Fluctuation in the soil nitrogen supply resulting from green manure plow-down are detected by ion exchange membranes and the nitrogen uptake of arugula (*Eruca sativa* L.)

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December 2016

A thesis submitted to McGill University in partial fulfillment of

the requirements for the degree of Doctor of Philosophy

ABSTRACT

Green manure crop mixtures contain legumes that capture N_2 from the atmosphere. When green manure is plowed down in agricultural soil, the decomposing green manure residues are a source of N fertilizer for the following crop. The challenge is to determine how quickly the green manure releases plant-available N and what proportion of the N requirements of the next crop can be met by green manure. Ion exchange membranes (IEMs) hold promise in evaluating the N supplied by green manure because these *in situ* measurement tools are placed in the same environment where roots grow, and they act as a sink for plant-available N. The objectives of my thesis were to 1) determine the pattern of plant-available N release from field pea-oat green manure under field conditions with IEMs, and relate this to the N demands of arugula (*Eruca sativa* L.); 2) to determine if IEMs could detect small changes in plant-available N dynamics in different soil types that were amended with green manure residues having a low C/N ratio; and 3) to determine how tillage practices that reduce the physical size of green manure residues may accelerate plant-available N release from green manure, as determined by IEMs, and meet crop N requirements; 4) to assess the potential contribution of root exudates from arugula on N mineralization after the incorporation of green manure mixture (peas-oats).

In a field experiment, in two soil types in Quebec, Canada, a green manure mixture (field pea and oat) contributed to the plant-available N concentration and arugula N uptake. The IEM-NO_3-N supply was greater than arugula N demand, suggesting that second crop or a winter cover crop could use the residual soil N released by the green manure. In an incubation experiment, I validated the use of IEMs by incorporating green manure residues with variable C/N ratios. The green manure residues with low C/N ratio (C/N = 8) showed immediate release of plant-available N, whereas residues with C/N = 12 ratio had a delay in releasing plant-available N. The delay

suggested that the C/N ratio of green manure residue plus analysis with IEMs was a good indicator of plant-available N dynamics. In addition, the different soil texture (clay loam and sandy loam) modulated the decomposition process and plant-available N concentrations. Greater tillage intensity reduced the percentage of residues with larger particle size. While the higher concentration of plant-available N was released with 4 passes of the cultivator, the maximum N uptake by arugula was reached with 2 passes of the cultivator. Residues remaining after the experiment continued to release plant-available N, suggesting that residual soil N would be left after arugula harvest. The cash crop root exudate demonstrated to play a role in N mineralization through the exudate and showed greater impact on microbial biomass when green manure residues were 0.5 – 1 mm, followed by residues size 2 – 4 mm, and bare soil.

In conclusion, the use of IEMs *in situ* and in incubation experiments could accurately assess the pattern of N release from green manure. This provides insight about the plant-available N dynamics and root input in soils receiving green manure and when N is available for subsequent crops. Farmers can use this information to select crops that will fully use the plant-available N, thus optimizing the N recovery from green manure crops.

RÉSUMÉ

Les mélanges des engrais verts contiennent des légumineuses qui capturent N2 de l'atmosphère. Lorsque l'engrais vert est enfoui dans les sols, les résidus en décomposition de cet engrais constituent une source de N pour la culture suivante. Le défi : déterminer la vitesse à laquelle l'engrais vert peut libérer de N disponible et la proportion des besoins en N de la culture suivante pouvant être satisfaite par cet engrais. Les membranes échangeuses d'ions (MEIs) sont prometteuses dans l'évaluation de N fournie par l'engrais, ces outils de mesure étant placés dans le même environnement où les racines se développent et agissent comme puits pour N disponible. Ma thèse visait à déterminer 1) le mode de libération de N disponible par l'engrais vert du petit pois-avoine sous les conditions de champs avec MEIs et relier ceci aux exigences en N de la roquette (Eruca sativa L.); 2) si MEIs peuvent détecter de petits changements dans la dynamique de N disponible dans différents types de sols recevant des apports des résidus d'engrais à faible rapport C/N; et 3) comment les pratiques de travail réduisant la taille des résidus d'engrais peuvent accélérer la libération N disponible par l'engrais vert, telle que déterminée par MEIS, et satisfaire les exigences en N de la culture; et 4) la contribution potentielle des exsudats racinaires de roquette sur N minéralisation après l'incorporation du mélange d'engrais.

Dans une expérience sur deux types de sols au Québec, Canada, un mélange d'engrais vert (de petit pois et d'avoine) a contribué à la concentration de N disponible et le N absorbé. La fourniture en NO3-N des MEIs était supérieure à la demande en N de la roquete: la possibilité d'insérer une culture additionnelle ou d'hiver qui utiliserait le N résiduel libéré par l'engrais. Dans une expérience d'incubation, j'ai validé l'utilisation des MEIs avec l'incorporation des résidus d'engrais à rapports C/N variables. La libération de N disponible a été immédiate par les

résidus d'engrais verts à faible rapport C/N (C/N = 8), mais plus lente chez des résidus à rapport C/N = 12. Ce retard signifierait que le rapport C/N de résidus d'engrais et l'analyse avec MEIs était un bon indicateur de la dynamique de N disponible. La texture différente du sol (limono-argileux et limono-sableux) a modulé le processus de décomposition et la concentration de N disponible. Une plus grande intensité du travail du sol a réduit le pourcentage des résidus à taille de particules plus large. Alors que la plus forte concentration de N disponible été libérée avec 4 passages du cultivateur, l'absorption maximale de N par la roquette a été atteinte avec 2 passages. Les résidus restants ont continué à libérer de N disponible: du N résiduel demeurerait dans le sol après la récolte. Les exsudats racinaires des cultures de rente semblent avoir joué un rôle dans la minéralisation d'azote, avec un plus grand impact sur la biomasse microbienne pour les résidus d'engrais verts de 0,5-1 mm, puis la taille des résidus de 2-4 mm et en dernier le témoin sans résidus.

En conclusion, l'utilisation des MEIs in situ et dans des expériences d'incubation pourrait évaluer avec précision le mode de libération de N par l'engrais vert, donnant un aperçu sur la dynamique de N disponible pour les plantes et les intrants des racines dans les sols recevant des engrais verts et lorsque N est disponible pour les cultures suivantes: information pour les agriculteurs dans le choix des cultures qui utiliseront pleinement le N disponible, optimisant ainsi la recouvrement de N à partir des cultures d'engrais vert.

PREFACE AND CONTRIBUTIONS OF AUTHORS

This thesis is composed of six chapters, preceded by a general introduction that provides the overall objectives of this thesis. Five chapters were written in the form of manuscripts, and the sixth chapter was the overall conclusions and future research recommendations. A statement of contributions to knowledge is also provided, as per the guidelines of the Graduate and Postdoctoral Studies Office, McGill University. The first chapter is a literature review that summarizes the decomposition and nitrogen contribution of green manure. Authentic research results are presented in Chapters two to five, which were written as scientific manuscripts, with connection paragraphs between each chapter to show the connections between each experiment. Chapter six consisted of general conclusions and future recommendations, which synthesized all the findings from previous chapters, and related them to the thesis objectives and hypotheses.

The candidate was the first author on all the manuscripts. Co-author included Dr. Joann K. Whalen. The thesis research was funded by Natural Science and Engineering Research Council of Canada (NSERC). The candidate received postgraduate award from the Institutional Committee of Scholarships and Financial Aid of Ecuador (SENESCYT). The candidate wrote the literature review in chapter one and undertook all the original research in chapters two to five, including designing of experiments, data collection, analysis and interpretation, and writing the manuscripts. Dr. Whalen provided financial support, advisory guidance on these research, and editorial assistance with the manuscripts.

The manuscript-based chapters are presented in the following order:

Chapter 1. León Castro, L., Whalen, J. K. Study of green manure decomposition and nitrogen availability pattern to meet nitrogen demand of crops.

Chapter 2. León Castro, L., Whalen, J. K. 2016. Ion exchange membranes as an indicator of soil mineral nitrogen availability and nitrogen uptake by arugula (*Eruca sativa* L.) in soils amended with green manure. Biological Agriculture and Horticulture 32: 206 - 220.

Chapter 3. León Castro, L., Whalen, J. K. 2016. Ion exchange membranes are sensitive indicators of ammonium and nitrate released from green manures with low C/N ratios. European Journal of Soil Biology 77: 4 - 8.

Chapter 4. León Castro, L., Whalen, J. K. 2016. Tillage intensity promotes different dynamics of nitrogen supply from green manure mixture to arugula (*Eruca sativa* L.) (In preparation for Renewable Agriculture and Food Systems)

Chapter 5. León Castro, L., Whalen, J. K. 2016. Decomposition of green manure mixture (field peas-oats) as affected by particle size and simulated root exudation (In preparation for Soil Biology and Biochemistry)

CONTRIBUTION TO KNOWLEDGE

One of the requirements for a Ph.D. thesis is a contribution to original scholarship. The research presented in this thesis provides the following original contributions to knowledge:

1. I quantified the plant-available N dynamics in fields under organic agriculture production on a working farm in Quebec, Canada. My research is the first that uses ion exchange membranes burial and retrievals on weekly bases to evaluate plant-available N dynamics for a short season crop, and to study the relationship between ion exchange membrane measurements and temperature, rainfall, and arugula N uptake in agroecosystems that rely on green manure as a readily-available source of N.

2. I designed a greenhouse experiment to evaluate the plant-available N release from green manure residue with low C/N ratios, in two soil types. Using ion exchange membranes, I showed that this tool is able to detect minor changes in the soil mineral N concentration, and also can detect the modulating effect of soil texture on the N mineralization of the green manure residues. These findings further confirm that ion exchange membranes are a sensitive tool for evaluating plant-available N dynamics.

3. I designed a field experiment to test the effect of tillage intensity on time release of plant-available N from green manure residue. My original finding was that 2 passes of rototiller would be sufficient to meet the N demand of arugula, where the possibilities were 1, 2, and 4 passes. Using ion exchange membranes, I provide evidence that IEMs buried on weekly bases are correlated well between soil mineral N and arugula N uptake. I also noted there were significant amounts of plant-available N released from the green manure residue following harvest of arugula (which absorbed about 20% of the N input from the green manure). This leads

me to suggest that farmers can use ion exchange membranes to detect post-harvest soil N levels in horticultural cropping systems and use this information to make decisions (e.g., to plant another crop and absorb the residual soil N, rather than let it be lost to the environment).

4. In a greenhouse experiment, I related N mineralization from green manure to its physical size (residue size < 4 mm was the best indicator). The relationship between N mineralization and residue size is poorly understood and seldom investigated in the literature, although my work suggests that residue size could be a rapid indicator of N mineralization potential. I also documented a priming effect of arugula root exudates and glucose on microbial biomass that was linked to N mineralization from newly-incorporated green manure residue. Practically, my research contributes to the development of in-field indicators, based on residue size and microbial biomass measurements, to predict N mineralization from green manure.

ACKNOWLEDGEMENTS

I am truly thankful to my supervisor Dr. Joann K. Whalen, for her guidance during this period in my studies. Dr. Whalen has the personal and professional attributes I strive for. I will always consider it a tremendous honor to have been under her tutelage. I am grateful to Hicham Benslim for the technical guidance, Hélène Lalande for providing me with her scientific insight, as well as Mr. Khosro Mousavi for providing the logistic provided during my experiments.

I would also like to thank the Secretariat for Higher Education, Science, Technology and Innovation of Ecuador for my postgraduate award, and providing me the opportunity to live my dream and reach one of my goals.

I am honored to have met and worked with such a brilliant lab group. Thank you all, your scientific inputs were of enormous value to me.

During my time in Canada, I had the chance to meet many wonderful people. I would like to thank to Amy, and Magda, for being the best roommates, I could not have gotten any luckier.

I dedicated this accomplishment to my family. Gracias, papá, mamá y hermanitas, sin su apoyo y amor nunca hubiera sido posible todo este trabajo. To my brother Nick, even though you are not with us, your words of encouragement and wisdom always will be remembered. Lastly but not least, I would like to thank to my fiancée Bethany for loving me and supporting me throughout my studies, especially during the difficult times.

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GENERAL INTRODUCTION

Green manure is any single or mixture of crops grown on agricultural land that will be terminated and incorporated to improve soil fertility or soil quality. When used to enhance soil fertility, green manure generally includes a legume that can fix atmospheric nitrogen (N_2), such that the decomposing green manure residue is a source of plant-available N for a subsequent crop (Janzen and Schaalje, 1992). Additionally, green manure crops scavenge mineral N and other elements from the soil in their biomass, and upon decomposition this supplies plant-available nutrients to following crops in the rotation. From the perspective of soil conservation, green manure also serves as a cover crop that enhances the formation of stable soil aggregates and protects soil from erosion (Baggs et al., 2000) and may be recommended as an alternative to fallowing, particularly in soils that are susceptible to wind and water erosion. Green manure crops contribute to carbon sequestration during their active growth (i.e., when root-derived photosynthates are metabolized by soil microorganisms) and when they are decomposed, as a portion of the carbon contained in green manure residues is transformed into stable soil organic carbon through physical, chemical and biochemical processes (Whalen et al., 2014).

Organic farmers in Canada rely on green manure as a primary N fertilizer (Agriculture and Agri-Food Canada, 2011), which may be supplemented by other fertilizers on the "Organic Production Systems Permitted Substances List" published by the Canadian General Standards Board (National Standard of Canada, 2006a; National Standard of Canada, 2006b). The number of organic farmers increased 4.4% from 2006 to 2011, with approximately 3,713 producers, representing 1.8% of all farms in Canada (Statistics Canada, 2012a). In Quebec alone, there were 1,037 certified organic farms that produced forage and green manure on their farmland, and the land area under these cropping systems represented 2.0% of total agricultural land area in

Canada in 2011 (Statistics Canada, 2012b). Conventional producers may also find green manure to be a cost-effective option to meet their N fertilizer requirements from an on-farm source. In recent years, conventional producers have seen considerable volatility in the price of inorganic N fertilizers, which is related to the price of natural gas (e.g. CH_4) used as a source of hydrogen and energy in the Bosch-Haber process ($N_2 + 3H_2 => 2NH_3$). For example, the usage of urea-N fertilizer increased at an annual growth rate of 4.8 % from 2010 to 2014 and reached a record price of $1000 per tonne in 2014 before fossil energy prices plunged to a low of $2.65 per gigajoule (GJ) of natural gas in 2013 (Agriculture and Agri-Food Canada, 2012a). Since fertilizer purchases represent 11.7 % of the cost of operation on conventional farms, risk-adverse producers may consider planting more green manure to meet their demands for N fertilizer (Agriculture and Agri-Food Canada, 2016)

Green manure is considered to be a source of N when it supplies plant-available NH_4^+ and NO_3^- to the next crops grown in the rotation. After terminating the green manure through natural means (e.g., senescence following a killing frost) or artificial methods (i.e., crimping, mowing or herbicide application), the crop residues are incorporated into the soil through tilling or left on the soil surface to be processed by soil macrofauna. The fragmenting and mixing of a green manure in the soil exposes the residues to the action of soil microorganisms. Saprophytic microorganisms hydrolyze the complex polysaccharides and proteins contained in the crop residue, utilizing the carbon-rich compounds as a source of energy and mineralizing NH_4^+, which may subsequently undergo ammonia oxidation and nitrification to yield plant-available NO_3^- (Gaskell and Smith, 2007; Whalen et al., 2014). Given the diversity of single and mixed crops used as green manure by farmers across Canada, it is challenging to predict how quickly the NH_4 will be mineralized from various green manures under different soil and climatic conditions. This

fundamental information is needed to develop decision-making tools that allow producers to select the correct time to terminate and plow down their green manure, such that they maximize the crop N uptake from the green manure fertilizer (Thorup-Kristensen et al., 2003; Thorup-Kristensen, 2006).

The factors that affect decomposition and N release from green manure were revised, particularly focusing on the biochemical composition of the green manure, climate conditions, biological activity and agriculture management methods. Methods to evaluate the N availability rate from green manure to subsequent crops were critically evaluated. The literature review is then followed by four experimental chapters that quantify 1) the soil N availability and N uptake by arugula after the incorporation of green manure mixture using ion exchange membranes as a tool; 2) the use of ion exchange membranes as sensitive indicators of ammonium and nitrate released from green manure with low C/N ratios; 3) the management of green manure mixture by tillage practice to produce different levels of N supply to arugula assessed with ion exchange membrane; and 4) effect of root exudates on N mineralization after the incorporation of green manure mixture.

The general objective of this thesis was to quantify the N plant availability after the incorporation of green manure.

The specific objectives were:

Objective 1: There are two objectives in this experiment, 1) use IEM-NH_4-N and IEM- NO_3-N concentrations as a tool to monitor the seasonal variability in soil mineral N of two soils (sandy clay loam, sandy loam) planted with field pea-oat green manure and after green manure incorporation, as related to climatic variables, e.g., soil temperature, soil moisture, rainfall, and

2) determine if IEM-NH_4-N and IEM- NO_3-N concentrations were related to the growth and N uptake of arugula, a short-season leafy herb commonly known as salad rocket, that was planted 2 wks after incorporation of the field pea-oat green manure.

Hypothesis 1-i. In weekly measurements the climatic conditions and management practices impact the IEM concentration adsorption

Hypothesis 1-ii. After the incorporation of green manure the plant available N adsorbed on IEMs increases throughout the arugula growing season

Objective 2: Evaluate IEMs as a sensitive indicator of NH_4-N and NO_3-N dynamics in two soils (sandy clay loam and sandy loam) amended with green manure residues having low C/N ratios (C/N=8 and C/N=12).

Hypothesis 2-i. Green manure residue with low C/N ratio <12 release the same concentration of plant N available

Hypothesis 2-ii. The N supply from residues (C/N=8 and C/N=12) measured with IEMs reveal a similar pattern

Objective 3: Determine whether N release from green manure is enhanced by increasing tillage intensity (i.e., more passes with the cultivator during the plow-down event), as well as the impact on microbial concentration.

Hypothesis 3-i. Four passes of tillage will result in greater N availability and green manure residues remaining at the end of the experiment.

Hypothesis 3-ii. Arugula meets the optimal N requirements when four tillage passes are used.

Objective 4: To assess the potential contribution of root exudates from arugula on N mineralization after the incorporation of green manure mixture (peas-oats)

Hypothesis 4-i. The net N mineralization is in the same magnitude in the presence of arugula and the glucose applied

Hypothesis 4-ii. The microbial biomass concentration performs the similar pattern in the three carbon sources, higher in residues size 0.5 to 1 mm, followed by 2 to 4 mm manure residue size, and lower in bare soil.

FOREWORD TO CHAPTER 1

Chapter 1 is a literature review that describes the decomposition pattern of green manure residues that are incorporated in the soil as a source of nitrogen for subsequent vegetables crops. The biotic and abiotic factors that influence the decomposition pattern are discussed. Methods of green manure incorporation are discussed. Finally, I describe the analytical methods and tools that might be appropriate to monitor the nitrogen mineralization in green manure-amended soils, and conclude with recommendations for future research that are the basis for the work presented in my Ph.D. thesis.

CHAPTER 1.

Literature Review

Study of green manure decomposition and nitrogen release in relationship to the nitrogen demand of crops

1.1 Abstract

Green manure is broadly defined as any plant that, when plowed down into an agricultural soil, decomposes and releases nitrogen (N) for the next crop. Green manure is the primary source of N fertilizer on organic farms in Canada. Common plant species used as green manure include peas (*Pisum sativum* L.), red clover (*Trifolium pratense* L.), oats (*Avena sativa* L.) and black lentil (*Lens culinaris* L.), which can be grown alone or in mixtures. Mixed green manures are preferred because they can achieve better nutrient recycling, as legumes fixed N and non-legumes uptake N. It is desirable to include legumes that fix atmospheric nitrogen (N_2) when selecting green manure crops because these bring a new N input to the agroecosystem when recently fixed N is released from the decomposing plant residue. It is challenging to predict how quickly the N will be mineralized from various green manures because each crop residue has a distinct chemical composition and the residue decomposition is affected by site-specific soil and climatic conditions. However, there are tools (e.g., ion exchange membranes and ^{15}N isotope) that respond to the variations in biotic and abiotic factors. Finally, management strategies need to be considered to optimize and synchronize the N supplied from green manure with the N required by the following cash crop, such as incorporation time and choosing the appropriate green manure crop.

1.2 Introduction

Green manure refers to a plant that is grown purposefully as a soil amendment, rather than to produce a marketable crop. Many green manures are selected for their ability to enhance soil fertility through the addition of plant-available nutrients. Legumes are well-known for their ability to furnish nitrogen obtained from the atmosphere via symbiotic nitrogen fixation. Many non-legume crops (e.g., rye grass (*Lolium perenne*), mustard (*Brassica campestris Moench*), and buckwheat (*Fagapyrum esculentum* L.) absorb appreciable quantities of soil mineral nitrogen during their growth, and this is released as plant-available N when the green manure decomposes (Table 1-1). Both crop types can be used, individually or in mixture, to enhance the soil N supply to subsequent marketable crops grown in the rotation. The recycling of soil N (in all green manures) and fixation of atmospheric N_2 (in green manures that include legumes) from green manure residues will reduce the amount of inorganic N fertilizer needed to support crop production (Doran and Smith, 1991). It should be noted that green manures are not a replacement for inorganic N fertilizers, since the N recovery from green manure by the following crop is often < 35% (Sharma and Behera, 2009, Sjursen et al., 2011, Vaisman et al., 2011).

Green manures can contribute an appreciable amount of plant-available N at key growth stages of the subsequent crop. Janzen and Schaalje (1992) reported that nearly 24% of the N applied in lentils was assimilated by barley after 45 days and 32% was recovered by barley grown to maturity. Likewise, Seo et al., (2006) observed 15% N recovery from hairy vetch through N uptake in silage corn grown in the first season after the green manure was incorporated. In contrast, green manures that do not contain legumes (e.g., oats, ryegrass, sorghum sudangrass, and buckwheat) mainly add organic matter and recycle soil N in their

abundant foliage and root biomass. The N content of non-leguminous green manure is limited by soil N availability. In two growing seasons, Vyn et al., (2000) reported N content in rye, oilseed radish, and oats varied from 12 to 31 kg N ha^{-1}, whereas red clover was between 67 and 98 kg N ha^{-1}. Further details about the N input that can be achieved with various green manures, grown alone or in mixtures, are provided in Table 1-1.

Although green manure only provides a fraction of the N required by the subsequent crop, the cumulative effects of repeated green manure applications in crop rotations are expected to have other significant benefits due to an increase in microbial activity and diversity (Dick, 1992). Green manure applications result in the build-up of N in transient pools (e.g., water-soluble organic N and mineral N) and pools with short-term stability (e.g., particulate organic matter and macro-aggregate fractions) that contribute to the soil N supply (Nyiraneza et al., 2010). Several agronomic methods are proposed to boost the N content of green manure and the amount of N in green manure residues that is transferred to the subsequent crop. These are: 1) association/mixture of green manure, 2) rotating with a cash crop, and 3) intercropping system.

1) Association/mixture of green manure: Legumes are more effective in green manure mixtures with non-legumes than a sole crop, because there is higher N concentration by the two crops; the N fixed by legume and the N uptake by the non-legume. Jannink et al., (1996) observed in a two year field experiment that pea grown alone produced residues containing 17 g N m^{-2}, whereas when it was grown together with oats and vetch mixture, the pea residues contained 20.3 g N m^{-2}. Another advantage of mixtures is that the deep root system of non-legumes has greater capacity of intercept/absorb more residual soil NO_3-N from the soil profile. Fowler et al., (2004) reported at 124 days after sowing NO_3-N losses by 0.005 mg N litre $^{-1}$ for

lupins compared to 0.061 mg N litre^{-1} for oats-lupins in a silt loam soil. Thus, optimization of the N supply by green manure can be accomplished by planting in mixture.

2) Rotation with a cash crop: A rotation system is designed mainly to build soil structure, recycle of nutrients, and maintain soil moisture; alternatively, this system can also act as a weed and pest suppressor. In a rotation system the incorporation of green manure increases the N availability for the subsequent crop. Bergkvist et al., (2011), reported in a barley cultivar an increment of soil mineral N by preceding white clover (24 kg N ha^{-1}), while 19 kg N ha^{-1} without green manure crops (Table 1-1).

3) Intercropping system: This system is used mainly in orchards. As the green manure grows between the rows of perennial crops such as apple or peach with the main purpose of maintaining soil cover. This practice has the benefit of protecting the soil from erosion, adding organic matter, weed suppression, reduction of insect population, and increase yields by the N input when legumes are included in the green manure mixture. In an apple cv. Royal Gala/EM26 cultivar using different green manure crops, Sanchez et al., (2007) reported in three years a yield increment from 32.6 to 57.9 t ha^{-1} from alfalfa (*Medicago sativa* L.) plus fescue (*Festuca arundinacea* Schribn.); from 34.4 to 57.0 t ha^{-1} from vetch (*Vicia sativa* L.); and from 37.1 to 54.4 t ha^{-1} from strawberry clover (*Trifolium fragiferum* L.).

1.3 Decomposition of green manure

The most common practice to incorporate green manure crops is tillage. Farmers use moldboard plows or chisels plows with sweeps, followed by disking, harrowing or other secondary operation, to terminate the green manure, physically fragment the residues and bring them into intimate contact with the soil. The increasing popularity of reduced tillage, which

minimizes the soil disruption and the fragmentation of crop residues (Madgoff and Weil, 2004), has consequences for the decomposition of green manures because it leaves the residues on the soil surface or shallowly incorporates them without much physical abrasion. Hence, crop residues are left mostly intact and with a larger particle size in reduced tillage systems. After this mechanical practice, the initial process of decomposition takes place. On the soil subsurface, the macrofauna, such as earthworms, millipedes, termites and isopods (primary fragmenters) break down plant residues through the consumption and burial of these materials. Comminution is a form of "external rumen digestion" whereby soil fragmenters mix residue fragments into the soil profile, increasing food availability for soil microfauna and mesofauna (Yang et al., 2012). At the beginning, soil microbes have access to simpler compounds (water-soluble compounds, mostly simpler sugars and peptides) that are assimilated and respired. Subsequently, more complex organic compounds in the residue are attacked by excretion of extracellular enzymes. The more labile organic compounds are degraded quickly (i.e., in a matter of days or weeks) whereas the recalcitrant organic compounds can remain undecomposed for months, years or longer periods of time. The relative lability of organic compounds within green manure residues may proceed in the following sequence: starch > pectin and proteins > hemicellulose > unprotected cellulose > lignin and protected cellulose > suberin and cutins. After these plant polymers are cleaved into monomeric units in the soil solution (e.g., phenols, amino acids), the byproducts are absorbed by microorganisms for intracellular hydrolysis. Amino acids and amides containing N undergo mineralization to release NH_4 and CO_2 as a product of respiration (Whalen, 2014). Mineralized NH_4 is released into the soil solution and can be used by plants or undergo ammonia oxidation/nitrification by microorganisms (Fig. 1-1).

1.4 Factors affecting decomposition

Soil type, temperature, water availability, green manure characteristics and management (e.g., planting density and timing, mowing) may influence the potential of N mineralization from green manure (Cabrera et al., 2005). Green manure decomposition results from the relationships between the plant material (biochemical characteristics) and the environmental conditions (soil, climate). Here the interaction of the biotic and abiotic factors that lead to N mineralization from green manure residues is explained.

1.4.1 *Climate*

Due to the lack of studies that looked particularly at the effect of temperature on N mineralization from green manure, it should be pointed out how temperature affects the crop residue decomposition and mineralization. Residue placement influences the soil temperature such that residues retained on the surface in no tillage system decrease the soil temperature (Nyborg and Malhi, 1989). Franzluebbers et al., (1994) observed that sorghum residues emitted similar amounts of CO_2 from no tillage and conventional tillage system, with values of 2.04 and 1.84 g CO_2-C m^{-2} day^{-1}, respectively. Changes in soil temperature, moisture levels, and other factors influence microbial activity, which alters decomposition rates (Bronick and Lal, 2005). Quemada et al., (1997) reported more N mineralization when temperature and water content increase from 0.054 g N m^{-2}, 10 °C, -4.96 MPa to 0.299 g N m^{-2}, 35 °C, -0.003 MPa from crimson clover applied on the surface.

In field conditions, the wetting and drying that occurs due to variation in rainfall affects microbial activity, soil aggregation and residue decomposition (Cosentino et al., 2006). Consequently, dry-wet cycles promoted the turnover of C derived from incorporated residues,

mainly due to the enhanced turnover of microbial products (Van Gestel et al., 1993). In accordance with Cabrera et al., (2005), drying and rewetting events are likely to impact more on surface residues than incorporated residues, which partially accounts for the lower decomposition and mineralization rates observed in surface residues.

1.4.2 *Residue quality*

The quality of green manure can be defined as the labile fraction content that is easy to decompose and mineralize, as well as the physical size fraction that is accessible to rapid colonization and degradation by decomposers (Broder and Wagner, 1988; Giller et al., 1997). Thus, both chemical and physical characteristics are part of residue quality.

1.4.2.1 *Physical characteristics*

The physical size of the residue and the contact between residue and the soil matrix are factors that influence decomposition pattern. The reduction of physical residue size is achieved by farmer practices (e.g., tillage, shredding, and mulching). Shredding and mulching reduce the particle size and leave a thick layer of residues at the soil surface, whereas tillage will mechanically reduce the particle size and mix the fragmented residues with the soil matrix and into the topsoil (0-15 cm layer). The mixing depth is a function of the tillage implement used, where inversion plowing effectively buries residues at the bottom of the plow layer (e.g., about 15-17 cm deep, depending on the setting used by the farmer) and harrows/rototillers result in a relatively even distribution of residues within the topsoil layer, to the harrow depth setting (e.g., from 5 to 12 cm deep, according to farmer practices). Some residues may remain at the soil surface when harrows and rototillers are used, depending on the quantity of residue that is present in the field.

Once in contact with the soil, residues are expected to decompose and mineralize relatively quickly with some differences according to particle size. For instance, using ^{15}N-labelled barley, Ambus and Jensen (1997) tested N mineralization of residues having two sizes, ground (< 3 mm) and cut (25 mm). The ground barley released 3.3 mg N kg^{-1} soil, due to faster N mineralization than the cut barley (2.7 mg N kg^{-1} released in 60 d incubation). Because the location of the residues through the soil profile is affected by tillage and other management practices like shredding and mulching, the N mineralization from residues differ in the soil profile. In a tillage system, Franzluebbers et al., (1994) observed N mineralization between 0.9 and 2.1 mg N kg^{-1} soil d^{-1} in the 0-5 cm soil depth, which was greater than the 0.6 to 0.9 mg N kg^{-1} soil d^{-1} at the 12.5 to 20 cm soil depth. Notably, microbial community is impacted by

particle size and location of residues. There is a higher diversity of microoorganisms in smaller residue fractions than in coarser residue fractions due to the higher surface area exposed for microbial colonization (Bending and Turner, 1999). The microbial activity is also expected to be greater, meaning that demand for carbon will result in more action of hydrolytic enzymes that release the amino acids and amides that microorganisms metabolize to acquire NH_4.

1.4.2.2 *Chemical characteristics*

Crops residues are composed by biochemical characteristics, which affect the assimilation by decomposers. After the incorporation of the green manure, the low molecular weight compounds of plant residues (e.g., amino acids, sugars, and soluble minerals) are susceptible to undergo to depolimerization, thus are intimately related in the first decomposition period (Martens, 2000). In a laboratory incubation study, using Italian ryegrass (*Lolium multiflorum* L.) green manure, Andersen and Jensen (2001) observed that the content of water soluble compounds decreased from 32 to 9% within the first 35 d. Microorganisms use carbon for energy and N for protein synthesis, hence the C/N ratio of the substrate influences the rate at which N (and C) are released and mineral N is made available for plant uptake (Hodge et al., 2000; Quemada and Cabrera, 1995). Crops residues with a high C/N ratio (< 25) induces immobilization and the increase of fungi as decomposers, whereas crop residues with a low C/N ratio (>25) promote the bacteria population. Using legumes, Marstorp and Kirchman (1991) reported N mineralization between 30 to 35% when C/N ratio ranged from 12-14, whereas mineralization decreased from 20 to 17% when C/N ratio was between 20 – 18. Green manure normally is incorporated as a young plant, therefore containing a low proportion of recalcitrant compounds (e.g., lignin) and a low C/N ratio (Thorup-Kristensen et al., 2003). However, lignin/N ratio can be used as an index of decomposition and reflects the delays in decomposition

as secondary cell walls form and ligno-cellulosic compounds are produced in the plant material. Not only do these compounds provide structural integrity to the living plant, but they impede the decomposition of crop residues. Yanni et al., (2011) observed that the lignin/N ratio was a good predictor of N mineralization potential, using corn residues in a 36 d study. This is due to the fact that early decomposition is fueled by labile organic compounds such as starch and peptides; once those supplies of energy and nitrogen are depleted, the microbial community will begin to synthesize hydrolytic enzymes to degrade recalcitrant, carbon-rich lignin.

1.5 Soil properties

Soil properties are the physical, chemical, and biological attributes of a soil that may affect decomposition. Briefly, the soil physical properties determine how residue volume can be accommodated in the soil matrix, how heat transfer occurs, and how water and air flows through pores around those residues, thus creating a physical environment for chemical and biochemical reactions. Soil chemical properties describe the soil reaction (e.g., due to pH and redox states) as well as the chemical binding that occurs between residue constituents and the soil organo-mineral surfaces. Soil biological properties refer to the action of soil microorganisms and fauna, which alter the size of residues and biochemically degrade the residues to release inorganic nutrients, such as NH_4 and $NO_{3,}$ into the soil solution. The diversity and activity of the soil biota, which function in the context of the soil physico-chemical environment, determine the speed at which residues are mineralized. Through their interactions with plant roots, the soil biota act to facilitate N uptake by the plant. Residue management is done with the goal of improving the overall soil fertility and productivity of crops growing on that soil.

1.5.1 *Soil physical properties*

Soil texture has a major influence on decomposition of residues and N mineralization. However, the role played in accordance to their relatively high inherent fertility, nutrient, water retention capacity, and microbial biomass (Kumar and Goh, 1999). In a 6 weeks experiment, Pare and Gregorich (1999) reported 12 and 15 % of N mineralized in clay soil and 6.5 and 11 % in sandy soil, from maize and soybean residues, respectively. Contrary, using wheat residues, Scott et al., (1996) reported higher N mineralization in sandy soils than loam soil. Therefore, the N mineralization in not based exclusively only in soil texture. For instance soil moisture influences the activity soil microorganisms that are intimately related with residue decomposition (Kieft et al., 1987). Using crop residues, Das et al., (1993) reported more N mineralized at field capacity than 50 % of the field capacity. The low water availability impacts negatively the microbial activity, causing a minimal intracellular water potential that resulted in low hydration and enzyme activity, thus affecting the mineralization of crop residues.

Soil compaction affects the total soil pore volume and changes the pore size distribution, resulting in a higher percentage of small pores. In these small pores, organic materials may be physically protected, and microorganisms may be inaccessible to predating protozoa and nematodes (Elliott and Coleman, 1988). In a 98 d experiment, Breland and Hansen (1996) reported a reduction of ^{15}N mineralization of white clover residues by 18 %, in sandy loam soil at bulk density of 1.4 g cm^{-3}, in addition the compaction increased the retention of ^{15}N added by 1 % in the microbial biomass. The authors attribute the results to the reduction of volume pore, resulting in an increment of the soil capacity to protect microbial biomass and metabolites against further degradation of crop residues.

1.5.2 *Soil chemical properties*

Soil chemical reactions such as pH, cation exchange capacity (CEC), and electrical conductivity (EC) directly affect the biological activity and action of hydrolytic enzymes that release NH_4 from organic compounds. The soil pH at extreme levels affects biological activity by interfering with enzyme catalysis, therefore it is important to maintain it at near neutral conditions for optimal biological functions and crop production. The N mineralizing bacteria are most active when soil pH ranged from 6.5 to 8, and decomposer fungi grow optimally from pH 5.5 to 6.5 (Whalen and Sampedro, 2010). The application of green manure residue can significantly increase soil pH because base cations (e.g., Na, Ca, and Mg) released from the water-soluble fraction of plant residues replace H^+ on reactive surfaces in the soil. Xiao et al., (2014) reported a change in the soil pH from 4.25 to 5.5 after the incorporation of vetch residues. Electrical conductivity can influence soil N availability because soil salinity creates a suboptimal environment for soil microorganisms (Sardinha et al., 2003), although, soil salinity does not affect enzymes (e.g., proteases, amidases, and deaminases) that are related with N mineralization (Pathak and Rao, 1998). Ninety days after the incorporation of *Sesbania*, Pathak and Rao (1998) reported a decrease of ammonification from 104 to 68 mg kg^{-1} when the soil salinity was >97 dSm^{-1}, whereas nitrifiers were completely inhibited at >16 dSm^{-1}.

1.5.3 *Soil biological properties*

Decomposer biota comprise communities of both microflora and fauna, these two living organisms perform a number of different roles in the detritus subsystem. Their activity ensures the decomposition of organic matter in soil. During the decomposition process the energy and

nutrient are transferred between the trophic levels; this interaction is known as food chain (Whalen and Sampedro, 2010).

1.5.3.1 *Soil fauna decomposers and accelerators of N mineralization*

The easier access to crop residues or decaying organic matter by saprophagous mesofauna and macrofauna (collembolan, mites, and earthworms) make them the first decomposer of the trophic level. In this group of organisms, the earthworms are a good example of the energy and nutrient transfer among the trophic groups. Earthworms and free-living soil microorganisms live in a mutualistic relationship in the soil food web. Free-living microorganisms function at a low metabolic level. When they are in contact with earthworms the microbial growth activates, due to the low molecular weight mucopolysaccharide secreted by earthworms. Likewise, the physical breakdown of the residues resulted in accessible size for microbial colonization and further mineralization (Fig. 1-1) (Whalen and Sampedro, 2010).

1.5.3.2 *Soil microorganisms and the role in N mineralization*

The primary decomposers (bacteria and fungi) soil bacteria are responsible for most nutrient transformations in the soil, such as, N transformations (e.g., chemoautotrophic and heterotrophic nitrification, dissimilatory NO_3 and NO_2 reduction and denitrification, symbiotic and asymbiotic N_2 fixation) (Beare et al., 1997). Most of these transformations depend directly on the availability of C or N derived from the breakdown of crop residues. Bacteria and fungi possess the capability of breaking down complex organic compounds by enzymatic activity. For instance fungi (e.g., Basidiomycetes) is responsible for the breakdown of complex polyaromatic

compounds such as lignin, humic acids, and phenolic acids; these compounds are present in higher percentage in plants with advanced maturity (Sánchez, 2009).

1.6 Green manure management to improve N supply to meet N crop demand by the subsequent crop

Crop nutrient demand varies throughout the growing season, therefore it is essential to complement nutrient supply with plant demand, especially with amendments that are considered slow release source of N (e.g., green manures). As the N mineralization from green manure is a function of multiple factors (described in the previous sections), it is challenging to predict the pattern of N release from green manure in relation to crop N demand. Some general rules can be helpful in describing the relationship between soil N availability and crop N uptake. First, it is important to determine at which stage of vegetative growth the crops demands more N. Most crops have the highest nutrient demands in the early growth stage, between 3 - 4 leaf and flowering, when they acquire the N that will be used to build developing fruits and seeds. Approximately 50 to 90 % of N in the plant at the time of flowering moves from the leaves to stem to develop the seed (Jones et al., 2011). Zotarelli et al., (2008) observed the N uptake pattern in a sweet corn (*Zea mays* var. rugose, cultivar 'Saturn'), which was very low during the first week 0.5 to 2 kg N ha^{-1}, followed by an increment between 6 -23 N ha^{-1} at fully emerged growth stage.

Second, the N supply by the green manure crops will depend on the decomposition pattern of the green manure residue. The N mineralization from green manure occurs rapidly, in that most N mineralization from green manure with low C/N occurs within 1-2 months of its incorporation (Thorup-Kristensen et al., 2003). For instance, Lenzi et al., 2009 reported more N

mineralization in tomato (*Lycopersicon esculentum* Mill. cv. *Palinuro*) –a short seasonal crop- from pigeon bean (64 kg N ha^{-1}, C/N = 15) than rye (0.6 kg N ha^{-1}, C/N = 24), brown mustard (6 kg N ha^{-1}, C/N = 20), flax (not det'd kg N ha^{-1}, C/N = 30) and oats-barley (14 kg N ha^{-1}, C/N = 28) (Table 1-1). Similarly, the termination (i.e., plow down) date is an important factor to consider because this relates to how long the green manure is left growing in the field and its state of maturity at the time of termination. McCauley et al., (2012) reported more N fixed by pod than flower termination time of lentils and peas (30 and 65 kg N ha^{-1}, respectively). In summary, the optimization of the use of green manure can be achieved by balancing the maximum N input from green manure with the speed that green manure residues will decompose, and also overlap the maximum N input from green manure with crop N demands. Thus, during the mineralization process there should be a balance between N immobilization vs. N mineralization, with higher N mineralization > N immobilization at the initial growth stage of the crop, when plant N demand is high, followed by N mineralization < N immobilization at the ripening growth stage when N demands decrease.

1.7 Field methods to assess soil N supply from green manure

From the previous sections, it is clear that we need to determine how much plant available N is present in the field, for the cash crop, throughout the growing season. We are also interested to know what proportion of the soil N supply is derived from green manure, since the idea is to give a N credit to the green manure crop. Here, I describe several *in situ* methods that are used to assess plant available N. The methods are appropriate for field studies and can predict the amount of organic N that will be mineralized from green manure residues to plant-available N forms during the growing season.

1.7.1 *Ion Exchange Membrane*

Ion exchange membranes (IEMs) are ion-selective membranes comprising cross-linked copolymers of vinyl monomers. Ions exchange membranes contains negative charged groups (e.g., $-SO_3^-$, $-COO^-$, $-PO_3^{2-}$, $-PO_3H^-$, $-C_6H_4O^-$) for cation exchange membranes, whereas anion exchange membranes are positively charged with groups, such as $–NH_3^+$, $-NRH_2^+$, $-NR_2H^+$, $-NR_3^+$, $-PR_3^+$; these groups fixed to the membranes permit the passage of opposite charge ions and repels the like charges (Xu, 2005). These membranes are used to adsorb/intercept ions (cations and anions) from soil solution applying the Electrostatic Attraction principle. Thus, when the membranes are placed in contact with soil solution, the soil ions displace the initial counter-ions on the membrane based on the principle of ion exchange. The diffusion gradient formed induces further movement of ions to the membrane surface, which continues until equilibrium is attained between ions in soil solution and ion exchange sites (Bremer et al., 2014). This dynamic of capture of the plant available ions result in a cumulative adsorption over time, which is differentiated from the static measurement by the soil extraction method.

The ions absorbed are reported as μg (or μmol) per cm^2 for the time of direct contact/burial (e.g., 1h, 1 week, 2 weeks) (Qian and Schoenau, 2002). Long term burial of membranes strips with a surface area between 8 to 15 cm^2 shown to be optimal (Ziadi et al., 1999; Qian and Schoenau, 2002). For instance Qian and Schoenau (1995) reported a closer relationship ($r^2 = 0.86$) between anion exchange membranes and canola N uptake at 2 weeks after burial as compared to a 1 h burial. The cumulative adsorption/ interception of ions over time allow quantification and description of the of soil N availability pattern, resembling the action of plant roots. Generally, the placement of IEMs in the same environmental and edaphic conditions where plant roots grow has shown a good correlation between IEM and N crop uptake (Ziadi et

al., 1999; Nyiraneza et al., 2011; Cambouris et al., 2014). In a field experiment, León and Whalen (2016) reported on a weekly basis sampling/retrieval in two soil types, a correlation between IEM-NO_3-N adsorbed on anion exchange membranes and arugula N uptake ($r = 0.86$, $p < 0.01$) in sandy clay loam and ($r = 0.71$, $p < 0.05$) in sandy loam. Despite the advantages of IEMs, there are some limitations that need to be considered. Soil moisture and temperature variation affect the ion adsorption on IEMs. For instance, the variation of soil moisture, particularly dry environments affects the diffusion flux of ions in soil solution impacting the plant root nutrient uptake, in the same way IEMs are influenced. Qian et al., (1996) reported a decrease of N, P, K and S (μg 10 cm^{-2}) adsorbed on IEMs as the soil moisture content decreased from 100% to 15% FC (field capacity). Likewise, the competition between IEMs and plant roots could be a limitation, as plant roots possesses the property of ion exchange capacity, in which the ions are actively absorbed from soil solution and from soil particles surface (Haynes, 1980; Qian and Schoenau, 2002); in this regards the burial time should consider the crop growing season. To exclude root competition a polyvinyl chloride (PVC) cylinder can be used (Huang and Schoenau, 1996). Overall the accuracy of results should be considered since the IEMs are exposed to variations of environmental conditions (explained above), this heterogeneity might cause large coefficient of variations, particularly in experiments conducted in the field. Therefore, is suggested an adequate sampling design to reduce this variability (Qian and Schoenau, 2002).

1.7.2 *^{15}N isotopes*

Isotopes refer to elements with nuclei having equal number of protons, or the same atomic number, but different numbers of neutrons. The stable isotope of ^{15}N is often used as a tracer to determine the transfer or transformation of a specific labelled compound or material (e.g., ^{15}N-NH_4 or ^{15}N as a component of plant residues).

When crop residues are a source of N, the ^{15}N tracer may be used to determine the ^{15}N contribution from the residue to the following crop (measured as the % ^{15}N atom abundance in the growing crop). Thus, nutrient contributions can be quantified using ^{15}N. However, one drawback of this method is that it is not economically feasible at the field scale due to the high cost of preparing enough green manure residues with the ^{15}N tracer. However, a subsample is possible to label, thus N contribution can be determined by labelling plant parts (roots and shoots). Finally, the price of analysis is costly, as an example, to analyze ^{15}N the sample is about $ 8.50 USD per sample (UC Davis Stable Isotope Facility, CA, USA), whereas the analysis of PRS (Plant Root Simulator) probe - a patent model of IEMs - for 17 different nutrients cost $3.50 USD per probe (Western Ag Innovations, SK, Canada).

1.8 Conclusions

The use of green manure as a N source is expected to contribute positively to crop nutrition on both organic and conventional farms, with greater benefits possible on organic farms that have limited options to fulfill their N fertilizer requirements. When used as N fertilizer, green manure residues with low C/N ratio are expected to provide more mineral N for the following crop. Green manure residues that are finely chopped and mixed with the soil should be more effective at delivering adequate N to the cash crop. The timing of the green manure termination and incorporation is selected to ensure the residue decay and N release are synchronous with crop N demands (i.e., peak N release should occur during the vegetative growth stage of the crop). As most prior studies concentrated on green manure in field crops such as maize and long-season vegetable crops such as tomato, there is little information on how green manure contributes to the N nutrition of short-lived annual vegetables such as arugula. First, a description of plant N availability throughout a growing season after the incorporation of

green manure is needed. The use of IEM would allow us to determine the N dynamics in response to soil types and climate variables such as rainfall, soil moisture and temperature. Second, although we know that low C/N ratio of green manure residue is preferable to get fast N mineralization, do all green manure residues with C/N ratio < 25 provide approximately the same amount of mineral N in a given period of time? Third, what is the optimal number of tillage passes needed to produce different size of green manure residues, and how does residue size affect plant available N concentrations and meet crop N demand under field conditions? Fourth, in what magnitude the cash crop plant root system plays a role in N mineralization after the incorporation of green manure mixture.

The following chapters of my thesis will investigate these questions in field and greenhouse experiments. The overarching hypothesis for the work is that N released from green manure residues is modulated by key abiotic factors, namely soil texture, soil moisture and temperature, as well as arugula root exudates and residue characteristics (physical size and chemistry, as indicated by the C/N ratio).

Table 1-1. Nitrogen mineralization from green manure, grown as a single crop or in a mixture. The dry matter content of the green manure, climate conditions for the study and method of incorporating the green manure into soil are provided.

Duration of the experiment	Crop	C/N ratio	Dry matter (t ha^{-1})†	N mineralization (kg ha^{-1})	Climate conditions	Incorporation method	Study
8 months	White clover (*Trifolium repens* L.)	13	1	7	Temperature -1 to 8 ℃	Mouldboard plough	Kirchmann and Marstorp, 1991
	Black medic (*Medicago lupulina* L.)	13	1	9			
	Subterranean clover (*Trifolium subterraneum* L.)	14	1	6			
One year	Red clover (*Trifolium pratense* L. cv. Rajah)		0.9	16	Temperature -4.1 to 19.3℃ 578 mm ‡	Mouldboard plough	Bergkvist et al., 2011
	White clover (*Trifolium repens* L. cv. Riespling)		0.8	22			
	Perennial ryegrass (*Lolium perenne* L. cv. Helmer)		0.2	10			
One year	Buckwheat (*Fagapyrum esculentum* L.)	26	2.1	20	Cold temperate climate	Tillage	N'Dayegamiye and Tran, 2001
	White clover (*Trifolium repens* L.)	24	0.6	15			
	Millet (*Echinocloa crus galli* L.)	35	3.4	15			
	Colza (*Brassica campestri* L.)	20	2	24			
	Mustard (*Brassica campestris* Moench)	23	2.3	14			
Two years	Rye (*Secale cereale* L.)	24	7	0.6	Temperature 10 to 33℃ Rainfall from 1 to 100 mm	Mouldboard plough	Lenzi et al 2009
	Brown mustard (*Brassica juncea* L.)	20	5.3	6			
	Flax (*Linum usitatissimum* L.)	30	1.2	-			
	Pigeon bean (*Vicia faba* L. var *minor*)	15	6.4	64			
	Oats-barley (*Avena sativa* L. and *Hordeum vulgare* L.)	28	5.8	14			
Two years	Phacelia (*Phacelia tanacetifolia* Benth)	15	5.5	22	Cold temperate climate	Mouldboard plough	Sørensen and Thorup-Kristensen, 1993
	Sunflower (*Helianthus annuus* L.)	16	5	19			
	Italian ryegrass (*Lolium multiflorum* Lam.)	13	4.8	38			
Six years	Feedpea (*Pisum sativum* L.)		3.4	23	Temperature 1 to 28℃ 357 mm ‡	Tillage	Biederbeck et al., 1998
	Black lentil (*Lens culinaris* Medik.)		1.8	38			
	Tangier flatpea (*Lathyrus tingitanus* L.)		2.4	30			
	Chickling vetch (*Lathyrus sativus* L.)		3	38			
Green manure mixture							
One year	white clover + rye grass		0.7	16	Temperature -4.1 to 9.3℃ 578 mm ‡	Mouldboard plough	Bergkvist et al., 2011
	red clover + rye grass		1	15			
One year	peas + oats		5.8	29	Temperature 15℃ 209 mm ‡	Tillage	Vaisman et al., 2011

† above ground biomass
‡annual mean precipitation

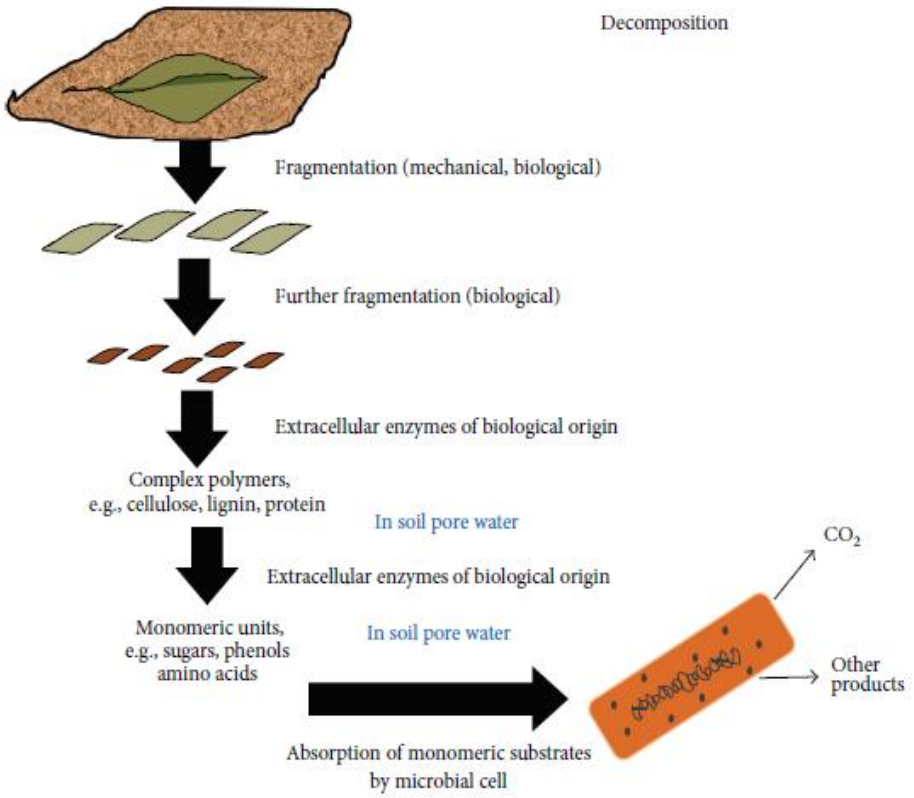

Figure 1-1. Conceptual model of the decomposition of green manure residue (represented by the leaf on the soil surface). Residue fragmentation occurs by biological or mechanical practices (e.g., tillage). Smaller residues are colonized by microorganisms, subsequently ingested by soil meso-fauna. Once the primary cell wall is degraded, compounds (cellulose, lignin, and proteins) contained in the secondary wall are released in soil solution. These complex compounds are hydrolyzed by extracellular enzymes into monomeric units like sugars, phenols, and amino acids. Finally these monomeric units are absorbed by microbial cells and undergo intracellular enzymatic hydrolysis, resulting in the production of CO_2 and other byproducts, including NH_4, the product of N mineralization (Whalen, 2014).

FOREWORD TO CHAPTER 2

The literature review revealed the interaction between factors (biotic and abiotic) that play a role during the decomposition process and green manure residues. In the next chapter, I used a green manure mixture and described the N mineralization pattern and the interaction with abiotic factors throughout one season growing arugula in two soils from Quebec, Canada.

This chapter was reformatted for this thesis from the publication: León Castro, L., Whalen, J. K. 2016. Ion exchange membranes as an indicator of soil mineral nitrogen availability and nitrogen uptake by arugula (*Eruca sativa* L.) in soils amended with green manure. Biol Agric Hortic 32: 206 – 220.

It should be noted that the first experiment has deficiencies in its design due to the lack of blocking, insufficient replication and the fact that there was no plant-free control plot. The field investigation was intended as a preliminary, observational study and the fact that it gave me some interesting results – which informed the subsequent studies – led me to include it in my thesis. The learning opportunity afforded by this experiment taught me how to design an experiment correctly, and the subsequent experimental chapters use random assignment, ample replication, and controls for each treatment.

CHAPTER 2.

Ion exchange membranes as a tool to monitor soil mineral nitrogen availability and nitrogen uptake by arugula (*Eruca sativa* L.) in soils amended with green manure

2.1 Abstract

Green manure crops are a common N source in organic agriculture. Producers need guidance on when to incorporate the green manure for efficient N uptake by the following crop. The objective of this study was to quantify the temporal dynamics of soil mineral N following green manure plow-down and N available to arugula (*Eruca sativa* L. sp.) also known as salad rocket, a fast-growing leafy herb used in salads. Green manure was a mixture of field pea (*Pisum sativum* L.) and oat (*Avena sativa* L. sp.) grown on fields, likewise fallow plots were allocated in sandy loam and sandy clay loam soils at an organic vegetable farm in southwestern Québec, Canada. Ion exchange membranes (IEMs) were used to monitor the ammonium (IEM-NH_4-N) and nitrate (IEM-NO_3-N) released following green manure plow-down. The IEM-NO_3-N concentration was positively correlated with rainfall ($r = 0.57$ to 0.95, $p < 0.05$) and was greater from 2-5 wks after green manure plow-down than in soils without green manure. Arugula N uptake and IEM-NO_3-N were positively correlated in the sandy clay loam ($r = 0.86$, $p < 0.01$) and sandy loam ($r = 0.71$, $p < 0.05$) soils. IEM-NO_3-N concentrations were found to be sensitive to weather, green manure decomposition and arugula growth, and could be a tool to monitor soil mineral N available for short-season vegetable crops.

2.2 Introduction

Green manure can be defined as any plant that, when plowed down into an agricultural soil, decomposes and releases nitrogen (N) for the following cash crop. Green manure is also reported to increase soil organic matter content and microbial activity, reduce N loss, control soil erosion and suppress weeds (Biederbeck et al., 1998; Blackshaw et al., 2001, Cheneby et al., 2010; Tosti et al., 2014). However, a green manure cannot fulfill all these functions simultaneously. For example, a rapidly-decomposing green manure is considered to be a source of plant-available N when it stimulates net N mineralization and releases mineral N (NH_4^+ and NO_3^-) into soil solution but it is unlikely to reduce N loss through immobilization at the same time. As a mineral N source, the N recovery from green manure via crop N uptake is affected by the speed at which green manure N is allocated to transient pools, e.g., water-soluble organic N and mineral N, or stabilized in particulate organic matter and macro-aggregate fractions (Nyiraneza et al. 2010). A sandy-loam soil amended with fast-decomposing pea green manure and planted with wheat (*Triticum aestivium* var. Norin 61) showed 9% apparent N recovery efficiency from the pea residues (C/N = 15.6) under greenhouse conditions (Zai et al., 2008). This is consistent with field experiments, where green manure seldom provides more than 20% of the crop N uptake (Sharma and Behera, 2009, Lu et al., 2013). Seo et al., (2006) reported that ^{15}N-labelled hairy vetch green manure (C/N = 14.1) contributed 15% of the N uptake in first-year silage maize and was the source of 3.5% of N uptake in silage maize in the following year. Similarly, Lu et al., (2013) reported that between 6.2 and 21.5% of the N uptake by upland rice was from ^{15}N-labeled ryegrass green manure (C/N = 19.4) after nine consecutive growing seasons.

Since green manure N must be transformed to soil mineral N to be accessible to crops, the challenge is to determine when, and how much, N is mineralized from green manure, considering the particle size and biochemical composition of the green manure residues, soil properties, climatic conditions and the interactions among these variables (Christensen, 1985; Trinsoutrot et al., 2000; Yanni et al., 2011; Whalen, 2014). Field studies show that N mineralized from green manure could be related to their particle size, which controls microbial colonization of the residue, the C/N ratio, an indicator of biochemical recalcitrance to decomposition, and soil microbial biomass (Jensen 1994a; Cookson et al., 2002). Soil texture also affects N mineralization since higher microbial activity is generally reported in clayey and loamy soils than sandy soils (Scott et al., 1996). Finally, Müller and Sundman (1988b) reported that ambient temperature and precipitation were important explanatory variables that accounted for 17 to 25% of the variation in N uptake by barley in plots that received green manure.

Agricultural producers who rely upon green manure as an N source have to select the right time to plow-down the green manure so it releases mineral N in synchrony with crop N needs. The seasonal pattern of N mineralization from green manure, like other organic residues, results in periods where N mineralization exceeds crop N demands, termed "excess-asynchrony" as well as periods when the N supplied by green manure is insufficient to meet crop N demands, termed "insufficient-asynchrony" (Crews and Peoples, 2005). For example, Thorup-Kristensen and Dresbøll (2010) reported that incorporating winter rye (*Secale cereale* L.) green manure in late spring (25 April) contributed 19 kg N ha^{-1} to barley N uptake but this increased to 46 kg N ha^{-1} when the winter rye green manure was incorporated in early spring (10 March). Hence, the fertilizer N value of green manure is affected by the date of its incorporation, since the plough-

down date provides information about the environmental conditions that control green manure decomposition, N mineralization and the soil mineral N available to the subsequent cash crop.

Organic vegetable producers who rely on green manure as a primary N source for short-season crops need to know the pattern of N release after the incorporation of green manure to decide upon a planting date that might allow the crop to achieve maximum N uptake from green manure. Vegetable crops gain appreciable benefits, e.g., high yields and N recovery, from green manure N because the marketable yield components represent a sink for absorbed N, e.g. 45-69% of absorbed N per marketable fresh weight yield in tomato fruits and lettuce leaves (Tei et al., 1999). Similarly, Lenzi et al., (2009) reported that green manures supplied up to 36% of the N recovered in dual-purpose market/processing tomatoes, and could be ranked for their N fertilizer value as: pigeon bean (*Vicia faba* L. var. minor) > oats + barley (*Avena sativa* L. and *Hordeum vulgare* L.) > flax (*Linum usitatissimum* L.) > rye (*Secale cereal* L.) > brown mustard (*Brassica juncea* L.). In two consecutive growing seasons, the soil NO_3-N concentration increased progressively following green manure incorporation and reached a peak after 54 d or showed a sharper peak after 33 d, due to contrasting weather conditions in their 2-yr study (Lenzi et al., 2009). This illustrates the need for in-field tools that can accurately evaluate the soil mineral N following green manure incorporation.

Ion exchange membranes (IEM), which adsorb mineral N (IEM-NH_4-N and IEM- NO_3-N) from the soil solution in an analogous manner as roots due to the Donnan exchange principle, are suitable for *in situ* soil mineral N monitoring because they are subject to the same environmental, e.g., moisture, temperature, and edaphic, ion competition, constraints as crops (Qian and Schoenau, 2002; Nyiraneza et al., 2009). The N uptake by grass forage was positively related to IEM- NO_3-N (R^2 = 0.88 and 0.92) in two years (Ziadi et al., 1999) and similar results

were reported with field potatoes (*Solanum tuberosum* L.) (Nyiraneza and Snapp, 2007), corn (*Zea may* L.) (Pare et al., 1995), canola and wheat (*Brassica campestris* 'Profit' and *Triticum aestivum*' Biggar') (Qian et al., 1996). While IEM could be appropriate to monitor soil mineral N in organic vegetable production systems that rely on green manure for N nutrition of short-season crops, the authors could find no published reports that investigated this possibility.

The objectives of this study were to (i) use IEM-NH_4-N and IEM- NO_3-N concentrations as a tool to monitor the seasonal variability in soil mineral N of two soils (sandy clay loam, sandy loam) planted with field pea-oat green manure and after green manure incorporation, as related to climatic variables, e.g., soil temperature, soil moisture, rainfall, and (ii) determine if IEM-NH_4-N and IEM- NO_3-N concentrations were related to the growth and N uptake of arugula, a short-season leafy herb commonly known as salad rocket, that was planted 2 wks after incorporation of the field pea-oat green manure. This timing of arugula planting after green manure plough-down is the standard practice on the commercial farm where the study was conducted.

2.3 Material and methods

2.3.1 General description of the experimental site

The experiment was conducted in 2013 on a certified organic farm that specializes in the production of vegetables, small fruits, flowers and seeds for local farmers markets and community sponsored agriculture baskets. The farm is located in Les Cedres, southwestern Quebec, Canada (45° 20'N, 74° 8' W), a region where during the growing season the average temperature ranges from 13.3°C in May to 15.6°C in September and annual precipitation is 794.7 mm, with 90.38 mm per month during the growing season (May to September), based on a 30-yr

average (Environment Canada, 2016). Two soil types, both classified as Gleyed humo-Ferric Podzols (Soil Classification Working Group, 1998), were present on the farm. The sandy clay loam contained 485 g kg^{-1} sand, 301 g kg^{-1} clay and 214 g kg^{-1} silt with soil pH of 7.7 (1:1 soil:water slurry), 18 g organic C kg^{-1} and 1.8 g total N kg^{-1}. Soil available nutrient concentrations, by Mehlich-3 extraction, were 145 mg P kg^{-1}, 251 mg K kg^{-1}, 2060 mg Ca $kg^{-1}$and 362 mg Mg kg^{-1}. The sandy loam had 650 g kg^{-1} sand, 112 g kg^{-1} clay and 238 g kg^{-1} silt, soil pH of 6.7, 17 g organic C kg^{-1} and 1.7 g total N kg^{-1}. Soil available nutrient concentrations were 138 mg P kg^{-1}, 176 mg K kg^{-1}, 1583 mg Ca kg^{-1}, and 191 mg Mg kg^{-1}. The previous crop at the experimental site was melon (*Cucumis melon* L.) in 2012.

2.3.2 Experimental design

A field experiment was set up as two-factor factorial design, green manure (field peas+ oats, none), soil texture (sandy loam and sandy clay). The soil response variables (IEM-NH_4-N, IEM-NO_3-N, microbial biomass nitrogen (MBN), and microbial biomass carbon (MBC)) were measured during four times periods; 1) green manure seeding (wk 1 to wk 7), 2) incorporation of green manure (wk 8 to wk 9), 3) arugula seeding (wk 10 to wk 14) and 4) arugula harvest (wk 14 to wk 19). In addition covariates such as nitrogen concentration in the arugula crop, rainfall, soil temperature, and moisture were fitted in a model (Equation 1) component using the R statistic software (R Development Core Team, 2008).

$$\text{Response Variables}_{ij} = u + \tau_i + \beta_j + Tem_i + Pre_i + Kpa_i + \varepsilon_{ij};\ i=1,2\ j=1,2 \quad \ldots\ldots\ldots\ldots (1)$$

Where τ with i levels (Green manure, none), β soil with$j$ levels (sandy loam and sandy), *Tem* is the soil temperature; *Pre* is rainfall; *Kpa* is soil moisture. Two fields with different soil

texture were selected for the study. Within each field there was one fallow control plot (3 x 5 m) and one green manure plot (3 x 5 m) (Fig. 2 - 1). Each plot was sub-divided into 57 units (0.13 x 1.6 m) randomly allocated for independent time-series analysis of three replicate sub-plots per week during a 19 wk sampling period.

2.3.3 Soil sampling and management practices

The initial sampling was done in 27 May 2013 (wk 1 of the study), to establish baseline conditions, was done within 1 d of cultivation of the ground. Before the experiment began both fields were cultivated with a disk harrow to a depth of 0.1 m to incorporate crop residues of melon from the previous year into the soil. Fallow control plots were not planted (fallow throughout the growing season) and kept relatively weed-free by cultivating every 7 d from 3 June 2013 (wk 2 of the study) to 15 July 2013 (wk 8 of the study). Thereafter, the fallow control was by hand using a Hula-Ho weeder before weeds reached flowering stage to avoid introducing more weed seeds into the plot. The green manure plots were planted on 3 June 2013 by broadcasting a mixture of field pea at 73 kg seed ha^{-1} and oat at 67 kg seed ha^{-1}. Six weeks later, the green manure was directly incorporated to a depth of 17 cm using a rototiller BCS 720E Harvester (Harvard, MA, USA). At the time of incorporation, the green manure was at the late vegetative growth stage; field peas were at vegetative stage, fourth node, leaf fully unfolded, more than one pair of leaflets (Knott, 1987), and oats were at Feekes growth stage 5, tillering, leaf sheaths strongly erected (Large, 1954). Before green manure incorporation, 30 plants (21 of peas and 9 of oats), were collected from a 3 x 5 m area of the field and homogenously ground/mixed to get a representative sample. The green manure mixture had a C/N ratio of 10 and contained 46.04% cellulose, 29.52% hemicellulose and 3.45% lignin (acid unhydrolyzable fraction) based on the method of Van Soest et al., (1991).

Two weeks following the plow-down event (wk 10 of the study), the green manure plot were seeded with arugula at a rate of 0.006 kg seed per plot (3 x 5 m) with a hand vegetable seeder that dropped two seeds every 10 cm. There were 9 rows and 10 rows, separated in the middle by 40 cm for tyre track, 15 cm between rows, and 30 cm of free space on both sides, representing 133 plants per m^2 in each plot. Planted plots were kept free of weeds by hand-weeding. Arugula was harvested by hand after 4 wks, corresponding to wk 14 of the study. Soil and climatic conditions were monitored until wk 19. Thus, the growing season was also divided into sampling periods that corresponded to key agricultural operations (Wk 1 to 7: plot preparation and green manure growth; Wk 8 to 9: incorporation of green manure; Wk 10 to 13 arugula growth; Wk 14 to 19 post-harvest period).

2.3.4 Environmental conditions and soil sampling

Each field separated by 25 m, had a weather station (Watch Dog 2000 Series, Spectrum Technologies Inc, Aurora, IL, USA) installed to monitor environmental conditions. Soil temperature, moisture, ambient temperature and precipitation were measured continuously and values were averaged at 30 min intervals from May to September using SM 100 probe sensors from the weather station, inserted to a depth of 0.1 m throughout the growing season. Once a week for 19 wk, a composite sample of four cores of soil from the 0-0.15 m layer taken with a hand-held soil probe (2.54 cm diam), approximately 500 g soil in total, was taken from three randomly selected sub-plots of the 57 independent sub-plots designated for weekly soil sampling. Composite samples were sieved (< 10 mm mesh) in the field to remove roots and rocks, stored in plastic Zip-lock bags and transported to the laboratory on ice, then sieved (< 2 mm) again and stored at -20 °C until thawed at room temperature (20 °C for 2 d) and weighed for determination of soil microbial biomass.

2.3.5 Soil microbial biomass analysis

Microbial biomass nitrogen (MBN) and microbial biomass carbon (MBC) were determined using the chloroform fumigation-direct extraction method (Voroney et al., 2008) with 10 g of field-moist soil, ethanol-free chloroform for the fumigation and 0.5 M K_2SO_4 as the extractant (1:4 soil:solution). MBN concentration was calculated as [(total extractable N after fumigation- total extractable N before fumigation)/0.54] (Voroney et al., 2008; Joergensen and Mueller, 1996). The MBC concentration was the difference between the extractable C, determined by a Sievers Innovox TOC analyzer (GE Analytical Instrument, Boulder, CO, USA) before and after soil fumigation, divided by an extraction efficiency value of 0.45 (Voroney et al., 2008).

2.3.6 Ammonium and nitrate concentrations on ion exchange membranes

Cation and anion exchange membranes (Ionics CR67-HMR (cation) and AR204-SZRA (anion), Durpro, Candiac, QC, Canada) were used to determine the IEM-NH_4-N and IEM- NO_3-N concentrations in soil solution as an indicator of plant-available N (Ziadi et al., 2000; Qian and Schoenau, 2002). Membrane sheets were cut in to strips of 0.028 x 0.055 m and stored in deionized water prior to use. One day before use, the membranes were saturated by shaking for 1 h in 1 M NaCl and transported to the field in a container filled with distilled water. In the field, one anion and one cation membrane were placed in each of the three randomly selected sub-plots of the 57 independent sub-plots designated for that week's sampling, following by a vertical placement (at 15 cm depth). After 1 wk in the field, the IEM were retrieved, rinsed with deionized water to remove soil added and placed in 25 ml tubes containing 1M KCl, in the laboratory the extraction was done by shaking for 1 h at 135 rpm in an orbital shaker. After

filtration through Whatman no. 5 filter paper, the filtered extractant concentration of IEM-NH_4-N and IEM-NO_3-N was analysed by the modified indophenol blue method (Sims et al., 1995) at 650 nm on a microplate reader (μQuant, Biotek, Winooski, VT, USA). Plant-available N values were reported in μg of NH_4-N or NO_3-N cm^{-2} $week^{-1}$ following Qian and Schoenau (2002).

2.3.7 Plant sampling

The arugula crop was sampled from July 29 to August 19, at wk 10, 11, 12 and 13 of the study by randomly selecting three plants from each of the three randomly selected sub-plots of the 57 independent sub-plots designated for that week's sampling. The sampling was done using a garden shovel at 0.20 m depth, arugula above ground biomass was separated from roots using scissors (fresh weight and dry weight after drying for 24 h at 60^{o}C) was recorded then a subsample of the dry plant tissue was ground (< 0.5 mm mesh) for digestion with concentrated H_2SO_4 and 30% H_2O_2 and nutrient analysis (N, P, K, Ca, and Mg) following Parkinson and Allen (1975). After determining the N and P concentrations on an Lachat Quik-Chem AE flow-injection autoanalyzer (Lachat Instruments, Milwaukee, WI, USA) and the K, Ca, and Mg concentrations by atomic absorption spectroscopy, the nutrient concentration per plant was calculated as: Plant above biomass (g) x nutrient concentration (mg g^{-1}). Cumulative nutrient uptake (g nutrient m^{-2}) of arugula was calculated by multiplying the mg nutrient $plant^{-1}$ by number of plants m^2. Table 2 - 3 shows the arugula nutrient concentration and cumulative nutrient uptake over the 4 wks growing period.

2.3.8 Statistical analysis

Soil response variables (IEM-NH_4-N, IEM-NO_3-N, MBN, and MBC) and N concentration and content in the arugula crop were analyzed for their response to the treatments

(green manure, and soil type) using two way-ANOVA. Covariables (arugula N concentration, rainfall, soil moisture, and soil temperature) were included in a general linear model approach. Tukey's HSD mean separation procedure was used to separate treatment means. The response of IEM- NH_4-N and IEM- NO_3-N concentrations to variation in rainfall, soil temperature and moisture and arugula N uptake was evaluated with Pearson correlation coefficients (r) for each soil type and sampling period. Statistical analyses were done using R 3.1.1 (R Development Core Team 2008).

2.4 Results and Discussion

During the 2013 growing season ambient factors were measured on a weekly basis, the soil temperature ranged from 14 to 24°C, soil moisture was 7 to 29 g water 100 g^{-1} soil and precipitation was from 7.20 to 67.50 mm (Fig. 2 - 2). These climate conditions were within the 30-year norms for this region (Environment Canada, 2016), and were favorable for the establishment and growth of the field pea-oat green manure as well as arugula production without supplemental irrigation. Due to the punctual nature of key field operations involved in organic vegetable production, weekly soil monitoring in the field occurred at four sampling periods during the growing season, as described in the next sections and illustrated in the Tables and Figures.

2.4.1 Week 1 to 7: Plot preparation and green manure growth

The IEM-NH_4-N and IEM- NO_3-N concentrations were responsive to field operations involved in preparing the plots for seeding of green manure and to soil temperature and rainfall fluctuations during green manure growth. At wk 2 with a harrow disk, the IEM-NH_4-N concentrations declined by 0.49 to 1.67 µg NH_4-N cm^{-2} wk^{-1} and IEM-NO_3-N concentrations

fluctuated from 11.05 to 19.78 µg NO_3-N cm^{-2} wk^{-1} in both soil types (Fig. 2 - 3), probably due to stimulation of ammonia oxidizers and nitrifiers following tillage. Cookson et al., (1998) reported an increment by 16 to 42% of microbial activity in systems where wheat residues were incorporated. No difference ($p > 0.05$) in the initial sampling of IEM-NH_4-N and IEM-NO_3-N concentrations was observed between fallow control and green manure plots (data not shown). From wk 2 to wk 7, during the green manure growth, the IEM-NH_4-N concentration ranged from 0.59 to 3.34 µg NH_4-N cm^{-2} wk^{-1} in the sandy loam and between 0.64 and 2.40 µg NH_4-N cm^{-2} wk^{-1} in the sandy clay loam (Fig. 2 - 3A). The IEM-NO_3-N decreased in both soil types by 1.20 and 2.31 µg NO_3-N cm^{-2} wk^{-1} in green manure plots and by 3.65 and 8.02 µg NO_3-N cm^{-2} wk^{-1} in fallow control plots. During this sampling period, the mean IEM-NH_4-N and IEM-NO_3-N concentrations were not different in fallow control and green manure plots of each soil type (Table 2 - 1) and temperature was the only climatic variable that was correlated with IEM-NH_4-N and IEM-NO_3-N concentrations (Table 2 - 2). Small fluctuations in a low MBN concentration, coupled with the continuous decline in MBC concentration during this period suggests microbial turnover (Fig. 2 - 4). It is considered that NH_4-N and NO_3-N were assimilated by weeds (fallow control) and the pea-oat crop (green manure); mainly by oats due to the extensive root area and great potential of absorption (Thorup-Kristensen, 2001), and it is likely that gaseous and soluble N losses occurred (Freney et al., 1983), but it was beyond the scope of this study to document those N transformations.

2.4.2 Week 8 to 9: Incorporation of green manure

Both fallow control and green manure plots were cultivated with a rototiller at the time of green manure incorporation. In this sampling period, the IEM-NH_4-N concentration decreased in both soil types (Fig. 2 - 3A) but there was no difference in the mean IEM-NH_4-N concentration

between fallow control and green manure plots (Table 2 - 1). In contrast, the IEM-NO_3-N concentration increased in both soil types (Fig. 2 - 3B), the green manure plots performed higher concentrations than fallow plots. This pattern in the short-term might be due mainly to the effect of tillage that affects greatly the green manure plots + the N input from green manure with low C/N ratio and the decomposition followed by the release of mineral N into the soil solution (Whalen, 2014; Li et al., 2015). Previously, Li et al., (2016) reported a NO_3-N concentration between 47 and 50 mg N kg^{-1}, seven days after the incorporation of leguminous (red clover and winter vetch). However, the IEM-N availability might also be attributed to the inherent soil mineral N concentration and to the rapid mineralization of small green manure residues (< 3 mm), based on the observation that finely-ground, homogeneous green manure residues can be transformed to mineral N forms in 1-2 wks (Jensen, 1994b). Despite, the scientific evidence provided, I acknowledge that the lack of blocking and random assigned treatment within blocks limits the assumption that the availability of IEM-N might be caused by inherent soils fertility, as it is known there are variations in the field, as gradient of fertility.

The plant yield and the nutrient concentration did not show any nutrient deficiency. Despite there is not literature existing about macro and micronutrient content in arugula along the growth period, it is been reported the optimal content in similar crops. For instance, Koudela and Petříková (2008) reported the nutrient content of five cultivars of lettuce (*Lactuca sativa* L.)-Bergamo, Dubacek, Frisby, Lollo Rossa and Redin; the ranges of nutrient concentration were: K (2.39 to 6.477 mg g^{-1}), Ca (0.20 to 0.75 mg g^{-1}), and Mg (0.11 to 0.41 mg g^{-1}). The values reported are within these ranges which lead us to assume that there was not limitation for nutrients uptake (Table 2 - 3).

In the fields in the present study, the incorporation of green manure residue appeared to stimulate N accumulation in MBN (Fig. 2 - 4). This observation needs to be tempered by the fact that the IEM- NO_3-N concentration was positively correlated with soil temperature ($p < 0.01$), rainfall ($p < 0.01$), and soil moisture ($p < 0.01$) (Table 2 - 2). These correlations might suggest that NO_3^--N loss occurred in wetter sandy clay loam and sandy loam soils. Similarly, Rosecrance et al., (2000) reported elevated NO_3^- leaching and denitrification from hairy vetch and hairy vetch-cereal rye green manures that peaked within 5 d after green manure termination. While IEM data cannot provide information about N loss pathways, it does indicate no appreciable increase in IEM- NH_4-N and IEM- NO_3-N concentrations within 2 wks of incorporating field pea-oat green manure in these soils.

2.4.3 Weeks 10 to 13: Arugula growth

During this period, the IEM-NO_3-N concentration was correlated with temperature ($p < 0.01$) and rainfall ($p < 0.01$) in green manure plots in the sandy clay loam. In the sandy loam soil, the IEM- NO_3-N concentration was correlated with rainfall ($p < 0.01$) in the green manure plots (Table 2 - 2). The higher IEM-NO_3-N concentration in green manure than fallow control of both soil types (Table 2 - 1) may indicate a priming effect due to arugula growth (Cheng, 2009) and the incorporation of green manure residue promoted the NO_3-N availability (Li et al., 2016). In green manure plots, arugula N uptake was positively correlated with the IEM-NO_3-N concentration in sandy clay loam (r= 0.86, $p < 0.01$) and sandy loam (r = 0.71, $p < 0.05$) soils during the same period of time (Table 2 - 2; Fig. 2 - 5). Thus, the IEM-NO_3-N concentration represents the plant-available NO_3-N that is accessible by arugula, consistent with other reports

that IEM- NO_3-N is an appropriate *in situ* indicator of plant-available N (Qian and Schoenau, 2002).

2.4.4 Weeks 14 to19: Post-harvest period

One week after arugula harvest, the IEM- NO_3-N concentration increased by 28.86 NO_3-N µg cm^{-2} wk^{-1} in the sandy loam and 22.86 µg NO_3-N cm^{-2} wk^{-1} in sandy clay loam (Fig. 2 - 3B) but thereafter no additional cultivation or weed control was done on the fallow control or green manure plots. In the sandy clay loam soil, there was a greater IEM-NH_4^+-N concentration and a lower IEM-NO_3-N concentration in green manure than fallow control plots during this period (Table 2 - 1). Although decomposition of arugula roots plus green manure residues could be a source of mineral N in the green manure plots, turnover of these organic residues appeared to be constrained by a relatively stable MBC and low MBN concentrations (Fig. 2 - 4). Fluctuations in IEM-NH_4-N and IEM-NO_3-N concentrations were correlated with soil temperature, rainfall and soil moisture, but no consistent pattern was detected (Table 2 - 2). In humid temperate region of Quebec, residual soil N accumulation of 20-30 kg N ha^{-1} in the post-harvest period is expected (De Jong et al., 2009) so the gradual decrease in IEM-NH_4-N and IEM-NO_3-N concentrations by wk 19 without any corresponding increase in MBN or MBC concentrations, this might suggests two different pathways, 1) part of the N allocated in the microbial biomass was allocated in the necromass pool, which is not measured by the chloroform fumigation-extraction method; or 2) the N was lost from these sandy loam and sandy clay loam soils after arugula harvest. The susceptibility of coarse-textured soils to N losses through leaching and gaseous emissions makes them prime candidates for best management practices and other interventions to control post-harvest N losses, as discussed by Rasouli et al., (2014).

2.5 Conclusions

The IEM-NH_4-N and IEM-NO_3-N concentrations reflected fluctuations in soil mineral due to green manure input, temperature, rainfall, and soil moisture conditions. The IEM-NO_3-N concentration was the best indicator of N uptake by arugula during its 4 wk growth period. The higher IEM-NH_4-N and IEM-NO_3-N concentrations remaining after arugula harvest, compared with sampling periods earlier in the growing season, indicates that these soils could support the N requirements of a second cash crop or a cover crop. Retaining organic N from green manure in the soil-plant system, where it could undergo internal N cycling to be plant-available for the next cash crop is preferable to its loss in the environment. The use of IEMs in a green manure system provides reliable information, making it a useful tool that might help farmers to take decisions about the planting time, maximizing the uptake of N supplied by green manure.

Table 2 - 1. Means and ANOVA effect of IEM- NH_4-N and IEM-NO_3-N, concentrations by sampling periods during the 2013 growing season in sandy clay loam and sandy loam soils at Les Cedres, Quebec. IEM = ion exchange membrane. NS, not significant, *, $p < 0.05$, **,$p < 0.01$

Soil type	Treatment	Wk 1 to wk 7[a]	Wk 8 to wk 9[b]	Wk 10 to wk 13[c]	Wk 14 to wk 19[d]
				IEM-NH_4-N (µg cm^{-2} $week^{-1}$)	
Sandy Clay Loam	Fallow	1.24 (0.22)	1.52 (0.47)	1.23 (0.16)	1.38 (0.17)
	Green manure	1.49 (0.23)	2.73 (0.46)	1.78 (0.26)	1.86 (0.24)
Sandy Loam	Fallow	2.08 (0.34)	3.88 (0.05)	2.50 (0.16)	1.38 (0.29)
	Green manure	1.84 (0.28)	3.79 (0.43)	2.43 (0.27)	2.38 (0.45)
				IEM-NO_3-N (µg cm^{-2} $week^{-1}$)	
Sandy Clay Loam	Fallow	11.82 (3.88)	10.31 (2.39)	11.20 (1.92)	12.47 (3.03)
	Green manure	8.10 (3.56)	9.75 (1.68)	11.73 (4.08)	11.43 (3.03)
Sandy Loam	Fallow	10.21 (1.51)	11.33 (4.56)	9.46 (1.64)	14.33 (3.73)
	Green manure	8.00 (2.43)	16.80 (4.06)	15.41 (3.30)	13.39 (4.38)
Source of variation	df[e]			ANOVA	
				IEM-NH_4-N	
Green manure (GM)	1	NS	NS	NS	*
Soil (S)	1	*	NS	*	NS
GM x S	1	NS	NS	NS	NS
				IEM-NO_3-N	
Green manure (GM)	1	NS	NS	NS	NS
Soil (S)	1	NS	NS	NS	NS
GM x S	1	NS	NS	NS	NS

Notes: [a]Plot preparation, green manure growth. Mean value with SE in brackets (n = 21). [b]Incorporation of green manure. Mean value with SE in brackets (n = 6). [c]Arugula growth. Mean value with SE in brackets (n = 12). [d]Post-harvest. Mean value with SE in brackets (n = 18). [e]df= degree of freedom.

Table 2 - 2. Correlation coefficient (r) between IEM-NH_4-N, IEM-NO_3-N, and measured climatic variables in fallow control and arugula N uptake in green manure plots in sandy loam and sandy clay loam soils during the growing season in Les Cedres, Quebec. IEM = ion exchange membrane.

		Sandy clay loam		Sandy loam	
		Fallow control	Green manure	Fallow control	Green manure
		Plot preparation, green manure growth, wk 1 to 7 (n = 21)			
IEM- NH_4^+-N	Temperature (°C)	NS	NS	0.62**	NS
IEM-NO_3^--N	Temperature (°C)	-0.53*	-0.61**	NS	NS
		Incorporation of green manure. wk 8 to 9 (n = 6)			
IEM- NO_3^--N	Temperature (°C)	0.76***	0.85*	0.64**	0.95***
	Rainfall (mm)	0.71***	0.85*	0.63***	0.95***
	Moisture (%)	NS	0.66*	NS	0.66**
		Arugula growth. wk 10 to 13 (n = 12)			
IEM- NO_3^--N	Temperature (°C)	0.72**	0.73**	0.69*	NS
	Rainfall (mm)	0.74**	0.75**	0.80***	0.57*
	Arugula N uptake(g m^{-2})	NS	0.86**	NS	0.71*
		Post-harvest. wk 14 to 19 (n = 18)			
IEM- NH_4^+-N	Temperature (°C)	-0.49*	NS	NS	-0.86***
	Rainfall (mm)	NS	0.51*	NS	NS
IEM- NO_3^--N	Temperature (°C)	0.79***	0.64**	NS	NS
	Moisture (%)	-0.65**	-0.51*	NS	-0.61*

Notes: NS, not significant (correlation coefficient not shown). * Significant at $p < 0.05$, ** Significant at $p < 0.01$, *** Significant at $p < 0.001$.

Table 2 - 3. Arugula yield, nutrient concentration, and cumulative nutrient uptake over 4 weeks in sandy clay loam and sandy loam soils with field pea +oat green manure during the 2013 growing season in Les Cedres, Quebec.

Week	Plant weight (g)	N (mg g^{-1})	P (mg g^{-1})	K (mg g^{-1})	Ca (mg g^{-1})	Mg (mg g^{-1})
		Sandy clay loam with green manure				
10	0.18 (0.06)[a]	4.34 (0.23)	0.65 (0.06)	3.46 (1.29)	1.35 (0.50)	0.30 (0.14)
11	2.05 (0.30)	3.90 (0.46)	0.51 (0.08)	4.22 (0.81)	2.01 (0.08)	0.30 (0.01)
12	4.10 (1.11)	3.03 (0.74)	0.28 (0.12)	2.68 (0.59)	2.09 (0.42)	0.35 (0.13)
13	4.45 (0.48)	3.97 (0.24)	0.21 (0.02)	1.96 (0.25)	1.56 (0.10)	0.13 (0.01)
Cumulative nutrient uptake (g m^{-2})		2.02 (0.27)	0.21 (0.10)	1.63 (0.48)	0.93 (0.17)	0.14 (0.04)
		Sandy loam with green manure				
10	0.18 (0.01)	4.45 (0.43)	0.70 (0.11)	4.27 (0.38)	0.83 (0.23)	0.16 (0.07)
11	2.51 (0.24)	3.76 (0.28)	0.43 (0.87)	7.18 (0.59)	0.36 (0.09)	0.05 (0.01)
12	6.50 (0.55)	4.19 (0.22)	0.12 (0.03)	0.99 (0.14)	0.73 (0.11)	0.13 (0.02)
13	6.10 (0.37)	5.58 (0.45)	0.14 (0.01)	1.13 (0.13)	0.75 (0.26)	0.08 (0.01)
Cumulative nutrient uptake (g m^{-2})		2.39 (15.86)	0.18 (0.13)	1.80 (1.47)	0.35 (0.10)	0.55 (0.02)

[a]Mean value with SE in brackets (n = 9).

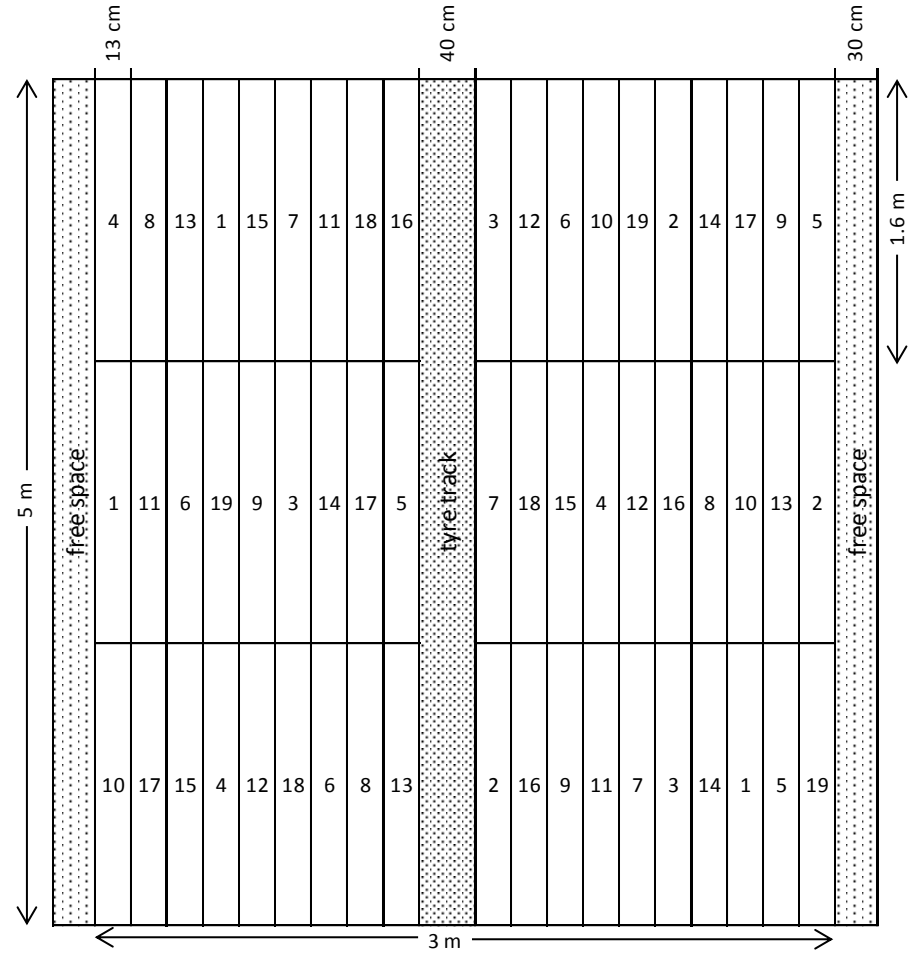

Figure 2-1. Experimental design deployed in each of the four plots used for this study – a fallow control on sandy loam, a fallow control on sandy clay, a green manure treatment on sandy loam and a green manure on sandy clay. Nineteen subplots were randomly assigned and replicated (n = 3) for time- series measurements of soil properties (IEM- NH_4-N, IEM- NO_3-N, microbial biomass C and N) in all plots from weeks 1 to 19 of the study.

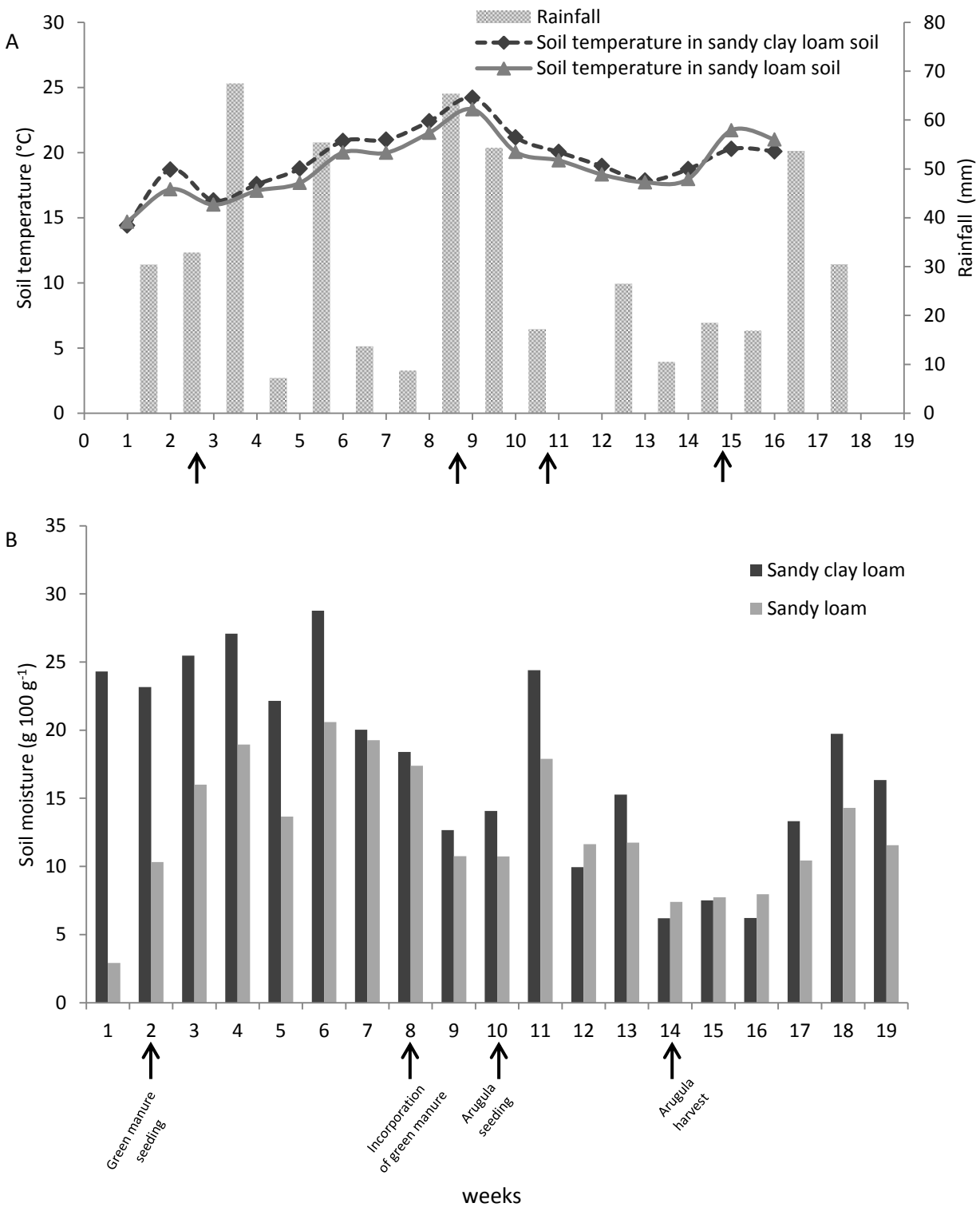

Figure 2-2. Weekly means of soil temperature and rainfall (A) and soil moisture (B) at 15 cm soil depth. In sandy loam and sandy clay loam soils during 19 wks of the growing season (May-October, 2013) in Les Cedres, Quebec. Timing of the field operations is indicated.

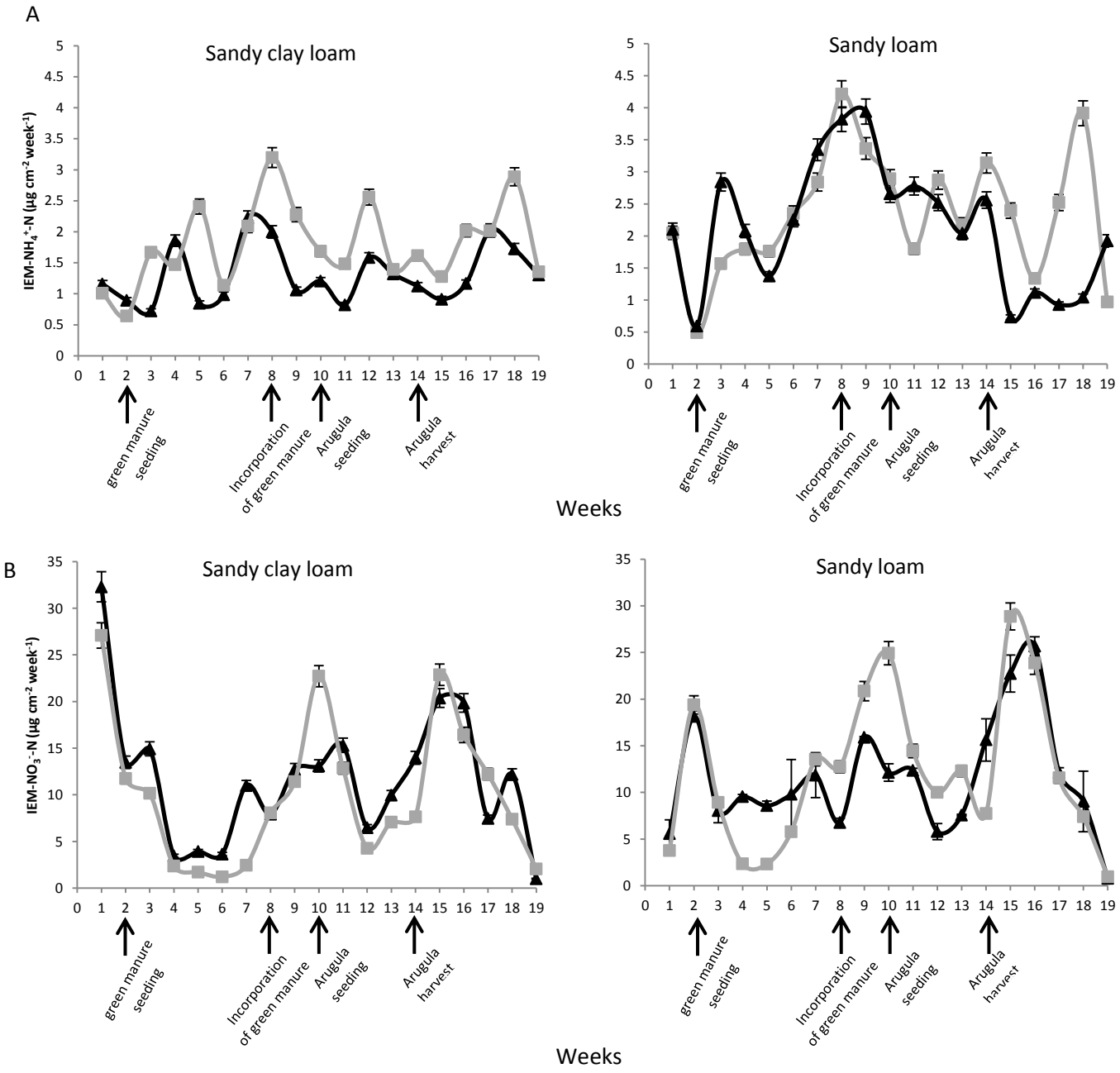

Figure 2-3. (A) IEM-NH4-N and (B) IEM- NO3-N concentrations in sandy loam and sandy clay loam soils during 19 wks of the growing season (May to October, 2013) in Les Cedres, Quebec. IEM = ion exchange membrane. Points and error bars represent standard error of three replicate sub-plots in the (▲) control with no green manure and (■) pea-oat green manure treatment. The timing of key field operations indicated.

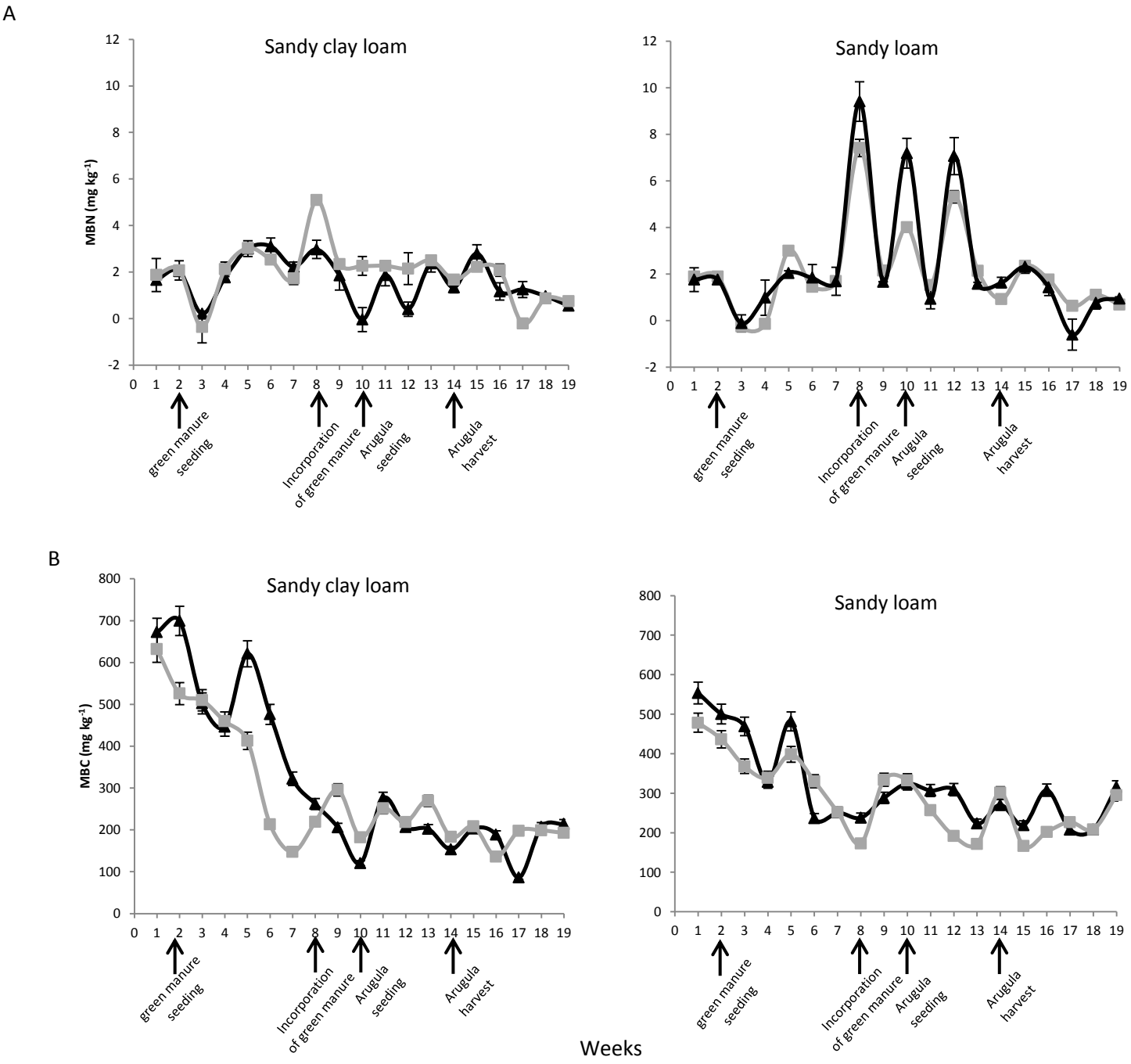

Figure 2-4. (A) Microbial biomass nitrogen (MBN) and (B) Microbial biomass carbon (MBC) concentrations in sandy loam and sandy clay loam soils during 19 wks of the growing season (May to October, 2013) in Les Cedres, Quebec. Points and error bars represent standard error of three replicate sub-plots in the (▲) control with no green manure and (■) pea-oat green manure treatment. The timing of key field operations indicated.

FOREWORD TO CHAPTER 3

In Chapter 2, I quantified the IEM-N concentration in two soil types, before and after the incorporation of mixed oat-pea green manure. During green manure growth, the N availability decreased in sandy soil and clay soil. After incorporation of green manure, the N availability peaked, and subsequently fluctuated with the environmental conditions and arugula growth. The N mineralization process after the incorporation of green manure was important for supplying ample NH_4 and NO_3 for the arugula crop. Chapter 3 investigates the N contributions from green manure, based on residue size, under controlled conditions during a 6 week study.

This thesis chapter was taken from the publication: León Castro, L., Whalen, J. K. 2016. Ion exchange membranes are sensitive indicators of ammonium and nitrate released from green manures with low C/N ratios. European Journal of Soil Biology 77: 4-8.

CHAPTER 3.

Ion exchange membranes are sensitive indicators of ammonium and nitrate released from green manures with low C/N ratios

3.1. Abstract

Green manure is a valuable nitrogen (N) source, but must undergo decomposition, mineralization and nitrification to release mineral N (NH_4-N and NO_3-N). The pattern of soil mineral N released from green manure is related to its biochemical composition (e.g., C/N ratio) and soil texture. The objective of this study was to investigate the use of ion exchange membranes (IEMs) as a sensitive indicator of N mineralization and nitrification from green manure (mixture of pea and oat residues) having C/N = 8 and C/N = 12, mixed into sandy clay loam and sandy loam soils. The green manures decomposed rapidly and released mineral N that was captured by IEMs inserted in the soil during a 6 wk greenhouse incubation. Net NH_4 and NO_3 produced after 6 wk was greater in soil mixed with green manure having C/N = 8, and in the sandy clay loam mixed with green manure having C/N = 12, than unamended control soil. The IEM- NH_4-N concentration peaked one week after the green manure incorporation, with 0.13 µg IEM- NH_4-N cm^{-2} wk^{-1} in the sandy clay loam and 0.19 µg IEM- NH_4-N cm^{-2} wk^{-1} in the sandy loam soil, and declined thereafter. While the IEM- NO_3-N increased steadily in the sandy clay loam during the 6 wk incubation, there was a plateau (green manure C/N = 8) or decline (green manure C/N = 12) in IEM- NO_3-N concentration in the sandy loam soil. Net N mineralization and nitrification rates corresponded to the temporal fluctuations in mineral N detected with IEMs, confirming that this tool is a sensitive indicator of NH_4-N and NO_3-N dynamics in soils amended with green manure having low C/N ratios.

3.2. Introduction

When a green manure crop is terminated and incorporated into the soil, it undergoes decomposition, mineralization and nitrification to release plant-available N (mineral N; NH_4-N plus NO_3-N) for the subsequent crop. Chemical composition of green manure, particularly the C/N ratio, is a good predictor of its N fertilizer value (Wagger, 1989; Kuo and Jellum, 2000; Diallo et al., 2006) because residue C/N ratio is correlated significantly ($r = - 0.88$) to soil NH_4-N and NO_3-N concentrations (Frankenberger and Abdelmagid, 1985). Residues from green manure with C/N ratios < 25 are expected to release mineral N in the first weeks after its incorporation, based on the pattern of N release from crop residues with C/N ratios from 16 to 9 (Müller et al., 1988; Baggs et al., 2000). Soil texture is another factor that modulates mineral N release from green manure residues due to the physical occlusion of partially-decomposed residues within aggregates and the binding of soluble products of decomposition (e.g., carbohydrates and proteins) onto clay surfaces (Six et al., 2000), both of which may impede the microbially-mediated processes of N mineralization and nitrification. Consequently, green manure-amended soils with higher clay content are expected to release less mineral N than those with low clay content.

The pattern of N release in green manure-amended soils can be evaluated by temporal sampling and analysis of the soil mineral N concentration, but these are static measurements that must be taken at short time-steps to reflect the dynamics of this plant-available N pool (Qian and Schoenau, 2002). Ion exchange membranes (IEMs) are an alternative and practical *in situ* method, as the continuously adsorption of ions on IEMs from soil solution reflects the temporal pattern of N release in green manure-amended soils and mimics ion capture/interception by roots (Ziadi et al., 1999; Qian and Schoenau, 2005; Ziadi et al., 2006; Nyiraneza et al., 2009). I

propose that IEMs are good indicators of the short-term dynamics of NH_4-N and NO_3-N produced through N mineralization and nitrification of substrates released from decomposing green manure. The objective of this study was to evaluate IEMs as a sensitive indicator of NH_4-N and NO_3-N dynamics in two soils (sandy clay loam and sandy loam) amended with green manure residues having low C/N ratios (C/N=8 and C/N=12).

3.3. Materials and methods

The experiment was conducted in an unheated greenhouse using field soils from an organic vegetable farm in Les Cedres, Quebec, Canada (45° 20'N, 74° 8' W). Soils were classified as Gleyed humo–ferric Podzols (Soil Classification Working Group, 1998) having sandy clay loam texture (485 g sand kg^{-1} and 301 g clay kg^{-1}, pH 7.7 and 18 g organic C kg^{-1}) and sandy loam texture (650 g sand kg^{-1}, 112 g clay kg^{-1}, pH 6.7 and 17 g organic C kg^{-1}). Field soils (0-15 cm depth) were collected with a shovel, passed through a <10 mm sieve to remove rocks and large plant residues, transported in sealed plastic bins and stored in a 4°C walk-in refrigerator until the experiment began. In the greenhouse prior start the experiment, soils were through a <3 mm mesh screen to eliminate residues from previous years. Green manures were a mixture of field peas (*Pisum sativum* L.) and oats (*Avena sativa* L.) grown in pots in the greenhouse for 5 wks. When harvested, field peas were in the vegetative stage, fourth node, leaf fully unfolded, more than one pair of leaflets (Knott, 1987), and oats were at Feekes growth stage 5, tillering, leaf sheaths strongly erected (Large, 1954). Plant residues were rinsed to remove dust and dried (60 °C for 24 h). A subsample was ground to pass a <1 mm mesh sieve and analyzed for the total C and N content on a Flash EA 1112 series C/N analyzer (Thermo Finnigan, Mississauga, Ontario, Canada). The field peas had a C/N ratio = 7, whereas oats had a C/N ratio = 10. Mixing equal quantities of residues from these crops generated a residue with

C/N = 8. Adding proportional amounts (33% field pea, 33% oats and 33% maize fiber from field-grown maize leaves collected at the tasseling growth stage having a C/N ratio = 17) produced a residue with C/N=12. The dried residue mixtures were uniformly chopped with pruning shears and fragments retained on sieves of 2 mm – 4 mm were used in the experiment.

Soil-residue mixtures were placed in plastic plant pot (10.5 cm diam. by 13 cm depth) with three drainage holes (0.5 cm diam.). Each experimental unit contained 1 kg (dry weight basis) of field-moist soil mixed with (a) 0.6 g kg^{-1} of green manure C/N=8, (b) 0.6 g kg^{-1} of green manure C/N=12 or (c) no green manure. The residue addition rate was based on a green manure input of about 3.6 t ha^{-1}, which is similar to the 3.7 t ha^{-1} of oat and pea reported by McKenzie and Spaner (1999). After adding and completely homogenizing the soil-residue mixture, soils were gently compressed to 1.36 g cm^{-3} for sandy clay loam and 1.54 g cm^{-3} for sandy loam, the bulk density of these soils under field conditions. Pots were arranged in a complete randomized design with four replications of each soil-residue mixture (6 factorial treatments representing 2 soils × 3 residue types) and each sampling date (once a week for six weeks) to allow for destructive sampling, for a total of 144 pots. The experiment simulated residue decomposition and N mineralization under field conditions in May-June, when green manure is generally terminated and incorporated in this region. The daily air temperature in the unheated greenhouse was, on average, 15.2 °C (SD± 1.86) with temperature increasing from a minimum of 13 to maximum of 22 °C during the study (monitored every 2-3 d with an indoor tube thermometer, Taylor Precision Products, Oak Brook, Illinois, USA). These values are similar to the air temperature (13 to 18°C) during these months, based on the 30 year average weather data from 1980 to 2010 (Environment Canada, 2016). Pots receiving the green manure treatment and the unamended control pots were subjected to this fluctuating temperature regime during the study.

Pots were freely drained and irrigated every 2-3 d to maintain moisture content at 11-15 g water 100 g^{-1} soil (Field Scout TDR 100 system, Spectrum Technologies Inc, Aurora, Illinois, USA) corresponding to about 40 – 50% water-filled pore space. This is an optimal moisture level for decomposition in sandy soils (Schomberg et al., 1994) that should not induce denitrification, which is negligible below 80% water-filled pore space (Dobbie and Smith, 1996).

Cation and anion exchange membranes (Ionics CR67-HMR (cation) and AR204-SZRA (anion), GE Water & Process Technologies, Trevose, Pennsylvania, USA) were used to monitor NH_4-N and NO_3-N concentrations in soil solution on a weekly basis. Ion exchange membrane strips of 2.8 x 5.5 cm were stored in deionized water until 24 h prior to use, when they were saturated by shaking with 1 M NaCl solution for 1 h and then placed in distilled water. Each pot had one cation and one anion exchange membrane buried to a depth of 10 cm. After 1 wk, the IEM was retrieved and replaced with a fresh IEM that was inserted into a new pot. After retrieval, IEMs were rinsed with deionized water to remove attached soil particles, then placed in a single conical screw cap tube with 25 ml of 1 M KCl. After extraction (shaking the tubes for 1 h in an orbital shaker, filtering through Whatman no. 5 filter paper), the concentration of IEM-NH_4-N and IEM- NO_3-N was determined by the modified indophenol blue method (Sims et al., 1995) at 650 nm on a microplate reader (μQuant, Biotek, Winooski, Vermont, USA). The IEM-NH_4-N and IEM- NO_3-N concentrations were reported as μg cm^{-2} wk^{-1} (Qian and Schoenau, 2002).

Soil from the beginning (wk 0) and end (wk 6) of the incubation was analyzed for the mineral N, microbial biomass nitrogen (MBN) and microbial biomass carbon (MBC) concentrations. Mineral N (NH_4-N and NO_3-N) extracted with 2 M K_2SO_4 (1:4 soil:solution) was analyzed with the modified indophenol blue technique (Sims et al., 1995). MBN and MBC

concentrations were determined by the chloroform fumigation-direct extraction method with 0.5 M K_2SO_4 as the extractant followed by persulfate digestion (Voroney et al., 2008). The MBN concentration was [(total extractable N after fumigation- total extractable N before fumigation)/k_{EN}] where k_{EN} is the extraction coefficient 0.54. The concentration of MBC was determined by subtracting extractable C (determined by a Sievers Innovox TOC analyzer, GE Analytical Instrument, Boulder, Colorado, USA) before and after soil fumigation with ethanol-free chloroform and adjusted by 0.45, the efficiency of extraction of microbial biomass (Voroney et al., 2008). After 6 wk, soils were sieved through 1-2 mm and 3-4 mm sieves to collect the undecomposed green manure residues that were either, undecomposed or not associated with organo-mineral complexes using the wet-sieving method. These residues were gently washed and weighed.

Data was normally distributed and had homogeneous variance, confirmed by the Shapiro-Wilk and Levene's tests, respectively. For each soil type, the effect of green manure C/N ratio on IEM- NH_4-N and IEM- NO_3-N concentration, corrected by subtracting the background IEM-NH_4-N and IEM- NO_3-N levels in the no green manure treatment, was determined using repeated measures (in time) one-way ANOVA. Post-hoc comparisons between C/N ratios (between-subject effects) and of C/N ratio x sampling time (within-subject effects) were evaluated with least significant difference (LSD) test. The effect of green manure C/N ratio on the mass of undecomposed residues, net N mineralization, net nitrification, MBN and MBC concentrations after 6 wks was analyzed using one-way ANOVA. When the main effects were significant ($p < 0.05$), treatment means were compared with LSD test. A paired sample t-test was conducted to compare the green manure residues remaining after 6 wks in the two soil types.

Analyses were performed with SAS Version 9.3 software (SAS Institute Inc., Cary, NC, USA, 2000).

3.4. Results

Soil mineral NH_4-N and NO_3-N concentrations were higher ($p < 0.05$) when mixed with green manure C/N=8 than C/N=12 in both soil types (Table 3 - 1). The MBC concentration increased in both soil types, indicating that soil mixed with green manures C/N=8 and C/N=12 supported greater ($p < 0.05$) microbial biomass than soils with no green manure (Table 3 - 1). In this study, the MBC and MBN concentrations should be taken as a relative measures and indicative of trends, rather than as definitive values because the microbial biomass C:N ≤ 6, lower than the expected C:N of 7 – 9 from microbial biomass (Griffiths et al., 2012). This suggests low extraction efficiency for MBC or that the standard correction factors were inappropriate for the soil type and experimental conditions. At the end of the incubation, more undecomposed residues (1-2 mm and 3-4 mm) were collected from soils mixed with green manure C/N = 12 than green manure C/N = 8 or the unamended control soil (Table 3 - 1).

The net IEM-NH_4-N concentration peaked in the first week of the incubation (from 0.13 to 0.19 µg IEM- NH_4-N cm^{-2} wk^{-1}), and more NH_4-N was captured from green manure C/N=8 than C/N=12 in both soil types ($p = 0.001$, sandy clay loam; $p = 0.001$, sandy loam). Thereafter, the IEM-NH_4-N concentration declined to as low as -0.02 µg IEM- NH_4-N cm^{-2} wk^{-1} after 6 wks (Fig. 3 - 1A). The green manure x time interaction was significant in sandy clay loam ($p = 0.001$), as IEM-NH_4-N concentrations were greater on 4 of 6 measurement days with green manure C/N = 8 than C/N = 12; however, the green manure x time interaction was not significant in sandy loam ($p = 0.14$) (Fig. 3 - 1A).

In the sandy clay loam, the net IEM-NO_3-N concentration increased steadily from 0.31 µg IEM- NO_3-N cm^{-2} wk^{-1} to 1.63 µg IEM- NO_3-N cm^{-2} wk^{-1}, with more NO_3-N released from the green manure C/N = 8 than C/N = 12 (p = 0.001) (Fig. 3 - 1B). Although the IEM-NO_3-N concentration was greater from green manure C/N = 8 than C/N = 12 on 5 of 6 measurement days, the green manure x time interaction was not significant in the sandy clay loam (p = 0.48) because both treatments followed the same pattern of NO_3-N release. A plateau or decline in the IEM-NO_3-N concentration was observed in the sandy loam soil, with values from -0.02 to 0.99 µg IEM- NO_3-N cm^{-2} wk^{-1} recorded during the incubation (Fig. 3 - 1B). Due to the divergent patterns of NO_3-N release from green manures mixed with the sandy loam soil, particularly in weeks 3 to 5 of the incubation, the green manure x time interaction was significant (p = 0.001).

3.5. Discussion

3.5.1. Residue chemistry and soil texture effects on N release from green manure

As expected, there was more N released from green manure with C/N=8 than green manure with C/N=12 and this is consistent with other reports (Müller et al., 1988; Baggs et al., 2000). Including soils with different clay content in this study allowed us to evaluate how soil texture modulates mineral N release from green manure residues.

First, when we considered residues having the same initial chemical composition, we found that the mass of residue remaining on the 3-4 mm sieve was greater in the sandy clay loam than the sandy loam (t-test: p = 0.04 for green manure C/N = 8; p = 0.05 for green manure C/N = 12). Similarly, more residues were collected on the 1 - 2 mm sieve in the sandy clay loam than the sandy loam (t-test: p = 0.02 for green manure C/N = 8; p = 0.03 for green manure C/N = 12). This suggests that there is a physical barrier, probably related to binding with clays, that slows

the decomposition of green manure in soils with higher clay content, consistent with Six et al., (2000).

Second, we posited that soil texture would constrain mineral N release from green manure because soluble products of decomposition (e.g., proteins) and N mineralization (e.g., NH_4-N) are known to bind to clay surfaces (Six et al., 2000). As the IEM-NH_4-N and IEM-NO_3-N concentrations were of the same magnitude for the sandy clay loam and sandy loam soils, this did not convince us that substrate availability was a limiting factor for N mineralization and nitrification. Further, the MBN and MBC concentrations in the two soil types were of the same magnitude, which implies that microbial processes and N immobilization might be similar. Finally, the net mineral N released from green manure C/N = 8 (corrected for the background net mineral N) after 6 wk was greater (p = 0.03) for the sandy clay loam (5.28 mg mineral N kg-1) than the sandy loam soil (3.92 mg mineral N kg^{-1}). This also occurred in the green manure C/N = 12, which had greater (p = 0.001) net mineral N released in the sandy clay loam (2.03 mg mineral N kg^{-1}) than the sandy loam (0.25 mg mineral N kg^{-1}). These results reflect a greater extractable mineral N concentration present in the sandy clay loam than sandy loam after 6 wks of greenhouse incubation. Of course, these results do not account for N losses through leaching, which were possible with our experimental set-up and would be more likely in the sandy loam than sandy clay loam soil. We suspect that N leaching was the dominant loss pathway, rather than denitrification, because soil moisture was at 51 to 64% water-filled pore space during the study.

We conclude that soil with a higher clay content can slow the decomposition of green manure residue, but early N release from labile fraction, most likely soluble substrates originating from green manure, were not influenced by soil texture (sandy clay loam and sandy

loam) or related to physical barriers, thus are equally susceptible to N mineralization and nitrification in both soils types.

In summary, we expect a similar amount of mineral N release from green manure having the same residue chemistry, but the fate of the mineral N – whether it is retained in the soil matrix, transferred to plants or released into the environment – depends on soil texture and edaphic factors.

3.5.2. Ion exchange membranes reveal dynamics of NH_4-N and NO_3-N release from green manure

The immediate release of IEM-NH_4-N in the first week following soil amendment with green manure residues is indicative of a rapid decomposition and solubilization of proteins contained in the residue, leading to N mineralization. We assume that turnover of microbial necromass following soil handling and rewetting was accounted for by subtracting the background IEM-NH_4-N concentration in the unamended control soil (no green manure added). As expected, there was more N mineralization in soils amended with green manure C/N=8 than C/N=12.

The decline in the IEM-NH_4-N concentration with time was attributed to the immobilization by growing microorganism (ammonia oxidizers and nitrifiers capable of converting NH_4-N to NO_3-N). The IEM-NO_3-N concentration increased with time, with more IEM-NO_3-N in soils amended with green manure C/N = 8 due to residue chemistry (Constantinides and Fownes, 1994; Mueller et al., 1998) and the fact that there was more NH_4 available for nitrifiers when green manure C/N = 8 was applied. As IEM-NH_4-N and IEM-NO_3-N concentrations were generally greater than 0 (i.e., higher than the background level without

green manure) particularly when green manure C/N=8 was used, this implies that there was a mineral N released in excess of microbial requirements such that net N mineralization + nitrification > net immobilization during the 6 wk greenhouse incubation. This corresponds to the higher net N mineralization and net nitrification of soils amended with green manure C/N = 8 than the unamended control soil (Table 3 -1). However, both soil types mixed with green manure C/N = 12 had similar net N mineralization as the unamended control soil, and there was no difference in the net nitrification of these treatments in the sandy loam soil (Table 3 - 1). Monitoring the IEM-NH_4-N and IEM-NO_3-N concentrations throughout the incubation period give a clearer picture of what occurred in the sandy loam soil mixed with green manure C/N=12. The results indicate that net N mineralization occurred in most weeks, but the IEM-NO_3-N concentration was less than the unamended sandy loam from weeks 3 to 6 of the study (Fig. 3 - 1), possibly due to NO_3-N leaching.

3.6. Conclusions

The IEMs used in this study are a sink for mineral N and thus represent a pool of NH_4-N and NO_3-N ions that may be available for microbes or for plant roots. We found them to be a sensitive *in situ* tool for weekly monitoring of soil mineral N dynamics that could detect differences in the decomposition of green manure residues with low C/N ratios. This information is complementary and consistent with the patterns of net N mineralization and net nitrification from green manure-amended soils.

Table 3 - 1. Net N mineralization and net nitrification, as well as the microbial biomass concentration (mg MBC kg^{-1} or mg MBN kg^{-1}) and the mass of green manure residues collected on sieves, from sandy clay loam and sandy loam soils amended with green manure, after 6 wk incubation period.

	Sandy clay loam			Sandy loam		
	no green manure	green manure C/N=8	green manure C/N=12	no green manure	green manure C/N=8	green manure C/N=12
	mg kg^{-1}					
NH_4-N	0.64(0.05)^{b}	0.98(0.04)^{a}	0.67 (0.03)^{b}	1.47 (0.02)^{b}	1.70 (0.03)^{a}	1.42 (0.02)^{b}
NO_3-N	3.41(0.41)^{c}	8.35(0.21)^{a}	5.41 (0.30)^{b}	5.27 (0.46)^{b}	8.96 (0.06)^{a}	5.47 (0.33)^{b}
MBC	123.7 (11.31)^{b}	164.70 (9.87)^{ab}	194.9 (6.29)^{a}	153.3 (0.96)^{b}	169.09(2.03)^{a}	159.5 (0.54)^{b}
MBN	20.6 (0.41)^{b}	44.0 (1.41)^{a}	47.0 (1.36)^{a}	25.0 (0.23)^{a}	44.6 (4.78)^{a}	39.0 (1.94)^{a}
	g kg^{-1}					
1-2 mm	0.13 (0.01)^{c}	0.20 (0.01)^{b}	0.40 (0.02)^{a}	0.13 (0.02)^{b}	0.15 (0.01)^{b}	0.33 (0.03)^{a}
3-4 mm	0.23 (0.02)^{c}	0.44 (0.01)^{b}	0.51 (0.02)^{a}	0.25 (0.02)^{c}	0.35 (0.02)^{b}	0.47 (0.01)^{a}

Number in the parentheses are standard error (n=4). Different superscript letters indicate significant difference (LSD, p <0.05) within a soil type that was either unamended (no green manure) or mixed with green manure having C/N ratios of 8 or 12
MBC microbial biomass carbon, *MBN* microbial biomass nitrogen
1-2 and 3-4 mm are the sieves used to collect undecomposed residues after 6wk

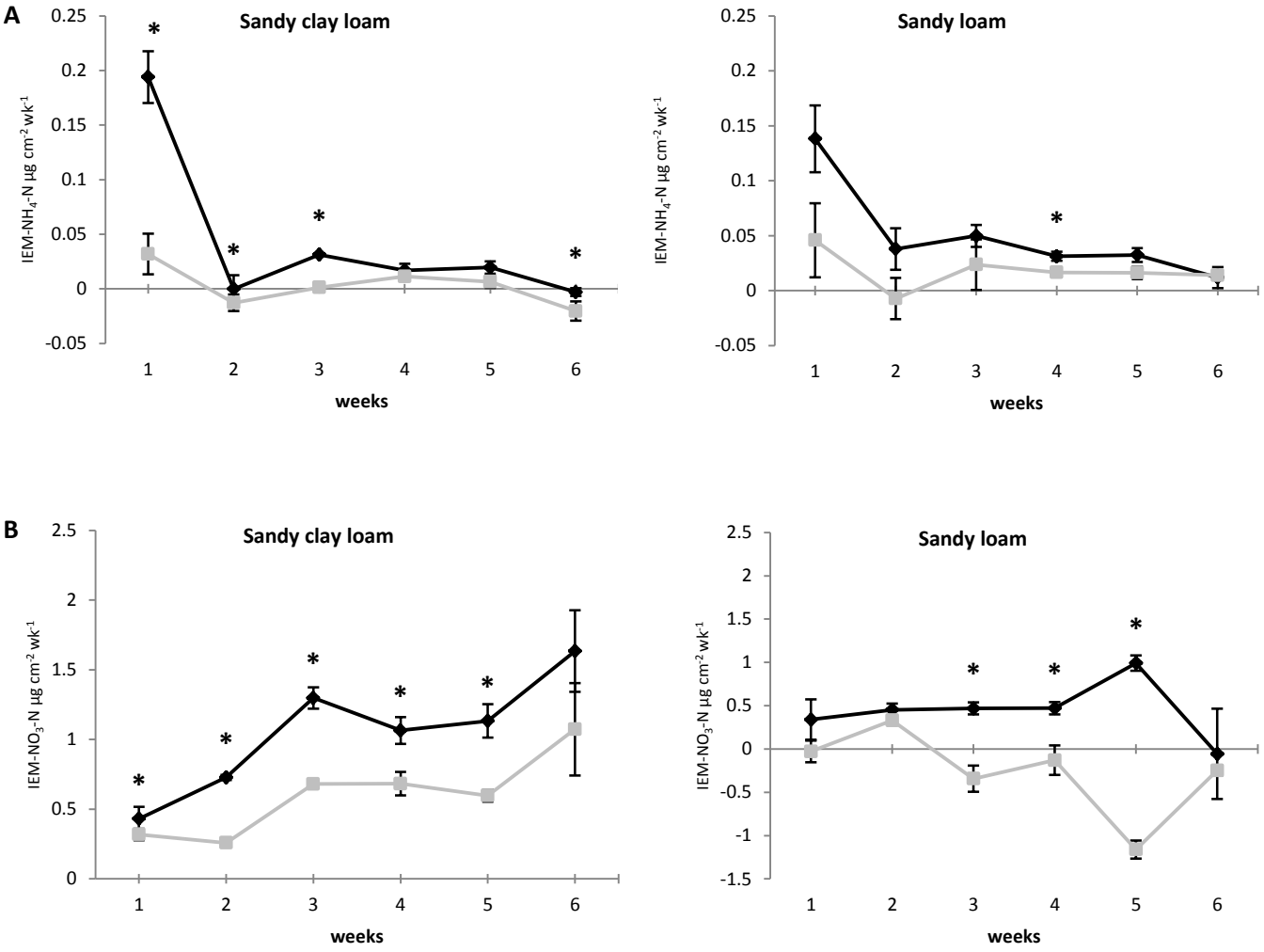

Figure 3-1. Net concentration of nitrogen release from green manure field peas and oats, as measured by the concentration of plant-available nitrogen adsorbed to ion exchange membranes as IEM-NH_4-N (A) and IEM-NO_3-N (B) buried in sandy clay loam and sandy loam soil for 6 wk. Values are the mean ± SE (n = 4) in the (♦) green manure C/N ratio = 8 and (■) green manure C/N ratio = 12. Data points with an asterisk (*) indicate significant difference between treatments in that week of the incubation (LSD, $p < 0.05$)

FOREWORD TO CHAPTER 4

In Chapter 3, I found that ion exchange membranes were a sensitive tool for detecting differences of the N mineralization pattern from green manure residues with C/N ratios of 8 to 12. The increase in plant-available N was attributed to decomposition of green manure residues having a particle size of 2-4 mm. A follow-up study was done to determine how tillage, an agriculture practice that reduces the particle size of organic materials, would affect the release of plant-available N from green manure. Chapter 4 describes a field study where I investigated how tillage could impact the green manure residue size and N supply to arugula crop.

CHAPTER 4.

Tillage intensity promotes different dynamics of nitrogen supply from a green manure mixture to arugula (*Eruca sativa* L.)

4.1 Abstract

Green manure decomposition releases mineral N for subsequent crops in the rotation, but the N mineralization process can be slower for green manure residues having larger particle sizes. To achieve smaller residue size of the green manure, the farmer might choose to cultivate more intensively by passing over the same field multiple times, but it is not known how many tillage passes are needed to optimize the plant N uptake. My objective was to quantify the mineral N dynamics following green manure incorporation with different numbers of tillage passes (1, 2 and 4 passes), and to relate the reduction in residue particle size to the crop N uptake. Factorial treatment (3 levels of tillage passes x 2 levels of green manure) were randomly assigned to field plots on a loam soil in southwestern Quebec, Canada. Plots were planted with a green manure mixture of peas (*Pisum sativum* L.) and oats (*Avena sativa* L.), while control was kept relatively weed-free by mowing and hand-weeding. Soil mineral N dynamics were monitored for six weeks after cultivation of green manure and control plots, and the N uptake by arugula (*Eruca sativa* L.) growing in all plots was measured from weeks 2 to 5 of the study. Green manure plots had 16 to 27% more soil mineral N. Greater tillage intensity increased the NO_3 concentration on ion exchange membranes from 1.94 to 18.7 µg cm^{-2} wk^{-1}, and the IEM-NO_3-N concentration was significantly higher with 2 and 4 than 1 pass. The smaller size of green manure residue achieved by 2 and 4 passes of the cultivator, had a positive impact on the mineral N supply, microbial biomass concentration and arugula N uptake. I conclude that the

incorporation of green manure by two passes could improve the optimization of N requirements of Brassicas, particularly arugula.

4.2 Introduction

Tillage is a common practice on Canadian farms, with more than 75% of agricultural land cultivated with plow and harrow. Although soil conservation practices like reduced tillage and direct seeding are gaining in popularity, particularly in semi-arid regions of Canada (Statistics Canada, 2012a), the reality is that most farmland is tilled. On farms where animal manure is an important fertilizer source, tillage is highly recommended to incorporate surface-applied manure as quickly as possible, to minimize nitrogen (N) losses through ammonia volatilization and reduce odors. Another reason to use tillage is to incorporate organic residues such as compost and crop residues into the soil and mechanically fragment the residue into smaller particles, thereby accelerating their colonization by microorganisms, decomposition of polymeric compounds contained in the residue, and ions/nutrient release (Whalen, 2014). Termination of a green manure crop is traditionally accomplished through a plow-down event, which ends the crop growth, reduces the physical size of the above- and below-ground residues, and brings the residue into contact with soil decomposers (fauna and microorganisms) that are responsible for nutrient recycling. Termination of green manure with tillage stimulates N mineralization and results at least one fold greater soil mineral N content than using no tillage/mulch systems for green manure termination (Groffman et al., 1987; Drinkwater et al., 2000, Halde et al., 2014).

Particle size reduction is the first step required to initiate residue decomposition, and may be accomplished with tillage equipment, mowing or stock chopping for crops with larger stalks or by the action of soil macrofauna in agroecosystems with low intensity, infrequent or no

tillage. Residues with a small physical size are more exposed to microbial attack than intact plant residues due to the greater surface area for microbial colonization (Angers and Recous, 1997). In a study with wheat straw residue, Ambus and Jensen (1997) reported total N mineralization of 10.4 % from residue < 3 mm diameter and 8.6 % from residue size of 25 mm, after 60 d. In the same study, the authors reported, more N mineralized (3.3 mg N kg^{-1}) from barley residues at < 3 mm than 25 mm (2.7 mg N kg^{-1}). They concluded that the contact between fine crop residues and soil is intimately related with N mineralization. Boström and Lofs-Holmin (1986) observed great decomposition by earthworms when they were provided with barley ground to < 0.2 mm than 10 mm residue size. These authors concluded that small residues were easier for the earthworm to digest, thus increasing the amount consumed and bringing the residue into contact with microorganisms within the earthworm gut and in their casts (mixture of residues and soil egested by earthworm). In this regards, the microbial biomass is positively impacted by the incorporation of smaller crop residues (Sørensen et al.,1996; Muhammad et al., 2010). After the incorporation of field peas, Jensen (1994c) reported microbial biomass nitrogen concentrations of 12.3 µg N g^{-1} soil when residue size was 10 mm, and this increased to 22.8 µg N g^{-1} soil for residue size < 3 mm. In agroecosystems relying on mechanical tillage to reduce the particle size of organic residues, it is hypothesized that more intensive tillage (i.e., more passes of the tillage equipment) will significantly accelerate the decomposition and nutrient release from residues such as green manure.

Green manure is an important N fertilizer in a variety of agroecosystems, particularly organic, including those producing vegetable crops, potatoes, cereal crops, and oilseed crops (Thorup-Kristensen, 1993; Vyn et al., 2000; Entz et al., 2001; Sincik et al., 2008). The N released from green manures can provide an average of 17% of the crop N requirements during

the growing season (Gardner and Drinkwater, 2009). To improve the N use efficiency from green manure, it is important to know how soon after the plow-down event that plant-available N is released from decomposing green manure residues, and how much plant-available N is captured by the subsequent crop. This can be evaluated in plant N uptake assays in greenhouse and laboratory studies, but may also be evident from non-plant testing methods. Ion exchange membranes (IEMs) hold promise in this regard because they can be deployed *in situ* and act as a sink for NH_4 and NO_3 ions, resembling plant roots, the adsorption responds to the same environmental conditions and edaphic factors that affect plant N uptake (Qian and Schoenau, 2002). A recent study in Quebec, Canada (León Castro and Whalen, 2016) monitored the N dynamics before and after the plow-down of field pea and oats. The concentration of IEM-NO_3-N was sensitive to arugula N uptake and ambient variables (i.e., rainfall, soil temperature and moisture). Furthermore, the reliance to IEMs can be validated by correlating the plant-available N dynamics (soil NH_4-N and NO_3-N concentrations) and the IEM-NH_4-N and IEM-NO_3-N values during the green manure decomposition process.

The objective of the study was to determine whether N release from green manure was enhanced by increasing tillage intensity (i.e., more passes with the cultivator during the plow-down event), as well as the impact on microbial concentration. Soil mineral N dynamics following the green manure plow-down was evaluated by monitoring the growth and N uptake by arugula, a short-season vegetable crop (maturity in 28 d), as well as the N adsorption by IEM.

4.3 Materials and methods

4.3.1 *Site description*

The experiment was conducted from June to August, 2015 at the Horticultural Research Center on the Macdonald Campus of McGill University (45° 24' N, 73° 56' W). The soil was of the Saint Bernard series (Soil Classification Working Group, 1998) and had a loam texture (400 g sand kg^{-1}, 230 g clay kg^{-1}) with pH of 7.7 and 27 g organic C kg^{-1}. Soil nutrient concentration was determined by Mehlich-3 extraction and contained 344 mg P kg^{-1}, 313 mg K kg^{-1}, 4371 mg Ca kg^{-1}, 643 mg Mg kg^{-1} and 786 mg Al kg^{-1}. The field selected for this study was previously cultivated with pepper and used for tomato production, two years before this study began. The experimental design was a randomized complete block design with two factors: fertilizer (without green manure, and green manure) and tillage with different number of passes (1, 2 and 4) with four replications per treatment. Accordingly, the field site (17 m x 25 m) was divided in 24 plots (3 m x 3 m), separated by a 1 m alley. Each plot was divided into 6 sub-plots (1 m x 1.5 m) for independent sampling in every week for six weeks.

On June 15th, the soil was cultivated and a green manure mixture of field peas (*Pisum sativum* L.) and oats (*Avena sativa* L.) was broadcast at the rates of 90 kg ha^{-1} and 83 kg ha^{-1}, respectively. Control plots were cultivated, but not planted. Since the cultivation stimulated weed seed germination, the control plots were kept weed-free by mowing the surface every 7 d with a string trimmer (Husqvarna 22cc, Stockholm, Sweden). One day before the tillage operations began; green manure was sampled in 1 m^2 quadrats to determine the biomass. The field pea was at the vegetative stage, fourth node, leaf fully unfolded, more than one pair of leaflets (Knott 1987), and oat was at Feekes growth stage 5, tillering, leaf sheaths strongly

erected (Large 1954). The C/N ratio of the green manure mixture was 15 and contained 40 % cellulose, 27 % hemicellulose and 5.5 % lignin (acid unhydrolysable fraction) based on the method of Van Soest et al., (1991).

Tillage treatment began 5 wks after the green manure was planted, on July 20th. A rototiller (Troy-Bilt ProLine CRT, Ohio, United States) set at a depth of 0.15 m was used to cultivate each plot either control and treatment. The same numbers of passes (1, 2, and 4) were applied to both control and green manure plots. The multiple passes occurred over a four day period for a total of 24 plots, requiring 5 h for 1 pass plots (area cultivated = 0.0108 ha), 9 h for 2 passes plots (area cultivated = 0.0108 ha), and 18 h for 4 passes plots (area cultivated = 0.0108 ha) using a rototiller. Obviously, this does not reflect a highly-mechanized system that would be used on a farm to reduce the tillage time and labour costs. Farm-scale tractors and appropriate tillage implements (e.g disc-subsoiler) could be expected to cultivate between 4.2 and 5.1 ha per hour (Ayres and Williams, 1976)

On August 3rd, arugula (*Eruca sativa* L.) was sowed by hand in all plots at distance of 0.10 m plant spacing and 0.15 m row spacing, total of 200 plants m^{-2}. During the next four wks the arugula was sampled once per wk by selecting 9 plants at random from each plot and cutting the plant at ground level. Plant material was weighed, oven dried at 60 ^{o}C for 24 h and milled to pass a 1 mm mesh screen, and a subsample underwent total N analysis with a Flash EA NC Soils analyzer (Thermo Scientific, Delft, Netherlands)

During the growing season, the precipitation, soil temperature and moisture were monitored every 30 min, from June to August, with an automated weather station (Watch Dog 2000 Series, Spectrum Technologies Inc, Aurora, IL). Soil probes sensors (SM 100, Series,

Spectrum Technologies Inc, Aurora, IL) were installed at 0.15 m depth to monitor temperature and moisture continuously during the study period.

Ion exchange membranes (Ionics CR67-HMR (cation) and AR204-SZRA (anion), Durpro, Candiac, QC, Canada) were used to quantify the IEM-NH_4-N and IEM- NO_3-N concentration in the soil solution, a measure of plant available N (Ziadi et al., 2000, Qian and Schoenau 2002). The membrane sheets were cut into 0.02 m x 0.05 m strips and placed in distilled water to avoid desiccation. Prior to use, the strips were saturated by shaking for 1 h in 1 M NaCl and transported to the field in container filled with distilled water. After green manure plow-down, one anion strip and one cation strip were buried in the planted row at 0.05 m between arugula plants. The placement of the strips was done at random, but no location was used more than once during the study. The strips were buried vertically from 0.10 - 0.15 m depth, making a slot in the soil using a garden shovel, after the placement of the membranes the slot was filled with soil by hand. One wk later, strips were retrieved, rinsed in the field with distilled water to remove soil particles and placed in centrifuge tubes containing 25 mL 1 M KCl.

4.3.2 *Soil sampling, residue recovery and N contribution from green manure*

Soil samples were collected starting in the 4th wk of the green manure growth period and then taken once a week for the next 6 wks (e.g., including one wk after arugula harvest). Soil sampling was done with a garden shovel in the 0 - 0.15 m layer, making one composite sample (from 5 locations) of approximately 1000 g field-moist soil from each plot. Composite samples were sieved at < 10 mm mesh to remove rocks and large crop residues. Soil samples were placed in Zip-lock bags and transported on ice to the laboratory. Half of the sample was sieved (< 2 mm) and stored at -20 ^{o}C until analysis. The other half was undisturbed and used to determine

the particle size of crop residues. The residue recovery from soil was achieved with a wet-sieving method based on Cambardella and Elliot (1993). Briefly, about 300 g of field-moist soil was placed on a nest of sieves with mesh sizes 2 mm and 4 mm. The sieves were immersed in water and sieved for 5 min at 20 movements per minute, the residues collected were weighed.

The relative N contribution from green manure and soil were calculated as follows (Sharma and Behera, 2009):

- Relative N contribution from green manure (%) = [(Total N uptake – N uptake without green manure) / total N uptake] x 100
- Relative N contribution from soil (%) = 100 - Relative N contribution from green manure (%)

4.3.3 *Nitrogen analysis in soil and ion exchange membranes*

For determination of soil mineral N and microbial biomass, soil samples were thawed at room temperature (20 ^{o}C for 2 d). Then mineral N (NH_4-N and NO_3-N) was extracted by shaking 5 g of field-soil with 50 mL of 2 M KCl for 60 min, filtering through Whatman no.42 filter paper and storing extracts in acid-washed plastic bottles. The IEM-NH_4-N and IEM-NO_3-N concentration were determined after extracting the adsorbed NH_4 or NO_3 from the IEM with 1 M KCl solution by shaking for 60 min and filtering through Whatman No. 5 filter paper. The NH_4 and NO_3 concentrations in soil extracts and IEM extracts were measured by the modified indophenol blue method (Sims et al., 1995) at 650 nm on a microplate reader (µQuant, Biotek, Winooski, VT).

The microbial biomass C and N were analyzed by a chloroform fumigation-extraction method (Voroney et al., 2008). Field moist soil samples (10 g) were extracted with 0.5 M K_2SO_4 (1:4 soil:solution). The calculation for MBN concentration was (total extractable N after fumigation- total extractable N before fumigation)/0.54 (Joergensen and Mueller, 1996; Voroney et al., 2008). MBC concentration was determined by the difference between the C extracted by a Sievers Innovox TOC analyzer (GE Analytical Instrument, Boulder, CO, United States of America) before and after fumigation, divided by $k_{EN} = 0.45$, where k_{EN} represents the efficiency of extraction of microbial biomass (Voroney et al., 2008).

4.3.4 *Statistical analysis*

The green manure and control plots were significantly different ($p < 0.05$) for all measured parameters. Therefore, the effect of number of passes and time after green manure plow-down on IEM- NH_4-N, IEM- NO_3-N, MBC and MBN concentrations due to green manure were corrected by subtracting the background concentration of each parameter in the corresponding replicate, and was determined using one-way repeated measures ANOVA. Least significant difference (LSD) test was used to determine significant differences between tillage passes (between-subject effects) and tillage passes x sampling time (within-subject effects). The relationships between IEM-NO_3-N and KCl-extractable NO_3-N, and arugula N uptake and IEM-NO_3-N were determined with Pearson's correlation coefficients. The effect of number of passes on N uptake by arugula due to green manure was determined by one-way ANOVA for each wk during the 4 week arugula growth period. Means separation tests were done with the LSD test at $p < 0.05$. Statistical analyses were performed with SAS Version 9.3 software (SAS Institute Inc., Cary, NC, USA, 2000).

4.4 Results and Discussion

The green manure mixture produced 3.6 t dry matter ha^{-1}. Weather conditions during the experimental period were typical for the June to August period in the area, with soil temperatures from 21 to 25°C, soil moisture between 4.2 and 26.6 g 100 g^{-1} and precipitation from 2.7 to 86.3 mm per week (Table 4 - 1). These values were consistent with the climate conditions experienced in this area in the last 30 years (Environment Canada, 2016).

4.4.1 *Nitrogen dynamics*

4.4.1.1 *Nitrogen concentration on IEMs*

Soil mineral N concentration increased after green manure was incorporated by tillage. Nitrogen mineralization showed changes after the incorporation of the green manure. In the 6 wks after green manure plow-down, the IEM-NH_4-N concentration decreased from 0.10 to 0.01 IEM-NH_4-N µg cm^{-2} wk^{-1} (Fig. 4 - 1A). The initial NH_4 concentration due to green manure was higher with 2 and 4 passes than 1 pass (p = 0.001), likewise the interaction tillage passes x time was significant different (p = 0.0001) (Table S4 – 1). This could be due to a stimulation in the green manure decomposition. In contrast, the IEM-NO_3-N concentration increased from 7.88 to 18.68 NO_3-N µg cm^{-2} wk^{-1} this increment might be due to the greater ammonia oxidation and nitrification rates occurred from wk 2 to 6 of the study. Similar pattern of NH_4 and NO_3 release was reported by Sarrantonio and Scott (1988) after the incorporation by tillage of hairy vech (*Vicia villosa* Roth), in which the soil mineral N increased by 55% in comparison to non-till system by 22%. Particularly, plots cultivated with 2 and 4 passes had higher NO_3 concentrations than those with 1 pass (p = 0.001). The greater NO_3 concentration in plots with more passes was sustained during the 6 wk study (p = 0.001) (Fig. 4 - 2B). This implies that the small physical

residue size and possible breakdown of soil aggregates due to more tillage passes was responsible for faster decomposition, N mineralization and nitrification.

4.4.1.2 *Nitrogen in soil by KCl extraction*

Soil NH_4-N showed a decreasing pattern in all treatments from 1.31 to -0.01 NH_4-N mg kg^{-1}, the highest concentration measured in the treatment with 4 passes (Fig. 4 - 2A), although the NH_4-N concentration showed no significant ($p = 0.94$) interaction between tillage passes x time (Table S4 – 1). On the other hand, soil NO_3-N increment ranged from 1.91 to 16.40 NO_3-N mg kg^{-1} (Fig. 4 - 2B), with numerically greater soil NO_3-N concentration in soils having 4 passes than 1 or 2 passes, and tillage passes resulted in a significantly ($p = 0.05$) different soil NO_3-N concentration with time (Table S4 - 1). The difference of N concentration among the tillage treatments was attributed mainly to the greater N mineralization and nitrification rates achieved with residue particle size reduction, although we cannot rule out the indirect priming effect of arugula roots on decomposition of green manure residues. I also note that the soil mineral N values were likely affected by the drier soil conditions after the first wk of the study. The significantly higher soil NO_3-N concentration with 2 and 4 passes, compared to 1 pass, in the 4^{th} and 5^{th} weeks after green manure incorporation (Fig. 4 - 2B) may reflect a lag time in the growth and activity of soil nitrifiers (Bending and Turner, 1999). Our finding is consistent with the report of Lupwayi et al.,(2006), where mineral N release from pea green manure peaked 5 wks after the plow-down event.

4.4.1.3 *IEMs as a reliable indicator of soil dynamics and plant N uptake*

Soil NH_4 and NO_3 concentrations are static measures of the extractable soil mineral N at a particular point in time, whereas IEM-NH_4-N and IEM-NO_3-N concentrations reflect the

cumulative NH_4 and NO_3 captured on IEM during a period of time. Although long term burials of IEMs are better correlated with crop N uptake than short-term burials (e.g., hours) (Qian and Shoenau, 1995), there are no reports where such measurements were taken on a weekly basis following green manure plow-down and prior to planting a short-season horticultural crop. My findings demonstrated a good correlation ($r > 0.63$) between the weekly IEM-NO_3-N and NO_3-N concentrations, as well as arugula N uptake and the IEM-NO_3-N ($r > 0.60$, $p = 0.05$) (Table 4 - 2). I conclude that the soil mineral N pool measured with IEM on a weekly basis is consistent with the results that can be obtained from soil chemical extractants, and may be a better predictor of crop N uptake. Hence, IEM deployment on a weekly basis seems to be appropriate to assess the soil mineral N dynamics in relationship to crop N uptake of soils receiving green manure inputs.

4.4.2 *Microbial biomass dynamics*

The MBC concentration increased with a greater number of passes, from 7.58 mg MBC kg^{-1} with one pass to 30.86 mg MBC kg^{-1} with four passes ($p = 0.03$). A similar pattern was observed with MBN concentrations. Throughout the experiment, the MBC and MBN concentrations were higher in plots that had 2 and 4 passes than 1 pass (Fig. 4 - 3).

Overall, the green manure residue mass was reduced by 15 % by 1 pass, 19% by 2 passes and 23 % by 4 passes. The low MBC and MBN particularly in 1 pass suggest that coarse residue demand more time for decomposition. This finding is consistent with Bending and Turner (1999), who studied two residues sizes (0.2 cm and 4 cm) of potato shoots (C/N = 10) and found that more microbial biomass was associated with fine residues. The delay in decomposition in coarse residues is due to the physical limitation (i.e., surface area) for microbial colonization, as

well as less exchange of water, nutrients and oxygen between the residue and soil matrix (Swift et al., 1979) and the presence of lignin barrier tissue (Whalen et al., 2014). On the other hand, smaller residues generated from the 4 pass treatment provide greater surface area for microbial colonization and degradation, thus enhancing the soil microbial biomass concentration in a shorter time (Fig. 4 - 3).

4.4.3 *Relative N contribution from green manure*

The relative N contribution by the arugula crop (after 4 wks) was between 5 and 10 % in plots with one pass, from 5 to 17 % in plots with two passes, whereas plots that received four passes recovered from 7 to 20 % of the green manure N in arugula biomass (Fig. 4 - 4). This indicates that recently-incorporated green manure supplies a relatively small percentage of the N required by arugula, regardless of the number of tillage passes. Still, the N contributions from recently incorporated green manure are consistent with previous studies. For instance, Seo et al., (2006) reported 15 % N recovered by maize as catch crop when hairy vetch was used as green manure. Likewise, Sharma and Behera (2009) reported 22 and 25 % of N contribution from cowpea and sesbania green manures, respectively.

The N uptake from green manure was higher in wks 3 and 4 than in the first two wks (Table 4 - 3). This might be due to greater N demand by the crop during this period of time, as Omirou et al., (2012) reported the highest NO_3 concentration in fully expanded leaves of arugula was recorded 15 d after planting. During the arugula growth period, the N uptake was enhanced by 13 to 21 % with 2 passes and 4 passes, compared to 1 pass. No visual N deficiency was seen. The arugula biomass showed an increasing pattern of N uptake, particularly in the green manure plots (Table 4 - 4). I am not aware of critical N values for arugula, but found that the N uptake of

arugula grown after green manure plow-down with 2 passes are around 30 g N kg^{-1}, which matched with the optimal N uptake reported by Chen et al., (2004) for turnip, Chinese cabbage, and spinach (Table 4 - 3).

The fact that decomposable residues remained at the end of the experiment, and the amount of residues (2-4 mm diameter) collected in green manure treatment increased, on average, by 50.3 and 84.9 g kg^{-1} between wk 2 and wk 4, respectively (Fig. 4 - 5), suggests that mineral N will continue to be released from the green manure residue in the coming weeks. It may take several weeks to build-up ample microbial activity to fully decompose the green manure residue (Bending et al., 1998), leading me to suggest that pea/oat is better suited as green manure for a horticultural crop with a longer growing period to take advantage of the residual N remaining in the green manure residues, but this remains to be determined. Given the residual soil mineral N concentration remaining after arugula harvest, I would further suggest that producers using this system should plant a second cash crop following arugula, or to plant a fall-seeded cover crop (e.g., fall rye, hairy vetch, winter rye, and winter wheat) that can absorb the N mineralized from green manure residue after arugula harvest in wk 6 of the study.

4.5 Conclusion

The number of passes reduced the size of green manure residues and stimulated N mineralization, nitrification, the size of the microbial biomass, and arugula N uptake. Two passes for the plow-down of pea/oat green manure supported better growth and N uptake by arugula, and was as effective as four passes, which might impact soil quality and structure negatively. The pea/oat green manure supplied up to 20% of the N required by arugula and continued to mineralize after this short-season crop was harvested. To increase N use efficiency from the

green manure residue, it is recommended to plant a second cash crop or a fall-seeded cover crop. The work presented here provides further evidence that IEMs evaluation on a weekly basis was a good indicator of soil mineral N dynamics and crop N uptake in cropping systems that use green manure as a source of N fertilizer.

Table 4 - 1. Weekly means of precipitation, soil temperature and moisture measured at 0.15m soil depth from growing season (July-August, 2015) in Ste-Anne-de-Bellevue.

	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6
Precipitation (mm)	86	20	26	3	49	10
Soil temperature (°C)	21	22	24	22	22	25
Soil moisture (g 100 g^{-1})	27	12	7	4	10	9

Table 4 - 2. Correlations of IEM-NO_3-N with NO_3-N during six weeks, and arugula N uptake during four weeks

	week 1		week 2		week 3		week 4		week5		week 6	
	IEM-NO_3-N		IEM-NO_3-N		IEM-NO_3-N		IEM-NO_3-N		IEM-NO_3-N		IEM-NO_3-N	
	r	*p*	*r*	*p*	*r*	*p*	*r*	*p*	*r*	*p*	*r*	*p*
NO_3-N	0.63	0.17	0.92**	<0.01	0.86*	0.02	0.90**	0.01	0.89**	0.01	0.95**	<0.01
Arugula N uptake	-	-	-	-	0.60	0.20	0.79*	0.05	0.79*	0.05	0.76	0.07

IEM-NO_3-N = NO_3-N adsorbed on ion exchange membrane, NO_3-N = NO_3-N concentration by soil extraction
*. Correlation is significant at 0.05
**. Correlation is significant at 0.01

Table 4 - 3. Effect of green manure mixture (field peas and oats) and number of passes with rototiller on arugula nitrogen uptake (g N kg^{-1})

Treatment	week 1	week 2	week 3	week 4	Cumulative N uptake
			g N kg^{-1}		
Control (C) 1 pass	3.45 (0.65)a	7.36 (2.35)ba	13.28 (1.77)c	66.89 (11.41)bc	90.98
Green manure (Gm) 1 pass	4.06 (0.82)a	10.83 (3.39)ba	22.35 (1.36)bc	86.48 (0.54)ba	123.72
C 2 passes	3.67 (0.42)a	7.23 (1.48)b	11.95 (2.48)c	68.18 (9.30)bc	91.03
Gm 2 passes	4.28 (0.96)a	15.22 (3.79)a	30.05 (1.22)ba	90.28 (0.86)a	139.83
C 4 passes	3.42 (0.57)a	8.48 (0.66)ba	41.21 (8.38)a	65.50 (4.07)c	118.61
Gm 4 passes	5.05 (0.62)a	12.41 (2.34)ba	38.86 (0.66)a	104.54 (5.16)c	160.86

Means (n = 9) with the same letter (s) (a, b, c, d) within the same column are not significant different at $p < 0.05$ (LSD)

Table 4-4. Fresh matter accumulation of arugula crop at different number of tillage passes

Treatment	week 1	week 2	week 3	week 4	Mean
			g plant^{-1}		
Control (C) 1pass	1.15a	2.28a	4.21c	22.21bc	7.46
Green manure (Gm) 1 pass	1.34a	3.00a	6.36a	27.79ba	9.62
C 2 passes	1.11a	2.14a	3.81c	19.81bc	6.72
Gm 2 passes	1.13a	4.07a	8.37bac	24.77bac	9.58
C 4 passes	1.10a	2.94a	12.06ba	19.28c	8.84
Gm 4 passes	1.38a	3.76a	10.56bc	31.99a	11.92

Means (n = 9) with the same letter (s) (a, b, c, d) within the same column are not significant different at $p < 0.05$ (LSD)

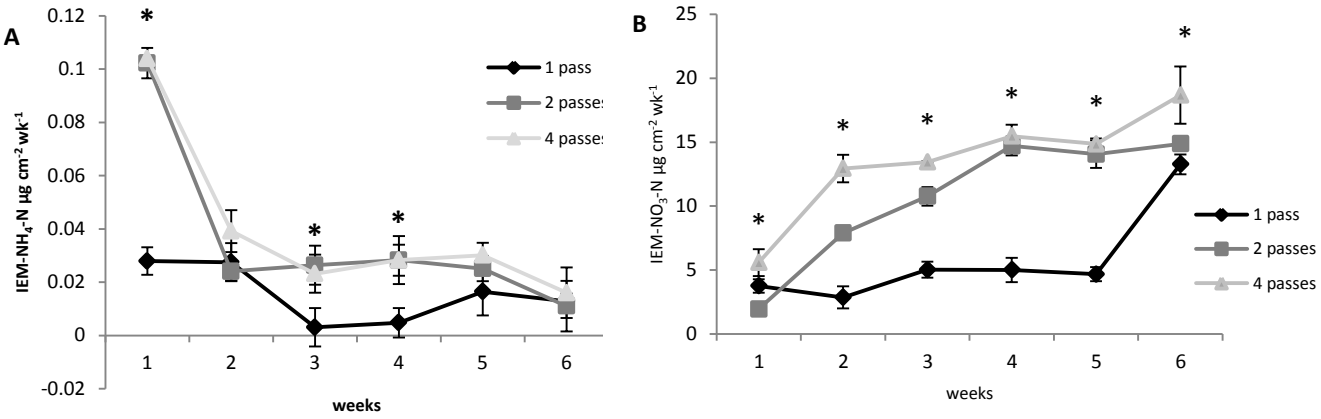

Figure 4 -1. Net concentration of four replicates from control-treatment of (A) IEM-NH_4-N and (B) IEM-NO_3-N after 3 tillage passes, during 6 weeks. Error bars represent standard error. Statistical significant difference among treatments is shown with an asterisk (LSD, $p < 0.05$)

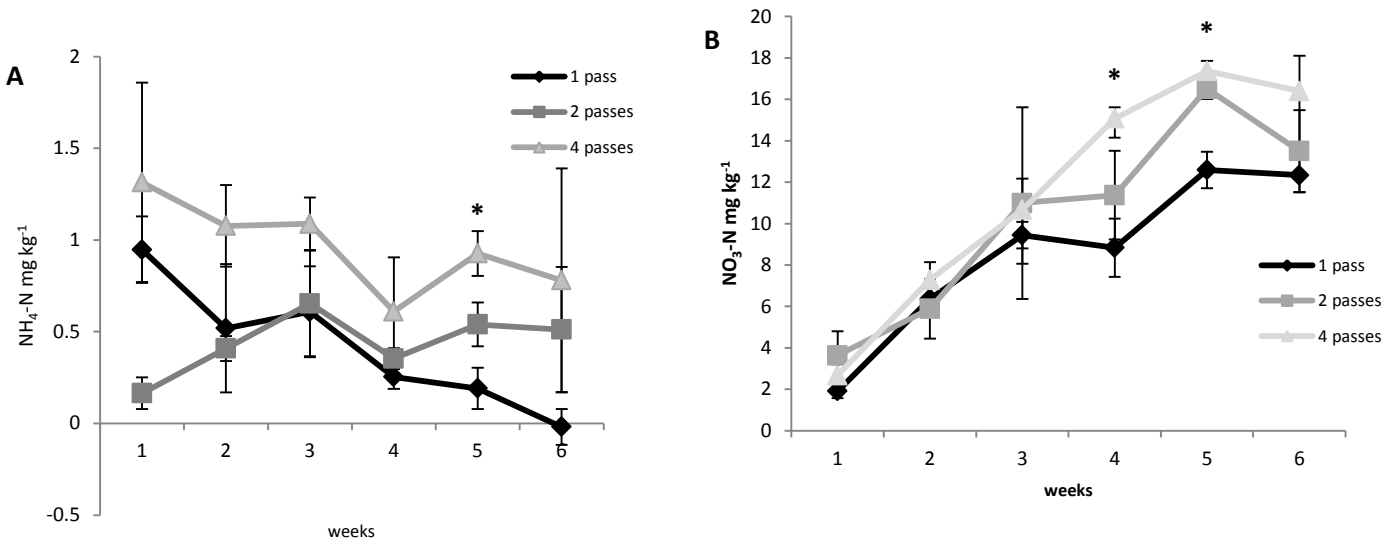

Figure 4 - 2. Net soil NH_4-N (A) and NO_3-N (B) of control-treatment from the incorporation of field-pea and oat in a field experiment. Error bars represent standard error. Statistical significant difference among treatments is shown with an asterisk (LSD, $p < 0.05$)

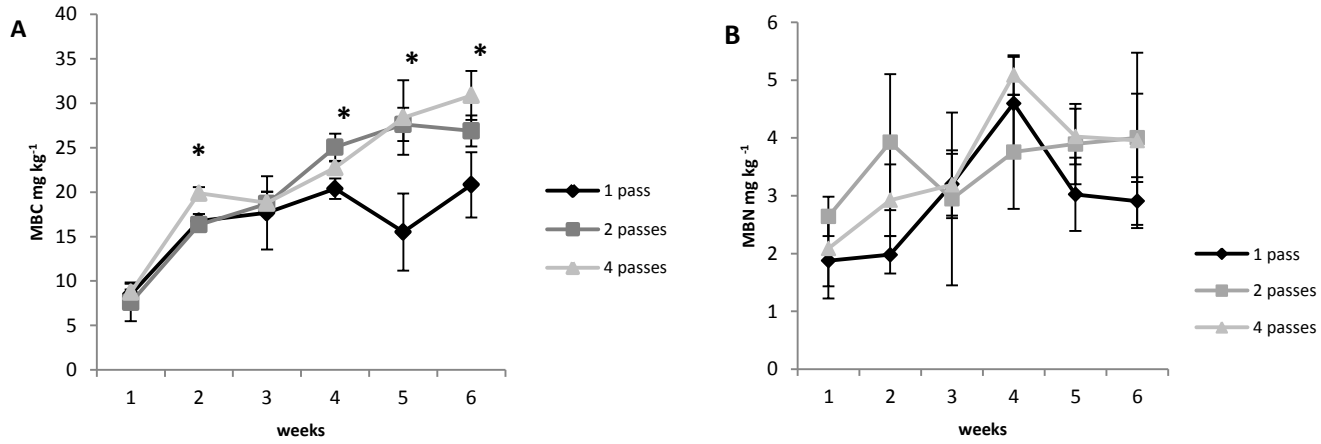

Figure 4 - 3. Net concentration of four replicates from control–treatment of soil (A) Microbial Biomass Carbon (MBC) and (B) Microbial Biomass Nitrogen (MBN) after 3 tillage passes, during 6 weeks. Error bars represent standard error. Statistical significant difference among treatments is shown with an asterisk (LSD, $p < 0.05$)

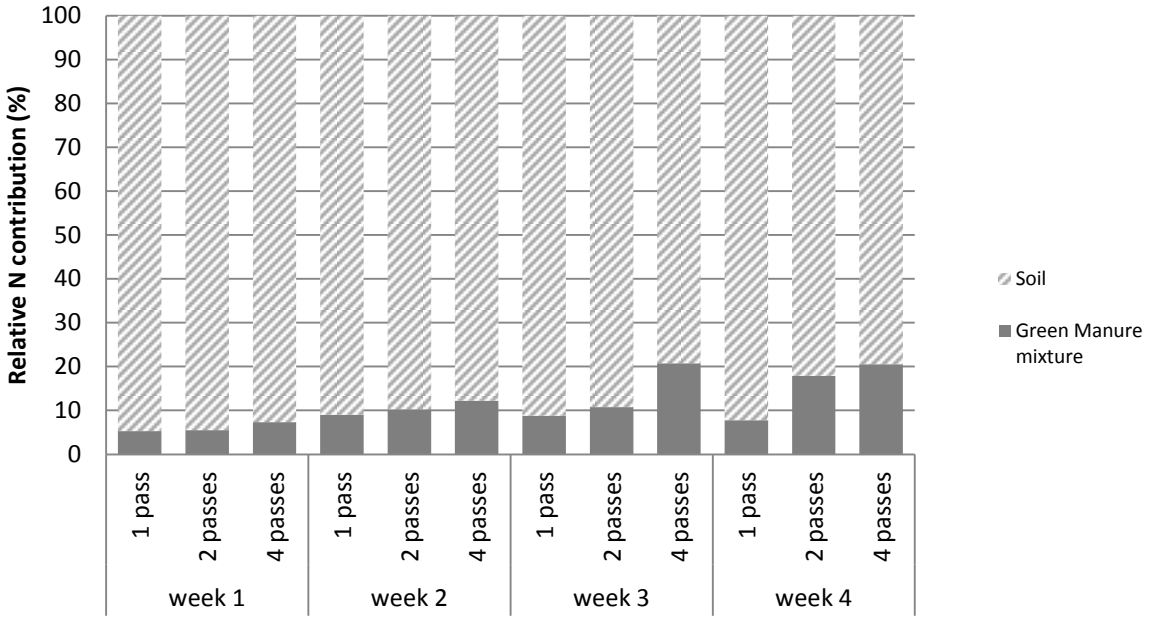

Figure 4 - 4. Relative N contribution from two sources in Arugula crop in a system with one, two and four passes to incorporate green manure mixture, peas (*Pisum sativum* L.) and oats (*Avena sativa* L.).

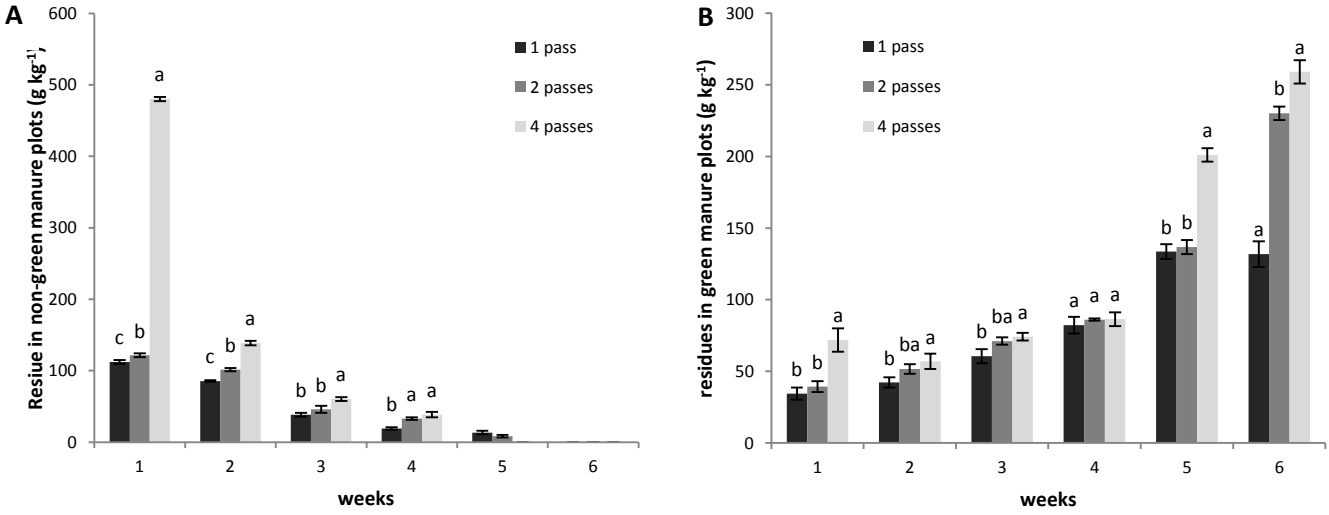

Figure 4 - 5. Residue with particle size 2 – 4 mm remaining in (A) non-green manure soil and (B) green manure amended soil in the 6 weeks following incorporation of green manure mixture (field peas and oats). Error bars represent standard error. Means (n = 4) with the same letter (s) (a, b, c) are not significant different at $p < 0.05$ (LSD)

FOREWORD TO CHAPTER 5

In chapter 4, I quantified the soil mineral N concentration after tillage passes. The N mineralization, nitrification, and green manure mixture residue size were affected by tillage intensity. Two and four passes resulted in an optimal N uptake by arugula. Likewise, the green manure mixture (field peas and oats) supplied up to 20% of arugula N requirements. Since arugula is not a passive recipient of NH_4 and NO_3, but actively participates in shaping the soil microbial community that is responsible for decomposition, N mineralization and nitrification processes, the feedback between arugula root system and green manure mineralization warrants investigation. Therefore, I conducted a greenhouse experiment to quantify the contribution of root exudates to N mineralization of green manure residues and the crop impact on microbial biomass during a six week growing period.

CHAPTER 5.

Decomposition of green manure mixture (field peas-oats) and nitrogen mineralization as affected by particle size and simulated root exudation

Abstract

Belowground plant roots are present in an ecosystem where green manure is mineralized after it is incorporated as a source of nitrogen (N). The residue decomposition is regulated by soil microbes that can be impacted by the carbon (C) released from root exudates. It is not known how the C contribution from arugula (*Eruca sativa* L.) root exudates impacts the N mineralization of green manure. The objective of this study was to quantify the N mineralization of green manure residue mixture composed of peas (*Pisum sativum* L.) and oats (*Avena sativa* L.) influenced by plant root exudates. The N mineralization was monitored for six weeks in a greenhouse experiment. Plant pots were set up for a two-way factorial experiment with three C sources (soil organic matter, arugula, glucose) and three green manure residue sizes (no green manure, 0.5 to 1 mm, 2 to 4 mm). The N mineralization and microbial biomass concentration was impacted by the three sources of C, and it was evident the significant ($p < 0.05$) increment one week after the arugula was planted. Similarly, the N increased after the application of glucose, but to a lesser degree. The C input by the arugula roots resulted in a 6% increase of plant available N in residues 0.5 – 1 mm, and 4% in residues 2 – 4 mm. Overall, the microbial biomass concentration was greatly impacted by residues sized 0.5 – 1 mm leading to an increase in plant available N. This study highlights the priming effect by root exudates and the impact on N mineralization from different sizes of green manure.

5.2 Introduction

Green manure residues play a major role supplying N for the following crop. The N supply begins immediately upon plant cell lysis whereby soluble NH_4-N and NO_3-N are released and after the residues are colonized by soil microorganisms, simple proteinaceous and non-proteinaceous forms of N are hydrolyzed by extracellular enzymes to release NH_4-N (Whalen, 2014). However, the velocity N is supplied by plant residues is regulated by their biochemical composition (Marstorp and Kirchman, 1991), and particle size (Ambus and Jensen, 1997). Thus, incorporating green manure residues with different biochemical and physical characteristics can differentially impact the microbial biomass. Seven days after incorporating brussels sprout (*Brassica oleracea* L vc. Peer Gynt) shoots (C/N = 15), microbial respiration increased by 1000 µl hr^{-1} mg^{-1} dw from residues size of 0.2 – 1 cm, whereas residues size of 0.2 – 4 cm reached 400 µl hr^{-1} mg^{-1} dry weight (dw); in the same experiment using rye grass (*Lolium perenne* L cv. Parcour) (C/N = 38) residues size 0.2 – 4 cm peaked to 350 µl hr^{-1} mg^{-1} dw (Bending and Turner, 1999). Residues with wider C/N ratios and/or greater size resulted undecomposed or minimum N mineralization , because microbes prefer to consume organic matter that demands the least energy input (Paterson, 2003).

Plants roots supply C via root exudation and rhizodeposition, which in turn promotes microbial activity (Jones et al., 2004; Gul and Whalen, 2013). Legumes, in particular will release flavonoids, aromatic acids, amino acids and dicarboxylic acids, which act as chemo-attractants for micro-organisms (e.g., rhizobia) in soil with low N fertility to stimulate N turnover (Dakora and Phillips, 2002). Root exudates are compounds characterized by complex mixtures of different low molecular weight compounds such as, organic acids, vitamins, sugars, amino acids, enzymes , gaseous molecules and root epidermal and cortical cells (Dakora and Phillips, 2002;

Farrar et al., 2003; Dennis et al., 2010). The total root exudate composition may be partitioned into sugars (50 – 70%), carboxylic acids (20 – 30%), and amino acids (10 – 20%) (Kraffczyk et al., 1984; Jones, 1998; Hütsch et al., 2002). About 30 to 60% of the total C fixed from photosynthesis can be translocated to the roots, and between 3 to 5% of the C is released in the form of sugars, mainly glucose (Pinton et al., 2001). Plant root exudates serve to shift microbial populations (Lynch, 1990), regulate soil pH (Jones et al., 2004), plant-microbe signaling (Gregory, 2006), and induce the priming effect (Kuzyakov, 2010). The priming effect, described by Kuzyakov (2010) as "the response after C input into the soil" was the focus of this study. There are different methods proposed to quantify the C released by root exudates that might impact the rate residues turnover (Meharg, 1994). Most studies assume a steady C release pattern in concert with plant growth stages, nevertheless, the rate by which root exudates are released varies based on different plant species, soil biotic and abiotic factors (Jones et al., 2004). In this regard, it is uncertain how arugula roots will supply exudates or C in cropping systems that incorporate green manure residues. I propose that ion exchange membrane (IEM) can be used as a sensitive tool to quantify plant-available N concentrations, temporally. Leon and Whalen (2016) detected different IEM-N release patterns when green manure residues with low C/N ratios (8 and 12) were used in two different soil types.

The objective of this study was to assess the potential contribution of root exudates from arugula to stimulate the decomposition and N mineralization from residues of a green manure mixture (peas-oats).

5.3 Materials and methods

5.3.1 Soil

Soil from the Saint Bernard series (Soil Classification Working Group, 1998) was collected from the Horticultural Research Center, Macdonald Campus, McGill University (45 ° 24' N, 73° 56' W). The soil texture was a loam (400 g sand kg^{-1}, 230 g clay kg^{-1}), contained the following concentration of Mehlich-3 extractable nutrients: 344 mg P kg^{-1}, 313 mg K kg^{-1}, 4371 mg Ca kg^{-1}, 643 mg Mg kg^{-1} and 786 mg Al kg^{-1}. It had a pH of 7.7 and 27 g organic C kg^{-1}. The soil was collected with a shovel at 0-15 cm depth, passed through a <10 mm sieve to remove rocks and large plant residue, and subsequently sieved through <2 mm mesh screen to thoroughly homogenize the soil.

5.3.2 Green manure preparation

The green manure mixture was field peas (*Pisum sativum* L.) and oats (*Avena sativa* L.) grown in the same field where the soil was collected. The vegetative stage of the field peas at harvest was fourth node, leaf fully unfolded, more than one pair of leaflets (Knott, 1987), and oats were at Feekes growth stage 5, tillering, leaf sheaths strongly erected (Large, 1954). The green manure mixture produced a dry biomass of 3.6 t ha^{-1}, and contained a C/N = 15. The cellulose (40 %), hemicellulose (27 %), and lignin (5.5 %) content (acid unhydrolysable fraction) were determined by the Van Soest et al., (1991) method. The green manure was ground and sieved; the residues sized 0.5 mm to 1 mm and 2 mm – 4 mm were collected separately.

5.3.3 Experimental design

Experimental units for this greenhouse study were plastic plant pots (10.5 cm i.d., 13 cm deep) that contained 1 kg (dry weight basis) of soil packed to a bulk density of 1.39 g cm^{-3} in the pot. Each experimental unit containing soil that was either planted with arugula or unplanted, and that had no green manure added or was mixed with 0.6 g kg^{-1} of green manure residue having small or large particle sizes. Thus, the experiment was designed as a two-way factorial with three carbon sources (from soil organic matter, arugula, and glucose) and three green manure residue sizes (no green manure, 0.5 to 1 mm, 2 to 4 mm). This resulted in the preparation of nine experimental treatments (Table 5 - 1). For the purpose of destructive sampling, separate pots were prepared for wk 1 to 6 of the experimental period. Four replicate pots from each treatment and sampling date (216 pots in total) were arranged on a greenhouse bench in a complete randomized design. The air temperature in the greenhouse was regulated by a heated pipe system whenever the temperature dropped below 14 ^{o}C, and was monitored every 2 – 3 d with a mercury indoor thermometer (Taylor Precision Products, Oak Brook, Illinois, USA). The soil was watered with distilled water to maintain water-filled pore space between 60-70%. The net IEM-NH_4-N, IEM-NO_3-N, NH_4-N, and NO_3-N contributions in the three carbon sources were calculated by subtracting the treatments with and without green manure residues addition.

5.3.4 Soil and residue analyses

On October 2nd, the pots were set up according to the aforementioned treatments. Soil samples from each pot were taken to establish a baseline. One week after the pots were set up, one arugula seed was sown in each pot and grown for four weeks. To determine the glucose concentration needed in the experimental units, the estimation was done by determining

arugula's photosynthetic rate and then calculating net CO_2 uptake. The photosynthetic rate of arugula was determined using the Li-Cor 6400XT portable photosynthesis system (Li-Cor, Inc., Lincoln, NE, USA). The measurements were made from wk 4 to wk 6 at 48 h intervals. The leaves selected were fully expanded. Two to three leaves were measured per plant. The measurements were done within 1.5 h. The amount of C assimilated through photosynthesis was calculated as,

1) Net uptake of CO_2 : µmol CO_2 m^{-2} s^{-1} converted to mg CO_2 cm^{-2} h^{-1} * leaf area (cm^{-2}) * number of leaves = mg CO_2 plant h^{-1}
2) mg CO_2 plant h^{-1} * 3 (number of hours needed to prepare the solution with glucose) = mg C plant 3 h
3) mg C plant 3 h * 3, 4 or 5% mg C exudate = mg C glucose needed

The percentages of glucose were from 3 - 5% of the total C uptake that is excreted by roots plant (Pinton et al., 2001).Thus, it was partitioned as 3% on the 4^{th} week, 4% on the 5^{th} week, and 5% on the 6^{th} week of the experiment. The glucose was prepared at 1 g dissolved in 1.1 mL of water at 25 ^{o}C (Sigma-Aldrich), and injected into the soil with a fine-needle polypropylene syringe at 48 h intervals in the daily treatment.

5.3.4.1 Ion exchange membranes preparation

Each week the plant available IEM-NH_4-N and IEM-NO_3-N in soil solution were determined with cation and anion membranes (Ionics CR67-HMR and AR204-SZRA, Durpro, Candiac, QC, Canada; Ziadi et al., 2000; Qian and Schoenau, 2002). In the laboratory, the membranes sheets were cut at 0.028 x 0.055 m, then were stored in a container filled with distilled water. Prior the usage, the membranes were saturated by shaking for 1 h in 1M NaCl

solution and subsequently placed in distilled water. In the greenhouse, one cation and one anion membrane were buried vertically to a depth from 0.07 to 0.12 m in each pot. The membranes were retrieved after one week, and a new membrane strip was buried in a new pot. The membranes retrieved from the pots were rinsed with deionized water to remove attached soil particles. Each rinsed membrane was placed in a single conical screw cap tube with 25 mL of 1 M KCl. The tubes were placed in an orbital shaker for 1 h at 135 rpm, and then filtered through Whatman no.5 filter paper. The extract concentration of IEM-NH_4-N and IEM-NO_3-N was analyzed by the indophenol blue method (Sims et al., 1995) at 650 nm on a microplate reader (μQuant, Biotek, Winooski, Vermont, USA). The IEM-NH_4-N and IEM-NO_3-N concentration were reported as μg cm^{-2} wk^{-1} (Qian and Schoenau, 2002).

5.3.4.2 Soil Mineral Nitrogen (SMN) extracted by KCl

Soil samples were analyzed for soil mineral N (NH_4-N and NO_3-N) weekly for the six weeks. Nitrogen was extracted from 5 g of field-soil using 50 mL of 2 M KCl for 60 min, and filtered through Whatman no.42 filter paper. Extracts were stored in acid-washed plastic bottles and were analyzed for NH_4-N and NO_3-N using the indophenol blue method (Sims et al., 1995).

5.3.4.3 Microbial Biomass

The soil microbial biomass nitrogen (MBN) and microbial biomass carbon (MBC) concentrations were analyzed on a weekly basis, from week 0 to week 6, and were determined using the chloroform fumigation-direct extraction method (Voroney et al., 2008). Briefly, 10 g of field-moist soil, ethanol-free chloroform for the fumigation and 0.5 M K_2SO_4 as the extractant (1:4 soil:solution) was used. The calculation for MBN was: (total extractable N of fumigated soil-total extractable N of non-fumigated soil)/0.54 (Voroney et al., 2008; Joergensen and

Muller, 1996). The extractable C from MBC was determined by the difference between fumigated and non-fumigated soil, divided by k_{EN}, where k_{EN} is the extraction coefficient 0.45 (Voroney et al., 2008). Samples were analyzed by a Sievers Innovox TOC analyzer (GE Analytical Instrument,Boulder, CO, USA).

5.3.4.4 Residue collection

At the end of each week, the undecomposed residues were collected from the experimental unit using a modified version of the method described by Magid and Kjærgaard (2001). The soil from each experimental unit was split into two samples of 500 g each, which were deposited into plastic containers of 1000 mL capacity. The containers were filled with distilled water until they reached capacity, and then were stirred for 2 min. The soil was left to sit for 24 h, after which the floating residues were aspirated by a vacuum filtration unit described by Gregorich and Beare (2008). The collected residues were dried at 60^{o}C for 24 h and weighed.

5.3.5. Statistical analysis

Statistical analyses were computed with SAS version 9.3 Software (SAS Institute Inc., Cary, NC, USA, 2000). A two-ways repeated measures ANOVA was computed to test the effect of carbon source and green manure residue size on IEM-NH_4-N, IEM-NO_3-N, NH_4-N, NO_3-N, MBC and MBN concentrations. Significant differences between green manure residues size, and green manure residues size x time were determined by Fishers protected least significant difference (LSD) means comparison test. Time was the repeated measure. A one-way ANOVA was used to test the effect of carbon source on the collected green manure residues, and the means were compared with the Fishers protected LSD. The CORR procedure was used to determine the Pearson correlation coefficient between IEM-NO_3-N and KCl-extractable NO_3-N.

5.4 Results and Discussion

Throughout the experiment, the temperature ranged from 13-22^{o} C, which is consistent with the typical temperatures of May – June in this region. The temperature range is consistent with the climate conditions reported the last 30 years (Environment Canada, 2016). In Quebec, green manure is commonly incorporated at this time of year.

5.4.1 *Nitrogen quantification on IEMs*

Soil NH_4-N adsorbed on ion exchange membranes showed a decreasing pattern in the three sources of carbon, suggesting active ammonia oxidizing bacteria, followed by the nitrification process. In wk 1, the IEM-NH_4-N from the 2 – 4 mm green manure had higher concentrations than 0.5 – 1 mm. The concentrations in the 2 – 4 mm green manure were 0.44, 0.24, and 0.91 µg cm^{-2} $wk^{-1}$for SMN, arugula and glucose treatment, respectively (Fig. 5 – 1A). This initial pattern of IEM-NH_4-N concentration might be related to the greater capacity of the residue (0.5 – 1 mm) to mix with soil particles, achieving the stabilization of the organic residue-derived N after contact with clay and other particles (Jensen, 1994b). In the following week (wk 2), the N concentration declined and was between -0.012 and 0.30 µg cm^{-2} wk^{-1} in both green manure residue sizes. From wk 2 to wk 6, the N concentration in green manure 0.5 – 1 mm was higher ($p < 0.05$) than the concentration in residues with 2 – 4 mm. The same pattern was observed in the three carbon sources and the concentration remained a constant rate between -0.03 to 0.06 µg cm^{-2} wk^{-1} (Fig. 5 – 1A). The increment of IEM-NH_4-N adsorption from green manure 0.5 – 1 mm might be caused by the intimate relationship soil matrix-residues, leading to a faster colonization of smaller than coarse residues and the increment of microbial activity (Angers and Recous, 1997; Ambus and Jensen, 1997).

On the other hand, the IEM-NO_3-N concentration increased gradually in the three sources of carbon from -8.66 to 8.64 µg cm^{-2} wk^{-1} (Fig. 5 – 1B). The IEM-NO_3-N concentration was higher when the 0.5 – 1 mm green manure residues were incorporated and a significant difference ($p < 0.05$) was detected in glucose and arugula in wk 3 and 4, respectively. In arugula and glucose the rise continues until wk 6, whereas in the SMN treatment the concentration declined at wk 5 and wk 6 (Fig. 5 – 1B). The overall net IEM-NO_3-N contributions from residues 0.5 – 1 mm were greater by10% in arugula and by 1.5% in glucose compared to SMN, whereas residues 2 – 4 mm showed a decrease of 10% and 16% in arugula and glucose, respectively (Fig. 5 - 2). Due to the low residues (0.5 – 1 mm) collected at the end of the experiment (Table 5 – 4), the increment in arugula and glucose suggests either stimulation provided by plant and glucose treatment, which promoted microbial activity due to low molecular weight input (process explained in the review by Richarson et al., 2009) or to the formation of aggregates by the mucilage contained in the exudates (Haichar et al., 2014).

5.4.2 *Nitrogen extracted by KCl*

The net NH_4-N extracted with KCl, demonstrated a fluctuating pattern (Fig. 5 – 3A). The N concentration in residue size 0.5 – 1 mm was higher in the three carbon sources, ranged between 1.66 – 0.28 mg kg^{-1}, whereas the N concentration from residue size 2 – 4 mm was lower with ranges between -1.01 to 0.72 mg kg^{-1}. The decreasing pattern of NH_4 concentration can be due to the rapid oxidation rate, the initial step in nitrification. From wk 4 to wk 6, particularly in the arugula treatment, there was an increase from 0.04 to 1.66 mg kg^{-1}, perhaps a response of the root carbon input (Fig. 5 – 3A). Højberg et al., (1996) reported an increment of NH_4-N oxidation rate by 0.15 µg N g^{-1} h^{-1} higher in barley (*Hordeum disticum* L.) rhizosphere compared to bulk soil.

The net concentration of NO_3-N fluctuated in the SMN, while the arugula and glucose demonstrated an increasing pattern. In the three sources of carbon, the concentration was higher in 0.5 – 1 mm green manure residues ranging from -2.48 to 7.92 mg kg^{-1}, whereas the NO_3-N concentration from residues sized 2 – 4 mm was between -2.60 to 6.65 mg kg^{-1} (Fig. 5 – 3B). There was a significant difference ($p < 0.05$) of NO_3-N concentration between the two residue sizes. The NO_3-N concentration increased by 6% in residues sized 0.5 – 1 mm, and 4% in residues sized 2 – 4 mm in arugula, while glucose showed negative values compared to SMN. I am not aware of previous studies reporting on the N contribution from arugula root exudates. However, Merbach et al., (1999) reported that between 5 – 6 % of ^{15}N assimilated by wheat (*Triticum aestivum*) was released in the root exudates. Thus, the increment of NO_3-N concentration in the presence of arugula in comparison with the SMN and glucose treatment suggest an induction of decomposition and mineralization of N-containing crop residues (Hodge et al., 2001; Pascault et al., 2013), or N released in the root exudates (Uren, 2007). It is important to consider that the variation of abiotic factors (e.g., temperature and moisture) might alter the N contribution (Inderjit and Weston, 2003; Jones et al., 2009). The non-availability of NO_3-N with the same magnitude in the glucose treatment may be related with the absence of real root hotspots where the soluble organic inputs concentrate the microbial activity and there is a high turnover rate (Kuzyakov, 2010). Herman et al., (2006) reported an increment of N mineralization in the rhizosphere 9.2 mg N kg^{-1} $d^{-1}$of the root of oats (*Avena barbata* Pott ex Link), whereas bulk soil was 1.0 mg N kg^{-1} d^{-1}.

The good correlation between IEM-NO_3-N and NO_3-N in wk 5 and wk 6 ($r = 0.70$, $p < 0.05$) (Table 5 – 2), can be related with the greater root exudate released at vegetative plant growth stage (Kuzyakov and Domanski, 2000), that impacted on microbial biomass and is

reflected in an increment of IEM-NO_3-N (Fig. 5 – 1B) and NO_3-N (Figure 5 – 2B) during these wks.

5.4.3 *Microbial biomass dynamics*

The MBC in the soil mineral nitrogen treatment showed a slightly decreasing pattern from 646.32 to 145.55 mg kg^{-1}. In wk 3, there was a significant difference ($p < 0.05$) between green manure residue size 0.5 – 1 mm and green manure residue size 2 - 4 mm (Fig. 5 – 4A). Overall, the MBC concentration increased by 10% in SMN, 14% in arugula, and 13% in glucose after the incorporation of green manure residues size 0.5 – 1 mm, whereas residues size 2 - 4 mm decreased by 7% in SMN, 12% in arugula, and 10% in glucose (Fig. 5 - 5A). Thus, microbial biomass responded to the accessible decomposition of residues size 0.5 – 1 mm.

The MBN concentration showed an increasing pattern for the SMN in the three treatments. Particularly, the soil mixed with green manure residues 0.5 – 1 mm showed the highest concentration at 10.57 mg kg^{-1}, followed by green manure residues 2 - 4 mm and bare soil with 8.45 and 7.37 mg kg^{-1}, respectively (Fig. 5 – 4B). These results are similar to the values reported by Jensen (1994b), after the incorporation of pea residues (*Pisum sativum* L., cv. "Bodil") the microbial biomass increased 25% in residues size < 10 mm and 75 % in residues size < 3 mm. The pattern might be related with the availability of easily degradable compounds (e.g., carbohydrates, water-soluble N) and afterwards a possible stabilization or immobilization can occur.

The MBC and MBN concentrations in the soil planted with arugula showed a decrease from wk 1 to wk 3 in the three treatments; after wk 4 there is a moderate increase in green manure sized 0.5 – 1 mm and 2 – 4 mm (Fig. 5 – 4). The rise was significantly different ($p <$

0.05) in residues sized 0.5 – 1 mm and 2 – 4 mm compared to bare soil. Based on the increase pattern of microbial biomass concentration is discarded the competition with roots plant for nutrients. Therefore, it is assumed that the plant root exudates promoted residue turnover and microbial biomass activity (Haichar et al., 2014; Cheng et al., 2014). Zhu and Cheng (2011) reported the effect of sunflower (*Helianthus annuus* L. var. Sunbright) and soybean (*Glycine max* L. var. Envy) root exudates on microbial activity, which resulted in an increment between 17 – 163 % of soil organic matter decomposition. The significant difference between three carbon sources and residue size of the residues collected at the end of each wk, suggests that priming effect promoted residue decomposition, particularly of the 0.5 – 1 mm size (Fig. 5 - 6).

In the glucose treatment there were two different patterns. The MBC concentration fluctuated between 143.83 – 308.58 mg kg^{-1}, whereas MBN increased from 1.52 to 12.03 mg kg^{-1} in the three treatments. However in wk 3, there was slight decline, followed by an increase in wk 4 (Fig. 5 – 4). Using a synthetic root exudate and switchgrass (*Panicum virgatum* L. Alamo) residues (2.5 mm), De Graaff et al., (2010) observed an increase of microbial respiration from 1.11 to 1.21 mg C g^{-1} soil, and residue decomposition increased 2 %. This pattern suggests that the addition of glucose led to a "real" priming effect, described as the increase of microbial turnover and the SOM turnover in a significant extend, after the labile C addition (De Graff et al., 2010; Kuzyakov, 2010). Overall, the MBN concentration by 8% in SMN, 20% in arugula, and 18% in glucose, while the residues 2 – 4 mm decreased the concentration by 20% in SMN, 9% in arugula, and 15% in glucose (Fig. 5 - 5B).The increment corresponded by the IEM-NO_3-N concentration reported in this study.

5.5 Conclusions

This study quantified the N contribution of green manure mixture (field-peas and oats) with residue size 0.5 – 1 mm and 2 – 4 mm. In the presence of arugula, the plant-available N concentration increased more than with SMN and glucose addition, and there was a higher microbial biomass concentration. I conclude that the increase in plant-available N was caused by a root priming effect. The addition of residues 0.5 – 1 mm resulted in an increment of 10% of more NO_3-N throughout the growth season, this N availability can be included in the N contributions from green manure. However, the dynamics of abiotic factors that influenced the release and C concentration of root exudate should be explored in future experiments.

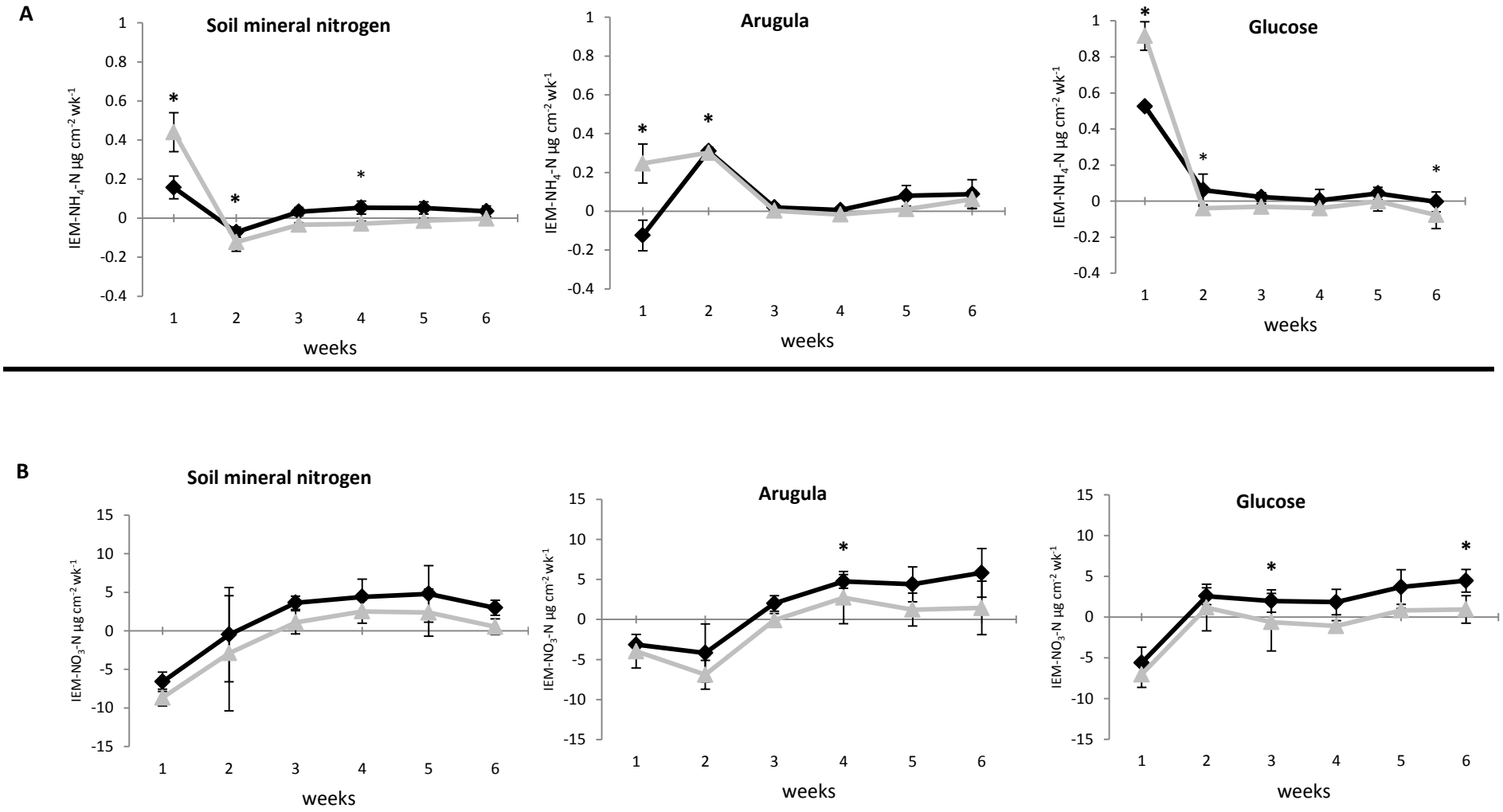

Figure 5 – 1: Net (A) IEM-NH_4-N and (B) IEM-NO_3-N concentration on ion exchange membranes from three sources of carbon (soil mineral nitrogen, arugula and glucose). Values are the mean of four replicates. Error bars represent standard error in the (◆) green manure residues 0.5 – 1 mm, (▲) green manure residues 2 – 4 mm.

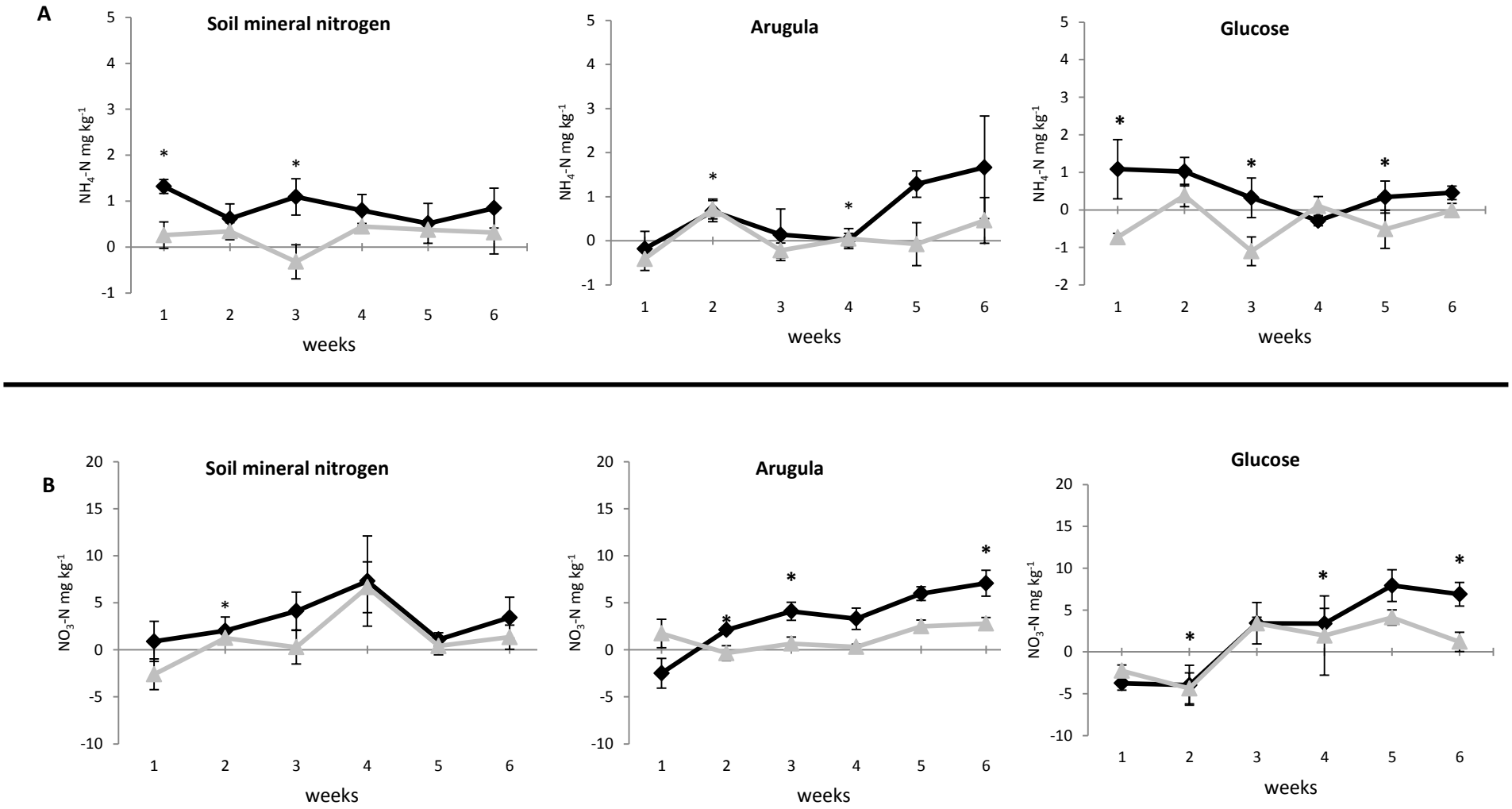

Figure 5 – 3: Net (A) NH_4-N and (B) NO_3-N concentration from three sources of carbon (soil mineral nitrogen, arugula and glucose). Values are the mean of four replicates. Error bars represent standard error in the (◆) green manure residues 0.5 – 1 mm, (▲) green manure residues 2 – 4 mm

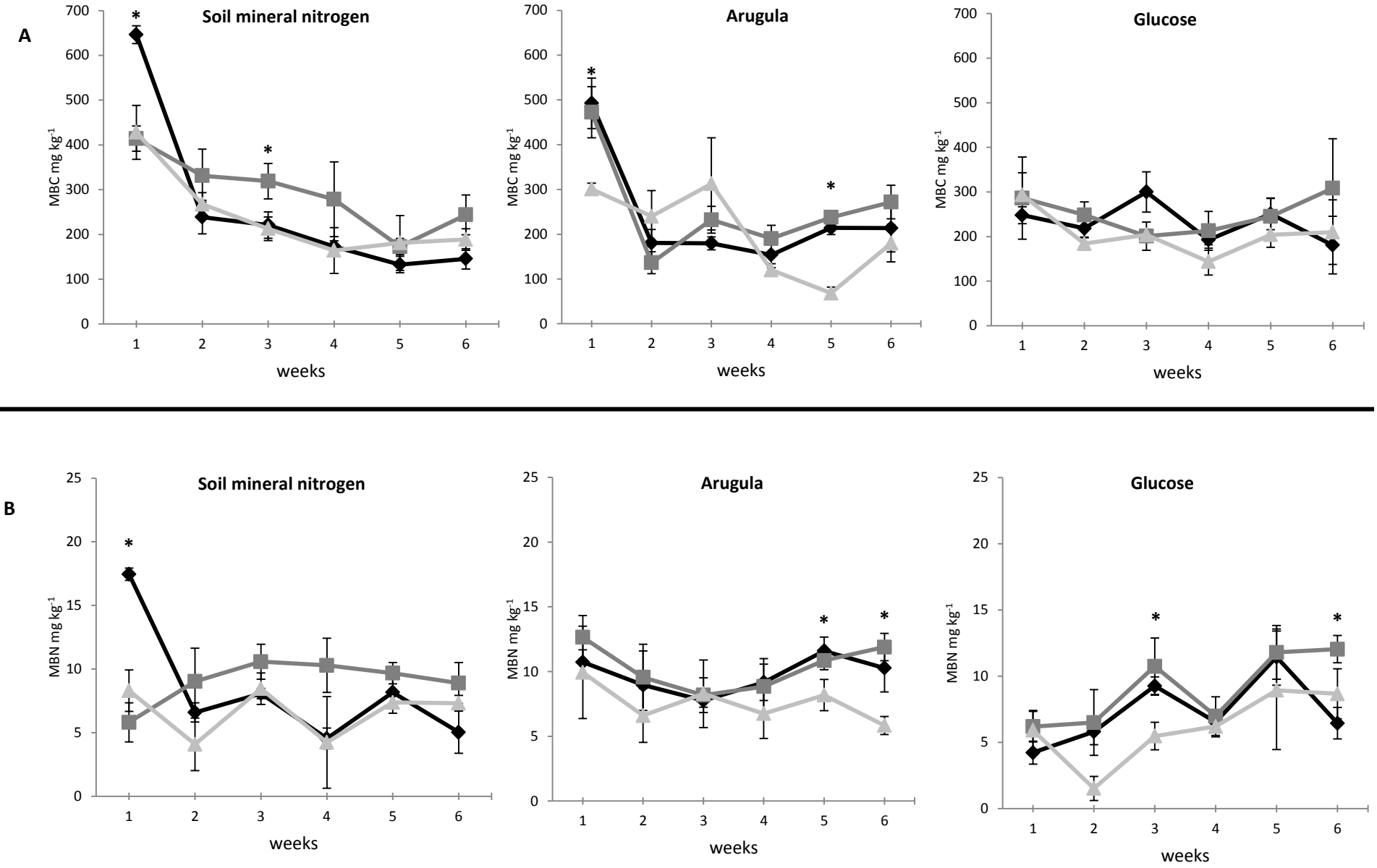

Figure 5 – 4: (A) MBC mg kg^{-1} and (B) MBN mg kg^{-1} concentration from three sources of carbon (soil mineral nitrogen, arugula and glucose). Values are the mean of four replicates. Error bars represent standard error in the (◆) bare soil, (■) green manure residues 0.5 – 1 mm, (▲) green manure residues 2 – 4 mm

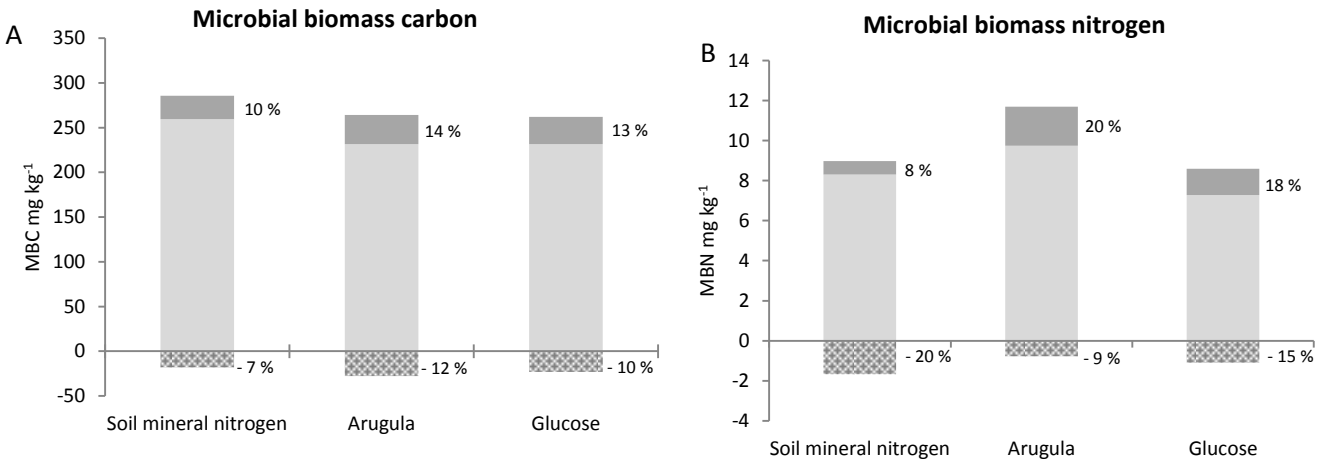

Figure 5 – 5. Net increment or decrease of (A) MBC and (B) MBN in three carbon sources after the incorporation of green manure residues ( ) 0.5 – 1 mm and ( ) 2 – 4 mm

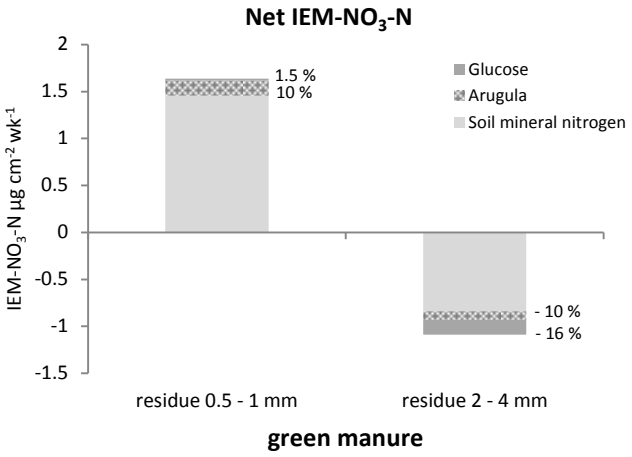

Figure 5 - 2. Net increment or decrease of NO_3-N adsorbed on ion exchange membrane in three carbon sources (soil mineral nitrogen, arugula, and glucose) after the incorporation of green manure residues 0.5 – 1 mm and 2 – 4 mm.

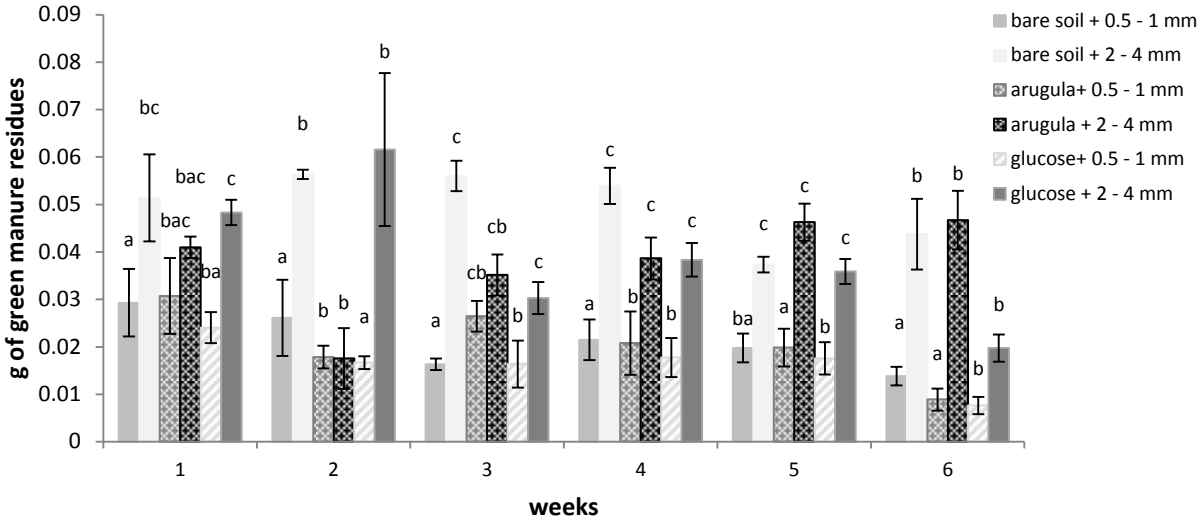

Figure 5 - 6. Green manure residues remaining in the 6 weeks following incorporation of green manure mixture (field peas and oats). Values are the mean of four replicates. Error bars represent standard error. The same letter (s) (a, b, c) are not significant different at $p < 0.05$ (LSD)

Table 5-1. Treatments tested corresponding to the experiment of different sources of carbon and green manure residues

1) Soil organic matter (bare soil)	4) Arugula + bare soil	7) Glucose + bare soil
2) Bare soil + green manure residues (0.5 mm to 1 mm)	5) Arugula + green manure residues (0.5 mm to 1 mm)	8) Glucose + green manure residues (0.5 mm to 1 mm)
3) Bare soil + green manure residues (2 mm to 4 mm)	6) Arugula + green manure residues (2 mm to 4 mm)	9) Glucose + green manure residues (2 mm to 4 mm)

Table 5 - 2. Correlations coefficient values (Pearson's) between IEM-NO_3-N and NO_3-N during six weeks

	week 1		week 2		week 3		week 4		week 5		week 6	
	IEM-NO_3-N		IEM-NO_3-N		IEM-NO_3-N		IEM-NO_3-N		IEM-NO_3-N		IEM-NO_3-N	
	r	*p*	*r*	*p*	*r*	*p*	*r*	*p*	*r*	*p*	*r*	*p*
NO_3-N	-0.65	0.05*	-0.34	0.36	0.36	0.33	0.30	0.42	0.70	0.03*	0.80	0.008*

IEM-NO_3-N = NO_3-N adsorbed on ion exchange membrane, NO_3-N = NO_3-N concentration by soil extraction

*. Correlation is significant at 0.05

CHAPTER 6.

GENERAL CONCLUSIONS

My thesis provides insights into the use of IEMs as a means of quantifying plant-available N in agroecosystems that rely on green manure as an N fertilizer. My IEM measurements were strongly correlated to arugula N uptake and soil mineral nitrogen extracted with KCl under field conditions, supporting the IEM method as a robust and sensitive indicator of plant-available N in humid temperate soils of Quebec (Chapter 2 and 4).

In field conditions plant-available N responded to tillage practice, likewise plant N uptake was enhanced by different number of tillage passes. The greater residual N reported at postharvest time suggests further studies for management practices to avoid losses (Chapter 2 and 4).

Controlled laboratory studies provide some insight into why IEM are a useful tool for NH_4 and NO_3 analysis in green manure-amended soil. First, they are quite responsive to minor differences in the C/N ratio of the residue (Chapter 3), as well as to root priming effect (Chapter 5).

The second major conclusion from this work was the utility of the physical size separation method, to determine residue size, since this measurement gave a good general indication of speed of residue decomposition. As decomposition must proceed before proteins and other organic N polymers are released into soil solution for subsequent mineralization, ammonia oxidation and nitrification, the proportion of residues of a particular size provides insight into the state of decomposition of a green manure. I am not aware of other research

groups that are using residue size as a rapid indicator of decomposition. The most useful residue size classes in this regard were < 2 mm (Chapter 3) and specifically the 0.5 to 1 mm size class (Chapter 5) warrants further investigation.

Future research recommendations

There are several research areas that merit further investigation, based on my thesis research. First, we need to investigate the fate of the plant-available N that is released following green manure incorporation. We know that about 20% of the N in a short-season crop was derived from the green manure, so what happens to the rest of the N in the green manure residue? It does not seem to accumulate in the microbial biomass, according to my measurements, but it could become part of the soil organic nitrogen pool or it could undergo other biological transformations (e.g., nitrifier-denitrification, denitrification) or it could be subject to abiotic reactions like NO_3 leaching, NH_4 fixation on clays, and NH_3 volatilization. A mass balance approach, particularly with the ^{15}N stable isotopes, would be helpful to answer this question.

I recommend that future studies of green manure residue decomposition and N mineralization be done in the presence of growing plants, as I did in my two field experiments. Of course, the plant effects cannot overcome physico-chemical barriers to N mineralization due to the residue characteristics and soil properties, so the future studies should include more types of green manure residue and soil to see how those factors modulate N mineralization.

Applied research on green manures should be relevant to decisions that are made on farms. For instance, my field studies showed a significant amount of plant-available N released from the green manure following arugula harvest, which suggests that the farmer should consider

planting a second cash crop or a cover crop to recover a greater proportion of the N input from the green manure. This is a good nutrient management practice that conserves the N fertilizer value of the green manure, using the fertilizing power of the green manure for the benefit of multiple crops rather than as a source of pollution to the air and water. I did not explore the soil N dynamics during the postharvest period, but this is an important area that deserves further consideration for agro environmental protection.

The farmer can use the information from the IEM-N indicator to make several decisions about managing their green manure. For instance, depending on the pattern of plant-available N release from the green manure (as measured by IEM-N) and the N required for the cash crop, the farmer has two options. First, they can modify the dates that the green manure is incorporated and the cash crop is planted. This will ensure that there is sufficient decomposition and N mineralization of the green manure residue to release enough plant-available N to support the N demand of the cash crop. Second, if the farmer has fixed the date for planting the cash crop and requires faster decomposition of the green manure, they can till the field with more than one pass. The labor cost for multiple tillage passes needs to be balanced against the N fertilizing power of the green manure and the market value of a high-yielding cash crop that has received sufficient N fertilizer. Although this thesis focused on IEM-N as an indicator of the plant-available N and crop N uptake, I would also note that the mass of green manure residue having a particle size of 2-4 mm responded to tillage practices, and could be another indicator of the N supply from green manure to crops. This possibility merits further investigation.

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APPENDICES – CHAPTER 4

Table S4 -1. Repeated measures of ANOVA for RCBD for IEM-NH_4-N, IEM-NO_3-N, NH_4-N, NO_3-N, concentration during six weeks sampling period

Source of variance	IEM-NH_4-N		IEM-NO_3-N		NH_4-N		NO_3-N	
	f- value	P value	f- value	P value	f- value	P value	f- value	P value
Block	19.73	0.001**	1.05	0.4367	1.42	0.34	0.40	0.69
Tillage passes (Tp)	188.53	0.0001**	168.20	0.0001**	1.91	0.26	6.48	0.05*
Time (T)	47.82	0.0001**	49.33	0.0001**	0.65	0.66	69.30	0.0001**
Tp x T	7.21	0.0001**	6.04	0.0001**	0.36	0.94	1.82	0.12

*, ** = Significant at $p < 0.05$ and $p < 0.001$, respectively.

Figure S4 -1.Field layout of a plot with experimental units the received green manure (treatment) and no green manure (control) with different number of tillage passes, during six weeks sampling period

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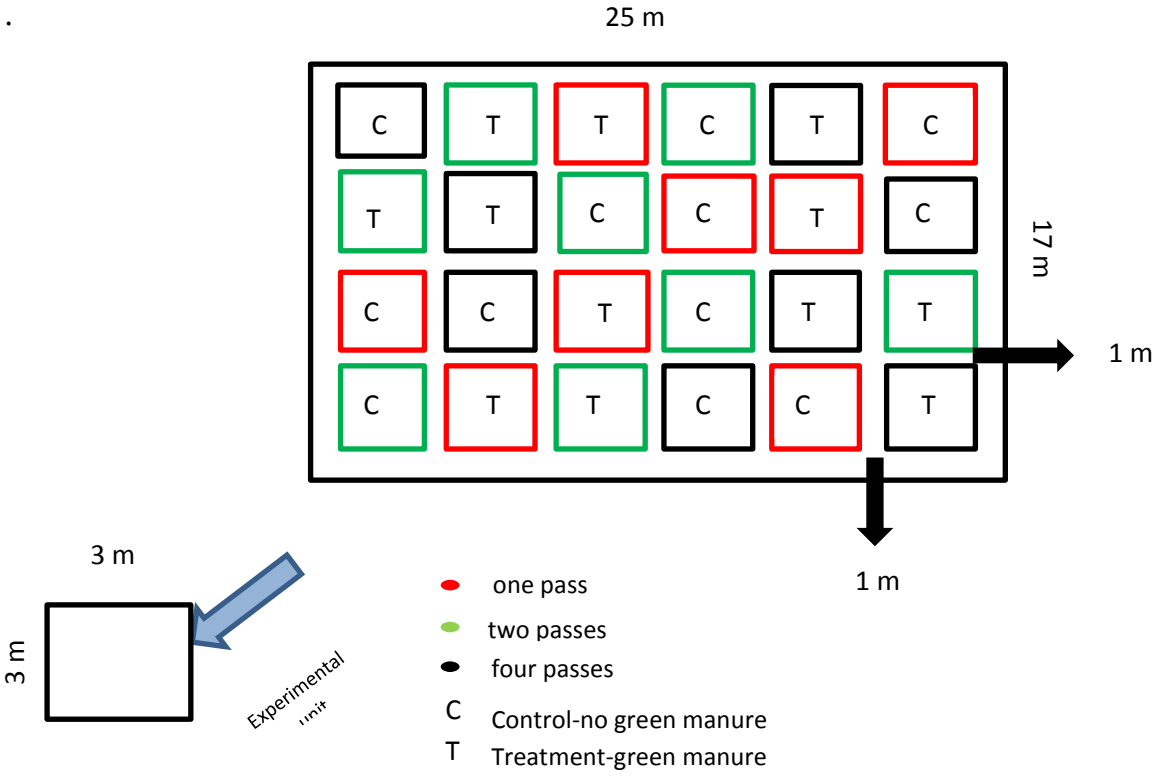

APPENDICES – CHAPTER 5

Table S5 - 1. Results of the ANOVA for repeated measures with f- values and p-values for the sources of carbon x residue size (between subject), and carbon sources and residue size x time (within subject)

Effect	IEM-NH_4-N		IEM-NO_3-N		NH_4-N		NO_3-N		MBC		MBN	
	f-value	P value	f-value	P value	f-value	P value	f-value	P value	f-value	P value	f-value	P value
Source of carbon (C)	0.35	0.70	2.92	0.07	1.71	0.20	15.33	0.001**	3.79	0.03	3.27	0.05*
Residue size (R)	8.31	0.009*	31.99	0.001**	10.26	0.004*	1.02	0.32	6.48	0.005*	7.48	0.002*
Time (T)	62.30	0.001**	9.06	0.001**	2.50	0.03*	18.60	0.001**	28.21	0.001**	4.86	0.001**
C x R	0.03	0.97	1.44	0.26	6.04	0.009*	41.22	0.001**	0.10	0.98	0.16	0.95
C x T	6.28	0.001**	2.26	0.02	1.29	0.24	1.50	0.15	4.52	0.001	2.74	0.004*
R x T	23.73	0.001**	0.68	0.63	1.10	0.36	2.38	0.04*	1.75	0.07	1.40	0.18
C x R x T	24.63	0.001**	0.34	0.96	1.66	0.10	3.20	0.001**	2.07	0.007*	1.94	0.01*

*, ** = Significant at $p < 0.05$ and $p < 0.001$, respectively