation	Function in Soil	Final product
B-1, 4-n-acetyl-glycosaminidase	NAG	Hydrolysis of chitin	N-acetyl-b-D-glucosamine (sugar)
Leucine amino peptidase	LAP	Hydrolysis of amino acid residues (N- terminus of peptides and proteins)	Leucine (other amino-acids)
B-1, 4-glucosidase	BG	Hydrolysis of cellulose	Glucose (sugar)
tobacco acid pyrophosphatase	TAP	Catalyze the hydrolysis of a phosphoric ester bond in a wide spectrum of molecules	Phosphate

Figure 1.2: The four treatments used and the ^{15}N mixing model experimental design of the greenhouse pot study.

The boxes in the figure contain:

^{15}N enriched Fertilizer

Total plant ^{15}N to determine which source it acquired its N from

4 Treatments:
High SOM/High Fertilizer
High SOM/Low Fertilizer
Low SOM/High Fertilizer
Low SOM/Low Fertilizer

Organic N from Soil Organic Matter

$$f_{fertilizer} = (\delta_{sample} - \delta_{soil}) / (\delta_{fertilizer} - \delta_{soil})$$

Table 1.4: Mean plant and soil values for each soil organic matter (SOM) and fertilizer treatment and analysis of variance results for variety and treatment factors. Analysis presented as mean ± standard error and associated p-values for variety and treatment. Bolded values indicate that the p-value significant and was below α = 0.05. Letters denote a significant difference between treatments within the row. There were no significant interactions between treatment and variety. Enzyme types are represented as Leucine amino peptidase (LAP), B-1, 4-n-acetyl-glycosaminidase (NAG), B-1, 4-glucosidase (BG), and tobacco acid pyrophosphatase (TAP).

Measurement	units	High SOM/ High fertilizer	High SOM/ Low Fertilizer	Low SOM/ High Fertilizer	Low SOM/ Low Fertilizer	Variety P-value	Treatment P-Value
Dry shoot biomass	g	4.46 ±.21 (a)	3.67 ±.24 (b)	3.24 ±.21 (b)	1.62 ±.19 (c)	0.2078	**<.0001**
Dry root biomass estimate	g	1.71±.15 (a)	1.59±.09(a)	0.61±.05 (b)	.37±.03 (c)	0.4197	**<.0001**
Root-shoot ratio	g	0.37±.04 (a,b)	0.44±.02 (a)	0.21±.02 (c)	0.28±.01 (b)	NA [1]	**0.0009**
Plant N	mg	194.9±6.03	127.7±5.39	110.1±4.21	38.6±3.73	0.5678	**<.0001**
Plant N from Fertilizer	mg	69.9±2.02	14.0±0.68	74.1±1.7	13.1±.48	0.6455	**<.0001**
Plant N from SOM	mg	125.0±5.27	113.7±5.55	36.1±4.45	25.4±4.4	0.3323	**<.0001**
RE_N	%	46.6±1.99	46.6±1.99	49.4±1.99	43.8±1.99	0.5476	**0.0120**
% N from Fertilizer	%	36.0±.96	11.0±.9	67.3±1.4	35.6±1.62	**0.0022**	**<.0001**
% N from SOM	%	64.0±.93	89.0±.9	32.7±1.4	64.4±1.62	**0.0022**	**<.0001**
LAP activity	nmol/h/g	65.8±6.93	84.5±8.78	12.7±3.47	30.6±3.97	0.4151	**<.0001**
NAG activity	nmol/h/g	3.77±.29	4.68±.44	1.47±.23	3.33±.31	0.1094	**0.0261**
BG activity	nmol/h/g	45.5±3.20	54.3±4.61	11.4±1.98	25.4±2.43	0.0912	**<.0001**
TAP activity	nmol/h/g	26.45±3.25	33.5±4.08	6.27±1.84	15.15±2.21	0.5084	**0.0007**
Total Soil inorganic N	mg/kg soil	3.85 (a)	2.34 (b)	1.7 (b,c)	1.13 (c)	0.1713	**0.0094**
Soil NO3$^-$	mg/kg soil	3.66 (a)	1.49 (a,b)	1.13 (b)	0.07 (c)	0.1582	0.4651
Soil NH4$^+$	mg/kg soil	1.02 (a)	1.02 (a)	0.88 (a,c)	1.09 (a,b)	0.9682	0.5445

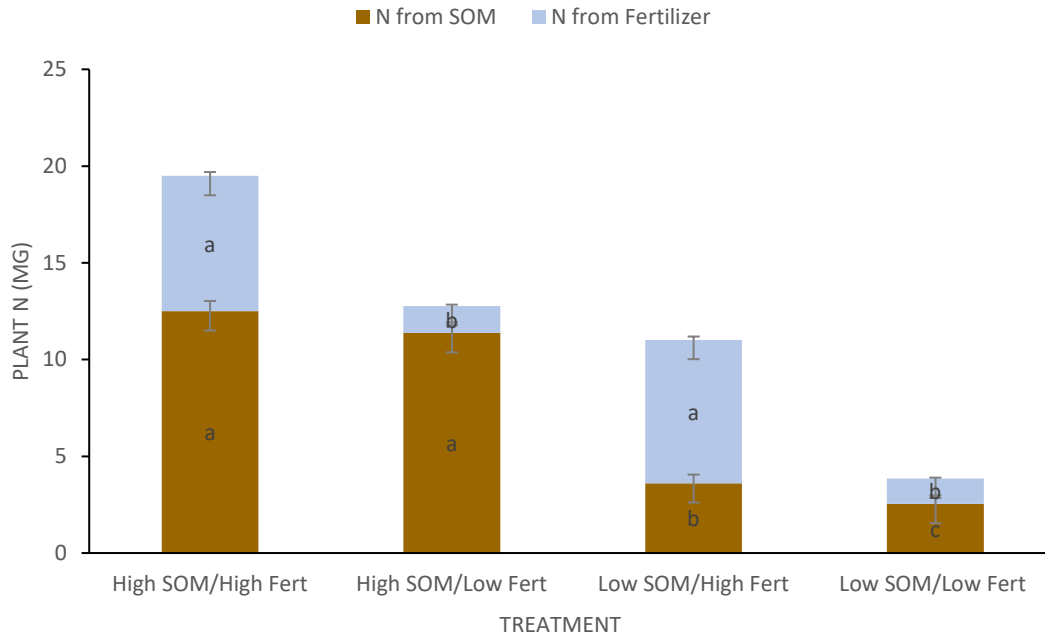[1] Not available: Sample size not large enough to support analysis

Figure 1.3: Average total plant nitrogen (N) from fertilizer and soil organic matter (SOM) by treatment averaged over variety.   Error bars represent standard error (n=4). Letters denote a significant difference between treatments for each N source (SOM or fertilizer) (p<0.05).
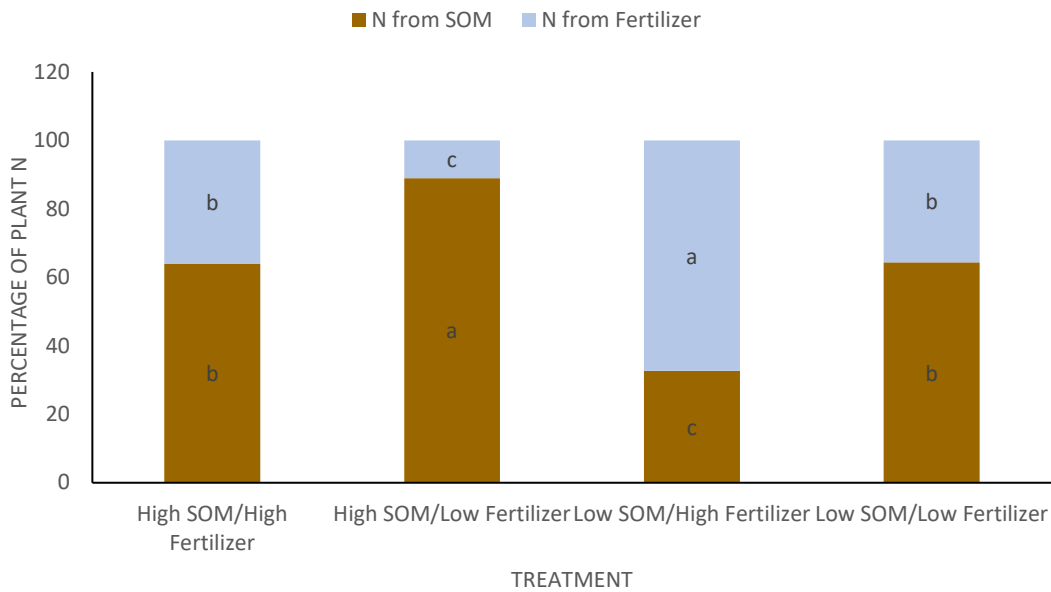

Figure 1.4: Percentage of plant nitrogen (N) obtained from fertilizer and soil organic matter (SOM) by treatment. Error bars represent standard error (n=4). Letters denote a significant difference between treatments (p<0.05).

Table 1.5: Average Total Plant Nitrogen (N) and Percent (%) of plant N from soil organic matter (SOM) by rapeseed variety.

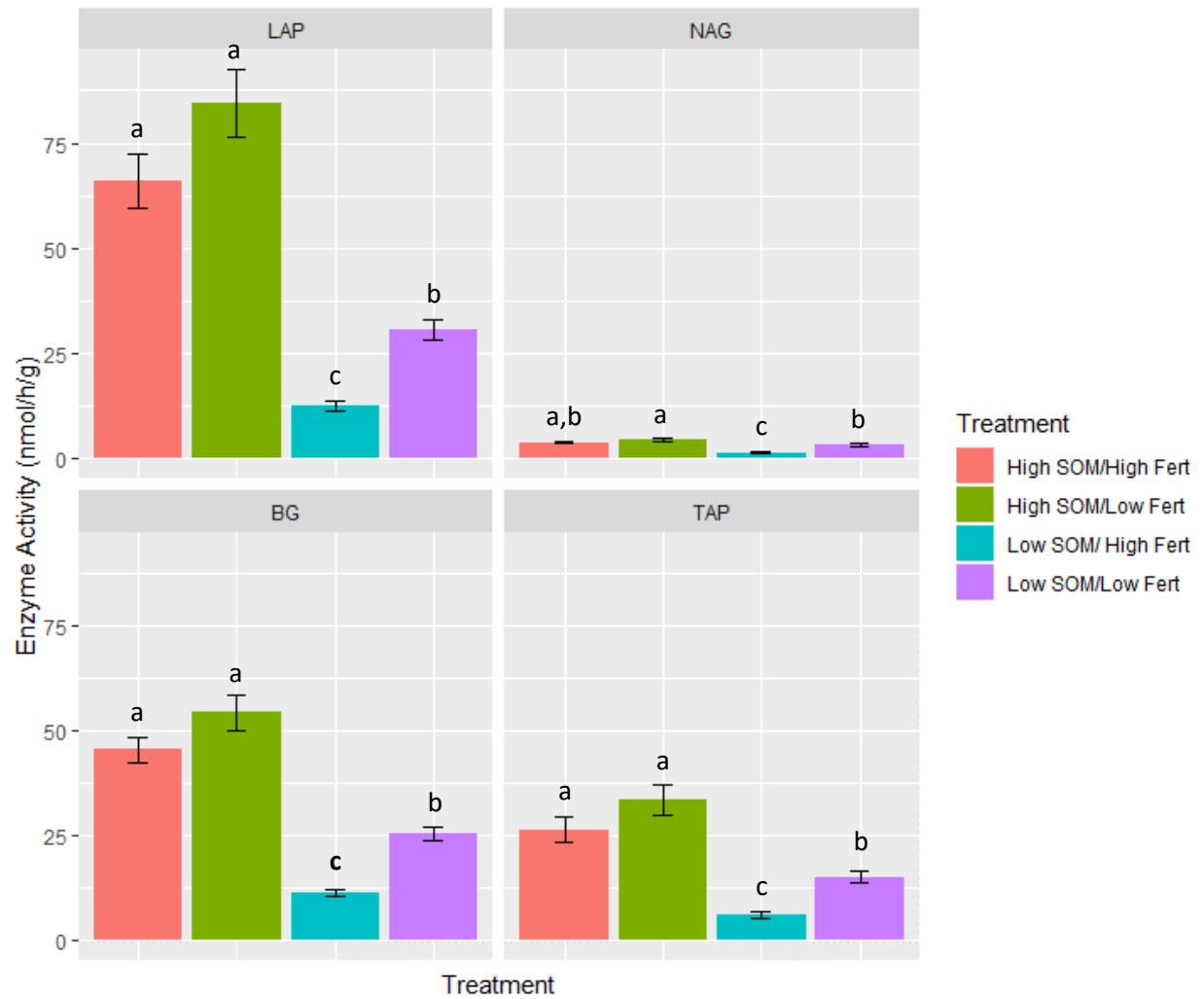| Variety Name	Plant N (mg)	% of N from SOM
BN-1	11.5	63
Czyzowska	11.9	61.5
Dong Hae 3	12.0	62.6
Ebony	11.4	62.9
N99-508	12.4	61.8
Noiza 531	12.2	63.8
Optima	11.4	61.5
US31-2	11.2	62.8
Topas	12.3	62.3
Tribune	11.4	62.9

Figure 1.5: Average activity for each soil enzyme measured. Error bars represent standard errors (n=4). Enzyme types are represented as Leucine amino peptidase (LAP), B-1, 4-n-acetyl-glycosaminidase (NAG), B-1, 4-glucosidase (BG), and tobacco acid pyrophosphatase (TAP). Letters indicate the significant differences between treatments for each enzyme (p<0.05).

References

Balint, Tatjana, and Zdenko Rengel. 2008. "Nitrogen Efficiency of Canola Genotypes Varies between Vegetative Stage and Grain Maturity." *Euphytica* 164 (2): 421–32.

Bates, Douglas, Martin Mächler, Benjamin M. Bolker, and Steven C. Walker. 2015. "Fitting Linear Mixed-Effects Models Using Lme4." *Journal of Statistical Software*.

Bell, Colin W, Barbara E Fricks, Jennifer D Rocca, Jessica M Steinweg, Shawna K. McMahon, and Matthew D Wallenstein. 2013. "High-Throughput Fluorometric Measurement of Potential Soil Extracellular Enzyme Activities." *Journal of Visualized Experiments*, no. 81: 50961.

Bingham, I. J., A. J. Karley, P. J. White, W. T.B. Thomas, and J. R. Russell. 2012. "Analysis of Improvements in Nitrogen Use Efficiency Associated with 75 Years of Spring Barley Breeding." *European Journal of Agronomy* 42: 49–58.

Bodirsky, B L, A Popp, I Weindl, J P Dietrich, S Rolinski, L Scheiffele, C Schmitz, and H Lotze-Campen. 2012. "N 2 O Emissions from the Global Agricultural Nitrogen Cycle-Current State and Future Scenarios." *Biogeosciences* 9: 4169–97.

Bottner, P, Marc Pansu, and Z Sallih. 1999. "Modelling the Effect of Active Roots on Soil Organic Matter Turnover." *Plant and Soil* 216 (1–2): 15–25.

Bowles, Timothy M., Philipp A. Raab, and Louise E. Jackson. 2015. "Root Expression of Nitrogen Metabolism Genes Reflects Soil Nitrogen Cycling in an Organic Agroecosystem." *Plant and Soil* 392 (1–2): 175–89.

Brandt, S. A., S. S. Malhi, D. Ulrich, G. P. Lafond, H. R. Kutcher, and A. M. Johnston. 2007. "Seeding Rate, Fertilizer Level and Disease Management Effects on Hybrid versus Open Pollinated Canola (Brassica Napus L.)." *Canadian Journal of Plant Science* 87 (2): 255–66.

Cassman, K. G., A Dobermann, and Daniel T. Walters. 2002. "Agroecosystems, Nitrogen-Use Efficiency, and Nitrogen Management." *AMBIO: A Journal of the Human Environment* 31 (2): 132–40.

Chamorro, Adriana M, Lía N Tamagno, Rodolfo Bezus, and Santiago J Sarandón. 2002. "Nitrogen Accumulation, Partition, and Nitrogen-Use Efficiency in Canola under Different Nitrogen Availabilities." *Communications in Soil Science and Plant Analysis* 33 (3–4): 493–504.

Conant, Richard T, Aaron B Berdanier, and Peter R Grace. 2013. "Patterns and Trends in Nitrogen Use and Nitrogen Recovery Efficiency in World Agriculture." *Global Biogeochemical Cycles* 27 (2): 558–66.

Coque, M., A. Martin, J. B. Veyrieras, B. Hirel, and A. Gallais. 2008. "Genetic Variation for N-Remobilization and Postsilking N-Uptake in a Set of Maize Recombinant Inbred Lines. 3. QTL Detection and Coincidences." *Theoretical and Applied Genetics* 117 (5): 729–47.

Craine, Joseph M, Carl Morrow, and Noah Fierer. 2007. "Microbial Nitrogen Limitation Increases Decomposition." *Ecology* 88 (8): 2105–13.

Damon, P. M., L. D. Osborne, and Z. Rengel. 2007. "Canola Genotypes Differ in Potassium Efficiency during Vegetative Growth." *Euphytica* 156 (3): 387–97.

Dawson, Julie C., David R. Huggins, and Stephen S. Jones. 2008. "Characterizing Nitrogen Use Efficiency in Natural and Agricultural Ecosystems to Improve the Performance of Cereal Crops in Low-Input and Organic Agricultural Systems." *Field Crops Research* 107: 89–101.

Doane, Timothy A, and William R Horwáth. 2003. "Spectrophotometric Determination of Nitrate with a Single Reagent." *Analytical Letters* 36 (12): 2713–22.

Drinkwater, L. E., and S. S. Snapp. 2007. "Nutrients in Agroecosystems: Rethinking the Management Paradigm." *Advances in Agronomy* 92 (04): 163–86.

Fan, Mingsheng, Fusuo Zhang, and Rongfeng Jiang. 2009. "Integrated Nutrient Management for Sustainable Agriculture in China." In *UC Davis: Department of Plant Sciences*.

Faucon, Michel Pierre, David Houben, and Hans Lambers. 2017. "Plant Functional Traits: Soil and Ecosystem Services." *Trends in Plant Science* 22 (5): 385–94.

Fierer, Noah, Jonathan W Leff, Byron J Adams, Uffe N Nielsen, Scott Thomas Bates, Christian L Lauber, Sarah Owens, Jack A Gilbert, Diana H Wall, and J. Gregory Caporaso. 2012. "Cross-Biome Metagenomic Analyses of Soil Microbial Communities and Their Functional Attributes." *Proceedings of the National Academy of Sciences of the United States of America* 109 (52): 21390–95.

Fog, K. 1988. "The Effect of Added Nitrogen on the Rate of Decomposition of Organic Matter." *Biological Reviews - Cambridge Philosophical Society* 63 (3): 433–62.

Fontaine, Sébastien, André Mariotti, and Luc Abbadie. 2003. "The Priming Effect of Organic Matter: A Question of Microbial Competition?" *Soil Biology and Biochemistry* 35 (6): 837–43.

Fox, John, Sanford Weisberg, Brad Price, Daniel Adler, Douglas Bates, and Gabriel Baud-Bovy. 2018. "Package 'Car.'" *R Documentation*.

Gallo, M., R. Amonette, C. Lauber, R. L. Sinsabaugh, and D. R. Zak. 2004. "Microbial Community Structure and Oxidative Enzyme Activity in Nitrogen-Amended North Temperate Forest Soils." *Microbial Ecology* 48 (2): 218–29.

Gan, Y., S. S. Malhi, S. Brandt, F. Katepa-Mupondwa, and C. Stevenson. 2008. "Nitrogen Use Efficiency and Nitrogen Uptake of Juncea Canola under Diverse Environments." *Agronomy Journal* 100 (2): 285–95.

Gardner, Jennifer B, and Laurie E Drinkwater. 2009. "The Fate of Nitrogen in Grain Cropping Systems: A Meta-Analysis of 15N Field Experiments." *Ecological Applications* 19 (8): 2167–84.

Garnett, Trevor, Vanessa Conn, and Brent N. Kaiser. 2009. "Root Based Approaches to Improving Nitrogen Use Efficiency in Plants." *Plant, Cell and Environment* 32 (9): 1272–83.

Geisseler, Daniel, and Kate M Scow. 2014. "Long-Term Effects of Mineral Fertilizers on Soil Microorganisms - A Review." *Soil Biology and Biochemistry* 75: 54–63.

Gruhn, Peter, Francesco Goletti, and Montague Yudelman. 2000. "Integrated Nutrient Management, Soil Fertility,and Sustainable Agriculture: Current Issues and Future Challenges." *International Food Policy Research Institute*, no. 32: 31.

Harker, K. N., J. T. O'Donovan, T. K. Turkington, R. E. Blackshaw, N. Z. Lupwayi, E. G. Smith, H. Klein-Gebbinck, et al. 2012. "High-Yield No-till Canola Production on the Canadian Prairies." *Canadian Journal of Plant Science* 92 (2): 221–33.

Harmon, M.E., K.J. Nadelhoffer, and J.M. Blair. 1999. *Measuring Decomposition, Nutrient Turnover, and Stores in Plant Litter. Standard Soil Methods for Long-Term Ecological Research*.

Hauck, R. D., and J. M. Bremner. 1976. "Use of Tracers for Soil and Fertilizer Nitrogen Research." *Advances in Agronomy* 28 (C): 219–66.

Huo, Changfu, Yiqi Luo, and Weixin Cheng. 2017. "Rhizosphere Priming Effect: A Meta-Analysis." *Soil Biology and Biochemistry* 111: 78–84.

Jilling, Andrea, Marco Keiluweit, Alexandra R. Contosta, Serita Frey, Joshua Schimel, Jörg Schnecker, Richard G. Smith, Lisa Tiemann, and A. Stuart Grandy. 2018. "Minerals in the Rhizosphere: Overlooked Mediators of Soil Nitrogen Availability to Plants and Microbes." *Biogeochemistry* 139 (2): 103–22.

Jingguo, Wang, and Lars R. Bakken. 1997. "Competition for Nitrogen during Decomposition of Plant Residues in Soil: Effect of Spatial Placement of N-Rich and N-Poor Plant Residues." *Soil Biology and Biochemistry* 29 (2): 153–62.

Ju, Chengxin, Roland J. Buresh, Zhiqin Wang, Hao Zhang, Lijun Liu, Jianchang Yang, and Jianhua Zhang. 2015. "Root and Shoot Traits for Rice Varieties with Higher Grain Yield and Higher Nitrogen Use Efficiency at Lower Nitrogen Rates Application." *Field Crops Research* 175 (April): 47–55.

Kant, Surya, Yong Mei Bi, and Steven J. Rothstein. 2011. "Understanding Plant Response to Nitrogen Limitation for the Improvement of Crop Nitrogen Use Efficiency." *Journal of Experimental Botany*. Oxford University Press.

Karamanos, R E, T B Goh, and D N Flaten. 2005. "Nitrogen and Sulphur Fertilizer Management for Growing Canola on Sulphur Sufficient Soils For Personal Use Only." *Canadian Journal of Plant Science* 11: 19.

Kessel, Bettina, Antje Schierholt, and Heiko C. Becker. 2012. "Nitrogen Use Efficiency in a Genetically Diverse Set of Winter Oilseed Rape (Brassica Napus L.)." *Crop Science* 52 (6): 2546–54.

Kögel-Knabner, Ingrid. 2002. "The Macromolecular Organic Composition of Plant and Microbial Residues as Inputs to Soil Organic Matter." *Soil Biology and Biochemistry* 34 (2): 139–62.

Kundu, D. K., and J. K. Ladha. 1995. "Enhancing Soil Nitrogen Use and Biological Nitrogen Fixation in Wetland Rice." *Experimental Agriculture* 31 (3): 261–78.

Kuzyakov, Yakov. 2002. "Review: Factors Affecting Rhizosphere Priming Effects." *Journal of Plant Nutrition and Soil Science* 165 (4): 382–96.

Lassaletta, Luis, Gilles Billen, Bruna Grizzetti, Juliette Anglade, and Josette Garnier. 2014. "50 Year Trends in Nitrogen Use Efficiency of World Cropping Systems: The Relationship between Yield and Nitrogen Input to Cropland." *Environmental Research Letters* 9 (10).

Li, Lu Jun, Xia Zhu-Barker, Rongzhong Ye, Timothy A Doane, and William R Horwath. 2018. "Soil Microbial Biomass Size and Soil Carbon Influence the Priming Effect from Carbon Inputs Depending on Nitrogen Availability." *Soil Biology and Biochemistry* 119: 41–49.

Ma, B L, and A W Herath. 2016. "Timing and Rates of Nitrogen Fertiliser Application on Seed Yield, Quality and Nitrogen-Use Efficiency of Canola." *Crop and Pasture Science* 67 (2): 167–80.

Mahal, Navreet K., William R. Osterholz, Fernando E. Miguez, Hanna J. Poffenbarger, John E. Sawyer, Daniel C. Olk, Sotirios V. Archontoulis, and Michael J. Castellano. 2019. "Nitrogen Fertilizer Suppresses Mineralization of Soil Organic Matter in Maize Agroecosystems." *Frontiers in Ecology and Evolution* 7 (March).

Moorhead, Daryl L., and Robert L. Sinsabaugh. 2006. "A Theoretical Model of Litter Decay and Microbial Interaction." *Ecological Monographs*.

Parkin, Isobel, Sally Vail, and Steve Robinson. 2017. "Development of a Germplasm Resource to Dissect Complex Traits in Brassica Napus."

Perchlik, Molly, and Mechthild Tegeder. 2017. "Improving Plant Nitrogen Use Efficiency through Alteration of Amino Acid Transport Processes." *Plant Physiology* 175 (1): 235–47.

Pinheiro, José, Douglas Bates, Saikat DebRoy, Deepayan Sarkar, Siem Heisterkamp, and Bert Van Willigen. 2018. "Nlme: Linear and Nonlinear Mixed Effects Models. R Package Version 3.1-137." *Retrieved from Https://Cran.r-Project.Org/Package=nlme*.

R Core Team. 2017. "R: A Language and Environment for Statistical Computing. Http://Www.R-Project.Org/." R Foundation for Statistical Computing, Vienna, Austria. 2017.

Ramirez, Kelly S., Joseph M Craine, and Noah Fierer. 2012. "Consistent Effects of Nitrogen Amendments on Soil Microbial Communities and Processes across Biomes." *Global Change Biology* 18 (6): 1918–1927.

Rathke, G. W., T. Behrens, and W. Diepenbrock. 2006. "Integrated Nitrogen Management Strategies to Improve Seed Yield, Oil Content and Nitrogen Efficiency of Winter Oilseed Rape (Brassica Napus L.): A Review." *Agriculture, Ecosystems and Environment* 117 (2–3): 80–108.

Raun, William R, and Gordon V Johnson. 1999. "Improving Nitrogen Use Efficiency for Cereal Production." *Agronomy Journal* 91 (3): 357–63.

Russell Lenth. 2019. "Emmeans: Estimated Marginal Means, Aka Least-Squares Means."

Schimel, Joshua P, and Jennifer Bennett. 2004. "Nitrogen Mineralization: Challenges of a Changing Paradigm." *Ecology*.

Sims, G. K., T. R. Ellsworth, and R. L. Mulvaney. 1995. "Microscale Determination of Inorganic Nitrogen in Water and Soil Extracts." *Communications in Soil Science and Plant Analysis* 26 (1–2): 303–16.

Smith, E G, B M Upadhyay, M L Favret, and R E Karamanos. 2010. "Fertilizer Response for Hybrid and Open-Pollinated Canola and Economic Optimal Nutrient Levels." *Canadian Journal of Plant Science* 90 (3): 305–10.

Sparks, D.L., A.L. Page, P.A. Helmke, R.H. Loeppert, D. W. Nelson, and L E Sommers. 1996. "Total Carbon, Organic Carbon, and Organic Matter." In *Methods of Soil Analysis, Part 3, Chemical Methods*, 1004–5.

Stahl, Andreas, Christine Bissuel-Belaygue, Rod Snowdon, Anne-Sophie Bouchet, Nathalie Nesi, and Anne Laperche. 2016. "Nitrogen Use Efficiency in Rapeseed. A Review." *Agronomy for Sustainable Development* 36 (2): 0–20.

Stahl, Andreas, Wolfgang Friedt, Benjamin Wittkop, and Rod J. Snowdon. 2016. "Complementary Diversity for Nitrogen Uptake and Utilisation Efficiency Reveals Broad Potential for Increased Sustainability of Oilseed Rape Production." *Plant and Soil* 400: 245–62.

Tilman, David, Kenneth G. Cassman, Pamela A. Matson, Rosamond Naylor, and Stephen Polasky. 2002. "Agricultural Sustainability and Intensive Production Practices." *Nature* 418 (6898): 671–77.

Zhou, Baoyuan, Xuefang Sun, Dan Wang, Zaisong Ding, Congfeng Li, Wei Ma, and Ming Zhao. 2019. "Integrated Agronomic Practice Increases Maize Grain Yield and Nitrogen Use Efficiency under Various Soil Fertility Conditions." *Crop Journal* 7 (4): 527–38.

Zhu, Shusheng, Jorge M. Vivanco, and Daniel K. Manter. 2016. "Nitrogen Fertilizer Rate Affects Root Exudation, the Rhizosphere Microbiome and Nitrogen-Use-Efficiency of Maize." *Applied Soil Ecology* 107: 324–33.

Chapter 2: The potential contributions of increased soil nitrogen availability from reduced and no-till practices on canola yields

Introduction

Canada produces about 1.5% of the food in the world. More than 80% of the arable land in Canada is in the prairies of Western Canada (Statistics Canada 2009). Soil organic carbon (SOC) has been reduced by approximately 15 to 30% from its native levels due to a century of cultivation. The loss of SOC has been associated with significant loss of soil fertility and production (Monreal and Janzen 1993). Degradation of soils has been recognized as an important issue for sustaining productivity and reducing the environmental impacts of these agroecosystems, and several key soil conservation practices have been adopted to address the problem (Janzen et al. 1998).

Conventional tillage (CT), where all crop residue is incorporated into the soil, contributes to soil degradation as CT disrupts the soil structure, breaks apart soil aggregates, and increases soil erosion from wind and rain (Lal 1993; Lafond et al. 2011; Janzen et al. 1998). Furthermore, CT contributes to the reduction of soil organic matter (SOM). The disruption of soil aggregates can expose organic materials to oxygen and increase microbial physical access and activity. Thus, CT can increase the rate of decomposition and rapidly reduce SOC (Campbell et al. 1996; Lafond et al. 2011) .

Conservation tillage, which includes both reduced till (RT) and zero till (ZT), has been shown to be effective in rebuilding SOC and soil quality (Lafond et al. 2011; VandenBygaart, Gregorich, and Angers 2003). There is some tillage involved in RT, but 30% or more of residue cover is left on the soil surface, while ZT or no till, is when the soil is undisturbed from harvest through planting. (Canola Council of

Canada 2019c). The benefits of reduced till and zero till (RT-ZT) on crop yield (Lafond et al. 2011),

reduced erosion (Janzen et al. 1998), and energy savings (Zentner et al. 2004) have been well

documented. The adoption of RT-ZT increases plant residue retention on the top of soil, and therefore

helps to accumulate snow, reduce surface evaporation, and retain moisture. This increase in soil

moisture in the water limited system of Canadian prairies can increase crop yield and with an increased

production, crop residue for subsequent cropping years will also increase. This increase in crop residue

increases carbon (C) inputs that will lead to increases in SOM (Campbell et al. 1996; Monreal and Janzen

1993; Janzen et al. 1998).

One potential benefit of RT-ZT that is rarely addressed is the increase in more stable soil nitrogen (N)

reserves that can support plant growth. The modern agricultural system is dependent on the application

of mineral N fertilizer, although only 30-50% the applied N fertilizer is harvested in that year's crop

(Garnett, Conn, and Kaiser 2009; Conant, Berdanier, and Grace 2013; Bodirsky et al. 2012). Even though

SOM is often equally important as a N source in cropping systems, few studies have focused on the

contribution of N sources other than current-year N fertilizer contributions (Gardner and Drinkwater

2009). Recent results from a greenhouse pot study suggest that N from SOM can substantially increase

rapeseed, (*Brassica napus*), also known as canola in Canada, productivity even under high N fertilizer

additions. In soil environments with high SOM and high fertilizer additions, rapeseed plants obtained the

majority (average of 64%) of their N from SOM. Mineral fertilizer additions were additive to overall N

uptake and did not replace SOM as a N source (Chapter 1).

Canola is one of the world's most important oilseed crops and the most profitable commodity for

Canadian farmers. Canola is widely used as a cooking and frying oil, the meal is a used as a high-protein

animal feed for livestock, and increasingly the oil is used for biofuel. Canola has become a major cash

crop in Canada and the number of acres has been steadily climbing (Canola Council of Canada 2016;

2019b). High yielding herbicide tolerant (HY-HT) crops were introduced in the late 1990's and quickly

adopted by farmers because of their high yields and superior weed control. These herbicide tolerant

varieties enabled farmers to adopt RT-ZT practices that have been adopted by the majority of Canadian

canola farmers (Canola Council of Canada 2019c). Canola is generally characterized to have high N

demands, but low N-fertilizer recovery efficiency (RE_N) (Kessel, Schierholt, and Becker 2012; Rathke,

Behrens, and Diepenbrock 2006). Studies have shown that canola crops grown in higher SOM soils

produce higher yields (K. N. Harker et al. 2012; Lafond et al. 2011). But most of the canola N use

efficiency research has focused on improving crop RE_N, and limited research has focused on the role of N

from SOM to support crop growth (Stahl, Friedt, et al. 2016; Kessel, Schierholt, and Becker 2012). The

impact of SOM on crop yields is widely ignored in evaluating the impact of RT-NT in canola cropping

systems.

The objective of this study was to estimate the potential increase in soil N availability in canola cropping

systems in the Canadian prairies due to the long-term adoption of RT-ZT practices and examine how this

increased soil N could have contributed to canola yield improvements.

Methods

Canola Yields

We used canola yield and acreage data reported by the Canola Council of Canada (CCC). A linear

regression was plotted to examine the linear response of canola yield gain over time. (Canola Council of

Canada 2019b). We used estimates from Morrison et al. (2006) to segregate genetic, agronomic, and environmental factors contributing to yield gains.

Soil organic carbon increases with conservation till methods

We compiled published studies that compared SOC levels of CT and longer-term RT-ZT agricultural systems. The studies supplied a large range of differences in SOC. We chose to use the increase in SOC value of 3.2 Mg C/ha from VandenBygaart et al. 2010. This study sampled 27 long-term agricultural study plots across Canada and analyzed them using identical lab protocols. This value is a good estimate of the increase in SOC under RT-ZT management compared to CT in Western Canada after 23.3 ± 2.7 years. This timeframe is close to the widespread adoption of RT-ZT management practices in canola cropping systems in Canada. We chose this value for our overall estimates due to the long term RT-ZT treatments, focus on Canadian agricultural areas, synthesis across a large number of studies, and standardized lab protocols (VandenBygaart et al. 2010).

Nitrogen availability from soil organic matter

To estimate the increased soil N due to the adoption of RT-ZT, we assumed that SOM has a C:N of 10:1 (Johnston, Poulton, and Coleman 2009). Cassman et al. (2002) estimated that 1-4% of total soil N is available and taken up by crops annually, with some estimates as high as 6% in richer soils of the Midwestern U.S. We used a relatively conservative estimate for our calculations that 2% of total soil N becomes available via mineralization each year (Cassman, Dobermann, and Walters 2002). The coarseness of this estimate of N availability is appropriate for the scale of our estimates that encompass

a large geographic region with many different soil types and reflects the continuing lack of reliable indicators of N availability from SOM.

As an upper threshold, we then assumed that this increased N availability contributed directly to increased N uptake and canola yield. As a lower threshold, we assumed that this soil N would be used with a similar efficiency to the recommended relationship between fertilizer additions required per unit of canola harvestable yield. Using these upper and lower bound assumptions, we estimated the potential canola yield increase that could be attributed to the increase in soil N availability and compared that yield increase to the yield increases attributed to management factors in the published literature.

Results

Canola farmer yield gain from 1995 to 2018

From 1995 to 2018, canola yields increased by 1262.4 kg/ha, which is an 82.6% yield increase (Canola Council of Canada 2019b) (Figure 2.1). This represents an annual increase of 54.9 kg/ha for this 23-year period or an average of a 4.35% increase in yield each year. The number of hectares grown in Canada increased by about 5.5 million from 1995 to 2018, a 2.5-fold increase (Figure 2.2). The average yield from 2016-2018 was 2,777.5 kg/ha (Canola Council of Canada 2019b).

Conversion from open pollinated to high yield herbicide tolerant hybrid canola varieties

From 1995 to 2010 canola growers made a rapid change from open pollinated (OP) conventional canola varieties to high yielding herbicide tolerant (HY-HT) hybrid varieties. In just 15 years HY-HT canola went

from being produced in 0% to 99% of hectares of canola grown in Canada (Figure 2.3). These HY-HT

varieties are resistant to glyphosate, glufosinate, or imidazolinone herbicides (Canola Council of Canada

2019b). These herbicide tolerant varieties allowed for better weed control and higher grain production

while needing to apply fewer herbicides over a conventional growing system (O'Donovan et al. 2006; K.

N. Harker et al. 2012).

From their introduction HY-HT varieties have shown yield advantages of 40-80% over OP types in the

Prairie Canola Performance Trials (CPT) that the CCC organize (Brandt et al. 2007). The CPT provide

comparative data on leading and newly introduced varieties and use a check variety so genetic yield gain

can be estimated. Historically the check was an OP variety, but in recent years has changed to a known

hybrid variety ("Canola Performance Trails" 2018; Morrison et al. 2016; Brandt et al. 2007). In 2016 and

2017 an evaluation of OP suitability for commercial production in Manitoba was conducted and found

that OP variety yield was were on average 20% lower than hybrid checks (Haplotech Inc 2017).

The CCC data were used to estimate what portion of the yield can be attributed to genetic

improvements in an analysis by Morrison et al. (2016) using data from 2003 to 2009. The analysis of

yield gain above the check over time suggested an improvement of 2.4% per year due to genetic gain

alone. Applying Morrison's improvement estimates to our current yield improvement estimate of 54.9

kg/ha per year, we have estimated that 59% or 32.4 kg/ha of this increase attributed to improvements in

crop genetics, and 28% or 15.4 kg/ha of yield gain per year were due to environment (precipitation and

increase in atmospheric CO_2), and the other 13% or 7.1 kg/ha was likely the result changes in agronomic

practices such as seeding density, crop rotations, fertilization rates, and tillage (Morrison et al. 2016).

Nitrogen Fertilizer

Nutrient studies have found that canola requires more macronutrients than cereal crops, generally N, phosphorus and sulfur fertilizer need to be applied each year (Elwin G Smith et al. 2010). Nitrogen is commonly the limiting nutrient in canola cropping systems and increased N fertilization has been shown to have a positive effect on yield (K. N. Harker et al. 2012; Elwin G Smith et al. 2010). The CCC recommends 1.3 to 1.6 kg of available N for each bushel of harvested seed (22.7 kg) (Candian Fertilizer Institute 2001; Canola Council of Canada 2019a). This suggests that the current average yield of 2,777.5 kg/ha crop would require approximately 159 to 196 kg N/ha of available N.

Reflecting the relatively low expected RE_N of canola, the actual N removal in harvested seed is considerably lower than recommended fertilizer N additions. On average, canola seed is typically about 3.9% N (Natural Resources Conservation Service 2020), which suggests that a harvest of the current average yield of 2,777.5 kg/ha crop would remove approximately 108 kg N/ha annually. Some studies have supported that the HY-HT varieties require more N for the high yielding production than traditional OP varieties (K. N. Harker et al. 2012; Elwin G Smith et al. 2010; Brandt et al. 2007) while other studies argue HY-HT are more efficient in N uptake and require less N application than OP varieties (Karamanos, Goh, and Flaten 2005). More recent recommendations for N application are .9 to 1.4 kg of available N per 22.7 kg of yield (*Canola Watch* 2012; Taurus 2016), which would reduce recommendations to approximately 110 to 171 kg N/ha of available N at current yield levels.

The Adoption of Reduced and Zero Tillage

Canadian farmers converted to HY-HT canola varieties because of the higher yield outputs, decrease of herbicide applications with superior weed control (K. N. Harker et al. 2012; Smyth et al. 2011; Morrison et al. 2016). This adoption of HY-HT crops was a major factor in the increased conversion to RT-ZT (Smyth et al. 2011). The adoption of RT-ZT technology has been one of the most significant contributors to the revolutionized cropping systems of the Canadian prairies (Baig and Gamache 2011). In 1991 only 30% of total Canadian cropping area used RT-ZT. After the adoption of HY-HT varieties in 1996, about 47% of total Canadian cropping acres used RT-ZT practices. RT-ZT acres continued to increase, in 2016 80% of Canadian cropping acres (Figure 2.4). In 2000 only 11% of canola growers used RT-ZT methods, which drastically increased to 65% in 2006, and to 87% in 2012. The CCC conducted a self-reported survey in 2012 of 996 canola growers in Western Canada, 40% claimed ZT, 30% RT, 17% direct seeding, and 12% CT (Canola Council of Canada 2019c). The reduction in weeds reduces competition for nutrients and water, resulting in higher yields in canola cropping systems (K. Neil Harker et al. 2008).

In a global meta-analysis, some crops such as cereals, maize, and rice have shown a yield lag in the first 1-2 years after adopting ZT practices, while, cotton, legumes, and oilseed crops didn't show a significant difference in yield in these first years. Cotton and oilseed crops continued to yield higher as ZT practices were continued with the biggest yield gains happening between 5-10 years (Pittelkow et al. 2015).

Increase of Soil Organic Carbon from Conservation Tillage

It has been well documented that conversion from CT to ZT practices can increase SOC levels (Eldor et al. 1996; VandenBygaart, Gregorich, and Angers 2003; Six et al. 2004). The increase in SOC in ZT systems

over CT systems depends on a great number of variables including soil type, crop rotation, nutrient inputs, location, and weather (Campbell et al. 1996; Shrestha et al. 2013; West and Post 2002). Comparisons of SOC under different tillage systems report a wide range of SOC improvement of .067-2.015 Mg C/ha in ZT systems (VandenBygaart, Gregorich, and Angers 2003; VandenBygaart et al. 2010; McConkey et al. 2003; Campbell et al. 1996; Lafond et al. 2011; West and Post 2002) (Table 2.1).

To estimate the potential corresponding benefits of SOC improvement on soil N availability, we chose to use the estimate from VandenBygaart et al. 2010 of a total stock change of 3.2 Mg C/ha in the top 15cm with the adoption of ZT (an annualized estimate of 0.14 Mg C/ha). The additional 3.2 Mg C/ha of SOC from conservation till practices has corresponded with an estimated increase in soil N of 320 kg N/ha in the top 15cm. Mineralization from SOM is an important source of N for plant uptake. Mineralization is strongly affected by soil, weather, and moisture conditions but generally 1-4% of available soil N will be mineralized and be available for crop uptake (Ortiz-Zayas et al. 2006). Crops starting in a soil with high SOM has been found to produce higher yields (K. N. Harker et al. 2012). If 2% of total soil N is available to plants via net N mineralization each year, that would increase N available for uptake by canola crops by 6.4 kg N/ha per year.

Using the estimate that canola seed is 3.9% N (Natural Resources Conservation Service 2020), if the increase in available N directly supported increased yield, it could contribute to an additional 164 kg/ha of canola yield each year. Using a more conservative estimate that integrates current, low RE_N estimates and the use of N by other plant parts, the CCC recommends 1.3 to 1.6 kg/N is needed for every 22.7 kg of canola yield. Therefore, the additional 6.4 kg N/ha per year from RT-ZT practices could provide N to support an increased yield of 91 to 112 kg of canola yield/ha per year. Morrison et al. (2016) estimated

that 13%, or 7.1 kg/ha of the 54.9 kg/ha annual yield gain has been contributed by all agronomic practices such as crop rotations, seeding density, fertilization rates, and tillage (Morrison et al. 2016). If 91 to 164 kg/ha of canola yield may be attributed to increased soil N availability, it is possible that all the recent annual canola yield increases may be due to the indirect benefit of increased soil N as a result of RT-ZT adoption.

Similar with general agronomic best management practices, it is recommended that soil tests be used to inform N fertilizer applications in canola cropping systems. The SOM percentage, and the estimate of N availability from this SOM, is factored into N fertilizer application recommendations to assure the correct amount of N fertilizer is applied for desired crop yield. For example, the CCC recommends accounting for 6 to 20 pounds of available N per percentage point of SOM (Canola Council of Canada 2019a). These recommendations suggest that soil N from SOM mineralization can replace and therefore reduce the need for N fertilizer additions. However, a recent greenhouse pot study suggested that N from SOM is additive, and not a replacement source for plant N in rapeseed plants. Even under high fertilizer additions, rapeseed plant biomass increased under high SOM relative to low SOM treatments. When available, the majority of total plant N uptake was from the SOM, even with high amounts of N fertilizer available and the uptake of N from SOM was largely independent of the amount of applied N fertilizer (Chapter 1).

Other Contributing Factors

Canola yields are impacted by many environmental variables. Precipitation and weather are important variables in canola crop yields. Morrison et al. (2016) estimates that 12 kg/ha of the 54 kg/ha or 22.2%, annual canola yield gains can be contributed to increased precipitation in the Canadian prairies. Early

spring precipitation (April-May) has a significant positive effect on grain yields (Morrison et al. 2016).

Crop yields tend to be higher in years with cooler summer temperatures (K. N. Harker et al. 2012;

Morrison et al. 2016). As CO_2 concentration increases in the atmosphere all C_3 crops yields, including

canola, are estimated to increase about .02% per ppm of CO_2 per year (Fischer 2015). Morrison et al.

(2016) estimates this CO_2 increase results in a 3 kg/ha of the 54 kg/ha of the annual yield increase

(Morrison et al. 2016) Other agronomic practices are also influential to canola yields such as seeding

rates, nutrient applications, and tillage depth (Morrison et al. 2016).

The effects of RT-ZT practices on SOC are influenced by environmental factors and cropping practices.

Crop rotation and cropping intensity has also been found to affect SOC and likely shifted over the same

time period of interest (Morrison et al. 2016; Shrestha et al. 2013; Campbell et al. 1996; McConkey et al.

2003; West and Post 2002).The SOC accumulation in the soil as a result of RT-ZT practices is dependent

on soil type and soil moisture and while SOC increased in RT-ZT adoption in Western Canada, no

increase in SOC was found in Eastern Canada (VandenBygaart, Gregorich, and Angers 2003).

There are many interacting, co-benefits to increasing SOM in cropping systems. Soil organic matter plays

an important role in the physical, chemical and biological properties of soil. The major role SOM plays in

the physical properties of soil is in structure and the stabilization of aggregates which makes cultivation

easier as well as increases water holding capacity. Chemically, SOM increases availability of micro and

macro nutrients while increasing soil's cation exchange capacity. The biological properties, such as

microbial biomass, of the soil are influenced by SOM. These properties contribute to the increase of N

mineralization and N availability to crops but are also independently beneficial for crop growth (Fageria

2012). In the controlled conditions of a greenhouse pot study, these other beneficial properties of SOM,

such as soil moisture content and availability of other nutrients, were controlled for and the N supplying

capacity of SOM was still a significant contributor to the increase in rapeseed biomass (Chapter 1).

It is also important to acknowledge that the increased soil N in RT-ZT soils likely reflects legacy N

fertilizer additions that were incorporated into SOM overtime. Less than half of the applied N fertilizer is

typically taken up by the current year's crop and the remainder can be lost from the system via leaching

or gaseous losses or can be retained in the soil (M. Yan et al. 2019). Overtime some of the excess N

fertilizer can become stabilized in the SOM, be re-mineralized by soil microorganisms, and contribute to

crop N supply (X. Yan et al. 2014; M. Yan et al. 2019). As a result of this accumulation of N fertilizer and

slow crop uptake RT-ZT practices may effectively increase the long-term RE_N of crops. It is important to

recognize that we do not fully understand the mechanisms behind crop N acquisition from these

different sources.

Conclusion

The conversion from CT to RT-ZT has been shown to have many benefits including reduced erosion,

moisture retention, and improved soil structure. The increase of crop residue left in the fields increases

C inputs and increases SOM. These improvements to soil quality have been shown to support larger crop

yields. The higher percentage of SOM provides stable soil N reserves that support plant growth.

Although SOM is an important source of N in cropping systems it is frequently ignored as a factor when

evaluating crop yield increases. While the other benefits of the improved soil quality are considered

important factors in yield improvements, N from SOM is not considered to be a direct contributor to

increased yields under full N fertilization. We examined how the increased soil N from the adoption of

RT-ZT practices could have contributed to canola yield improvements. We found that 59% of annual

yield increases can be attributed to genetic improvements, and 13% of yield increases are due to

changes in agronomic practices. We attributed an increase of 3.2 Mg C/ha of SOC or 320 kg N/ha per

year to RT-ZT practices. While increasing SOC has many co-benefits to production, we demonstrate that

it is possible that all annual yield improvements could be attributed to increased soil N alone as a result

of RT-ZT adoption. The variability in N mineralization makes it difficult to estimate its contribution to

crop yields, but soil N is important for crop growth and can serve as an additive resource to the available

N from fertilizer sources and could be an underestimated factor in canola yield improvements.
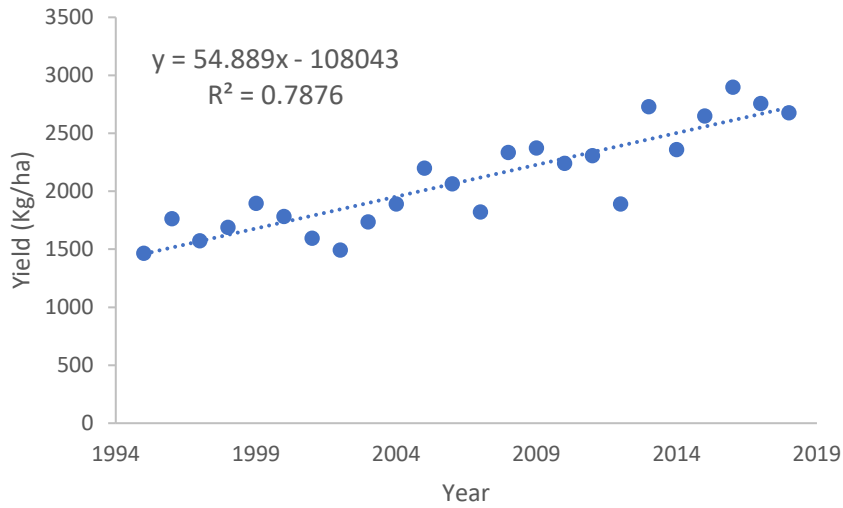
Figure 2.1. Average annual canola yield (kg/ha) and annual rate of increase for Canada from 1995 to 2018 (Canola Council of Canada 2017).
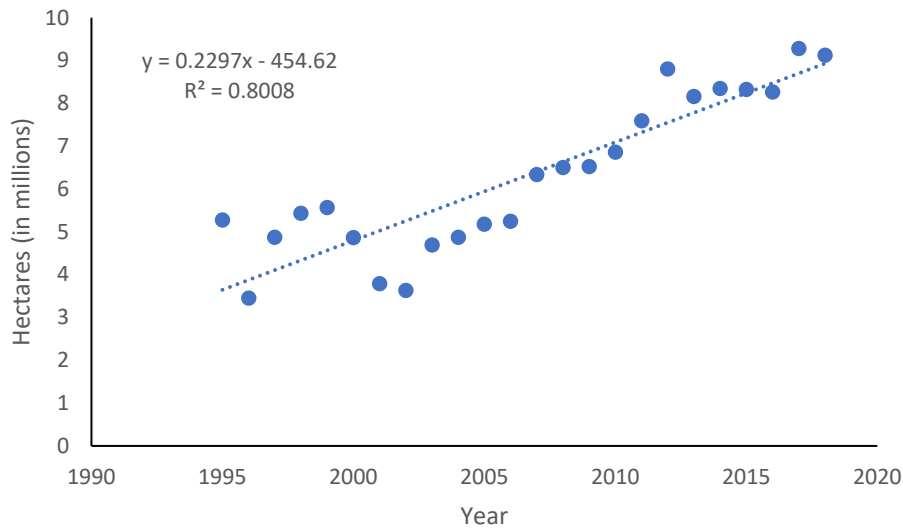

Figure 2.2. Annual hectares of canola grown in Canada and annual rate of increase from 1995 to 2018 in millions of hectares (Canola Council of Canada 2019b).
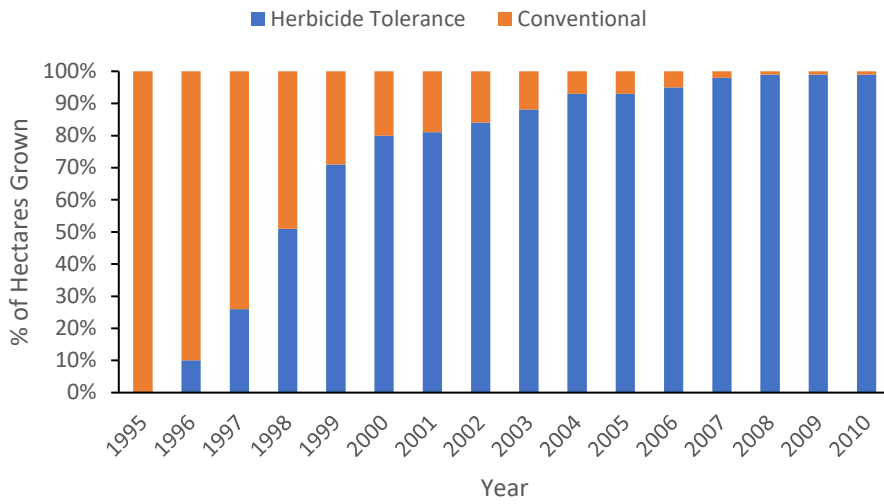
Figure 2.3: Percentage of hectares of Canola grown in Canada by from 1995 to 2010 by conventional or herbicide tolerant crops (Canola Council of Canada 2017).
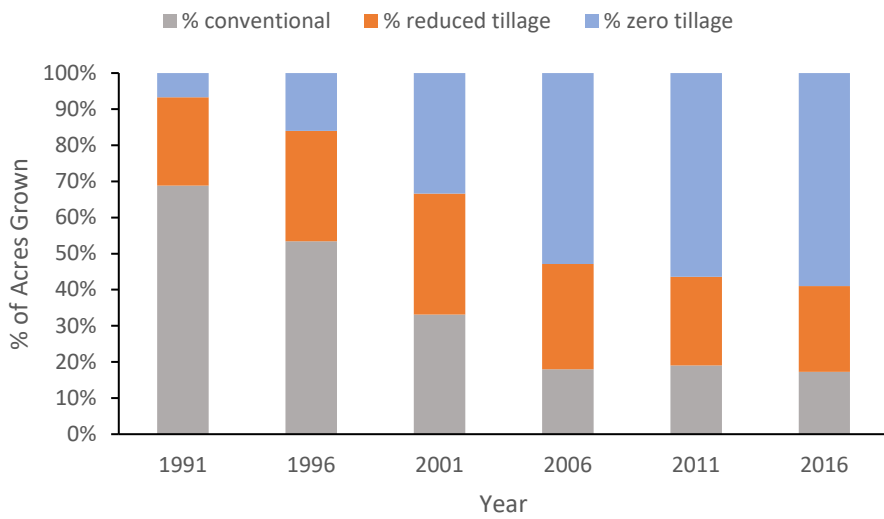

Figure 2.4: Percentage of Canadian cropping acres by year and tillage type (conventional, reduced tillage, and zero tillage) (Statistics Canada 2017; Baig and Gamache 2011).

Table 2.1: Change in soil organic carbon (SOC) overall, by year, study depth, years of reduced and zero conservation tillage (RT-ZT), study location, and sources of studies comparing conventional and conservation till practices in agricultural lands.

Δ SOC (Mg C ha-1)	Δ SOC (Mg C/ha-1/year)	Study Depth (cm)	Years of no till	Location	Source
2.9 ± 1.3	0.26	7.5-37.5	11	Western Canada	VandenBygaart et al. 2003
3.2 ± 1.3	0.14	15	23.3 ± 2.7	Western Canada	VandenBygaart et al. 2010
1.6-8.5	0.07-.51	15	16.5	Saskatchewan, CA	McConkey et al 2003
4.5	0.41	15	11	Saskatchewan, CA	Campbell et al. 1996
40.3	2.02	15	20	Saskatchewan, CA	Lafond et al. 2011
8.55	0.57	22	15	Worldwide	West and Post 2002

References

Baig, Mirza N, and Peter Gamache. 2011. "The Economic, Agronomic and Environmental Impact of No-Till on the Canadian Prairies." In *Alberta Reduced Tillage Linkages*, 2:176.

Bodirsky, B L, A Popp, I Weindl, J P Dietrich, S Rolinski, L Scheiffele, C Schmitz, and H Lotze-Campen. 2012. "N 2 O Emissions from the Global Agricultural Nitrogen Cycle-Current State and Future Scenarios." *Biogeosciences* 9: 4169–97.

Brandt, S. A., S. S. Malhi, D. Ulrich, G. P. Lafond, H. R. Kutcher, and A. M. Johnston. 2007. "Seeding Rate, Fertilizer Level and Disease Management Effects on Hybrid versus Open Pollinated Canola (Brassica Napus L.)." *Canadian Journal of Plant Science* 87 (2): 255–66.

Campbell, C. A., B. G. McConkey, R. P. Zentner, F. Selles, and D. Curtin. 1996. "Long-Term Effects of Tillage and Crop Rotations on Soil Organic C and Total N in a Clay Soil in Southwestern Saskatchewan." In *Canadian Journal of Soil Science*, 76:395–401. Agricultural Institute of Canada.

Candian Fertilizer Institute. 2001. "Nutrient Uptake and Removal by Field Crops."Retrieved from https://www.canolawatch.org/wp-content/uploads/2012/02/CFI_nutrient_uptake_for_wcanada_2001.pdf

Canola Council of Canada. 2016. "What Is Canola?" Agricultural and Agri-Food Canada. 2016.Retrieved from https://www.canolacouncil.org/oil-and-meal/what-is-canola/

Canola Council of Canad. 2019a. "Nitrogen Fertilizer Management." Canola Encyclopedia. 2019. Retrieved from https://www.canolacouncil.org/canola-encyclopedia/fertilizer-management/nitrogen-fertilizer-management/

Canola Council of Canad. 2019b. "Statistics - Canola Council of Canada." 2019. Retrieved from https://www.canolacouncil.org/markets-stats/statistics/.

Canola Council of Canad. 2019c. "Tillage, Canola Encylopedia." 2019. Retrieved from https://www.canolacouncil.org/canola-encyclopedia/field-characteristics/tillage/

"Canola Performance Trails." 2018. Retrieved from https://www.canolaperformancetrials.ca/Booklet-2018.pdf

Canola Watch. 2012. "How Much Fertilizer Does Canola Need?," 2012. Retrieved from https://www.canolawatch.org/2012/02/09/how-much-fertilizer-does-canola-need/

Cassman, K. G., A Dobermann, and Daniel T. Walters. 2002. "Agroecosystems, Nitrogen-Use Efficiency, and Nitrogen Management." *AMBIO: A Journal of the Human Environment* 31 (2): 132–40.

Conant, Richard T, Aaron B Berdanier, and Peter R Grace. 2013. "Patterns and Trends in Nitrogen Use and Nitrogen Recovery Efficiency in World Agriculture." *Global Biogeochemical Cycles* 27 (2): 558–66.

Eldor, Paul A., Keith H. Paustian, E. T. Elliott, and C. Vernon Cole. 1996. *Soil Organic Matter in Temperate Agroecosystems*. *Soil Organic Matter in Temperate Agroecosystems*. CRC Press.

Fageria, N K. 2012. "Role of Soil Organic Matter in Maintaining Sustainability of Cropping Systems."

Communications in Soil Science and Plant Analysis 43 (16): 2063–2113.

Fischer, R. A. 2015. "Definitions and Determination of Crop Yield, Yield Gaps, and of Rates of Change." *Field Crops Research* 182 (October): 9–18.

Gardner, Jennifer B, and Laurie E Drinkwater. 2009. "The Fate of Nitrogen in Grain Cropping Systems: A Meta-Analysis of 15N Field Experiments." *Ecological Applications* 19 (8): 2167–84.

Garnett, Trevor, Vanessa Conn, and Brent N. Kaiser. 2009. "Root Based Approaches to Improving Nitrogen Use Efficiency in Plants." *Plant, Cell and Environment* 32 (9): 1272–83.

Haplotech Inc. 2017. "Evalutation of OP Lines and Varieties for Suitability for Commercial Production in Manitoba." Winnipeg. Retrieved from https://canolagrowers.com/wp-content/uploads/2018/02/MCGA-2017-study-report-Nov

Harker, K. N., J. T. O'Donovan, T. K. Turkington, R. E. Blackshaw, N. Z. Lupwayi, E. G. Smith, H. Klein-Gebbinck, et al. 2012. "High-Yield No-till Canola Production on the Canadian Prairies." *Canadian Journal of Plant Science* 92 (2): 221–33.

Harker, K. Neil, John T. O'Donovan, George W. Clayton, and John Mayko. 2008. "Field-Scale Time of Weed Removal in Canola." *Weed Technology* 22 (4): 747–49.

Janzen, H H, C A Campbell, R C Izaurralde, B H Ellert, N Juma, W. B. McGill, and R P Zentner. 1998. "Management Effects on Soil C Storage on the Canadian Prairies." *Soil and Tillage Research* 47 (3–4): 181–95.

Johnston, A. Edward, Paul R. Poulton, and Kevin Coleman. 2009. "Chapter 1 Soil Organic Matter. Its Importance in Sustainable Agriculture and Carbon Dioxide Fluxes." In *Advances in Agronomy*, 101:1–57.

Karamanos, R E, T B Goh, and D N Flaten. 2005. "Nitrogen and Sulphur Fertilizer Management for Growing Canola on Sulphur Sufficient Soils For Personal Use Only." *Canadian Journal of Plant Science* 11: 19.

Kessel, Bettina, Antje Schierholt, and Heiko C. Becker. 2012. "Nitrogen Use Efficiency in a Genetically Diverse Set of Winter Oilseed Rape (Brassica Napus L.)." *Crop Science* 52 (6): 2546–54.

Lafond, Guy P., Fran Walley, W. E. May, and C. B. Holzapfel. 2011. "Long Term Impact of No-till on Soil Properties and Crop Productivity on the Canadian Prairies." *Soil and Tillage Research* 117: 110–23.

Lal, Rattan. 1993. "Introduction Tillage Effects on Soil Degradation, Soil Resilience, Soil Quality, and Sustainability." *Soil & Tillage Research*. Vol. 27.

McConkey, B. G., B. C. Liang, C. A. Campbell, D. Curtin, A. Moulin, S. A. Brandt, and G. P. Lafond. 2003. "Crop Rotation and Tillage Impact on Carbon Sequestration in Canadian Prairie Soils." *Soil and Tillage Research* 74 (1): 81–90.

Monreal, C. M., and H. H. Janzen. 1993. "Soil Organic-Carbon Dynamics after 80 Years of Cropping a Dark Brown Chernozem." *Canadian Journal of Soil Science* 73 (1): 133–36.

Morrison, M. J., K. N. Harker, R. E. Blackshaw, C. J. Holzapfel, and J. T. O'Donovan. 2016. "Canola Yield Improvement on the Canadian Prairies from 2000 to 2013." *Crop and Pasture Science* 67 (3–4): 245–52.

Natural Resources Conservation Service. 2020. "Crop Nutrient Tool | USDA PLANTS." 2020. Retrieved from https://plants.usda.gov/npk/main

O'Donovan, John T., K. Neil Harker, George W. Clayton, and Robert E. Blackshaw. 2006. "Comparison of a Glyphosate-Resistant Canola (Brassica Napus L.) System with Traditional Herbicide Regimes." *Weed Technology* 20 (2): 494–501.

Ortiz-Zayas, J R, E Cuevas, O L Mayol-Bracero, L Donoso, I Trebs, D Figueroa-Nieves, and W H McDowell. 2006. "Agroecosystems, Nitrogen-Use Efficiency, and Nitrogen Management." *Biogeochemistry* 79 (1–2): 132–40.

Pittelkow, Cameron M., Bruce A. Linquist, Mark E. Lundy, Xinqiang Liang, Kees Jan van Groenigen, Juhwan Lee, Natasja van Gestel, Johan Six, Rodney T. Venterea, and Chris van Kessel. 2015. "When Does No-till Yield More? A Global Meta-Analysis." *Field Crops Research* 183 (November): 156–68.

Rathke, G. W., T. Behrens, and W. Diepenbrock. 2006. "Integrated Nitrogen Management Strategies to Improve Seed Yield, Oil Content and Nitrogen Efficiency of Winter Oilseed Rape (Brassica Napus L.): A Review." *Agriculture, Ecosystems and Environment* 117 (2–3): 80–108.

Shrestha, B M, B G Mcconkey, W N Smith, R L Desjardins, C A Campbell, B B Grant, and P R Miller. 2013. "Effects of Crop Rotation, Crop Type and Tillage on Soil Organic Carbon in a Semiarid Climate." *Canadian Journal of Soil Science* 93 (1): 137–46.

Six, Johan, Stephen M. Ogle, F. Jay breidt, Rich T. Conant, Arvin R. Mosier, and Keith Paustian. 2004. "The Potential to Mitigate Global Warming with No-Tillage Management Is Only Realized When Practised in the Long Term." *Global Change Biology* 10 (2): 155–60.

Smith, Elwin G, Bharat M Upadhyay, M Lucila Favret, Rigas E Karamanos, E G Smith, and M L Karamanos. 2010. "Fertilizer Response for Hybrid and Open-Pollinated Canola and Economic Optimal Nutrient Levels." *Candadian Journal of Plant Science* 90 (3): 305–10.

Smyth, Stuart J, Michael Gusta, Kenneth Belcher, Peter W B Phillips, and David Castle. 2011. "Environmental Impacts from Herbicide Tolerant Canola Production in Western Canada." *Agricultural Systems* 104: 403–10.

Stahl, Andreas, Wolfgang Friedt, Benjamin Wittkop, and Rod J. Snowdon. 2016. "Complementary Diversity for Nitrogen Uptake and Utilisation Efficiency Reveals Broad Potential for Increased Sustainability of Oilseed Rape Production." *Plant and Soil* 400: 245–62.

Statistics Canada. 2009. "Human Activity and the Environment : Annual Statistics. Section 1: Food in Canada." *Catalogue No. 16-201-X*, no. 16: 1–168.

Taurus. 2016. "Crop Nutrient Uptake and Removal Chart."Retrieved from https://resources.taurus.ag/download-taurus-crop-nutrient-uptake-removal-chart

VandenBygaart, A. J., E Bremer, B. G. McConkey, H H Janzen, D A Angers, M R Carter, C F Drury, G P Lafond, and R. H. McKenzie. 2010. "Soil Organic Carbon Stocks on Long-Term Agroecosystem Experiments in Canada." *Canadian Journal of Soil Science* 90 (4): 543–50.

VandenBygaart, A. J., E. G. Gregorich, and D. A. Angers. 2003. "Influence of Agricultural Management on Soil Organic Carbon: A Compendium and Assessment of Canadian Studies." *Canadian Journal of Soil Science* 83 (4): 363–80.

West, Tristram O., and Wilfred M. Post. 2002. "Soil Organic Carbon Sequestration Rates by Tillage and Crop Rotation: A Global Data Analysis." *Soil Science Society of America Journal* 66 (6): 1930–46.

Yan, Ming, Genxing Pan, Jocelyn M. Lavallee, and Richard T. Conant. 2019. "Rethinking Sources of Nitrogen to Cereal Crops." *Global Change Biology* 26 (1): 191–99.

Yan, Xiaoyuan, Chaopu Ti, Peter Vitousek, Deli Chen, Adrian Leip, Zucong Cai, and Zhaoliang Zhu. 2014. "Fertilizer Nitrogen Recovery Efficiencies in Crop Production Systems of China with and without Consideration of the Residual Effect of Nitrogen." *Environmental Research Letters* 9 (9).

Zentner, R P, G P Lafond, D A Derksen, C N Nagy, D D Wall, and W E May. 2004. "Effects of Tillage Method and Crop Rotation on Non-Renewable Energy Use Efficiency for a Thin Black Chernozem in the Canadian Prairies." *Soil and Tillage Research* 77 (2): 125–36.

Conclusion

Nitrogen (N) management is one of the biggest challenges in sustainable agriculture. More than 50% of

the N fertilizer applied to crops globally is lost into the environment. Soil organic matter (SOM) has been

shown to be an important source of N for most crops, but intentionally managing SOM as a N source has

been underutilized. Our studies have highlighted the importance of SOM as a N source in both a

controlled pot study and in a field setting. Our greenhouse study showed that SOM remained an

important N source to support crop growth even when ample N fertilizer was available. Fertilizer

additions were additive to overall N uptake but did not replace SOM as an N source. Plants in high SOM

environments had an additional source of N that contributed to increased plant biomass. We also saw a

decrease in microbial enzyme activity in high fertilizer environments relative to low fertilizer

environments, suggesting that N fertilizer additions have a direct effect on microbial nutrient dynamics.

Although we found limited differences in N uptake and growth patterns among our chosen rapeseed

varieties, targeting diversity in belowground traits as well as including some high yielding hybrid

varieties could have increased our chances to see varietal differences.

To understand the broader potential implications of soil N to support crop productivity, we then

estimated the potential additive effect of SOM on biomass production on a field scale. We estimated the

potential yield contributions of soil N from SOM increases to canola yields following the adoption of

conservation tillage practices on the Canadian prairies. In our literature review we found that all

agronomic management practices are estimated to contribute 7.1 kg/ha per year of the total 54.9 kg/ha

annual yield increase observed over 23 years. We estimated that 91 to 112 kg of rapeseed yield/ha per

year could be due to the indirect benefit of increased soil N as result of the adoption of conservation till

practices. While there are many potential co-benefits of conservation tillage adoption, our estimates

strongly suggest that the contribution of N mineralization to canola yield improvements is underestimated.

Nitrogen mineralization is highly variable, it is impacted by soil type, temperature, moisture, microbial community composition, and many other factors. This complexity makes it very difficult to predict or estimate available N, making it a difficult source of N to rely on for farmers. Soil is a very dynamic system and it is important to acknowledge that we do not fully understand all the mechanisms that control crop uptake from these available N sources. This research highlights key knowledge gaps, including how plant-SOM-microbe interactions influence the observed variability in N mineralization estimates and understanding how N fertilizer additions impact soil microbial activity. Even without a full understanding of the mechanisms, this research supports adopting an integrated approach to N management that actively manages both N fertilizer and SOM as N sources in both crop breeding and agronomic management as an essential part of improving agricultural sustainability.