Norwegian University
of Life Sciences

Master's Thesis 2016 30 ECTS
Plant Science Department, Ås

Soil particulate organic matter contribution of hairy vetch ecotypes as winter annual legume cover crops

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Agroecology

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Abstract

Efficient cover crop management provides numerous benefits to the agroecosystems including soil organic matter (SOM) contribution. In the cold climates of the upper Midwest, options of cover crop species that can withstand harsh winters including freezing temperatures, variable snowfall, and limited time for establishment, are few. The particulate organic matter (POM) is a potential fertility index. The objective was to investigate hairy vetch (*Vicia villosa*) ecotypes as promising winter annual legume cover crops by quantifying their contribution to the particulate organic fraction of SOM, compared to winter rye (*Secale cereale*) cereal cover crop, and to evaluate C:N ratios of contributed POM by each cover crop treatment to demonstrate the differences in POM quality after early spring incorporation of fall-planted winter cover crops. A field trial was conducted over two locations with different soil types and land use history. Grand Rapids had sandy loam soil and previous land use was >25 years old apple orchard, and Lamberton had silty loam soil and organic row-cropping production systems prior to this study. POM was separated by light (1.6 g cm^{-3}) density fractionation of soil samples collected before incorporation of cover crops and one month after incorporation, to assess the quantity and to analyze its C and N ratios. Strong weed competition led to weak emergence of cover crops and reduced biomass in both locations. Average POM quantity of combined treatments increased after incorporation of cover crops in Lamberton, but was not significant in Grand Rapids due to the high amount of litter content in soil of previous land use. Cover crop treatments in Lamberton had contributed lower C:N ratio POM quality.

Keywords:

Soil organic matter – particulate organic matter – winter cover crop – fertility management – density fractionation – soil texture – land use

Acknowledgement

Thank God for guidance and care-

I would like to thank all my supervisors; Alexander Liebman[1], Alexander Wezel[2], Julie Grossman[3], and Tor Arvid Breland[4] very much for teaching and supporting me all the way since the start of the thesis work. Many thanks to Cathrine Strømø for her patience and kind support, and Sharon Perrone and Thanwalee Sooksa-nguan for their assistance in the lab work.

Special thanks to my parents Hoda Ghaly and Mohamed Said for everything.

I would also like to thank all my family and friends; Amr Ahmed, Andreas Gmeiner, Armand McCoy, Bill Giese, Cécile Cordier, Claire Holdreith, Erin Ball, Hassan Hossam, Julia Kloehn, Mahmoud Said, Mariam Wael, Mona Ghaly, Morris Knopf, Mostafa Said, Robbi Knopf Holdreith, Tove Bang, and everyone else.

Tusen takk.

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Ås, October 2016

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Abbreviations and acronyms

CC	Cover crop
CLO	Red clover
NCROC	Northcentral Research and Outreach Center
NIN	Non-inoculated treatment
NOP	National Organic Program
POM	Particulate organic matter
POM-C	Particulate organic carbon
POM-N	Particulate organic nitrogen
POX-C	Permanganate oxidizable carbon
SOM	Soil organic matter
SPT	Sodium polytungstate
SWROC	Southwest Research and Outreach Center
USDA	United States Department of Agriculture
V1n and V2n	Hairy vetch ecotype 1 and 2 non-inoculated
V1w and V2w	Hairy vetch ecotype 1 and 2 inoculated
WIN	With inoculation treatments

Tables and figures:

1. Introduction

1.1 Cover crops in organic agriculture in the United States

This brief literature presents a short overview about the current role and growth of organic farming in the United States, focusing on cover cropping practices and those employed in the Upper Midwestern region of the US. In addition, to present background information on how cover crops contribute to soil organic matter pools.

1.1.1 Organic agriculture in the United States

Organic agriculture in the United States relies on ecologically established practices such as crop rotation, biological pest control, and omission of synthetic chemicals in crop and animal production. The United States had less than a million of acres of certified organic farmland when the Organic Foods Production Act was established by the congress in 1990 (OFPA, 1999). The U.S. Department of Agriculture created a national organic standard in 2002 to unify the varying definitions of organic agriculture between the states. At that time, the total certified organic land had reached two million acres, and had doubled by 2005 from 2002. In 2011, the total organic farmland was estimated to be 5.4 million acres. The organic agriculture trend however is still very low in comparison to the conventional agriculture numbers; only about 0.8% of all U.S. cropland is certified organic.

Organic agriculture involves numerous practices, like intercropping, incorporating legume plants, crop rotations, etc., that can enhance and protect the farming ecosystems. Organic management contributes to growing healthy crops and controls unwanted plants or insects in an integrated strategy. Growing compatible plants in space proximity works in several ways, including better use of the soil's resources and minerals, and protection against pests. Intercropping, aims to efficiently use the soil's available resources, contributing to higher yield and reduced soil erosion (Magagula et al., 1979; Laloy and Bielders, 2010). Due to the diversity that compatible intercropping provides, plant pest populations are often reduced, since cash crops are separated spatially by biotic diversity and may provide habitat to include beneficial natural enemies (Srinivasa et al., 2012). Another feature is the allelopathy contribution of the selected crops against weeds.

Crop rotation is another aspect of organic cropping systems of a different dimension, where farmers change the type of plants grown in a field temporally. Crops may be alternated in different growing seasons, and or within the same field throughout the growing season, a "crop succession" depending on the crop type. Organic farmers often use diverse crops in the cycle, to increase

income from specialty crop market like fruits and vegetables, and to increase soil quality. In nitrogen management, crop rotation is most effective when integrating green manure cover crops for their nitrogen fixation ability and low carbon to nitrogen ratio biomass. Cover cropping can be featured in crop rotation or intercropping where it dedicates the land to maintain soil health.

When the crop selection includes leguminous plants, soil fertility may be enhanced as leguminous plants biologically fix nitrogen (Bekunda and Woomer, 1996). Biological nitrogen fixation, discovered by Beijerinck in 1901, is done by microorganisms that contain and utilize nitrogenase. This enzyme is found in some prokaryotes such as free-living azotobacter, and rhizobium that lives in symbiosis with most legume plants (Postgate, 1982). These microbes transform atmospheric nitrogen (N_2) into ammonia (NH_3). Legume plants form symbiosis with rhizobia, where rhizobia create nodules in the legume plant roots, establishing a site of exchange in which the plant transfers photosynthetically derived carbon in exchange for soluble nitrogen (NH_3). As a major component for chlorophyll used in photosynthesis, as well as amino acids for building proteins and biomass, nitrogen is a critical limiting element for plant growth and physiology (Vance, 2001).

1.1.2 Cover crops in organic agriculture

A cover crop can be defined as a crop grown for other purposes besides harvestable outputs, often temporally in rotation with cash crops. Efficient cover crop management has shown to provide numerous benefits to agroecosystems including contribution to soil organic matter, soil erosion reduction, and help reduce nitrate leaching from soils during fallow periods (Dabney et al., 2001). One of the most important reasons organic growers use cover crops is that they can help a grower manage soil nutrients (Lal et al., 1991) and, if a legume, are a valuable source of nitrogen via nitrogen fixation. Organic farmers are interested in growing cover crops because they also control weeds through competition and allelopathy, improve soil organic matter and improve overall soil quality. The roots of the cover crops hold soil particles and form aggregates that aid in water infiltration. In extreme precipitation events, this may reduce flooding, thus limiting topsoil runoff. The aboveground shoots of cover crops act as a shelter to protect the soil from the physical impacts of raindrops, machinery and wind (Reeves, 1994; Pierce and Lal, 1994).

Organic farmers consider many purposes of growing cover crops, while also considering many soil and climatic conditions. In the context of the upper Midwest, cover crops are incorporated off-season where the active growing season is short and busy, starting late May or June and ending in late August or September. The climates of the upper Midwest narrows down the options of cover crop species that can survive the harsh winter seasons. During winter, the average annual minimum temperature ranges from -30°C to -40°C in the upper Midwest, killing most cover crops. Winter

cover crops are planted in the fall and will grow until winter when they then enter dormancy. In the following spring, the cover crops break dormancy and continue to grow but at a faster rate than before the cold dormancy, and if a legume, resulting in additional nitrogen fixation. Grass cover crops can scavenge soil nitrates and convert them into biomass. The quantity of nitrogen taken up by the cover crop and the timing rely on the available nitrogen in the soil, the climate, the species of cover crop used, seeding density and date, and turnover time (Shipley et al., 1992; Clark et al., 1994). Cover crop capacity to add nitrogen or scavenge nutrients from deeper soil layers, depends mainly on whether the cover crop is leguminous or cereal, respectively. Legume cover crops, via N fixation, usually add more nitrogen to the soil in the spring than non-legume cover crops (Clark et al., 1997; Vaughan and Evanylo, 1998). When residual soil nitrates are high, legume root nodulation and nitrogen fixation rates are reduced (Shertz and Miller, 1972; Streeter, 1985). In the case of low amount of residual soil nitrogen, cover crop growth can be limited. Rye can scavenge soil nitrates better than legume cover crops (Shipley et al., 1992; Wagger et al., 1998; Isse et al., 1999). The abilities of cover crop to scavenge nitrate from the soil or fixing atmospheric nitrogen into the farming system have to be balanced when utilizing cover crops as nutrient management practice.

Some farmers also use mixtures of cover crops to obtain combined benefits. Mixtures of legume and grass cover cropping can scavenge more nitrates and produce sufficient amount of vegetation from nitrogen fixation (Clark et al., 1994; Ranells and Wagger, 1997). Common mixtures include a legume and grass such as, red clover and oats, or hairy vetch and winter rye. Organic farmers finally have to follow National Organic Program (NOP) regulations to become organic certified, which states crop rotation should be applied to include cover-cropping practices. National Organic Program regulations also prohibit use of synthetic nitrogen fertilizers and thus, legume cover crops provide a needed source of biologically fixed nitrogen. Hairy vetch (*Vicia villosa*) is a winter hardy annual legume that can survive freezing winters. Winter rye (*Secale cereale*) is a winter hardy annual grass that is easy to establish and can survive cold winters. These cover crops, with a main focus on hairy vetch ecotypes, are the main foci of this research.

1.1.3 Soil organic matter

Soil organic matter originates from living organisms that undergoes decomposition. It consists of living soil organisms, and partially decomposed residues of plants and animals. The partially decomposed organic residues are the source of food for the living organisms (FAO, 2005). When organic residues are well decomposed, they transform into non-humic substances that assist in aggregation and provide nutrients for soil organisms and plants. Humic substances are very stable and serve as nutrient buffer. Soil organic matter provides plants and soil organisms with nutrients

as soluble nutrients are released through microbial degradation. Soil organic matter also improves water-holding capacity, as organic matter holds up to 90% of its weight in water, and influences the structure of the soil by forming aggregates, protecting the soil surface and controlling erosion (Bollag et al., 1992; Lal, 2004; Apezteguia et al., 2009). Soil organic matter also helps water infiltration and reduces runoff. The total SOM is the main source for plant nutrients in organic farming, but measuring it has proven to be difficult and thus has found little use as a management tool (Wander et al., 1994). Soil quality and soil organic matter increases when tillage is reduced, or when soil organic carbon is augmented using manure, cover crops and crop rotations, especially with legumes (Reicosky et al., 1995; Drinkwater et al., 1998). Differences in the amount of total soil organic matter caused by specific agricultural practices may not be detected in the short term, especially in soils having high carbon contents, like the upper Midwest due to its native prairie lands (Sikora et al., 1996).

Soil organic matter has been partitioned into two main pools; active (labile) and stable. The stable fraction serves as a source of slowly decomposing nutrients and is essential for the soil nutrient balance over the long run (Stevenson, 1994). The labile fraction is particularly important in managing soil productivity, as it indicates management changes with greater sensitivity than total soil organic matter (Wander et al., 1994). Labile pools of organic matter are more sensitive to management and thus may be measured in the short term to determine predicted impacts of management on long-term changes in soil organic matter. In sustainable agricultural systems, long-term fertility is developed through the use of organic residues under minimum tillage systems and reduced fertilizers inputs. The soil labile organic matter pool depends on the organic residues used, and its decomposition is mainly affected by climatic and microbial conditions. The fraction of the soil organic matter that is actively decomposing, the labile pool, can be more useful to measure as a management tool than the total soil organic matter (Wander et al., 1994). The soil labile organic matter has been found to be elevated in farming systems depending on organic fertilization compared to systems using synthetic fertilizers (Wander et al., 1994; Willson et al., 2001). This small fraction of the soil organic matter provides a steady source of nutrients to plants and soil organisms. The soil labile organic matter pool can increase and decrease during different stages of crop rotation. It is assessed in organic farming systems to determine the effects of the practices used (i.e. cover crops, tillage method, and compost type).

Particulate organic matter (POM) fractionation is a method used to determine quantities of labile carbon and nitrogen in the soil. The particulate organic matter, composed of partially decomposed organic materials, was found to be an energy source for soil organisms (Janzen et al., 1992; Stevenson, 1994; Christiensen, 2001). Measuring carbon and nitrogen POM, together with

information of the recently added plant residues into the soil, it enables us to read the potential of N mineralization in legume based organic fertility management (Willson et al., 2001). The particulate organic matter can be assessed either by density or by size fractionation methods used to determine the impacts of the organic farming practice. The importance of POM in the soil ecosystem has been underlined in many studies (Gregorich and Janzen, 1996; Karlen and Cambardella, 1996); however, the dynamics of POM are not well understood (Gale and Cambardella, 2000).

1.1.4 Fractionation of particulate organic matter

Soil organic matter occurs in a biologically resistant state often contained within mineral colloids (Paul and Juma, 1981; McGill et al., 1981). ^{14}Carbon isotope dating showed that novel soils contain a stable organic carbon pool with a turnover time that can be as long as thousand years (Campbell et al., 1967; Guillet, 1982). The other soil organic carbon pools are younger in age, not older than several decades (Ayanaba and Jenkinson, 1990). Time required for decomposition of similar compounds in liquid microbial cultures ranged from hours to days. These indicate that soil provides significant protection against microbial decomposition (Sorensen and Paul, 1971). Mathematical models have described accumulation processes of organic matter and its turnover times under field conditions over 1 to 100 year time scales (Jenkinson and Rayner, 1977; van Veen and Paul, 1981; Jenkinson, 1990). These models simulated the nature of organic matter by partitioning it in pools with different turnover times. Decomposition simulation of soil organic matter showed different rates of decay due to the chemical nature of organic materials and physical conservation by adsorption of substrates to surface, and their location inside soil aggregates in locations inaccessible to microorganisms. The strong association between soil organic matter and soil minerals requires a selective fractionation method to separate the organic fraction from the mineral portion. Studies of soil organic matter have used chemical extractants or physical methods to fractionate soil organic matter (Stevenson and Elliott, 1989). Chemical fractionation methods have not proven useful in explaining dynamics of soil organic matter (Oades and Ladd, 1977; Duxbury et al., 1989). Fresh and partially decomposed organic materials which have short turnover times of months to years and accumulates as separate, non-mineral particles, are mainly regulated by their chemical composition (Waid, 1974). Physical fractionation by size or by density separation however is less destructive than chemical fractionation. Physical fractionation techniques differ depending on amount of energy used to disrupt or disperse soil particles, and the fractionation process whether based on size or density.

Density separation has been used to separate light fraction mainly consisting of undecomposed plant residue and partially decomposed products (Spycher et al., 1983) and to separate organo-

mineral compounds. Light fraction was defined by Greenland and Ford in 1964 as material lighter than 2 g cm^{-3}, and more recently was set to a lower density of 1.6 g cm^{-3} to exclude mineral materials from the light fraction (Scheffer, 1977; Ladd et al., 1977). Sodium polytungstate density separation medium is a non-toxic, non-flammable, high density, low viscosity solution alternative to sodium iodide, zinc chloride and zinc bromide, which is reusable and environmentally friendly (Drik and Susan, 1995). At density 1.8 g cm^{-3}, it showed 152% increased light fraction carbon recovery than sodium iodide (NaI) density solution (Conceicao et al., 2007). The use of sodium polytungstate poses no apparent danger or toxicity like zinc bromide and zinc chloride, and features much lesser viscosity than zinc chloride (Traverse, 1988; Chemiekaarten, 1994).

1.2 Research objectives

The overall research project "Assessing nitrogen contribution and soil biological effects of promising winter annual legume cover crops for Minnesota", aims to identify species of winter annual legumes that contribute to soil carbon and nitrogen pools in cold and variable Minnesota climates. Researching options of legume cover crops for the upper Midwestern farmers to help improve nutrient cycling and availability, and soil quality in order to develop resilient and sustainable agroecosystem. The main objectives of the project are: (1) Quantify total nitrogen contribution of promising legume cover crops, and the amount of nitrogen derived from soil and atmosphere. (2) Assess the impact of hairy vetch ecotypes, as well as other legumes, on biological soil quality parameters including; labile organic matter pools, and microbial carbon and nitrogen biomass.

The research thesis scope was to separate and evaluate the light fraction particulate organic matter (POM) using density fractionation protocol of Wander et al. (2006), and to elucidate the impact of different cover crop treatments before and after cover crops incorporation. The trial took place over two locations of different soil types and previous land use; silty loam with row-cropping system, and sandy loam with apple orchard system. Hypotheses were:

(1) Planting cover crop species in the fall season followed by spring termination and incorporation of cover crop biomass will increase the quantity of POM in soil as newly added plant residues start to decompose, increasing the soil labile organic matter.

(2) The POM contributed after incorporating hairy vetch cover crop ecotypes will have lower C:N ratios compared to the POM contributed by winter rye cover crop, As legume cover crop roots

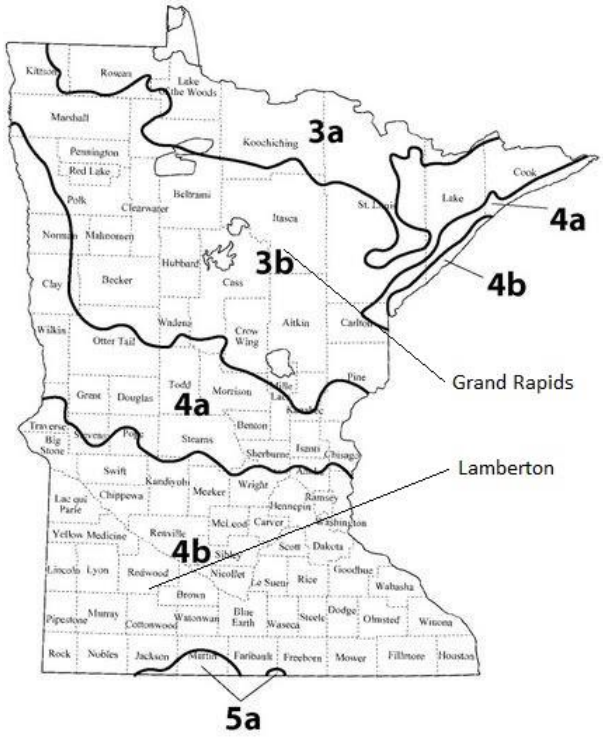
make symbiosis with nitrogen fixing microorganisms like rhizobia, they will contribute more nitrogen to the soil than non-legume cover crops.

2. Materials and Methods

2.1 Experimental design and field location

The research experiment began in September 2014 with fall seeding of cover crops, and will last for three growing seasons, terminating in early fall 2017 with sweet corn harvest following fall 2016 planted cover crops.

Figure 2.1: USDA plant hardiness zones of Minnesota, 2012. (Source: extension.umn.edu)



Field experiments were established at two research stations (Figure 2.1), North Central Research and Outreach Center (NCROC) in Grand Rapids, Itasca County, Minnesota, which is the northern-most research and outreach center in the continental U.S. with a USDA cold hardiness zone of 3b. And Southwest Research and Outreach Center (SWROC) in Lamberton, Redwood country, Minnesota, with a 4b hardiness zone to include a wider range of the upper Midwest. Both research sites were organically certified and managed according to USDA NOP regulations. The previous land use of Grand Rapids was apple orchard production as shown in (Table 2.1), and row crop production for Lamberton. Soil types were sandy loam and silty loam for Grand Rapids and Lamberton.

Table 2.1: Field locations and settings.

Station	SWROC	NCROC
Location (geographical coordinations)	Lamberton, MN (44.2311°N, 95.2642°W)	Grand Rapids, MN (47.2372°N, 93.5302°W)
Previous land use	Row-cropping production, (25+ years)	Apple orchard, (25+ years)
Incorporation style of cover crops	Tandem disc	Rototiller
Soil type	Silty loam	Sandy loam
Number of treatments (actual)	24	20

2.1.2 Cover crop treatments

The experimental design of both field sites was a randomized complete block design, with 4 blocks, initially containing 16 treatments at each site (Appendix 1). Treatment plots of 25 ft (7.6 m) by 10 ft (3 m) included three ecotypes of hairy vetch (*Vicia villosa*) varieties shown in previous experiments to be winter hardy, winter rye (*Secale cereale*) as a non-legume control, red clover (*Trifolium praetense*), and Austrian winter pea (*Pisum sativum*). While varieties of vetch are relatively undefined, seed was sourced from three seed companies in the upper Midwest, Welter Seed Company, Buckwheat Growers, and Albert Lea Seed Company. Alleys were 18 ft (5.5 m) between each plot row, and four rows of sweet corn of 30 inch (76.2 cm) spacing within the 16 rows of cover crops of 7.5 inch (19 cm) spacing within each plot. Trials were with and without rhizobium inoculation treatment, except for winter rye, which received no inoculant. Seeding rates are shown in (Table 2.2). Trials were planted in September 2014 (Table 2.3) to overwinter until early spring 2015. Some treatments had high weed competition resulted in weak establishment and others did not survive the winter, eliminated: pea, V3, and the inoculated red clover, and red clover in Grand Rapids. This decreased the total amount of treatments to 6 in Lamberton: winter rye, red clover, inoculated vetch 1 and 2, and non-inoculated vetch 1 and 2, and five treatments in Grand Rapids: winter rye, inoculated vetch 1 and 2, and non-inoculated vetch 1 and 2.

Table 2.2: Seeding rates.

Cover crops	Vetch 1	Vetch 2	Vetch 3	Red clover	Pea	Rye
Seed rate (kg ha^{-1})	28	28	28	12	84	118

2.1.3 Soil sampling

Cover crop and weed biomass were sampled in June 2015 (Table 2.3) before cover crop termination, by removing all biomass from four squares of 1 m², separating cover crops and weeds and pooling samples. Soil samples were also taken at this time as cores of 0 – 20 cm deep and were pooled across 12 randomly selected locations in each plot. Cover crops were then terminated when vetch flowering was approximately 90% complete by using rototiller at NCROC in Grand Rapids and a tandem disk at SWROC in Lamberton. A second set of samples were taken approximately one month after cover crop termination to allow for partial

Table 2.3: Operation dates.

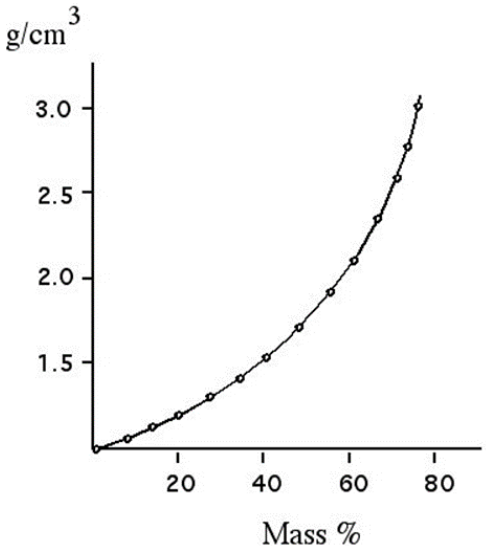
Operation	*Location and date*	
	Lamberton	Grand Rapids
Planted cover crops	22 Sept. 2014	24 Sept. 2014
Sampled cover crop biomass	9 June 2015	11 June 2015
Sampled soils (Pre-term)	9 June 2015	11 June 2015
Flail-mowed cover crops	11 June 2015	12 June 2015
Tillage time	11 June 2015	17 June 2015
Sweet corn planted	19 June 2015	29 June 2015
Sampled soils (Post-term)	8 July 2015	15 July 2015

decomposition and nutrient mineralization. Soil samples were sieved at <2 mm, dried using controlled environment storage room at 65°C and stored until sample processing for POM analyses.

2.2 Particulate organic matter procedures

Air dried soil samples (20 grams) were placed in a 250 ml centrifuge bottles (Nalgene brand), and 40 ml of sodium polytungstate (SPT) solution ($Na_6.(H_2W_{12}O_{40}).H_2O$) (GeoLiquids Inc., Prospect Heights, IL 60070 USA) with density set at 1.6 g cm^{-3} was added. The density function to adjust the density of SPT solution is non-linear, as shown in (Figure 2.2). It is prepared by adding powder SPT to de-ionized water, starting by approximately 400 grams of SPT to 700 ml de-ionized water on a magnetic stirrer, at 20°C, giving density of about 1.6 g cm^{-3}. Continual adjustment with water droplets is necessary to reach the density required. Bottles were then shaken on an orbital shaker at 200 rpm for 1 hour, ensuring the light fraction of POM to be dispersed in the solution. Then bottles were tilted to submerge soil particles left on the sides. Suspensions were set aside for 1 hour to avoid mechanical occlusion of light fraction POM from trapping under soil particles during the centrifuging step. Bottles were then centrifuged at 5000 rpm for 30 min at 20°C, the same temperature as SPT preparation. The supernatant with floating particles of the centrifuged suspension was poured into a Millipore filter funnel equipped with a 1.0 µm polycarbonate filter membranes of 47 mm diameter. A manifold vacuum system was used to take out air from the filter funnels, speeding the filtration process of collecting the light fraction POM from the density solution. The vacuumed bottom flask with SPT solution was removed and kept for later recycling, and replaced with an empty bottom flask. With vacuum turned on again, the filtered material was washed with 0.5M calcium chloride ($CaCl_2$) to clean residual SPT from the collected material. The materials were finally rinsed with de-ionized water to clean the calcium chloride and excess chemicals. Each polycarbonate filter was assigned a numbered tin plate and weight was recorded prior using the filters. Filters were carefully removed with all the collected supernatant, and transferred onto assigned tin containers. The tins and filters were dried overnight at 50°C. After recording dried tins and filters with the filtered materials (POM) weights, the particulate organic

Figure 2.2: Aqueous SPT density/mass concentration. (Source: sometu.de/spt)

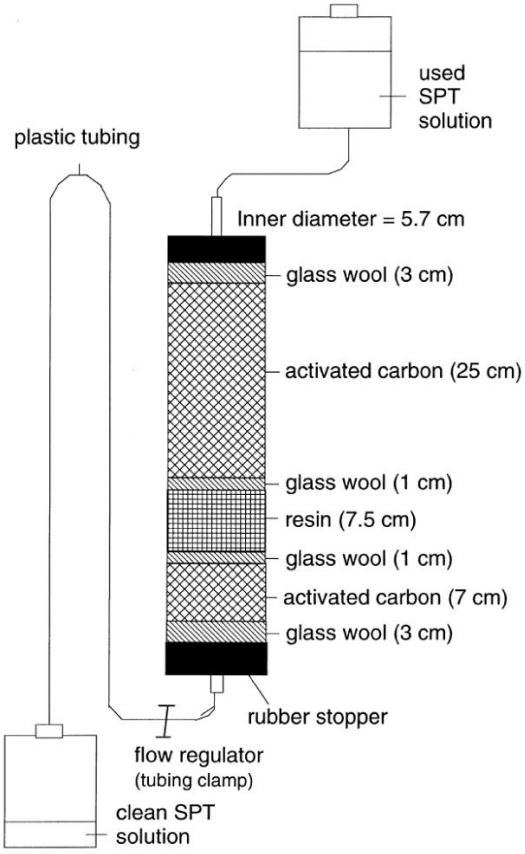


materials were scrapped from the polycarbonate filters into a 7 ml ball grinder vial, with two stainless steel grinder balls for grinding using Geno Grinder 2010 at 1000 rpm for 10 min, to make sure the POM was homogenous. 20 mg of POM was weighed on a microscale in a tin foil for total carbon and nitrogen analyses. Total carbon and nitrogen were determined on an elemental analyzer (vario PYRO Cube, Elementar Analysensysteme GmbH Donaustr 7, 63452 Hanau, Germany). The actual weight of particulate organic carbon and nitrogen were calculated by the total carbon and nitrogen ratios found in POM.

2.2.1 Sodium polytungstate recycling

Although SPT is more expensive than other density solutions, it is reusable. When using SPT for soil organic matter fractionation procedures, small amounts of carbon may be exchanged between soil and the SPT solution. This contamination should be removed from the density solution before reusing in soil organic matter studies. A modified version of recycling procedures used by geologists and paleontologists (Savage, 1988) is used to clear out the carbon from the SPT solution. We followed the recycling procedure of Six et al. (1991), on recycling of sodium polytungstate used in soil organic matter studies. We have built a filter column as in (Figure 2.3), filled with activated charcoal (Darco1 S-51, 4±12 mesh; Norit Americas, Atlanta, GA) and ion exchange resin in H+ form. Before using the filter column, it had to be rinsed with 2 liters of de-ionized water to clear out any cations present in the charcoal, and then followed by another rinse of 5 liters of 1M sodium chloride (NaCl) to change the resin to Na- form and 2 liters of deionized water to clear excess NaCl. The aqueous SPT solution is recycled at density of 1.1 - 1.4 g cm^{-3}. Once done with SPT recycling, column was rinsed with 1 liter de-ionized water and closed by rubber stopper with water inside to prevent residual SPT from drying and clogging the filter column. The recycled SPT solution becomes diluted and would require evaporation of excess water to attain the required density for next use. Using glass or plastic container with large surface area to dry the recycled SPT solution in a drying oven at 70°C. This recycling procedure cancels the high price disadvantage of the SPT, making it a favorable density agent available for separation studies.

Figure 2.3: Diagram of sodium polytungstate recycling column. (Six et al., 1991)



2.3 Permanganate oxidizable carbon

Permanganate oxidizable carbon (POX-C) test was first used by Loginow et al. (1987) to fractionate soil organic carbon via oxidation by potassium permanganate. Weil et al. (2003) did further development to the work and updated the procedures to measure the active carbon fraction of total soil organic carbon. A detailed methodology can be found at: http://lter.kbs.msu.edu/protocols/133. This active carbon measurement is considered fast and inexpensive compared to particulate organic carbon (POM-C) extraction. It can be modified and applied for use on field or at low cost soil test centers for growers who do not have access to a research setting (Idowu et al., 2008). Permanganate oxidizable carbon measurement of the treatments was done by Liebman (2016, unpublished). The POX-C measurement was used to check its correlation to POM-C.

2.4 Statistical analyses

Statistical analyses were performed on R (R Core Development Team, Version 3.3.1, 2016). One-way analysis of variance test (ANOVA) was performed on biomass of cover crops, and multi-way ANOVA was performed to test the effects of different cover crops on the labile organic C and N fractions of soil samples (0 - 20 cm depth) collected before and after incorporation of cover crops. The two locations were evaluated separately as environmental factors were different regarding temperature, incorporation style of cover crops, soil type, and land use history. Inoculation treatment of legume cover crops was regarded as independent factor. Particulate organic matter weight, POM-C, POM-N, and POX-C were tested as response variables for cover crops and time points.

3. Results

3.1 Cover crop biomass

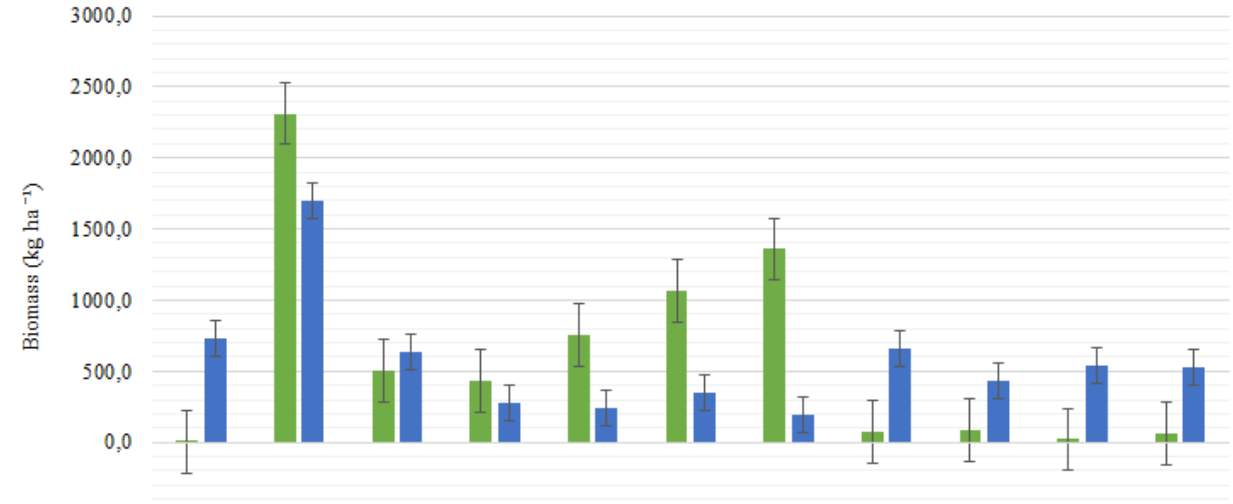
One-way ANOVA test showed significance of biomass of cover crop treatments in both locations (Table 3.1). At the southwestern location (Lamberton), cover crop biomass yield was higher than the northern location (Grand Rapids). Inoculation treatment of hairy vetch ecotypes was not significant. Termination timing of cover crops can be critical regarding above and below ground biomasses. In this experiment, cover crops were terminated on the latest in attempt to provide significant results.

Table 3.1: One way ANOVA results showing significance of cover crop biomass as response variable to cover crop treatments.

	Lamberton			*Grand Rapids*		
	Df	F value	*P* value	Df	F value	*P* value
Biomass of cover crops	5	13.17	<0.001*	4	25.17	<0.001*

Red clover emerged weak due to strong weed competition in both locations, and did not survive winterkill in Grand Rapids. Hairy vetch ecotypes had strong weed competition in Grand Rapids that led to low amounts of biomass production. Winter rye is the most cold tolerant, easy to establish and most productive among winter regions cereal cover crops (Dabney et al., 2001). As shown in (Figure 3.1), average rye biomass exceeds biomass production of hairy vetch ecotype 1 and 2.

Figure 3.1: Average biomass of cover crops and weeds in both locations.



	CLO	RYE	V1n	V1w	V2n	V2w	RYE	V1n	V1w	V2n	V2w
	Lamberton						Grand Rapids				
Cover crop	2,1	2312,4	504,0	427,0	755,3	1062,5	1358,4	76,7	82,3	19,8	57,1
Weed	725,8	1703,5	635,5	272,2	242,2	351,4	192,4	662,5	428,0	537,5	531,1

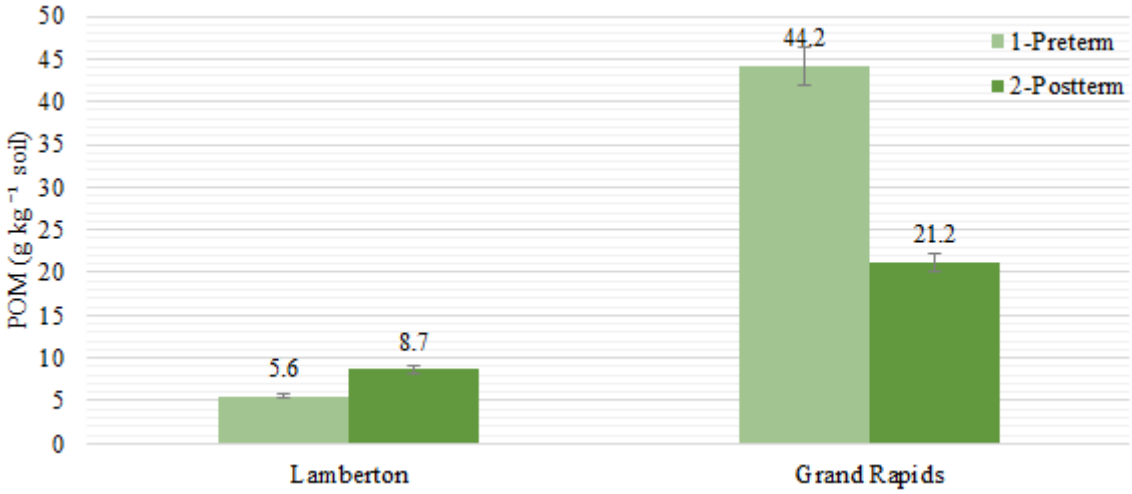
3.2 Particulate organic matter

Multi-way ANOVA test showed significance of average POM weight of cover crop treatments after incorporation of cover crop biomass in Lamberton (p<0.01), shown in (Table 3.2), and (p<0.1) in Grand Rapids. There were no significant interactions of POM to cover crop treatments, and cover crop treatments and time factors in both locations. After incorporation of cover crop biomass, average POM level in Lamberton soil increased as shown in (Figure 3.2). In Grand Rapids, average POM level decreased with less significance. Treatments with error weight record, i.e. sand content with POM filtered (Highlighted under appendix 8), were removed to obtain more significance, however did not affect the results.

Table 3.2: Multi-way ANOVA test results showing significance of POM as response to cover crops and time.

					---Lamberton---				
		Cover crop			Time			Cover crop x Time	
	Df	F value	*P* value	Df	F value	*P* value	Df	F value	*P* value
POM	5	0.7483	0.5927	1	10.3905	0.0026*	5	0.7391	0.5992
					---Grand Rapids---				
		Cover crop			Time			Cover crop x Time	
	Df	F value	*P* value	Df	F value	*P* value	Df	F value	*P* value
POM	4	0.6953	0.60111	1	3.4250	0.07409	4	0.7265	0.58084

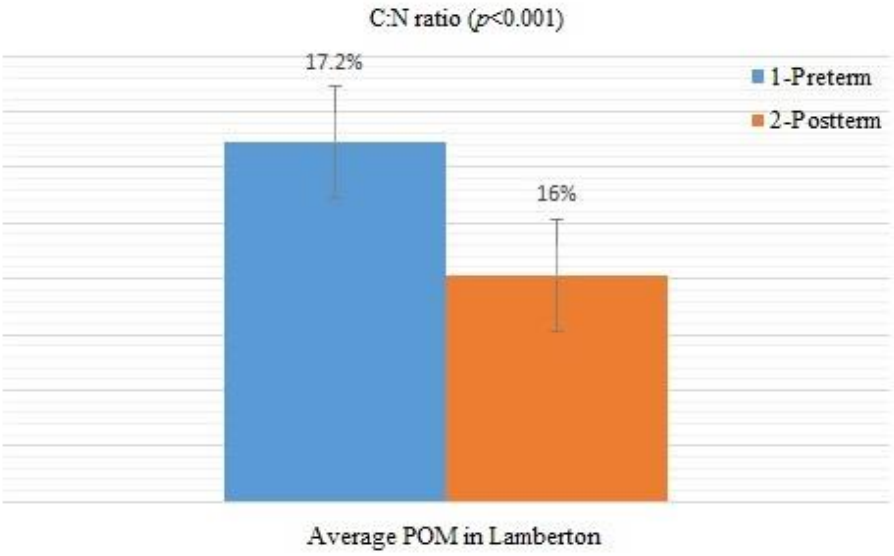
Figure 3.2: Average POM change of combined cover crop treatments across time at both locations; average POM increased in Lamberton (p<0.01) and decreased in Grand Rapids with weak significance (p<0.1).



3.3 Particulate organic carbon and nitrogen

Particulate organic carbon (POM-C) and nitrogen (POM-N) were not significant when tested as independent variables to cover crop treatments, time, and cover crop treatments and time factors in both locations (Appendix 3). The C:N ratios obtained from POM analyses were significant to time in Lamberton (Figure 3.3) but not in Grand Rapids.

Figure 3.3: C:N content ratios of POM before and after incorporation of cover crop biomass in Lamberton.

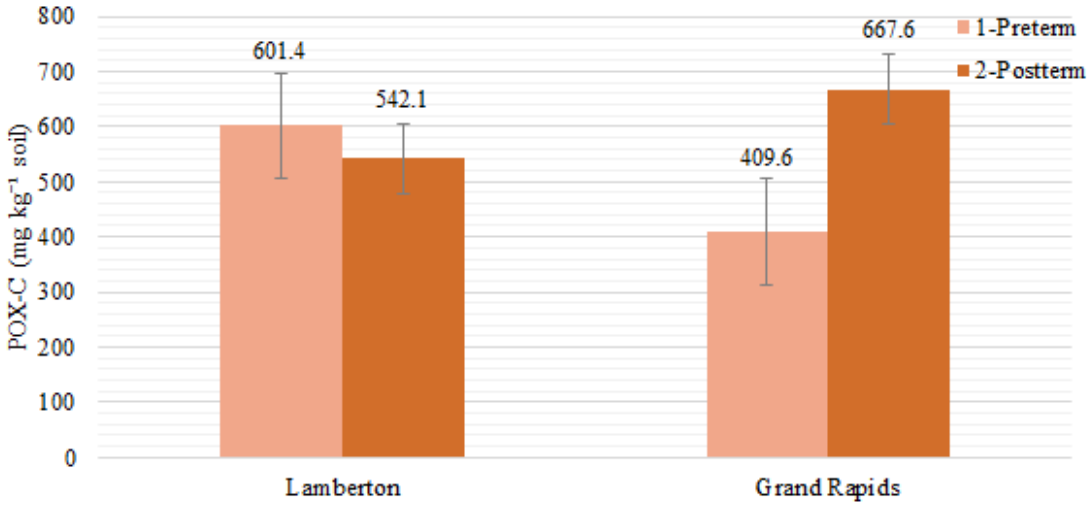


Averages of POM-C and POM-N, over time in both locations are in appendix 4.

3.4 Permanganate oxidizable carbon

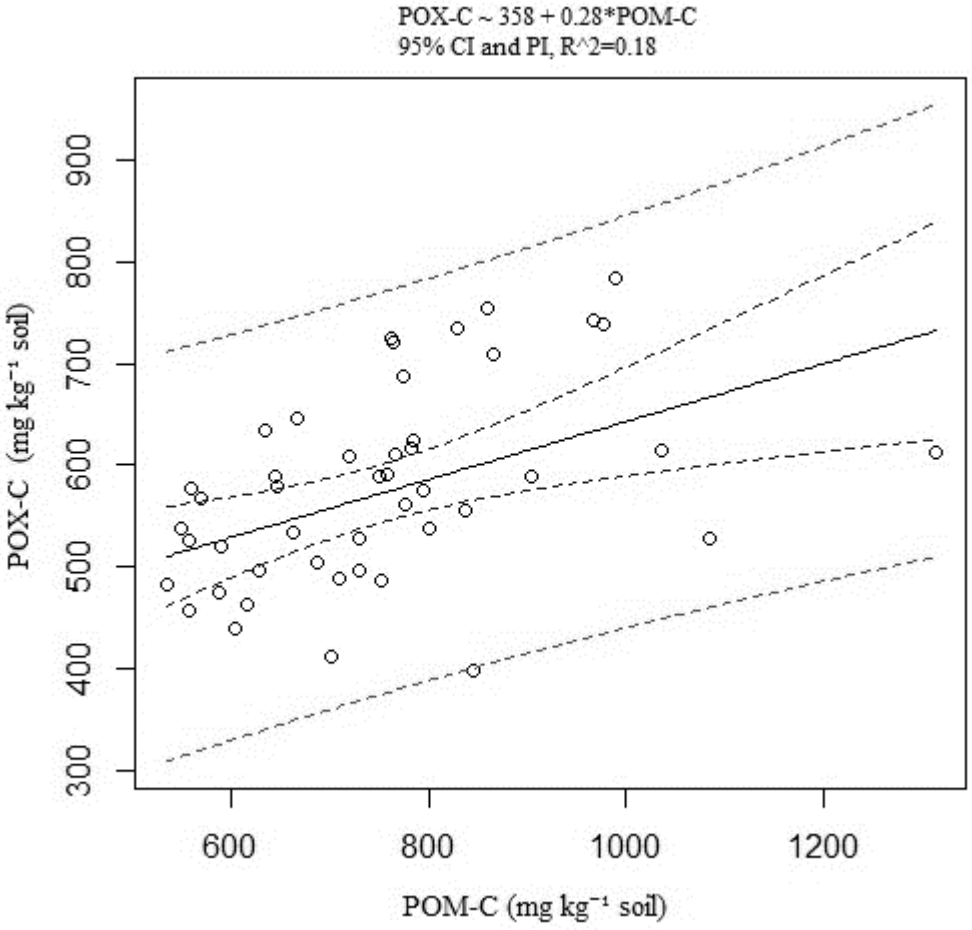
Permanganate oxidizable carbon test of cover crop treatments was time significant in both locations (Appendix 2), but not across cover crop treatments. Average change of POX-C (Figure 3.4) slightly decreased in Lamberton, and increased in Grand Rapids.

Figure 3.4: Average change of POX-C over time points for Lamberton, and Grand Rapids.



POX-C measurement correlated positively with POM-C in Lamberton (Figure 3.5). In Grand Rapids, there was no significant correlation between POX-C and POM-C measurements.

Figure 3.5: Regression plot showing correlation between POX-C and POM-C in Lamberton. (p<0.05)



4. Discussion

4.1 Cover crop biomass

Results shown in (Figure 3.1), aboveground cover crop biomass production varied across both locations might be due to weed pressure and mean temperature differences. In Grand Rapids, the previous land use was an organic apple orchard with grass pasture that might have contributed to weed establishment, unlike in Lamberton, which had row-cropping system. Although, planting time (Table 2.3) of cover crops in Lamberton was only two days ahead of Grand Rapids, Grand Rapids is one level higher on the hardiness scale. Winter survival can be unreliable when cover crops are planted in late September. The low cover crop and high weed biomass yield may have decreased the chance to identify differences of average POM contributed, and the C:N content ratios of POM contributed by each cover crop.

4.2 Particulate organic matter

The average level of particulate organic matter content in soil was different across both locations (Figure 3.2), possibly due to the previous land use effect and soil texture. The previous land use of Lamberton was similar to cover cropping management in terms of crop cultivation, and soil tillage. Organic cropping management balances nutrients input (i.e. incorporation of cover crops biomass) to compensate nutrients utilized by crops during production season, this nutrient recycle may point out to an equilibrium level of input and output of soil labile organic matter. The silty loam soil of Lamberton may have also played a role in the average level of POM content in soil and its increase after incorporation of cover crop biomass, as fine textured soil particles with clay contents can protect plant residues and POM from decomposition (Six et al., 2002; Cotrufo et al., 2013). In Grand Rapids, the results of POM change after incorporation of cover crops biomass was not significant ($p<0.1$), mainly due to the previous land use effect that was apple orchard with grass pasture. Grasslands and uncultivated soils have high carbon and nitrogen contents compared to cultivated arable soils, due to higher incorporation of particulate organic matter, absence of soil tillage and lesser exposure to erosion (Christensen, 1992; Elliott et al., 1993; Jenkinson, 1988; Lugo and Brown, 1993: cited in Hassink 1997). It is possible that the trees had high amount of litter waste that contributed to the high content of POM found in Grand Rapids soil, making it insignificant to test the contribution of cover crop biomass to POM when incorporated in soil. Moreover, in the POM filtration process, sample solutions of Lamberton soils were filtered at a faster rate than of Grand Rapids, which could also refer to the high litter content found in Grand Rapids soil. The soil texture of Grand Rapids may have also assisted in the average level of POM

in soil, as mean POM increases proportionally with sand contents (Liang et al., 2003). Cambardella and Elliott (1993) suggested that under cultivated land, decrease of soil aggregates was related to loss of POM found in soil. Underlining that the roots of cover crops help aggregation of soil particles, this also might had an effect after incorporation of cover crops where living roots ceased to hold particles in aggregates.

Limitations

In POM separation procedures, dry soil samples collected from Grand Rapids had bigger volume per 20 grams than soils collected from Lamberton, due to different soil properties. This might had an effect on levels of particulate organic matter across both locations. Four Grand Rapids soil samples included amounts of sand particles (Highlighted under appendix 8) in the POM filtration step, because soil samples of Grand Rapids did not create a firm pellet after centrifugation, causing loose sand particles to get on filters with the supernatant. Excluding sample with error weight however did not affect the results. The net weight of POM recovered from soil samples may have contained fine mineral soil particles, as the mechanism of pouring the density solution containing the POM supernatant onto the funnel filter may have included other particles than POM. Results of POM measurement in both locations may have been influenced by systematic error during soil samples measurement in the laboratory, in which pre-term soil samples were measured before post-term soil samples with no sample randomization.

4.3 Particulate organic carbon and nitrogen

Particulate organic carbon and nitrogen measurements were obtained by ratios of total carbon and nitrogen found in POM content. Particulate organic carbon negatively correlated with POM weight (Appendix 5), and POM-N positively correlated with POM weight, as the mean C:N ratio of POM decreased after incorporation of cover crops biomass in soil in Lamberton (Figure 3.3). Possibly indicating that incorporation of cover crops biomass contributed low C:N ratio POM to the soil. Particulate organic carbon and nitrogen were not significant when tested as independent variables to cover crop treatments and time, might be due to reduced statistical power when C:N ratio was divided into two measurements, POM-C and POM-N, and high weed biomass interfered with cover crop biomass effect on soil POM quality. As discussed earlier, POM measurement was not significant in Grand Rapids, which affected its C:N ratio.

POM-N

A study by St Luce et al. (2013) on particulate organic matter as good predictors of soil nitrogen supply following legume and non-legume crops in western Canada suggested that POM-N might not be responsive to preceding cover crops on a short-term basis. A large portion of soil organic nitrogen is protected physically and chemically from microbial decomposition; therefore, it is the labile soil organic nitrogen, including POM-N, which mainly contributes to the soil nitrogen supply (Haynes, 2005). However, predicting soil N supply of POM-N can be challenging due to land use and management history, soil properties, and environmental conditions (St Luce et al., 2011).

POM-C and POX-C

Studies have found positive relationship between POM-C and POX-C (Wuest et al., 2006; Mirsky et al., 2008). In Lamberton, POM-C was positively correlated with POX-C measurement (Figure 3.5), which decreased after cover crop biomass incorporation (Figure 3.4). This may hint to the reduction of C:N ratio after cover crops biomass incorporation in soil.

5. Conclusion

In agreement with the research questions, planting cover crop species in the fall season followed by spring termination and incorporation of cover crop biomass increased the average POM in soil in Lamberton. However, in Grand Rapids, the high amount of POM level in soil masked out the effect of cover crops biomass incorporation on average POM level in soil. Incorporation of hairy vetch ecotypes and winter rye contributed to lower C:N ratio POM in Lamberton. Particulate organic matter C:N ratio was not affected by cover crop treatments or time in Grand Rapids due to presumed high litter content found in soil from previous land use. This research is an important step in confirming other related studies and toward developing new research questions relevant to soil texture effect on POM levels in soil, and advances in POM measurement techniques.

6. References

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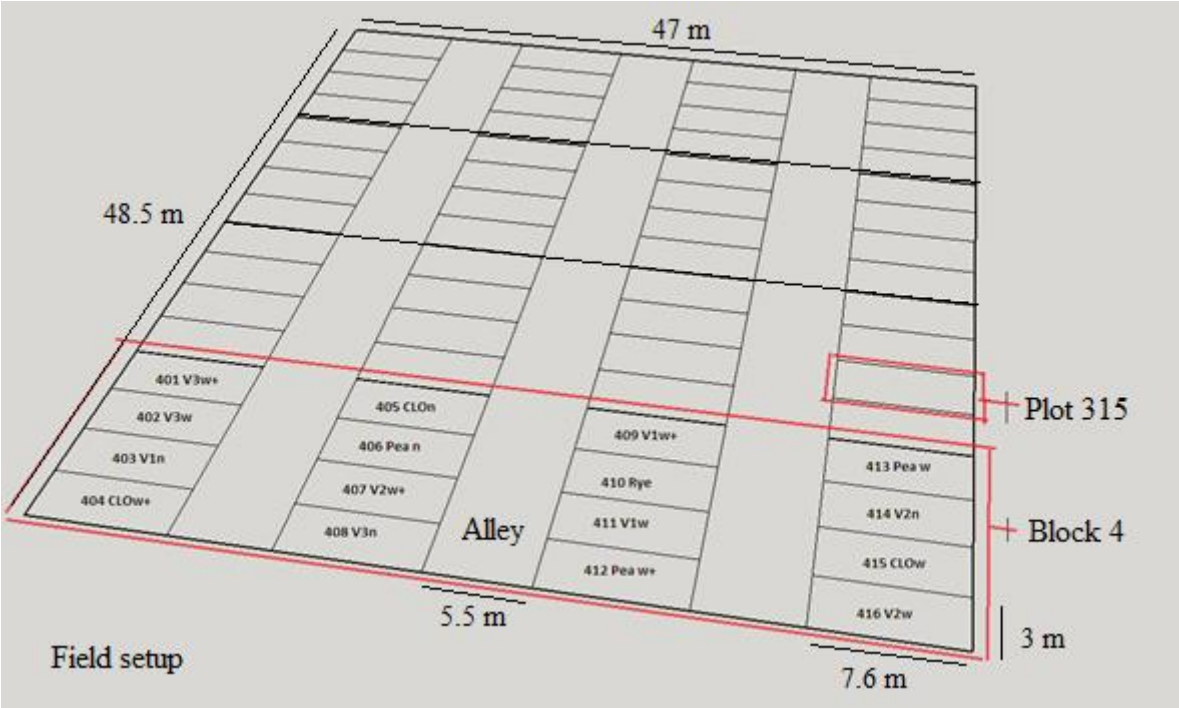
7. Appendices

7.1 Appendix 1

Draft of field setup with plot numbers and original treatments. V1n: vetch ecotype 1 non-inoculated. V1w: vetch ecotype 1 with inoculation. (+): application of nitrogen fertilizer (Initially planned but canceled). CLO: red clover.



7.2 Appendix 2

Multi way ANOVA test results showing significance of POX-C as response variable to cover crop treatment and time at both locations.

---Lamberton---									
	Cover crop			Time			Cover crop x Time		
	Df	F value	*P* value	Df	F value	*P* value	Df	F value	*P* value
POXC (mg kg^{-1} soil)	5	1.3202	0.2782	1	4.2439	0.0468*	5	1.3552	0.2647
---Grand Rapids---									
	Cover crop			Time			Cover crop x Time		
	Df	F value	*P* value	Df	F value	*P* value	Df	F value	*P* value
POXC (mg kg^{-1} soil)	4	0.6572	0.6264	1	16.6610	<0.001*	4	0.3662	0.8307

7.3 Appendix 3

Multi-way ANOVA test results showing significance of POM-C and POM-N as response variables to cover crops and time at both locations:

---Lamberton---

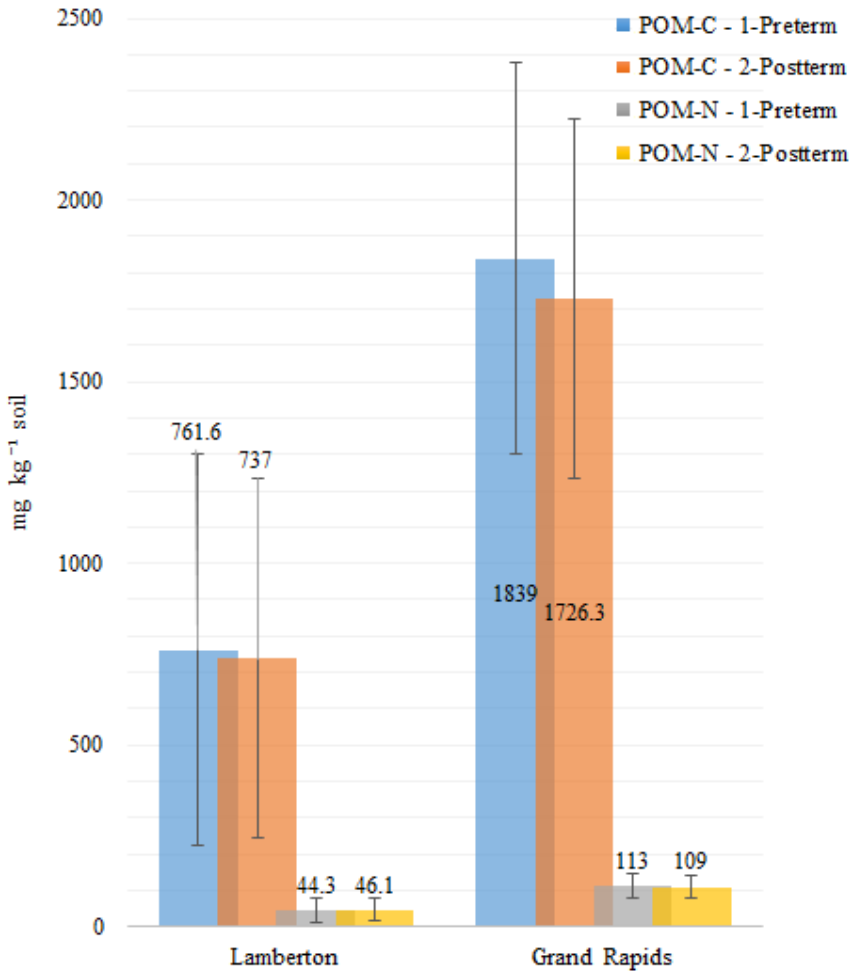
	Cover crop			Time			Cover crop * Time		
	Df	F value	*P* value	Df	F value	*P* value	Df	F value	*P* value
POM-C (mg kg^{-1} soil)	5	0.7680	0.579	1	0.2751	0.6031	5	0.7046	0.6237
POM-N (mg kg^{-1} soil)	5	0.8023	0.5555	1	0.3797	0.5416	5	0.9873	0.439
C:N %	5	2.3592	0.0596	1	18.2461	<0.001*	5	2.6256	0.04*

---Grand Rapids---

	Cover crop			Time			Cover crop * Time		
	Df	F value	*P* value	Df	F value	*P* value	Df	F value	*P* value
POM-C (mg kg^{-1} soil)	4	0.7486	0.5667	1	0.1182	0.7334	4	0.5326	0.7128
POM-N (mg kg^{-1} soil)	4	1.0114	0.4172	1	0.0259	0.8733	4	0.5184	0.7228
C:N %	4	1.1279	0.3620	1	0.0568	0.8133	4	0.3381	0.8501

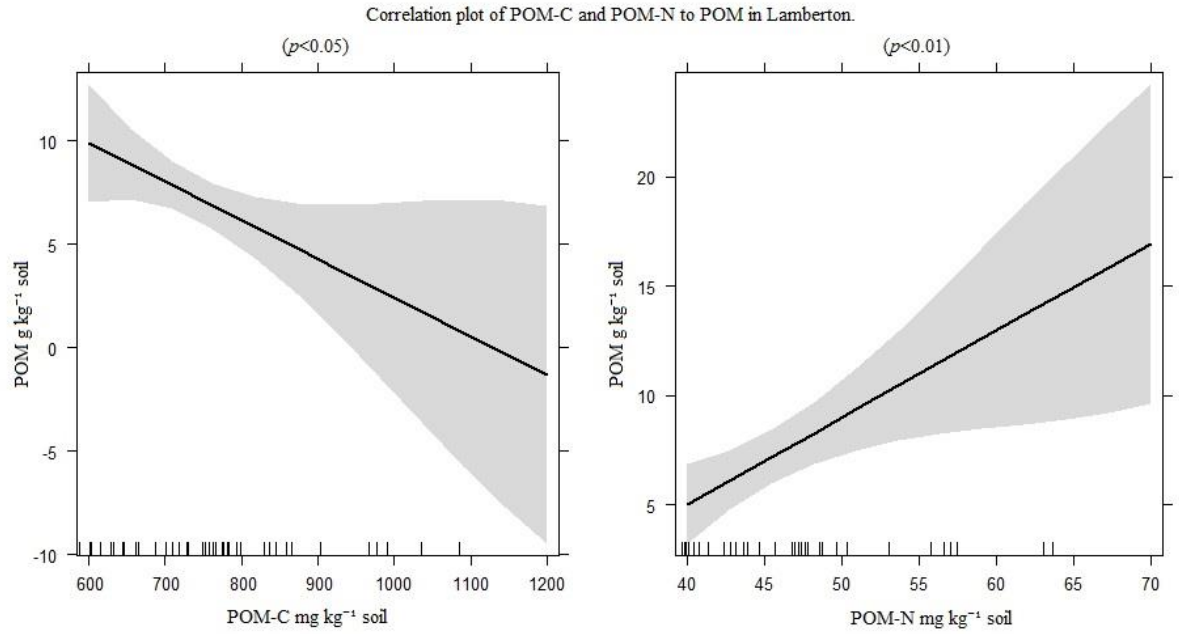
7.4 Appendix 4

Average levels of POM-C and POM-N over time, in both locations.



7.5 Appendix 5

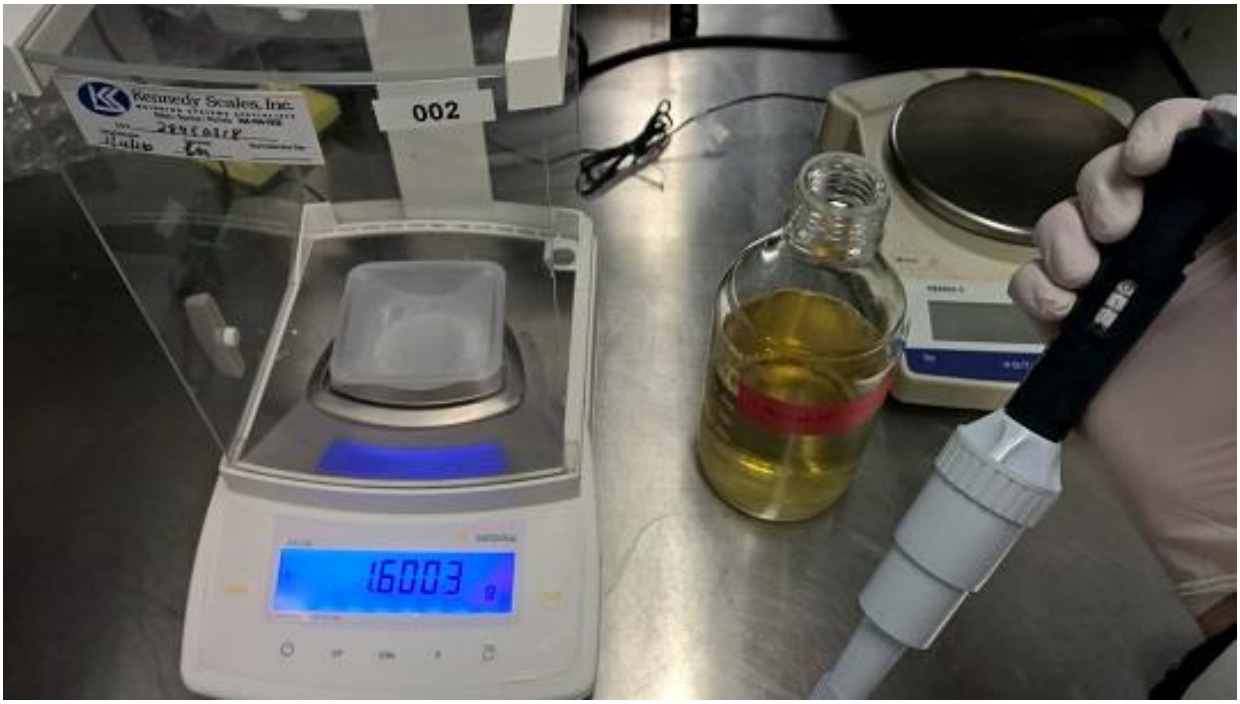
Correlation was significant when POM-C and POM-N tested together to POM in Lamberton.



7.6 Appendix 6

Weighing of dry soil sample of cover crop treatments.

Adjusting the SPT solution density using a microscale for the required density.



Collecting the supernatant from SPT solution in the filtration step after centrifuging.

Collecting dried POM from filter to be grinded using steel ball grinder. Making it homogenous for elemental analyses.

Weighing homogenous POM on a sensitive microscale to analyze its elements.

7.7 Appendix 7

Raw data collected from South West Research and Outreach Center (Lamberton, MN)

		Soil Procedures					POM Procedures	
PlotID	Timepoint	CC	Inoc	Bottle wt(g)	Dry Soil wt(g)	Tin + Filter wt(g)	Tin+Filter+Dry POM wt(g)	POM+Debris wt(g)
105	1- Preterm	CLO	NIN	73,93	20	2,681	2,7478	0,0668
106	1- Preterm	V1w	WIN	73,92	20	2,6273	2,8098	0,1825
108	1- Preterm	V2n	NIN	74,01	20,01	2,678	2,7538	0,0758
109	1- Preterm	V1n	NIN	73,97	20,01	2,6609	2,7448	0,0839
110	1- Preterm	RYE	NIN	73,99	19,99	2,7077	2,9292	0,2215
114	1- Preterm	V2w	WIN	73,89	20	2,6423	2,7497	0,1074
202	1- Preterm	V2w	WIN	73,89	20,01	2,66	2,7579	0,0979
203	1- Preterm	V1n	NIN	73,95	20	2,697	2,7858	0,0888
207	1- Preterm	RYE	NIN	74	20	2,7055	2,8057	0,1002
209	1- Preterm	CLO	NIN	73,96	20	2,6663	2,8088	0,1425
212	1- Preterm	V1w	WIN	73,82	20,01	2,6405	2,8044	0,1639
216	1- Preterm	V2n	NIN	73,88	20	2,6672	2,8217	0,1545
301	1- Preterm	V1w	WIN	73,92	20	2,6768	2,773	0,0962
302	1- Preterm	V2n	NIN	73,93	20	2,704	2,786	0,082
306	1- Preterm	CLO	NIN	74	20	2,668	2,8235	0,1555
308	1- Preterm	V1n	NIN	73,94	20	2,64	2,7324	0,0924
311	1- Preterm	RYE	NIN	73,97	20,01	2,639	2,8051	0,1661
316	1- Preterm	V2w	WIN	73,87	14	2,6482	2,7232	0,075

403	1- Preterm	V1n	NIN	73,86	20	2,6765	2,744	0,0675
405	1- Preterm	CLO	NIN	73,91	20	2,6756	2,7534	0,0778
410	1- Preterm	RYE	NIN	73,97	20	2,6525	2,7535	0,101
411	1- Preterm	V1w	WIN	73,95	20	2,6731	2,7627	0,0896
414	1- Preterm	V2n	NIN	73,79	20	2,6639	2,7464	0,0825
416	1- Preterm	V2w	WIN	73,87	20	2,6501	2,7444	0,0943
105	2- Postterm	CLO	NIN	73,95	20,01	2,642	2,8131	0,1711
106	2- Postterm	V1w	WIN	73,88	20,01	2,6774	2,7937	0,1163
108	2- Postterm	V2n	NIN	74,02	20,01	2,6809	2,7763	0,0954
109	2- Postterm	V1n	NIN	73,98	20	2,7064	2,9923	0,2859
110	2- Postterm	RYE	NIN	73,99	20	2,6672	2,9743	0,3071
114	2- Postterm	V2w	WIN	73,85	20	2,6664	2,8225	0,1561
202	2- Postterm	V2w	WIN	73,89	20,01	2,7079	2,9916	0,2837
203	2- Postterm	V1n	NIN	73,96	20,01	2,6613	2,7998	0,1385
207	2- Postterm	RYE	NIN	73,99	20	2,6967	3,005	0,3083
209	2- Postterm	CLO	NIN	73,96	20,01	2,6415	3,0062	0,3647
212	2- Postterm	V1w	WIN	73,81	20,01	2,6605	2,781	0,1205
216	2- Postterm	V2n	NIN	73,8	20,01	2,628	2,8995	0,2715
301	2- Postterm	V1w	WIN	73,95	20	2,6843	2,8058	0,1215
302	2- Postterm	V2n	NIN	73,94	20,01	2,6681	2,7618	0,0937
306	2- Postterm	CLO	NIN	74,02	20	2,7004	2,828	0,1276
308	2- Postterm	V1n	NIN	73,97	20,01	2,6385	2,7792	0,1407
311	2- Postterm	RYE	NIN	73,99	20,01	2,6593	2,7574	0,0981
316	2- Postterm	V2w	WIN	73,89	20	2,6632	2,8454	0,1822
403	2- Postterm	V1n	NIN	73,89	20,01	2,6744	2,7719	0,0975
405	2- Postterm	CLO	NIN	73,96	20	2,6668	2,7855	0,1187
410	2- Postterm	RYE	NIN	74	20	2,6395	2,746	0,1065
411	2- Postterm	V1w	WIN	73,96	20	2,688	2,8178	0,1298
414	2- Postterm	V2n	NIN	73,82	20,02	2,6574	2,8066	0,1492
416	2- Postterm	V2w	WIN	73,88	20	2,6584	2,834	0,1756

POM Analyses

PlotID	Timepoint	CC	POM wt(mg)	%C	%N	C:N Ratio
105	1- Preterm	CLO	20,015	17,64348	0,93038	18,96374
106	1- Preterm	V1w	20,003	6,00994	0,37215	16,14917
108	1- Preterm	V2n	20,001	18,95708	1,05133	18,03154
109	1- Preterm	V1n	19,997	15,86625	0,91177	17,40156
110	1- Preterm	RYE	20,001	5,45188	0,33494	16,27734
114	1- Preterm	V2w	20	10,38003	0,64196	16,16923
202	1- Preterm	V2w	20,011	15,37687	0,97690	15,74050
203	1- Preterm	V1n	20,008	14,16630	0,89316	15,86079
207	1- Preterm	RYE	19,989	16,53593	0,91177	18,13604
209	1- Preterm	CLO	20,002	9,83914	0,56753	17,33672
212	1- Preterm	V1w	20,014	9,76187	0,59544	16,39429
216	1- Preterm	V2n	20,001	12,81835	0,74430	17,22194
301	1- Preterm	V1w	20,049	17,89246	1,16297	15,38508

302	1- Preterm	V2n	20,054	18,67376	1,14437	16,31798
306	1- Preterm	CLO	20,03	12,57796	0,73500	17,11287
308	1- Preterm	V1n	20,046	16,81067	1,03272	16,27803
311	1- Preterm	RYE	20,022	7,63263	0,42797	17,83430
316	1- Preterm	V2w	20,034	20,74290	1,06994	19,38703
403	1- Preterm	V1n	20,029	21,05198	1,20949	17,40561
405	1- Preterm	CLO	20,041	21,54994	1,20949	17,81733
410	1- Preterm	RYE	20,021	15,11072	0,77222	19,56801
411	1- Preterm	V1w	20,031	20,19341	1,27462	15,84269
414	1- Preterm	V2n	20,016	21,00046	1,13506	18,50158
416	1- Preterm	V2w	19,996	21,95347	1,20949	18,15096
105	2- Postterm	CLO	20,04	6,25034	0,40937	15,26831
106	2- Postterm	V1w	20,024	10,35428	0,55823	18,54848
108	2- Postterm	V2n	20,024	12,32897	0,77222	15,96572
109	2- Postterm	V1n	19,989	4,62766	0,30703	15,07256
110	2- Postterm	RYE	20,026	4,67917	0,26981	17,34246
114	2- Postterm	V2w	20,024	10,18256	0,62335	16,33511
202	2- Postterm	V2w	20,04	5,96702	0,40006	14,91518
203	2- Postterm	V1n	20,045	13,99459	0,92108	15,19374
207	2- Postterm	RYE	20,023	7,04881	0,40937	17,21879
209	2- Postterm	CLO	20,023	3,38274	0,23259	14,54348
212	2- Postterm	V1w	20,029	12,13150	0,72570	16,71705
216	2- Postterm	V2n	20,035	9,69318	0,58614	16,53734
301	2- Postterm	V1w	20,01	11,32445	0,73500	15,40742
302	2- Postterm	V2n	20,006	13,81429	0,92108	14,99799
306	2- Postterm	CLO	20,033	12,03706	0,79082	15,22093
308	2- Postterm	V1n	20,018	11,12698	0,70709	15,73634
311	2- Postterm	RYE	20,03	14,87891	0,87456	17,01308
316	2- Postterm	V2w	20,048	8,24221	0,52101	15,81960
403	2- Postterm	V1n	20,004	13,23046	0,81873	16,15966
405	2- Postterm	CLO	20,012	9,59015	0,58614	16,36156
410	2- Postterm	RYE	20,007	10,50882	0,60475	17,37722
411	2- Postterm	V1w	20,015	8,57705	0,53962	15,89460
414	2- Postterm	V2n	20,013	10,51740	0,63266	16,62415
416	2- Postterm	V2w	20,008	8,62856	0,60475	14,26806

POM -C and -N in Soil

PlotID	Timepoint	CC	gPOMC/20g soil	gPOMC/kg soil	gPOMN/20g soil	gPOMN/kg soil	gPOM/kg soil
105	1- Preterm	CLO	0,01179	0,58885	0,00062	0,03105	3,34
106	1- Preterm	V1w	0,01097	0,54833	0,00068	0,03395	9,125
108	1- Preterm	V2n	0,01437	0,71844	0,00080	0,03984	3,79
109	1- Preterm	V1n	0,01331	0,66569	0,00076	0,03825	4,195
110	1- Preterm	RYE	0,01208	0,60377	0,00074	0,03709	11,075
114	1- Preterm	V2w	0,01115	0,55741	0,00069	0,03447	5,37
202	1- Preterm	V2w	0,01505	0,75228	0,00096	0,04779	4,895
203	1- Preterm	V1n	0,01258	0,62873	0,00079	0,03964	4,44

207	1- Preterm	RYE	0,01657	0,82891	0,00091	0,04570	5,01
209	1- Preterm	CLO	0,01402	0,70097	0,00081	0,04043	7,125
212	1- Preterm	V1w	0,01600	0,79943	0,00098	0,04876	8,195
216	1- Preterm	V2n	0,01980	0,99017	0,00115	0,05749	7,725
301	1- Preterm	V1w	0,01721	0,85852	0,00112	0,05580	4,81
302	1- Preterm	V2n	0,01531	0,76356	0,00094	0,04679	4,1
306	1- Preterm	CLO	0,01956	0,97647	0,00114	0,05706	7,775
308	1- Preterm	V1n	0,01553	0,77487	0,00095	0,04760	4,62
311	1- Preterm	RYE	0,01268	0,63319	0,00071	0,03550	8,305
316	1- Preterm	V2w	0,01556	0,77654	0,00080	0,04005	3,75
403	1- Preterm	V1n	0,01421	0,70948	0,00082	0,04076	3,375
405	1- Preterm	CLO	0,01677	0,83658	0,00094	0,04695	3,89
410	1- Preterm	RYE	0,01526	0,76229	0,00078	0,03896	5,05
411	1- Preterm	V1w	0,01809	0,90326	0,00114	0,05701	4,48
414	1- Preterm	V2n	0,01733	0,86558	0,00094	0,04678	4,125
416	1- Preterm	V2w	0,02070	1,03531	0,00114	0,05704	4,715
105	2- Postterm	CLO	0,01069	0,53365	0,00070	0,03495	8,555
106	2- Postterm	V1w	0,01204	0,60138	0,00065	0,03242	5,815
108	2- Postterm	V2n	0,01176	0,58739	0,00074	0,03679	4,77
109	2- Postterm	V1n	0,01323	0,66189	0,00088	0,04391	14,295
110	2- Postterm	RYE	0,01437	0,71755	0,00083	0,04138	15,355
114	2- Postterm	V2w	0,01589	0,79380	0,00097	0,04859	7,805
202	2- Postterm	V2w	0,01693	0,84473	0,00113	0,05664	14,185
203	2- Postterm	V1n	0,01938	0,96695	0,00128	0,06364	6,925
207	2- Postterm	RYE	0,02173	1,08533	0,00126	0,06303	15,415
209	2- Postterm	CLO	0,01234	0,61613	0,00085	0,04236	18,235
212	2- Postterm	V1w	0,01462	0,72986	0,00087	0,04366	6,025
216	2- Postterm	V2n	0,02632	1,31355	0,00159	0,07943	13,575
301	2- Postterm	V1w	0,01376	0,68762	0,00089	0,04463	6,075
302	2- Postterm	V2n	0,01294	0,64701	0,00086	0,04314	4,685
306	2- Postterm	CLO	0,01536	0,76670	0,00101	0,05037	6,38
308	2- Postterm	V1n	0,01566	0,78208	0,00099	0,04970	7,035
311	2- Postterm	RYE	0,01460	0,72872	0,00086	0,04283	4,905
316	2- Postterm	V2w	0,01502	0,74907	0,00095	0,04735	9,11
403	2- Postterm	V1n	0,01290	0,64486	0,00080	0,03991	4,875
405	2- Postterm	CLO	0,01138	0,56883	0,00070	0,03477	5,935
410	2- Postterm	RYE	0,01119	0,55940	0,00064	0,03219	5,325
411	2- Postterm	V1w	0,01113	0,55623	0,00070	0,03500	6,49
414	2- Postterm	V2n	0,01569	0,78409	0,00094	0,04717	7,46
416	2- Postterm	V2w	0,01515	0,75728	0,00106	0,05308	8,78

			POX-C	Biomass	
PlotID	Timepoint	CC	POX-C mg/kg soil	CC kg/ha	Weed kg/ha
105	1- Preterm	CLO	519,8294	6,4000	492,2995
106	1- Preterm	V1w	538,7877	248,1997	247,2997
108	1- Preterm	V2n	608,0223	357,1996	278,4997

109	1- Preterm	V1n	645,4584	481,8995	597,8994
110	1- Preterm	RYE	438,8525	1273,9987	3363,3965
114	1- Preterm	V2w	458,0816	1000,9990	644,4993
202	1- Preterm	V2w	485,8017	318,2997	577,6994
203	1- Preterm	V1n	496,7172	486,1995	313,8997
207	1- Preterm	RYE	734,2519	2843,7971	46,2000
209	1- Preterm	CLO	410,8970	0,4000	481,5995
212	1- Preterm	V1w	537,0911	307,2997	412,1996
216	1- Preterm	V2n	784,0287	748,3992	306,2997
301	1- Preterm	V1w	754,3412	609,6994	57,0999
302	1- Preterm	V2n	720,6316	954,9990	150,4998
306	1- Preterm	CLO	739,4342	0,5000	1008,2990
308	1- Preterm	V1n	688,1173	473,5995	852,0991
311	1- Preterm	RYE	633,4302	2065,4979	--
316	1- Preterm	V2w	561,0728	1087,7989	123,7999
403	1- Preterm	V1n	488,0574	575,6994	780,1992
405	1- Preterm	CLO	555,3543	1,2000	923,2990
410	1- Preterm	RYE	724,7086	3073,3968	--
411	1- Preterm	V1w	588,7455	543,9994	373,0996
414	1- Preterm	V2n	709,1722	962,6990	234,2998
416	1- Preterm	V2w	614,1572	1846,1981	60,5999
105	2- Postterm	CLO	482,9786		
106	2- Postterm	V1w	--		
108	2- Postterm	V2n	475,6423		
109	2- Postterm	V1n	534,8975		
110	2- Postterm	RYE	260,7741		
114	2- Postterm	V2w	575,1685		
202	2- Postterm	V2w	397,3879		
203	2- Postterm	V1n	742,1903		
207	2- Postterm	RYE	527,9118		
209	2- Postterm	CLO	462,2940		
212	2- Postterm	V1w	528,8097		
216	2- Postterm	V2n	612,1064		
301	2- Postterm	V1w	503,9129		
302	2- Postterm	V2n	578,5056		
306	2- Postterm	CLO	611,1000		
308	2- Postterm	V1n	616,0724		
311	2- Postterm	RYE	497,5238		
316	2- Postterm	V2w	588,5806		
403	2- Postterm	V1n	588,7064		
405	2- Postterm	CLO	566,7109		
410	2- Postterm	RYE	577,2576		
411	2- Postterm	V1w	525,2375		
414	2- Postterm	V2n	624,1788		
416	2- Postterm	V2w	590,7360		

7.8 Appendix 8

Raw data collected from North Central Research and Outreach Center (Grand Rapids, MN)

		Soil Procedures				POM Procedures		
PlotID	Timepoint	CC	Inoc	Bottle wt(g)	Dry Soil wt(g)	Tin + Filter wt(g)	Tin+Filter+Dry POM wt(g)	POM+Debris wt(g)
106	1- Preterm	V1w	WIN	73,85	20	2,6765	2,8974	0,2209
108	1- Preterm	V2n	NIN	73,9	20	2,7038	5,1614	2,4576
109	1- Preterm	V1n	NIN	73,88	20	2,648	5,1085	2,4605
110	1- Preterm	RYE	NIN	73,75	20	2,6787	3,5173	0,8386
114	1- Preterm	V2w	WIN	73,94	20,01	2,6412	3,2256	0,5844
202	1- Preterm	V2w	WIN	73,98	20,01	2,6721	3,4468	0,7747
203	1- Preterm	V1n	NIN	73,95	20,01	2,6803	3,4801	0,7998
207	1- Preterm	RYE	NIN	73,93	20	2,6491	3,0786	0,4295
212	1- Preterm	V1w	WIN	74,06	20,01	2,7054	3,0046	0,2992
216	1- Preterm	V2n	NIN	74,03	20,01	2,6573	2,9477	0,2904
301	1- Preterm	V1w	WIN	74,06	20	2,7082	3,0149	0,3067
302	1- Preterm	V2n	NIN	73,93	20,01	2,6521	3,076	0,4239
308	1- Preterm	V1n	NIN	73,83	19,99	2,6801	2,7876	0,1075
311	1- Preterm	RYE	NIN	74	20	2,6765	2,765	0,0885
316	1- Preterm	V2w	WIN	74,09	20	2,6666	2,751	0,0844
403	1- Preterm	V1n	NIN	73,96	20	2,6915	3,979	1,2875
410	1- Preterm	RYE	NIN	73,93	20	2,6783	2,7843	0,106
411	1- Preterm	V1w	WIN	73,95	20	2,693	6,5011	3,8081
412	1- Preterm	V2n	NIN	73,84	20	2,6758	4,5968	1,921
416	1- Preterm	V2w	WIN	74,01	20	2,6563	3,0584	0,4021
106	2- Postterm	V1w	WIN	73,94	20	2,6471	2,8518	0,2047
108	2- Postterm	V2n	NIN	74	20	2,6538	3,1579	0,5041
109	2- Postterm	V1n	NIN	73,86	20	2,6448	2,7265	0,0817
110	2- Postterm	RYE	NIN	73,96	20	2,6486	2,8965	0,2479
114	2- Postterm	V2w	WIN	73,96	20	2,6256	2,7356	0,11
202	2- Postterm	V2w	WIN	73,93	20	2,6378	3,0426	0,4048
203	2- Postterm	V1n	NIN	74,01	20,01	2,6918	3,0251	0,3333
207	2- Postterm	RYE	NIN	73,97	19,99	2,7012	3,5648	0,8636
212	2- Postterm	V1w	WIN	74	19,99	2,68	3,1147	0,4347
216	2- Postterm	V2n	NIN	73,88	20	2,6801	3,3727	0,6926
301	2- Postterm	V1w	WIN	73,89	20,01	2,6851	4,1853	1,5002
302	2- Postterm	V2n	NIN	73,94	20	2,6695	2,7448	0,0753
308	2- Postterm	V1n	NIN	73,98	20	2,6331	2,7363	0,1032
311	2- Postterm	RYE	NIN	73,95	20	2,6648	2,8106	0,1458
316	2- Postterm	V2w	WIN	73,8	20	2,6459	2,8928	0,2469
403	2- Postterm	V1n	NIN	73,86	20	2,6641	2,7391	0,075
410	2- Postterm	RYE	NIN	73,84	20	2,6412	3,0894	0,4482
411	2- Postterm	V1w	WIN	73,9	20	2,6505	3,0688	0,4183
412	2- Postterm	V2n	NIN	73,9	20,01	2,6601	3,2608	0,6007
416	2- Postterm	V2w	WIN	73,77	20,01	2,6582	3,6646	1,0064

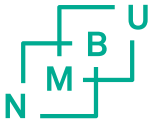
POM Analyses

PlotID	Timepoint	CC	POM wt(mg)	%C	%N	C:N Ratio
106	1- Preterm	V1w	20,019	10,91234	0,49310	22,13002
108	1- Preterm	V2n	20,024	2,86760	0,20468	14,00993
109	1- Preterm	V1n	20,021	1,10755	0,07443	14,88031
110	1- Preterm	RYE	20	4,08676	0,26981	15,14681
114	1- Preterm	V2w	20,006	4,62766	0,27911	16,57982
202	1- Preterm	V2w	20,021	3,44284	0,21399	16,08899
203	1- Preterm	V1n	20,026	6,81699	0,51171	13,32202
207	1- Preterm	RYE	20,031	10,15681	0,62335	16,29379
212	1- Preterm	V1w	20,004	9,81338	0,70709	13,87857
216	1- Preterm	V2n	20,039	12,76684	0,82804	15,41818
301	1- Preterm	V1w	20,035	6,73972	0,50241	13,41492
302	1- Preterm	V2n	20,032	5,39178	0,42797	12,59836
308	1- Preterm	V1n	20,05	24,33169	0,93968	25,89349
311	1- Preterm	RYE	20,02	24,04836	0,99551	24,15692
316	1- Preterm	V2w	20,035	27,79170	0,94899	29,28564
403	1- Preterm	V1n	20,125	2,73882	0,13025	21,02688
410	1- Preterm	RYE	20,003	24,64077	0,93968	26,22242
411	1- Preterm	V1w	20,065	2,86760	0,17677	16,22202
412	1- Preterm	V2n	20,068	1,83733	0,12095	15,19087
416	1- Preterm	V2w	20,001	10,49165	0,66987	15,66213
106	2- Postterm	V1w	20,032	12,80977	0,58614	21,85448
108	2- Postterm	V2n	20,042	8,97199	0,61405	14,61115
109	2- Postterm	V1n	20,042	9,37551	0,65127	14,39583
110	2- Postterm	RYE	19,987	11,22143	0,61405	18,27443
114	2- Postterm	V2w	20,046	18,65659	0,76291	24,45446
202	2- Postterm	V2w	19,997	7,25486	0,49310	14,71272
203	2- Postterm	V1n	20,044	8,41392	0,55823	15,07256
207	2- Postterm	RYE	19,985	6,13014	0,44658	13,72680
212	2- Postterm	V1w	20,046	9,27249	0,65127	14,23764
216	2- Postterm	V2n	19,991	9,61591	0,71639	13,42269
301	2- Postterm	V1w	20,055	2,31812	0,15816	14,65639
302	2- Postterm	V2n	19,991	15,66878	0,76291	20,53814
308	2- Postterm	V1n	20,023	19,60959	0,84665	23,16151
311	2- Postterm	RYE	20,077	13,74560	0,61405	22,38513
316	2- Postterm	V2w	19,964	7,81293	0,40937	19,08538
403	2- Postterm	V1n	20,078	23,14687	0,94899	24,39113
410	2- Postterm	RYE	20,055	10,06236	0,66987	15,02129
411	2- Postterm	V1w	20,031	10,66336	0,70709	15,08065
412	2- Postterm	V2n	20,016	8,74018	0,53032	16,48106
416	2- Postterm	V2w	20,052	8,05333	0,53962	14,92406

POM -C and -N in Soil

PlotID	Timepoint	CC	gPOMC/20g soil	gPOMC/kg soil	gPOMN/20g soil	gPOMN/kg soil	gPOM/kg soil
106	1- Preterm	V1w	0,02411	1,20412	0,00109	0,05441	11,045
108	1- Preterm	V2n	0,07047	3,51949	0,00503	0,25121	122,88
109	1- Preterm	V1n	0,02725	1,36113	0,00183	0,09147	123,025
110	1- Preterm	RYE	0,03427	1,71358	0,00226	0,11313	41,93
114	1- Preterm	V2w	0,02704	1,35180	0,00163	0,08153	29,22
202	1- Preterm	V2w	0,02667	1,33219	0,00166	0,08280	38,735
203	1- Preterm	V1n	0,05452	2,72258	0,00409	0,20437	39,99
207	1- Preterm	RYE	0,04362	2,17780	0,00268	0,13366	21,475
212	1- Preterm	V1w	0,02936	1,46779	0,00212	0,10576	14,96
216	1- Preterm	V2n	0,03707	1,85014	0,00240	0,12000	14,52
301	1- Preterm	V1w	0,02067	1,03173	0,00154	0,07691	15,335
302	1- Preterm	V2n	0,02286	1,14096	0,00181	0,09056	21,195
308	1- Preterm	V1n	0,02616	1,30457	0,00101	0,05038	5,375
311	1- Preterm	RYE	0,02128	1,06308	0,00088	0,04401	4,425
316	1- Preterm	V2w	0,02346	1,17076	0,00080	0,03998	4,22
403	1- Preterm	V1n	0,03526	1,75216	0,00168	0,08333	64,375
410	1- Preterm	RYE	0,02612	1,30577	0,00100	0,04980	5,3
411	1- Preterm	V1w	0,10920	5,44237	0,00673	0,33549	190,405
412	1- Preterm	V2n	0,03530	1,75877	0,00232	0,11578	96,05
416	1- Preterm	V2w	0,04219	2,10924	0,00269	0,13467	20,105
106	2- Postterm	V1w	0,02622	1,30899	0,00120	0,05990	10,235
108	2- Postterm	V2n	0,04523	2,25665	0,00310	0,15445	25,205
109	2- Postterm	V1n	0,00766	0,38219	0,00053	0,02655	4,085
110	2- Postterm	RYE	0,02782	1,39180	0,00152	0,07616	12,395
114	2- Postterm	V2w	0,02052	1,02376	0,00084	0,04186	5,5
202	2- Postterm	V2w	0,02937	1,46860	0,00200	0,09982	20,24
203	2- Postterm	V1n	0,02804	1,39910	0,00186	0,09282	16,665
207	2- Postterm	RYE	0,05294	2,64898	0,00386	0,19298	43,18
212	2- Postterm	V1w	0,04031	2,01075	0,00283	0,14123	21,735
216	2- Postterm	V2n	0,06660	3,33149	0,00496	0,24820	34,63
301	2- Postterm	V1w	0,03478	1,73405	0,00237	0,11831	75,01
302	2- Postterm	V2n	0,01180	0,59020	0,00057	0,02874	3,765
308	2- Postterm	V1n	0,02024	1,01069	0,00087	0,04364	5,16
311	2- Postterm	RYE	0,02004	0,99821	0,00090	0,04459	7,29
316	2- Postterm	V2w	0,01929	0,96625	0,00101	0,05063	12,345
403	2- Postterm	V1n	0,01736	0,86464	0,00071	0,03545	3,75
410	2- Postterm	RYE	0,04510	2,24879	0,00300	0,14971	22,41
411	2- Postterm	V1w	0,04460	2,22679	0,00296	0,14766	20,915
412	2- Postterm	V2n	0,05250	2,62301	0,00319	0,15915	30,035
416	2- Postterm	V2w	0,08105	4,04192	0,00543	0,27083	50,32

			POX-C	Biomass	
PlotID	Timepoint	CC	POX-C mg/kg soil	CC kg/ha	Weed kg/ha
106	1- Preterm	V1w	473,2988	47,8999	375,4996
108	1- Preterm	V2n	579,0434	1,6000	313,0996
109	1- Preterm	V1n	645,2856	80,3999	644,9992
110	1- Preterm	RYE	583,9786	1054,9988	197,4998
114	1- Preterm	V2w	693,9638	170,6998	719,1992
202	1- Preterm	V2w	176,4720	28,0000	533,5994
203	1- Preterm	V1n	258,6522	27,2000	406,2995
207	1- Preterm	RYE	546,1929	2025,0976	84,8999
212	1- Preterm	V1w	585,5040	74,0999	483,8994
216	1- Preterm	V2n	386,1249	3,8000	709,8992
301	1- Preterm	V1w	327,1841	156,5998	343,0996
302	1- Preterm	V2n	270,6857	27,9000	439,7995
308	1- Preterm	V1n	366,1725	51,6999	949,8989
311	1- Preterm	RYE	578,7206	885,5990	456,0995
316	1- Preterm	V2w	433,8051	20,5000	486,2994
403	1- Preterm	V1n	218,3159	147,8998	650,6992
410	1- Preterm	RYE	165,9146	1472,0983	31,8000
411	1- Preterm	V1w	110,8481	50,8999	510,7994
412	1- Preterm	V2n	399,2959	45,8999	688,8992
416	1- Preterm	V2w	393,2032	9,5000	387,0995
106	2- Postterm	V1w	672,9292		
108	2- Postterm	V2n	670,2119		
109	2- Postterm	V1n	683,8047		
110	2- Postterm	RYE	665,3716		
114	2- Postterm	V2w	626,6208		
202	2- Postterm	V2w	503,9766		
203	2- Postterm	V1n	652,8242		
207	2- Postterm	RYE	657,7730		
212	2- Postterm	V1w	508,9767		
216	2- Postterm	V2n	583,0583		
301	2- Postterm	V1w	676,2038		
302	2- Postterm	V2n	543,8485		
308	2- Postterm	V1n	803,4844		
311	2- Postterm	RYE	766,6316		
316	2- Postterm	V2w	661,3308		
403	2- Postterm	V1n	524,6092		
410	2- Postterm	RYE	654,2634		
411	2- Postterm	V1w	533,8705		
412	2- Postterm	V2n	506,0853		
416	2- Postterm	V2w	1456,7573		



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