THE INFLUENCE OF ECOLOGICAL CROPPING PRACTICES ON AGGREGATE STABILITY: RESULTS FROM TWO LONG-TERM STUDIES

BY

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ABSTRACT

Stainsby, April E. M. Sc. University of Manitoba, May 2019. The influence of ecological
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Agricultural cropping practices, including crop rotations with annual grains and perennial forages, cover crops, and no-till and organic management influence soil processes. Aggregate stability is a soil property that relates to many different physical and biological functions of the soil, and as such is an important indicator of soil health. It was hypothesized that aggregate stability would be improved through the following cropping system interventions: 1) including two years of alfalfa (*Medicago sativa* L.) in an organic annual grain rotation; 2) adding composted manure to long-term organic systems; 3) including four years of perennial forages in rotation with annual grains as a one-time intervention for system rehabilitation; and 4) including cover crops in a no-till crop rotation. Furthermore, the first two cropping system interventions were compared to conventional rotations and long-term grasslands. This research took place at two long term studies, a rotation study in southern Manitoba and a cover crop study in south eastern Saskatchewan. The rotation study included an annual grain rotation, consisting of wheat (*Triticum aestivum* L.) - flax (*Linum usitatissimum* L.) - oat (*Avena sativa* L.) - hairy vetch (*Vicia villosa* Roth) / barley (*Hordeum vulgare* L.) green manure, and a perennial forage and grain rotation (wheat-flax-two years of alfalfa). Composted manure was added to the organic forage grain rotation every four years. The cover crop study used black medic (*Medicago lupulina* L.) as a self-regenerating cover crop. Black medic produces large amounts of seed and regrows each spring, to grow under the crop and continues to grow in the fall after harvest. It was grown in a no-till wheat-flax-canaryseed (*Phalaris canariensis* L.) rotation at two nitrogen fertilizer levels. Aggregate stability samples were taken in both wheat and flax phases of the rotations at both sites in the spring of 2017 and 2018. A wet sieving procedure using stacked sieves with five mesh sizes was used to determine mean weight diameter (MWD) of stable aggregates. At both study sites grassland areas had higher MWD and generally more 1-6.3mm aggregates and fewer 0.25-1mm aggregates than the arable treatments. In a few cases the rotations with a perennial forage component had similar AS to the grasslands. The perennial forage increased MWD under organic management at 10-20cm depth. Manure additions did not affect AS, and in most cases neither did the perennial forage in rotation. The presence of alfalfa in the alfalfa intervention increased AS but the number of years in alfalfa did not. The black medic cover crop increased MWD with low nitrogen fertilizer in the wheat phase but not the flax phase of rotation. It was concluded that long term grasslands and cover crops were the most effective ways to improve AS at these sites.

FOREWARD

References are in the style of the Canadian Journal of Plant Science.

1. GENERAL INTRODUCTION

Improving soil health has become an important focus in agriculture worldwide as a way to build resilient agricultural systems. It is a response to negative impacts of agriculture on soil quality and ecological functioning/biodiversity and an alternative to reliance on external inputs to support intensive agricultural production. The soil health movement seeks to improve agricultural productivity and ecological functioning/reduce ecological harms through increasing soil functioning using natural processes.

Organic and natural systems agriculture share this goal of increasing soil health, which is tied to improved productivity and ecological benefits. These farming systems seek to use natural processes that can be integrated into agricultural production systems to improve soil and ecosystem function. Organic farms on the northern prairies use different practices than conventional farms, including more use of forages, green manures, and composts (Nelson et al. 2010). The effects of organic farming practices on soil properties, especially physical properties have not received much attention.

A commonly used definition of soil health is “the capacity of a soil to function within ecosystem boundaries to sustain biological productivity, maintain environmental quality, and promote plant and animal health" (Doran and Parkin, 1994), which was originally written to define soil quality. In this context quality does not refer to inherent properties of the soil but to the ability of a soil to fulfill a certain function. It applies the subjective idea of health to a holistic view of the soil as a dynamic living system. Aggregate stability (resistance of aggregates to erosive forces) is an important measure of soil health as it is a soil property that relates to many functions and processes in the soil. For example, aggregates and the pores in between them are key to soil structure, which influences water and air movement through the soil and plant rooting ability as well as compaction (Tisdall and Oades 1982). Stable aggregates on the soil surface reduce susceptibility to erosion and surface crusting. Aggregates and pores of different sizes provide habitat for soil organisms. Aggregates also physically protect organic materials from decomposition which increases their residence time in the soil, making them important sites of carbon storage in the soil (Six et al. 2004). These multiple functions make aggregate stability a useful indicator of soil health, or soil quality.

Aggregates, especially macroaggregates, are sensitive to agricultural management practices (Oades, 1984). Cropping practices typically influence aggregate stability by altering organic matter inputs to the soil and the level of soil disturbance. The present study focuses on practices used in organic management systems, such as adding perennial crops into annual rotations, one-time use of perennial crop phases for soil rehabilitation, cover crops, and manure, with long-term grasslands as an ecological benchmark. All of these management practices have the potential to increase organic matter input to the soil, and all, except for manure, increase the proportion of the year that living roots are growing in the soil to different degrees, grading from cover crops which add living roots for part of the season, to perennial phases which keep roots in the soil for a number of years, to long term grassland or pasture interventions which could last for decades.

The effects of cropping practices on soil properties are best assessed at sites where they have been established for many years, such as in long-term studies. This study utilized two long term experiments, the Glenlea Long-term Rotation in Manitoba, which compares organic and conventional management, and the Indian Head Long-term Black Medic (*Medicago lupulina* L.*)* Rotation, a cover crop study in Saskatchewan.

The broad objective of this thesis is to better understand how alternative cropping practices in organic and no-till systems impact aggregate stability. At the Glenlea rotation the objectives were to see if manure additions and including perennials in an organic annual grain rotation increased aggregate stability, and to compare these organic rotations to conventionally managed controls and long-term grassland plots. The second study at Glenlea aimed to determine if four years of alfalfa (*Medicago sativa L.)* inserted into an organic annual grain rotation for the purpose of system rehabilitation improved aggregate stability. At the Indian Head rotation the objective was to see if a self-regenerating black medic cover crop increased aggregate stability at two different nitrogen fertilizer rates in a no-till system. The hypotheses were that including perennials in rotation, adding manure, four consecutive years of alfalfa, and cover crops would increase aggregate stability at the two sites. This would be due to greater organic matter input to the soil, longer periods of soil cover, a greater proportion of the year with roots in the ground, changes in the soil biotic community, and in the case of the perennials, reduced disturbance.

2. LITERATURE REVIEW

2.1 Introduction

The first part of this review looks at how aggregates are formed, focusing on biotic factors, as these particularly are manipulated by organic cropping practices. The second section is a discussion of these cropping practices and a review of the literature on their impacts on aggregate stability (AS) and other measures of soil structure. The last section reviews long-term experiments.

2.2 Functions of Aggregates

Soil aggregates and the pores between them are an important component of soil structure. The pores between aggregates and within aggregates facilitate aeration, water infiltration and water holding capacity in the soil as well as plant rooting ability. Having good aggregation with stable aggregates can reduce compaction and, at the soil surface, stable aggregates can reduce erosion and surface crusting.

Aggregates and pores of different sizes provide important habitat for soil organisms, can influence the microbial community, and may in fact increase soil microbe diversity through the 'incubation' effect of isolation inside aggregates (Rillig et al. 2017). Aggregates are also sites of carbon storage in the soil. Aggregates contain organic materials from plants and soil organisms and can prevent or slow the decomposition of these materials (Kögel-Knabner 2017). Organic materials can be chemically stabilized, tightly bound to inorganic compounds on aggregates, so that they are difficult to remove, or they can be surrounded by inorganic materials that act as a physical barrier and restrict access to them (Jastrow and Miller 1998). In these ways aggregates can slow decomposition and increase the residence time of organic carbon in the soil.

2.3 Aggregate Formation

2.3.1 General

In most soils the main binding agents of aggregates are organic materials. An exception to this is oxisols, where the main binding agents are oxides, not organic compounds (Tisdall and Oades 1982), and these oxide-bound aggregates have different properties (Oades and Waters 1991). The present review focusses on soils where the dominant binding agents are organic materials. In these soils, aggregates are formed when inorganic soil particles bind to organic materials. A fragment of plant matter or particulate organic matter may become coated in mucilage as it is decomposed by microbes, and become encrusted with inorganic materials such as clay particles and minerals that will bind to it (Oades 1984; Six et al. 2004). This mineral encrusted fragment would become the core of a microaggregate (Six et al. 2004).

Tisdall and Oades (1982) developed a theory of aggregate hierarchy, based on their observations that water stable macroaggregates of a couple millimeters in size were not randomly organized but made up of many smaller and denser aggregates. They proposed a model with four stages of aggregation, starting with the formation of stable particles (0.2 - 2 µm) and ending with aggregates >2mm, each having different properties and binding agents. The smallest size group of aggregates would bind together to form aggregates of the next larger size class, and so on. Oades and Waters (1991) found evidence for hierarchy in aggregate formation in a study where macroaggregates (>250µm) sequentially broke down into microaggregates (20-250µm) and then into particles <20µm. This was based on the idea that the microaggregates formed first, and then combined to form the macroaggregates. Oades (1984) proposed that macroaggregates would be formed first by roots and hyphae and then the microaggregates would form inside the macroaggregates as the labile organic materials inside the macroaggregates bound together and became stabilized with clay particles and microbial materials. This lead to the idea that microaggregates could be stabilized within macroaggregates (Oades 1984; Elliott et al. 1988). Angers et al. (1997) traced $^{13}C^{15}N$ labelled wheat (*Triticum aestivum* L.) straw residue as it decomposed in soil. They found that initially there was a large amount of the labelled material in the stable macroaggregate fraction (> 0.25μm), though it decreased quickly, and that more of the labelled material eventually ended up in the 50-250μm aggregate fraction, which supported the hypothesis of microaggregate formation within macroaggregates. It is likely that depending on the soil and management practices, over time, aggregates of all size classes are being created and destroyed, and that microaggregates are being formed within macroaggregates as well as combining to form other macroaggregates (Jastrow et al. 1998).

Different sizes of aggregates are held together by different types of binding agents which have been categorized based on longevity of the binding agents in the soil and types of compounds involved (Tisdall and Oades 1982). In general, macroaggregates are considered to be aggregates with a diameter >250μm and microaggregates <250μm. Persistent binding agents are recalcitrant organic compounds, such as humic substances that bind microaggregates. Transient binding agents include microbial and plant polysaccharides which serve to bind macroaggregates. Temporary binding agents include roots and hyphae and also bind macroaggregates (Tisdall and Oades 1982). They are temporary because they will start to decay when the roots or hyphae die, and thus their capacity as binding agents is quite brief (Tisdall and Oades 1982).

As the different binding agents suggest, micro and macroaggregates are associated with different forms of organic matter (OM). Elliott (1986) found that macroaggregates contained more labile OM and total OM than microaggregates. Macroaggregates were also found to have a higher C:N ratio, and more N, P, and C than microaggregates (Elliott 1986). Microaggregates are considered to contain older, more recalcitrant OM (Elliott 1986). Zhang et al. (2013) found that microaggregates had more soil organic carbon and microbial biomass carbon than macroaggregates.

Aggregates are dynamic, they are continually forming and disaggregating over different periods of time. Rillig et al. (2017) described the cycle of aggregation as consisting of formation, stabilization, and disintegration. Aggregates have different residence times in the soil, with different rates of turnover (Six et al. 2004). Macroaggregates have shorter residence times in the soil than microaggregates, with an estimated average of 27 days and potentially as few as five days (Six et al. 2004). Microaggregates are very stable and resistant to disturbance including agricultural management practices (Oades 1984). Macroaggregates however can be very responsive to agricultural management practices. Additions of organic materials can increase the number of macroaggregates (Oades 1984) while fallow periods can reduce them (Tisdall and Oades 1982). Macroaggregates have larger pores which can act as planes of weakness that can result in the aggregates crumbling when they are disturbed (Oades and Waters 1991), for example by tillage. Increased aggregate stability can lead to lower turnover rates of aggregates, which can also slow turnover rates of OM in the aggregates (Pulleman et al. 2005). Thus aggregate stability and formation is important in soil carbon cycling and sequestration.

Many factors affect the formation, stabilization, and maintenance of aggregates. Very important among these are soil organisms, including plants, soil macrofauna, fungi and bacteria. These organisms have many important effects on soil structure as do the organic compounds derived from them. A discussion of how these organisms affect aggregation follows.

2.3.2 Roots and Hyphae

Roots and fungal hyphae are important in aggregate formation and macroaggregate stability (Tisdall and Oades 1982; Jastrow, Miller and Lussenhop 1998). In a field restored prairie site, using wet-sieving, Jastrow et al. (1998) found a strong effect of roots and hyphae on water-stable macroaggregates, and attributed this to both direct and indirect effects. Roots and hyphae physically entangle soil particles, which makes them very important in the formation of macroaggregates. Roots also affect soil structure via pore formation (Oades 1993) and indirectly through their effects on soil moisture (Oades 1993), while both roots and hyphae indirectly contribute to the formation and stability of aggregates via exudates and products released during their degradation (Six et al. 2004).

Roots and hyphae form macroaggregates through physical entanglement of soil particles (Oades 1993). The role of hyphae has been described as the 'sticky string bag' effect, where mucilage on hyphae interacts with particles of silt and clay (Oades and Waters 1991).The living roots and hyphae entangling the soil are protected from decomposition by being encrusted with inorganic soil particles (Oades and Waters 1991). When the roots and hyphae die, they fragment, allowing access to microbes that will decompose them. Eventually they will not be long enough to hold aggregates together, and their stabilizing effects on aggregates will decline as the roots/hyphae decompose (Oades and Waters 1991). Thus the stabilizing effect of roots and hyphae is temporary (based on their lifetime in the soil), and relies on their presence every year or the proportion of macroaggregates will be reduced (Oades 1984).

Roots exert a strong effect on soil structure through the creation of biopores. Roots create large biopores, which can be very long (Oades 1993). Roots preferentially grow through existing pores rather than through aggregates (Oades 1993).

Living roots affect soil moisture by absorbing water from the soil. The resultant drying of the soil can affect aggregation, particularly in clay and loam soils (Oades 1993). Different types of root systems have different drying effects on the soil. Monocots stabilize aggregates better than dicots, and the fibrous root systems of grasses particularly form granular, well aggregated soils (Oades 1993). Rillig et al. (2002) studied the influence of plant species on aggregation and found that after two years the soils under monocultures of five different plant species had different proportions of water-stable aggregates (1-2mm), with greater aggregation found in soils with grass species. Greater proportions of water-stable aggregates were positively correlated with root length and AMF hyphal length (Rillig et al. 2002). Different crop plants, i.e., peas (*Pisum sativum* L.), ryegrass (*Lolium rigidum* Gaud.*),* and wheat, can also have different effects on aggregate size distribution (Materechera et al. 1992). In some cases, the presence of roots can decrease the proportion of large macroaggregates and unstable macroaggregates while still increasing aggregate stability overall (Materechera et al. 1992; Denef et al. 2002).

In a mesocosm study, Kohler et al. (2017) found an effect of mycorrhizal species on aggregate stabilization and formation. They also found that hyphae could form and stabilize aggregates in soil from which roots had been excluded, that outside the rhizosphere was where most of the aggregation from hyphae occurred, and that the main mechanism for this was hyphal entanglement (Kohler et al. 2017).

Blankinship et al. (2016) compared the effects of plant roots, microbes and moisture level on aggregate formation. In the field, plant removal resulted in reduced stable aggregate size. In both dry and irrigated plots, large macroaggregates decreased by 13.1% and 23.1 % respectively. In a lab incubation experiment under sterile conditions, water-stable macroaggregates increased with moisture levels after one week and after six months, and this was independent of carbon inputs from the plants. The proportion of water-stable macroaggregates was highest at intermediate moisture levels in the live soil treatment. At intermediate moisture levels, the live soil had greater macroaggregation than the sterilized one, whereas at high moisture levels the sterile soil had a greater proportion of macroaggregates and at low moisture levels they were similar to each other (Blankinship et al. 2016).

Roots are also very important in aggregation in that they are a key source of organic materials for the soil ecosystem, both via rhizodeposition and decomposition of dead roots. The roots contribute directly by producing exudates and mucilages which are involved in aggregation and also indirectly by being consumed by organisms that will produce other organic binding materials (Kögel-Knabner 2017).

2.3.3 Microbes: Chemical Mechanisms of Aggregation

Chemical mechanisms of aggregation involve organic compounds that originate from plants and microbes binding with inorganic materials, particularly clay minerals. Positively charged organic molecules can act as cation bridges, bridging between negatively charged clay particles (Six et al. 2004). Microbes contribute to aggregation by transforming and decomposing plant and other residues into organic binding substances. These can be both parts of the decomposing microbes (residue) or substances they excrete.

2.3.3.1 Origin of Soil Organic Matter

Organic compounds that form and stabilize aggregates are part of the larger pool of soil organic matter (SOM). Initially, OM in the soil comes mostly from plants, either above ground biomass, root biomass, or root exudates/rhizodeposition (Kögel-Knabner 2017). Rhizodeposition materials contain more readily decomposable compounds than plant tissues, which contain more recalcitrant structural material. This affects decomposition as different types of microbes are likely to consume these different materials (Kögel-Knabner 2017). There has recently been a shift from considering most SOM to be plant residues to understanding the importance and prevalence of microbe-processed contributions to SOM and that most SOM has been microbe processed (Cotrufo et al. 2015; Kallenbach et al. 2016; Kögel-Knabner 2017). Kallenbach et al. (2016) studied microbes and SOM formation and found that the SOM produced by microbes was chemically diverse and stable and that microbes contributed to the organic compounds and aggregate formation in incubated soil systems even with only glucose as an input (no other plant derived compounds). The initial soils in the incubation study had no carbon or microbes. Microbes and simple substrates, mono and dimeric sugars, were added, to see if chemically diverse stable SOM would be formed by microbes with just simple sugars, in the absence of any other more complex plant inputs. More recalcitrant and complex plant derived compounds were added to control treatments (Kallenbach et al. 2016). Pictures of a mixture of kaolinite and sand, initially, and after inoculation and weekly glucose additions for 15 months, showed dramatic changes in soil structure and colour (Figure 1 A in Kallenbach et al. 2016). After 15 months the soil appeared well aggregated, very different than the initial structure. These visually observed changes in the soil were accompanied by increases in microbe-derived proteins and lipids and decreases in plant derived carbohydrates. The organic compounds in the soil after incubation with only simple sugars were similar in chemical diversity to those in the soil incubated with more complex plant compounds and became similar to the chemical diversity of the soil incubated with a mixture of plant compounds. In treatments with more recalcitrant and diverse plant compounds, the amount of lignin declined to 1% while microbial proteins and lipids increased, bringing into question the idea of recalcitrant plant compounds persisting in the soil.

Overall, the conclusion was that SOM consists of diverse chemical compounds of microbial as well as plant origins and that these microbial residues can be formed from simple sugars (Kallenbach et al. 2016).

The chemical composition and origin of organic materials is not the only important factor in SOM quality, as it is also affected by decomposition. The rate at which organic matter decomposes in the soil is not just related to its inherent chemical properties (molecular structure), but is also affected by environmental and biological conditions (Schmidt et al. 2011). Schmidt et al. (2011) found that some plant compounds that are considered inherently recalcitrant, such as lignin, had a shorter residence time in the soil than the bulk SOM and that other compounds (of both microbial and plant origins) such as proteins and saccharides had a longer residence time in the soil. Cotrufo et al. (2015) also found that labile organic compounds (often processed by bacteria) could become stable and have long residence times in the soil, as part of the stable SOM.

2.3.3.2 Microbes and Aggregates

Aggregates are partly held together by microbial derived components of SOM (Kallenbach et al. 2016). SOM from microbes consists of residue (dead microbe fragments) and exudates, which include enzymes, exopolysaccharides, lipids, and glycoproteins (Kögel-Knabner 2017). Additions of residue or other organic materials to the soil leads to increased aggregation by stimulating soil microbes, which decompose the residue and create compounds that increase aggregation such as bacterial and fungal extracellular polysaccharides (Tang et al. 2011). Some of the labile organic carbon that is produced by microorganisms and roots can act as bonding compounds which can form macroaggregates by bonding soil particles together (Degens, 1997).

Microaggregates can be stabilized by bacteria, polysaccharides, organic residues, and inorganic materials (Tisdall 1994). Both microbial biomass and microbial biomass carbon have been correlated with aggregation in soils (Degens 1997). Carbohydrate C has been found particularly to increase aggregation, specifically between clay particles, and particularly that of microbial rather plant origin (Degens 1997).

Sarker et al. (2018) compared the effects of ten different types of organic inputs on wet aggregate stability to test the importance of the initial molecular composition of the organic materials. More readily decomposable organic matter additions (alfalfa litter, meat powder) caused the highest initial increase in aggregate stability, but did not persist at that level. The more cellulose-rich OM additions caused a slightly lower but more persistent increase in aggregate stability. The aggregate stability of the compost treatment was lower, but was persistent and continued to increase throughout the 300 day duration of the study. Biochar had the least effect, which was very minimal. The initial rapid response to labile OM additions was attributed to bacteria consuming these and releasing extracellular polysaccharides which increased aggregation. There was not a significant correlation between N or C:N of the OM inputs and water stable aggregation over the three different soils tested and the 300 days of the experiment (Sarker et al. 2018).

Tang et al. (2011) compared the effects of bacteria and fungi on aggregate stability. They incubated soils with and without maize residue, fungicide and bactericide for 40 days. Much more macroaggregation occurred with residue than non-residue treatments, and fungicide and bactericide both reduced macroaggregate stability. The fungicide decreased aggregate stability to a greater degree than did the bactericide, emphasizing the importance of fungi. The treatment with bactericide had more fungal biomass and lower macroaggregation than the control without bactericide, suggesting that bacteria were also involved in aggregate stability. Their results suggested that both bacteria and fungi contribute to aggregate stability and that plant residue is important for macroaggregate formation (Tang et al. 2011).

Soil texture can affect which organisms will be involved in aggregation. Bacteria and fungi (microbes) are more important in stabilizing aggregates in fine textured soils than in sands, through production of organic materials which are involved in stabilizing microaggregates (Six et al. 2004). Bacteria are in general more associated with the formation of microaggregates (Tisdall 1994), through their exudates and biomass residue. Zhang et al. (2013) found that microbial diversity within aggregates increased as aggregate size decreased in a clay loam soil. In an experiment comparing the effects of microbial communities from unaltered grasslands and those that had experienced anthropogenic disturbance, Duchicela et al. (2012) found that after soils from disturbed sites had been inoculated with soil biota from the unaltered sites the aggregate stability of the disturbed soils increased by 9.7%.

2.3.3.3 Glomalin

Discussions of the mechanisms by which arbuscular mycorrhizal fungi (AMF) contribute to aggregate stability often mention a substance called glomalin, which was discovered by Wright et al. (1996). An immunofluorescent material on the surface of AMF hyphae, spores and roots was detected and extracted from hyphae by heating in citrate for 90 minutes. Because of the harsh conditions required to extract glomalin and its persistence and abundance in the soil, it was suggested that it functioned as a soil glue that could be very important for aggregation in soils and was described as a glycoprotein produced by AMF hyphae (Wright and Upadhyaya 1998). Glomalin content of the soil has been correlated with aggregate stability and percent carbon

(Wright and Upadhyaya 1998). Glomalin and aggregate stability have also been studied in soils under different crop rotations, where glomalin was correlated with aggregate stability across all treatments, and both glomalin and aggregate stability were highest under perennial grass (Wright and Anderson 2000). Though glomalin has been correlated to aggregate stability many times, the specific mechanism for this has not been proven, largely because little is known about the protein glomalin (Purin and Rillig 2007).

Due to questions around the nature of the operationally defined extract glomalin, the extract has been referred to as glomalin related soil protein (GRSP). Rillig (2004) made a distinction between glomalin and GRSP. He described GRSP as pools of soil carbon that are operationally defined, as distinct from glomalin which is a protein of AMF origin that may or may not be related to the GRSP extract. There is evidence that the mycorrhizal protein glomalin exists, but it is not strongly proven that GRSP is related to/contains glomalin (Rillig 2004). A putative gene for the glomalin protein has been sequenced and identified (Purin and Rillig 2007).

GRSP has not been fully chemically characterized, but has been found to be composed of a diverse mixture of substances, including many proteins and other substances, such as phenolic compounds, fatty acids and waxes (Gillespie et al. 2011). It does not contain as much glycosylated material as would be expected if it was mostly glycoprotein, and contains multiple kinds of glycoprotein (Gillespie et al. 2011). In addition, none of the proteins identified through BLAST were of mycorrhizal origin, although many proteins remained unidentified (Gillespie et al. 2011). In their conclusion, Gillespie et al. (2011) did not question the existence of glomalin but rather the extraction process. Others had previously posited that GRSP extract contained non-AMF material, including non-AMF proteins, and humic materials (Purin and Rillig 2007).

Despite the uncertainties around GRSP, it is still useful as a measurable fraction of persistent soil OM that is correlated with aggregate stability (Rotter et al. 2017), and many papers continue to be published using it in relation to soil quality.

2.4 Ecological Cropping Practices

2.4.1 Organic Agriculture and Aggregate Stability

Organic management can change soil quality as compared to conventional management through several mechanisms. These include absence of chemical fertilizers and pesticides on organic farms, and possible additions such as manure or compost. Some organic systems may have more tillage than conventional ones, and their fertility may be greater or lesser, with consequent impacts on crop biomass and yield. Also, organic farms are more likely to have green manures or forage phases and to utilize forage legumes and cover crops in their crop rotations (Nelson et al. 2010), which are often longer and more complex than rotations on conventional farms. All of these practices can affect soil health, though the effects can be variable as management practices and soil and climatic conditions differ between farms. Despite this variability, studies have found organic farms over-all to provide greater environmental sustainability and social wellbeing as well as good profitability even with lower yields in many crops than conventional farms (Reganold and Wachter 2016). Farms using regenerative agriculture techniques, which are similar to organic practices (cover crops, no insecticide or other pesticide use, grazing and no-till), can be more profitable despite lower yields than conventional farms, while decreasing pest pressures (LaCanne and Lundgren 2018).

When studies compare organic and conventional systems impacts on soil structure/health they often focus on the effects of specific organic interventions such as manure additions or green manures. Because of this, the present review is divided into sections based on specific management practices. Though it is important to focus on the specific mechanisms/interventions, some studies that focus on overall differences between organic and conventional cropping systems will be discussed briefly first.

A study of soils from hundreds of organic and conventional farms from across the US found higher mean total percent SOM, fulvic acid, humic acid and percent humification in organic than conventional farms (Ghabbour et al. 2017). There was a large range in percent SOM in both systems, and they considered the higher percent humification in organic farms to suggest greater sequestration of carbon by the soils (Ghabbour et al. 2017). At the Glenlea Long-term rotation, organic management without or with minimal inputs lowered levels of soil carbon and available P in the soil compared to conventional systems (Welsh et al. 2009; Bell et al. 2012). Though organic management, even where no manure had been added for 19 years, resulted in higher levels of microbial biomass P (Braman et al. 2016). This suggests that soil biology differences between organic and conventional production may play an important role in nutrient supply, in this case with P. Systems with organic fertilizer inputs and long rotations can also have more efficient nitrogen cycling, with less N loss compared to a conventional continuous grain system (Ross et al. 2008).

Differences in abundance and diversity of AMF between organic and conventional farms is perhaps one mechanism to explain differences in aggregate stability in soils, as AMF have an important role in aggregate formation (Tisdall 1994). At Glenlea, arbuscule colonization and spore abundance of AMF were greater in the organic than conventional systems (Entz et al. 2004). In eleven sites across the UK, Gosling et al. (2010) found both spore numbers and percent root colonization were higher overall in organically managed soils than conventionally managed soils. Both organic and conventional fields were tilled. Manoharan et al. (2017) found AMF diversity to be greater in organic than conventional systems, but the highest in grasslands. Application of phosphorous fertilizer can decrease phylotype diversity (Sheng et al. 2013) while nitrogen fertilizer can have an effect on the AMF community composition (Avio et al. 2013).

2.4.2 Perennial Forages and AS

2.4.2.1 Perennials in Rotation

Crop rotation is an important management practice in Manitoba. It is used particularly for pest and weed management, to improve soil structure and nutrient cycling, to reduce erosion, and increase crop yields. Well-designed crop rotations are particularly important in organic agriculture, where they are a key strategy for pest control and nutrient management. Organic crop rotations often include green manure or pasture phases, particularly with legumes to increase nitrogen fertility. Adding perennial forages into simplified annual grain rotations can have many benefits, both agronomic and ecological, including increased yields in subsequent crops, weed control, biological nitrogen fixation, improve soil quality, reduced nitrate leaching, increased soil carbon sequestration, and provision of wildlife habitat (Olmstead and Brummer 2008).

Including perennial forages in a rotation allows for increased root production, living plant cover for a greater portion of the year, and reduced tillage during the perennial phase, all of which can impact soil health and particularly aggregate stability. A key way that perennials increase AS is through increasing soil organic carbon. King and Blesh (2018), in a meta-analysis, found that rotations with perennial components increased SOC (6.2%) compared to grain only rotations. Whereas interventions that did not lead to higher total C input into the system such as increasing diversity of annuals in grain only rotations did not affect SOC (King and Blesh 2018). Previous studies at the Glenlea Long-term Rotation showed that the organic perennial system (two years of alfalfa and two years of grain) did not add more C to the soil than the annual rotation despite the deep rooted alfalfa (Bell et al. 2012), and depleted plant available P more than the organic annual rotation (Welsh et al. 2009). This could be in part due to the lack of external nutrient input to these systems, harvest and removal of the alfalfa, and the very short term alfalfa, only about 18 months. Despite this, the perennial rotations at Glenlea had on average higher microbial biomass carbon (MBC) and microbial biomass phosphorus (MBP) than annual rotations (Braman et al. 2016). Additionally, unlike the annual and conventional treatments, the organic perennial treatments were similar in MBC, soil respiration and qCO2 (microbial metabolic quotient) to long-term grasslands (Braman et al. 2016).

Perennials such as alfalfa and grass-legume mixes as components of annual rotations have been found to increase AS. Including two years of alfalfa as a green manure in an organically managed four year rotation in Nebraska had the greatest effect on AS at 15-30 cm, where it increased the mean weight diameter (MWD) of water stable aggregates by 55 to 100% compared to organic and conventional annual rotations (Williams et al. 2017). It also led to 30% higher AS in the top 15cm of soil compared to a conventionally farmed control, although diversifying the conventional rotation by adding wheat increased MWD more. From 30-100 cm there was no difference between treatments. The green manure had no effect on dry AS or bulk density, although Proctor bulk density, which is a measurement of soil compactibility, was lower with green manure than the conventional two year rotation (Williams et al. 2017). Loaiza et al. (2018) in Switzerland found that a two year ley period of grass and clover following a four year annual crop rotation increased the proportion of large aggregates in intensively tilled systems, both organic and conventional, but did not increase MWD.

Romano et al. (2017) studied best practices in organic production systems in a long term study at a certified organic farm in West Virginia and found that adding three years of sod (orchard grass-red clover grazed with sheep) in rotation following four years of grain increased wet AS (as geometric weight diameter, GMD) in the first but not the fourth crop after sod. Having the sod in rotation also increased dry AS (GMD) and OM, and decreased bulk density in the first year after sod but not the fourth year (Romano et al. 2017). Throughout the three years of the sod period, wet AS (GMD) increased as did dry AS (GMD), SOM, and bulk density (Pena-Yewtukhiw et al. 2018). The increases tended to be in the first two years after sod, and increases in AS were attributed to both increasing organic matter and lack of tillage during the sod part of the otherwise tilled rotation. Interestingly, in terms of length of sod intervention, aggregation was improved after only two years, with not much difference seen in the third year (Pena-Yewtukhiw et al. 2018).

2.4.2.2 Rehabilitating Soil with Several Years of Perennials/Pasture

Instead of being a regular part of a rotation, pasture also has been used for soil remediation, where it can be grown for several years, but as a one-time event. It can have positive effects in addition to adding OM and improving soil structure. Entz et al. (2001) observed that a six year alfalfa stand effectively removed deep-leached N from subsoil in Manitoba. Hoyt (1990) looked at the long term effects of two to six years of hay crops on wheat yield (no N fertilizer added), and found that wheat yield was higher after alfalfa (66-114%) and alfalfa-bromegrass (*Bromus inermis* Leyss) mixture than control plots (wheat – fallow) for the first nine crops after the hay crops had been ploughed in. Yields of wheat after alfalfa or alfalfa-bromegrass mixture were higher than after bromegrass alone. The number of years a forage crop had been grown for did not affect wheat yield. Since nitrogen fertilizer was not added it makes sense that much of the yield increase was due to N fixation by the alfalfa, but it is interesting that yields still increased by an average of 12% over the nine years after bromegrass, so likely OM additions and other physical properties were playing a role as well (Hoyt 1990).

Alfalfa is used as a perennial to rehabilitate soils, but its effectiveness may depend on the number of years it is grown and initial soil conditions. Guidi et al. (2017) looked at the effect of one year of alfalfa to improve degraded soils in a ploughed organic three year rotation in Italy. Some aggregate properties were improved after one year of alfalfa in the most degraded soils (higher mass of 1-2mm aggregates, MBC and total OC) but not in the initially less degraded soils, where AS decreased after one year of alfalfa (Guidi et al. 2017). In soils that had been intensively tilled for many years, Angers (1992) found that a five year period of alfalfa increased the MWD of water stable aggregates while five years of silage corn and fallow did not affect MWD. Much of the increase in MWD was due to more >2mm aggregates and fewer 0.25-1mm diameter aggregates under alfalfa. SOC also increased in soils under alfalfa and decreased in those under corn and fallow (Angers, 1992). In the Loess Plateau in China, Guo et al. (2010) converted cropland that had been in long-term wheat production to perennial pasture (alfalfa, sweet clover [*Melilotus officianalis* L.], and natural regeneration) to reduce soil erosion. After five years, wet aggregate stability was higher and bulk density lower in all treatments, and, similar to Angers (1992), there were more macroaggregates and fewer small aggregates. Each class size of aggregates also had higher SOC after the five years of perennials, and the amount of water stable aggregates positively correlated with SOC (Guo et al. 2010).

2.4.2.3 Long-term Grassland/Pasture Compared to Arable Systems

Long-term grasslands or perennial pastures can improve soil properties and serve as benchmarks with which to compare arable systems. They have been particularly important in studying soil carbon dynamics (Janzen 2005). Grace et al. (1995) in a long-term study in Australia found that while increased frequency of pasture phases in rotation led to higher SOC, permanent pasture had the highest SOC. Frequency of fallow decreased SOC, and SOC decreased over the 68 years of the study to some degree in all treatments. At Glenlea, MB, in heavy clay soils, 18 year old restored grasslands had higher SOC than cropped sites in the top 120cm of soil, while at another site, on a sandy loam soil, a nine year restored grassland site did not have more SOC compared to cropped sites (Bell et al. 2012). One mechanism by which grasslands can increase SOC is by increasing root production. Acharya et al. (2012) found higher root biomass in 17 year grasslands compared to one to four year grass phases, which had higher root biomass than annual crops with no grass phase. They also found that CO_2 emissions did not increase with age of grasslands (1-4 years), even as underground biomass increased, suggesting that they were retaining more carbon (Acharya et al. 2012). This is supported by Kong and Six (2010) and Rasse et al. (2005) who observed that root derived carbon persisted longer in the soil than shoot derived carbon.

Soils under long term pasture often have improved soil structural properties. Wright and Anderson (2000) found that soils that had been undisturbed under crested wheatgrass (*Agropyron cristatum* (L.) Gaertn.) for 30 years had higher AS then those under several two to four year annual grain rotations. In Alberta, soil structural properties including porosity and fractal aggregation were generally higher in native grasslands than planted pastures and lowest in annual croplands, although there were sites that did not follow this pattern (Hebb et al. 2017). A 12 year old pasture in New Zealand had higher MWD down to 25cm and more larger aggregates

(8-2mm) than long term fallow or tilled crop treatments (Vezzani et al. 2018). The pasture also had more SOC and total N in all aggregate class sizes down to 15cm than other treatments, as well as higher microbial biomass and metabolic diversity (Vezzani et al. 2018).

Pasture does not always have higher AS than arable systems. In treatments that had been in place for 70 years in the Netherlands, organic tilled 5-6 year rotations (potatoes, sugarbeet, wheat, 2-3 years of grass) had similar AS to grazed fertilized pasture in the top 10cm, while the organic system had higher AS than pasture at 10-20cm depth (Pulleman et al. 2003). Both had higher AS than the conventional rotation. The high AS in the organic rotation was partly explained by compaction in the heavily tilled arable soils. The organically-managed soils contained many large clods which survived wet sieving and contributed to the high MWD, but these clods were low in SOM compared to the large water stable aggregates from the pasture. The pasture had higher SOM than the other two systems in the top 10cm, but was similar to organic at 10-20cm, while both were higher than conventional (Pulleman et al. 2003).

2.4.2.4 Arbuscular Mycorrhizal Fungi

Effects of crop rotations on AMF are varied, largely depending on the specific crops. AMF colonization and spore numbers were not significantly different between the annual and perennial rotations at Glenlea (Welsh 2007).

Alfalfa forms associations with arbuscular mycorrhizal fungi, and this can influence its effect on soil structure, especially since AMF hyphae are involved in forming particularly macroaggregates. Teng et al. (2010) found that alfalfa's remediation effect in removing PCBs from contaminated agricultural soil was increased when inoculated with AMF (and/or rhizobium). In a study using plants from the tall grass prairie, later-succession plants were found to have a stronger response to AMF than early succession plants (Koziol and Bever 2015). Alfalfa, being a perennial, and maintained for four years, will likely function as a later succession plant and may benefit from this enhanced response to AMF more than an annual crop species would, potentially leaving more inoculum in the soil for the next crop in the rotation.

2.4.3 Cover Crops and AS

Cover crops function to have plants growing in the field during the off-season, when there is no crop being grown. Cover crops can reduce soil erosion, retain nutrients, increase biodiversity, reduce weed competition, and increase crop productivity (Schipanski et al. 2014). They can also act as a green manure, fixing nitrogen if they contain legumes and adding organic matter to build the soil, thereby improving soil structure. There is on average sufficient thermal time and moisture in south-central Manitoba to support the growth of a legume cover crop after winter wheat, and enough heat on average in southwest Manitoba and southern SK, though moisture is low (Thiessen Martens and Entz 2000).

There are many mechanisms by which cover crops increase aggregation including providing soil cover and contributing living roots to the soil (Rasse et al. 2005). The roots also influence rhizosphere processes and soil organisms (particularly bacteria and fungi) which create organic compounds that act as binding agents and form and stabilize aggregates (Lal 2015). Whether or not the cover crop associates with AMF is also relevant, since AMF hyphae have an important role in aggregation (Lal 2005). Kabir and Koide (2000) also found a positive correlation between AMF hyphae and water stable aggregation.

An important way in which cover crops increase AS is through increasing SOC. A meta-analysis showed cover crops and perennials increased SOC levels compared to grain only controls (King and Blesh 2018). SOC concentrations were 12.5% higher with cover crops compared to grain only rotations. The cover crops increased both total C and root C into the system. By comparison, there was no effect of higher species diversity alone in a grain only rotation, and including legumes in a previously cereal only grain rotation decreased SOC. Blanco-Canqui et al. (2011) found that SOC increased in the top 7.5 cm of soil with cover crops after 15 years of no-till management. SOC was also increased by winter and spring triticale and spring lentil cover crops, but not winter lentil and spring pea cover crops, relative to fallow at 0-7.5cm but not at 7.5-15cm. Continuous winter wheat had similar or higher SOC than the cover crops (Blanco-Canqui et al. 2013). Sainju et al. (2003) found that soils under a rye cover crop had larger C pools (organic C, PCM, MBC) than a legume cover crop.

Cover crops can also reduce bulk density (Blanco-Canqui et al. 2011) or have no effect on bulk density (Blanco-Canqui et al. 2013), including no effect on bulk density after 35 years of cover crops (Nouri et al. 2019). Nunes et al. (2018) found that cover crops increased organic matter, and there was more benefit of cover crops in no-till. Cover crops can increase water filtration (Blanco-Canqui et al. 2011), reduce soil erosion (Blanco-Canqui et al. 2013) and improve soil hydraulic properties and other physical properties (Nouri et al. 2019).

Blanco-Canqui et al. (2011) looked at the effects of cover crops (hairy vetch [*Vicia villosa* Roth], then sunn hemp [*Crotalaria juncea* L.] and late-maturing soybean [*Glycine max* L.]) after 15 years in a no-till winter wheat-sorghum system in Kansas and found that MWD of aggregates was 80% higher with cover crops relative to no cover crop at 0-7.5cm. At 7.5-15cm only soybean cover crops had higher MWD than no cover. They also looked at aggregate size classes individually, and found that in the 0-7.5cm depth there were more large aggregates under cover crops, and cover cropped soils had fewer of the smallest sized aggregates. At 7.5-15 cm soybean had more 2-4.75mm aggregates than no cover crop, otherwise they were all the same. Results of this study suggested that greater aggregation was due to higher SOC with cover crops (Blanco-Canqui et al. 2011).

Hermawan and Bomke (1997) found that after one winter season plots with fall rye and annual ryegrass cover crops had higher aggregate stability (MWD and more 2-6mm diameter aggregates) relative to bare plots (and those with a spring barley cover crop) in the top five cm of soil. Thus, the seasonal destruction of aggregates was reduced under cover crops in a high precipitation region. After spring tillage, an effect of the cover crops was still seen, with cover cropped plots having more 2-6mm and .25-2mm aggregates than the bare soil and the bare soil having more >6mm aggregates, or clods, which they considered a sign of poor soil structure/low AS. Again, increases in AS were associated with increases in soil organic carbon (Hermawan and Bomke 1997).

Kabir and Koide (2000) compared fall seeded cover crops of winter wheat and dandelion (chosen because it is highly mycorrhizal and perennial) to winter fallow plots over one winter. The cover crops were disked in spring and all plots seeded to maize. Fifty four days after maize emergence, the soils under both cover crops had a higher percentage of water stable aggregates than the winter fallow. Water stable aggregation was also positively correlated with mycorrhizal colonization. The authors attributed the increased AS to the increase in AMF as there was no difference in maize root length. It was interesting that the effect of the cover crop on water stable aggregation was still seen after spring tillage and 54 days of plant growth. On the other hand, Blanco-Canqui et al. (2013) did not find an effect of cover crops nine months after termination.

Blanco-Canqui et al. (2013) looked at several different spring and winter cover crop species (winter lentil and triticale, spring lentil, pea and triticale) to replace fallow in a winter wheat-fallow no-till system in Kansas. They also compared them to a continuous wheat system. After five years, wet AS (GMD) was increased after cover crops relative to fallow (0-7.5cm). Winter triticale had the greatest effect, then spring triticale, lentil and peas, whereas winter lentil did not have an effect, and continuous wheat was similar to cover crops and higher than fallow. Dry aggregate size distribution (GMD) was also higher with spring lentil and triticale than fallow, but highest in continuous wheat. In an irrigated, conventional corn and sunflower rotation in Spain, García-gonzález et al. (2018) studied the effect of vetch and barley winter cover crops in place of winter fallow on a number of soil characteristics including AS. Over 10 years, they found more large water stable aggregates with barley than the vetch cover crop or fallow. This was attributed to the larger root system of barley as compared to vetch, and is in agreement with Oades (1993) who reports that grasses stabilize aggregates better than dicots.

Adding cover crop residue to soils without a cover crop can also increase AS. In a pot study, Liu et al. (2005) added chopped root and shoot material from annual ryegrass, fall rye and double-dose fall rye cover crops to subsoil from fallow plots of tilled vegetable fields and incubated them for periods of 2, 4, and 8 weeks. In each incubation period, all treatments increased MWD and had a higher percentage of water stable 2-6 mm aggregates compared to control. The double dose of fall rye had the highest MWD of the treatments after 8 weeks and the highest percentage of 2-6mm water stable aggregates after 4 weeks. They suggested that a mechanism by which these plants increased aggregation was through addition of dilute acid extractable polysaccharides and, therefore, a chemical as opposed to a physical effect.

In some studies cover crops had no effect on AS. For example, Nunes et al. (2018) found that four years of grass-legume cover crops in no-till and tilled corn (*Zea mays* L.) monocultures improved many indicators of soil health but did not affect wet AS. Mendes et al. (1999) found no difference in dry aggregate size distribution after red clover and triticale cover crops compared to fallow in Oregon. Five years of hairy vetch, crimson clover, and rye winter cover crops were also found to not significantly differ from a fallow control in terms of dry MWD in Georgia (Sainju et al. 2003). Although they did find differences within certain class sizes of aggregates, for example there were more 2-0.85mm aggregates with rye than legume cover crops. They also found less total soil in smaller aggregate class sizes than larger aggregate class sizes. Nouri et al. (2019) looked at both wet and dry AS after 34 years of hairy vetch and winter wheat cover crops and NT and CT in cotton fields in Tennessee. Wet AS was similar between cover crops at both depths, but dry AS (GMD and MWD at 0-15cm soil depths) was higher in vetch and wheat than no cover. At 15-30cm soil depth, there was no difference in dry GMD and in dry MWD vetch was higher than wheat which was higher than no cover.

2.4.3.1 Self-Regenerating Cover Crop Systems

One of the barriers to adoption of cover crops is the expense of seeds and additional labour. A way to address this is to use self-regenerating cover crops. Self-regenerating annual medics and subterranean clovers have been used in Australia since the 1950s in legume-cereal ley systems, which alternate years of pasture legume and cereals (Puckridge and French 1983). The use of legume pastures, along with phosphorus fertilizer, increased fertility and improved soil aggregate stability, porosity and water retention and infiltration and increased cereal yields and livestock production (Puckridge and French 1983). Black medic has been utilized as a self-regenerating cover crop in the Canadian prairies. Black medic is a low growing leguminous weed that grows under the crop during the growing season, and remains after the crop is harvested. It produces large numbers of seeds in the fall and re-grows from the seed bank again early in the spring (Braul 2004). At Indian Head, SK, an experimental black medic cover crop increased grain yield relative to control under low nitrogen inputs (20% of recommended N) but had no effect with higher N rates (60% and 100%) (May and Entz 2016). Its success in low nitrogen environments suggests it may be useful in low-input organic agriculture. Lupwayi et al. (2016) found a positive effect of legume crops, both green manures and pulses, on the following crops in rotation for three subsequent years. The effect of the pulse crops was strongest on the crop following three years later (Lupwayi et al. 2016). Turmel et al. (2011) found no effect of the medic cover crop at Indian Head on percent colonization of flax (*Linum usitatissimum* L.) roots by AMF, though there was high AMF colonization over all, perhaps due to no-till and the use of mycorrhizal crops. The black medic cover crop did have an effect on soil bacteria however. Medic increased bacterial diversity and changed the bacteria community structure (Lupwayi et al. 2017).

2.4.4 Manure Additions and AS

The source of nutrients for crops is a key difference between organic and conventional agriculture. While conventional systems can rely on inorganic fertilizer, organic systems have traditionally used nutrient sources such as manure, compost, and green manures. Animal manure is a very important nutrient source in organic systems as it can contain all necessary nutrients, is often available, and can be used on large scales. Manure can affect soil properties both by adding organic matter to the soil directly and indirectly by increasing plant growth.

Many studies compared AS in organic and conventional systems with the main difference between the two systems being manure inputs in organic and inorganic fertilizer in conventional. In a South Dakota corn soybean rotation, Ozlu and Kumar (2018) found that cattle manure increased wet AS in the top 10cm of soil relative to inorganic fertilizer, but had no effect below 10cm (disked to 6cm). A 70 year long-term study in the Netherlands compared organic and conventional management in tilled 5-6 year rotations including 2-3 years of pasture (Pulleman et al. 2003). Higher wet MWD and percent water stable macroaggregation was found with organic management which coincided with the use of manure while the conventional system used inorganic fertilizer. The authors acknowledged that the study did not allow for determining whether effects seen were due to manure additions or lack of inorganic fertilizer and pesticides (Pulleman et al. 2003). Williams et al. (2017) looked at the effect of organic agriculture on soil physical properties in a 40 year study in Nebraska. They compared organic systems with manure or green manure to a conventional system. Manure increased MWD of water-stable aggregates in the top 15cm by 50% compared to conventional controls and the green manure treatment by 30%. The manure, which was incorporated to 10cm, only increased AS in the top layer; the treatments were all similar below 30cm. Romano et al. (2017) in a study at a certified organic farm in West Virginia found 2.25 Mg/ha composted manure applied every second year to a corn, soy, wheat, kale (*Brassica oleracea* L.)/cowpea (*Vigna unguiculata* (L.) Walp.) rotation that included cover crops increased wet AS (GWD) relative to organic plots with no manure.

Wortmann and Shapiro (2007) distinguished between different size classes of aggregates and found that composted and raw manure increased stable large macroaggregates (>2mm) and small macroaggregates (0.25-2mm) and decreased microaggregates (0.053-0.25mm) in the top 25mm of soil. Manure and compost affected the size distribution of aggregates, but the total amount of soil in aggregates remained the same. They also found no effect of three years of manure application (totalling 200 Mg/ha) on aggregate size distribution four years after the last application in a tilled soil.

Along with increasing aggregate stability, manure can decrease bulk density (Romano et al. 2017), increase SOM and SOC (Pulleman et al., 2003; Romano et al. 2017; Ozlu and Kumar, 2018) and increase water infiltration (Williams et al. 2017). Williams et al. (2017) found no effect of manure treatment on bulk density, but they found that it lowered Proctor bulk density, which specifically measures compactibility of the soil. In studies where wet AS was increased by manure, dry AS was also increased by manure (Pulleman et al. 2003) or not affected at all (Williams et al. 2017).

2.4.5 Tillage and AS

2.4.5.1 General

Tillage is an agricultural management practice that can strongly impact soil structure. Tillage can be done in a variety ways and intensities which can change its effects. No-till cropping is widely used in Canada, with 56.4% of land prepared for seeding in 2011 being no-till (Stats Canada 2011). No-till minimally disturbs the soil and retains residue on the surface, thus there is interest in how it affects aggregation. Tillage can disrupt aggregates directly. It also brings more soil to the surface where it is exposed directly to erosive forces such as rain drops (Tisdall and Oades 1982). There are also many indirect effects of tillage on soil aggregation through changes in organic matter and soil biota. Many studies have compared aggregate stability in tilled and no-till sites, including pastures and native grasslands.

Beare et al. (1994) measured wet aggregate stability in the 13th year of a long-term field study (sorghum-winter rye double crop). They found more stable macroaggregates in no-till (NT) soils than conventionally tilled soils (CT) (moldboard plough, disk, and rotary tilled) at 0-5cm depth and no difference at 5-15cm depth. They noted that residue inputs were similar for both NT and

CT. They suggested that the reason macroaggregate stability in CT was not reduced compared to NT at deeper depths could be because of more microbial activity at depth in CT due to residue incorporation, and the development of more stable aggregates at depth because they are not exposed to erosive forces on the surface (Beare et al. 1994). Helgason et al. (2010) found that aggregation was higher under no-till management in all size classes of aggregates except the 1-2mm size class. Du et al. (2015) found that after seven years, no-till treatments had more macroaggregate formation and soil organic carbon associated with both micro and macroaggregates compared to rotary till and mold-board plough treatments. Six et al. (1998) found that aggregate stability was much higher under NT than CT (moldboard plough, cultivator, and rotary rod-weeder), and much higher under native sod than NT. They suggested that macroaggregates would be formed at the same rate in no-till and tilled sites (by fresh residue added to the soil in both cases), but that the total number of macroaggregates would be lower in CT due to continuous aggregate disruption from tilling (Six et al. 1998). More specifically, Six et al. (1999) hypothesized that increased rates of macroaggregate turnover, due to tillage, cause a reduction in the formation of microaggregates because the microaggregates are forming within the macroaggregates. This also leads to less SOM being incorporated and stabilized in the microaggregates (Six et al. 1999).

2.4.5.2 Soil Biota

Tillage can alter the temperature and moisture conditions in the soil (Six et al. 1998). These changes in the abiotic conditions affect the microbial life and rates of decomposition of organic residues involved in aggregation (Beare et al. 1992; Six et al. 1998). Tillage can also directly disrupt soil organisms, notably mycorrhiza, by fragmenting their hyphae. It can affect soil organisms habitat, by, for example, disrupting the network of biopores in the soil in which larger soil organisms live and which supply them with air and water (Oades 1993).

Beare et al. (1992) looked at the effect of NT and CT on fungi (mostly saprotrophs) and found significantly higher hyphal lengths and densities in NT than CT. The fungal communities were significantly different in the two tillage systems, but the fungal species richness was not (Beare et al. 1992).

Zhang et al. (2013) compared soil organisms in large macroaggregates, small macroaggregates, and microaggregates, under different tillage conditions. They found that across all aggregate sizes, total microbial biomass as well as bacterial and AMF biomass and total microbial and nematode diversity were higher in NT and reduced tillage than in CT (moldboard ploughing to 20cm). Under NT, nematode abundance, microbial biomass C and soil moisture were also higher. They also measured SOC and found that reduced tillage had higher SOC than CT in all aggregate sizes, and that NT had higher SOC than reduced tillage only in microaggregates (Zhang et al. 2013).

Earthworms are affected by agricultural management practices. Their numbers tend to increase with less tillage and more residue inputs (Pulleman et al. 2005). Pulleman et al. (2005) compared pasture to organic and conventional systems (both tilled) and found that the system most conducive to earthworm activity, the pasture, had the most stable microaggregates. There was not a significant difference between the other two systems.

2.4.5.3 Soil Organic Matter and Residue Stratification

Tillage can lead to a reduction in OM in the soil, which can lead to a loss of aggregates (Tisdall and Oades 1982). The reduction in OM is partly from aggregates being directly broken apart, which makes the OM that was previously protected susceptible to decomposition (Oades and Tisdall 1982). CT (moldboard plough) and disruption of macroaggregates also lead to less SOM being incorporated and stabilized in microaggregates (Six et al. 1999). More OM accumulates under pasture, because of greater plant biomass and continuous plant cover, than in annual cropping systems; hence they tend to have more stable aggregates (Tisdall and Oades 1982). Annual systems, regardless of whether they are tilled or not, may have limited effectiveness in improving aggregation due to less root development than perennials (Eynard et al. 2004). Devine et al. (2014) and Eynard et al. (2004)'s results both support the idea that pasture land is superior to both CT and NT annual systems in terms of promoting aggregate stability.

Another change with no-till is the placement of the residue. In no-till, residue becomes stratified, in one layer at the surface rather than being distributed throughout the till layer. Devine et al. (2014) compared aggregate stability at sites that had been in CT (moldboard plough and disk) and NT for 30 years to a forest newly established on long-term pasture land in Georgia. They found that aggregate stability was higher in NT compared to CT only at the 0-5cm depth. The forest sites had higher aggregate stability than the NT soils from 5-28cm, and higher aggregate stability than the CT to a depth of 28cm. They also measured SOC and found that NT and forest soils both had more SOC compared to CT in the top 5 cm, whereas at deeper depths the forest soil had significantly more carbon than both NT and CT. It was interesting that after 30 years of NT, there was only a change in the top 5cm of soil compared to CT, due to difference in litter stratification (Devine et al. 2014).

In a South Dakota study, fields that had been under NT management for an average of 10 years had just 5% higher wet AS than CT (chisel plough and disk) in the top 5cm only (Eynard et al. 2004). There was a larger difference in dry AS (35%) but again only in the top 5cm. In all cases, the grass pastures had more stable aggregates than the annual systems. There was also a difference in structure types, with aggregates under grasslands having a finer granular structure and those under annual systems having some large blocky and platy structure. Again, organic matter stratification in NT meant that structure was usually only improved in the surface 5cm or so of soil (in the time scale of ten years) (Eynard et al. 2004).

Degree of stratification can be considered as an indicator of soil quality since OM on the surface performs important functions such as reducing erosion (Franzluebbers 2002). Litter decomposes more slowly on the surface and is affected by climate (Six et al. 1999; Franzluebbers 2002). A study looking at SOC found that at warmer sites in the southern US, NT sites had more SOC in the surface soil and CT (disking or conventional shallow tillage) sites had more SOC below the surface down to the plow layer (Franzluebbers 2002). At colder and more northerly sites in BC/Alberta, no difference was found in SOC in the surface soil between NT and CT. This was attributed to shallow tillage and the cold and dry climate. Stratification ratios of SOC (amount of SOC in the surface layer compared to SOC lower in the till layer) tended to be higher in drier and colder areas and with higher initial soil carbon levels. This suggested that NT may be more important and more of a quality indicator in warmer areas that have trouble accumulating SOC and therefore benefit from slower decomposition (Franzluebbers 2002).

2.5 Long-term Experiments

Janzen (2009) described long term sites as " ‘listening places’, places where, in patient quiet, we press our ears to the earth and listen for its pulse". These sites are highly valued for the important and unique opportunities they provide to scientists to study long term effects of agricultural management on soils (Janzen and Ellert 2017). They are important for understanding changes in ecosystems, and looking at their response to management practices or stresses, which could give ideas about the resilience of different systems (Janzen and Ellert 2017). Long-term studies allow the study of slowly changing ecosystem properties where the full extent of change/impact of management practices may only be seen over long periods of time (decades) (Janzen and Ellert 2017). Changes such as those in soil carbon and nitrogen flows and stocks can be tracked over time (Janzen 2009), and long term sites have been particularly important in looking at the long term effects of cropping practices on soil carbon (Janzen and Ellert 2017). They have been used in the Canadian prairies to monitor carbon loss since very close to the onset of colonizer agriculture (Janzen 2001). An important aspect of planning long term experiments it that it is necessary to think far ahead, to questions future researchers might ask (Janzen and Ellert 2017).

The oldest agricultural long term field experiment is at Rothamstead, in the UK. The oldest experiments were started between 1843 and 1856 by John Lawes and Joseph Gilbert (Johnston and Poulton 2018). They were not initially intended to be long-term experiments, but their unique value was recognized early on and some, including ones started later, are still going today. In addition to the surviving plots, there are extensive archives of soil and plant samples. This site has supported studies on soil fertility to sustain yields long-term, including the importance of SOM, as well as ecological studies on long-term grasslands, and soil pollutants and carbon sequestration (Johnston and Poulton 2018).

Perhaps the most well-known organic long term experiment is the DOK (D – biodynamic, O – organic, K – conventional [konventionell in German]) trial in Switzerland. Started in 1978, it compares farm management systems: biodynamic, organic, conventional with manure, conventional with mineral fertilizer only, and an unfertilized control (Mäder et al. 2002). The seven year ley rotations include root and grain crops. After 21 years in these treatments, yields were lower in organic systems (average 20%), as was nutrient input (34-51%), showing that the organic systems were more efficient in nutrient use. Most differences in the soil were biological. The organic plots had a higher percentage of AMF colonization, greater earthworm biomass, higher density of epigaeic arthropods, and greater microbial and weed diversity (Mäder et al. 2002). Soils under organic management had lower susceptibility to erosion than those managed conventionally (Siegrist and Schaub 1998). Percent root length colonization by AMF was highest in the unfertilized control, followed by the organic systems (manure) and lowest in conventional (manure and/or inorganic fertilizer) (Mäder et al. 2000). Similarly, higher AMF infection potential was found in soils from organic/biodynamic than conventional plots in a pot study (Mäder et al. 2000). Oehl et al. (2004) found higher AMF diversity and spore abundance in organic than conventional systems at the DOK experiment. The AMF species community was different between organic and conventional, with organic having more of the species typically found in unmanaged ecosystems (Oehl et al. 2004).

North Carolina State University's 'Farming Systems Research Unit' trial, started in 1998, is interesting because, in addition to three year organic and conventional rotations, it includes a 15 year crop and livestock rotation with annual crops and perennial pastures, plantation forestry plots, and fields that have been left to natural succession processes (Mueller et al. 2006). In the first five years they found that dry AS was higher in the crop/livestock and natural succession fields than in the organic and conventional systems, and the organic system had the lowest bulk density whereas the crop/livestock system had the highest, which they attributed to compaction from animals and lack of tillage (Mueller et al. 2006).The Mediterranean Arable Systems Comparison Trial (MASCOT), in Italy, compares organic and conventional farming systems in a Mediterranean environment (Barberi and Mazzoncini 2006). It was started in 2001, with a five year rotation of sugar beet, wheat, sunflower, pigeon bean, and durum wheat, with red clover green manure. In the first three years, they found that yield of some organic crops was lower and some higher than conventional (Barberi and Mazzoncini 2006). Other organic long term studies of note include the Rodale Institute Farming Systems Trial and the Minnesota Variable Input Crop Management Systems trials. Our very own Manitoban organic long-term experiment, the Glenlea Long-term Rotation, is the oldest organic conventional comparison trial in Canada and was started in 1992.

Also of interest is a long term study on 'Multifunctional Woody Polycultures', called 'Agroforestry for Food', established at the University of Illinois in 2015. It is a replicated alley cropping study with treatments combing rows of different species of trees and shrubs that produce edible nuts and fruit with 'alleys' of hayed land between them (Lovell et al. 2018). This agroforestry study is notable in that it is in a temperate climate and the trees are grown for production of food rather than timber or biomass. Other long-term alley cropped agroforestry sites include the Restinclieres Estate Farm in France and the Horticulture and Agroforestry Research Center at the University of Missouri (Lovell et al. 2018).

3. INFLUENCE OF PERENNIALS IN ANNUAL GRAIN ROTATIONS AND MANURE ON AGGREGATE STABILITY IN A 25 YEAR ORGANIC SYSTEM COMPARED TO RESTORED GRASSLAND

3.1 Abstract

To improve organic farming systems it is important to understand how organic cropping practices influence soil properties. Aggregate stability (AS) is an important soil physical property that relates to many soil functions. The purpose of this study was to look at the effects of perennial forages and manure in organic grain rotations on aggregate stability. It was hypothesized that aggregate stability would be improved by both adding composted manure and a perennial forage component to an organic annual grain rotation. These three organic treatments were also compared to conventional controls as well as long-term grassland plots. This study took place in the 25th year of a long-term rotation experiment at Glenlea, Manitoba. The site included forage grain (2 year alfalfa-wheat-flax) and grain only (wheat-flax-oat-soybean or hairy vetch) rotations under conventional and organic management as well as restored prairie grassland plots. Composted manure was added every four years to half of the organic forage grain rotation. Wet AS was measured using the Yoder method, with a wet sieve shaker with stacked sieves to determine mean weight diameter (MWD) of stable aggregates. Both wheat and flax stages of the rotations were sampled. The highest aggregate stability was observed in the grassland treatment though in some cases aggregate stability was similar between the grassland and arable rotations containing perennial phases. Including perennials in rotation increased MWD at 10-20cm depth in the organic system. Aggregate stability in the wheat phase was similar among arable treatments down to 20cm depth and flax at 0-10cm. Aggregate stability was highest in the

grassland plots, though the flax plots (10 – 20 cm depth) which contained perennial phases (conventional, organic, and organic + manure) were similar in MWD to the grassland.

3.2 Introduction

Agricultural cropping practices, including crop rotations with annual grains and perennial forages, manure additions, and long-term perennials/pastures and organic management, can have a large influence on soil processes. There is interest in how such cropping practices used in organic agriculture affect soil health. Aggregate stability (AS) is an important soil property that relates to many physical and biological functions and processes in the soil. A well aggregated soil with stable aggregates creates pore networks in the soil that improve water infiltration and aeration as well as root growth (Tisdall and Oades 1982). Soils with good structure are less susceptible to compaction, and stable aggregates on the surface reduce the risk of erosion. Aggregates and pores of many sizes provide habitat for soil organisms. Aggregates are also important in SOC cycling and increase the residence time of organic materials in the soil, and microaggregates are important storage sites of carbon in the soil (Six et al. 2004). Thus, aggregate stability is important to provide good soil structure for plant growth, but also for reducing soil erosion, water runoff, and retaining SOC. These multiple functions make aggregate stability a useful indicator of soil health or soil quality.

Cropping practices that could potentially improve aggregate stability in organic field crops include having perennial forages in rotation and adding composted manure as an organic nutrient source. Studies have found these practices to have varying but usually positive effects on AS (Williams et al. 2017; Romano et al. 2017; Loaiza et al. 2018), especially in the case of manure (Pulleman et al. 2003; Ozlu and Kumar 2018). Soil types differ in the way that aggregates form and how readily they form. Thus to understand the effect of cropping practices on AS it is important to look at the effect of cropping practices on AS at many different sites in many soil types.

The effects of these practices on the soil are best demonstrated at long-term sites. At the Glenlea Long-term Rotation (GLTR), in Glenlea, MB, cropping practices and rotations under both organic and conventional management have been in place since 1992. Two main interventions have been used to improve the basic organic no-input four year annual grain rotation. These are adding perennial forages into the grain rotation and adding manure to these. The Glenlea site also has long-term grassland plots which imitate the native tall grass prairie ecosystem and, with diverse species and year round cover, provide an example of these soils in their optimal state.

The objective of this study was to see if cropping practices used in organic systems could improve AS and to compare these management systems to both conventional controls and long-term grasslands in heavy clay soils in the Red River Valley of Manitoba. The specific questions being addressed were: 1) does adding a perennial forage component to an organic annual grain rotation increase AS; 2) does adding composted manure to an organic forage-grain rotation increase AS; 3) how does the organic system with the highest AS compare to the standard conventional rotation in terms of AS; and 4) how do all of these systems compare to a long-term grassland in terms of AS. The first hypothesis was that inclusion of a perennial forage component in an annual grain rotation would increase AS due to having growing plants in the soil for a greater proportion of the year and less frequent tillage. A second hypothesis was that additions of composted manure would increase AS due to organic matter additions and increased productivity as a result of the manure. The third hypothesis was that organic management would increase AS relative to conventional because of increased arbuscular mycorrhizal fungal colonization in the organic systems. Lastly, it was hypothesized that the long-term grasslands would have the highest AS due to the mixed perennial root systems and lack of disturbance.

3.3 Methods

3.3.1 Study Site

This study took place at the Glenlea Long-term Rotation, at the University of Manitoba's Glenlea Research Station, south of Winnipeg, Manitoba (49° 38'25" N, 97° 8'28" W, 230 m above sea level). The long-term field study was established in 1992 on Gleyed Humic Vertisols (Bell et al. 2012) of the Scanterbury, Red River series and Gleyed Black Chernozem of the Hoddinott series (CANSIS, n.d.). These heavy clay soils (71% clay, 23% silt, and 6% sand in the top 18cm) in the Red River Valley have 2.59% organic carbon and a pH of 6.5 in the top 18cm (Bell et al. 2012). The Glenlea Long-term Rotation compares organic and conventional management, with two crop rotations under each management system. The study has a completely randomized design, rotations are fully phased, and there are three replicates. Each main plot is 4 by 28 metres in size. The organic and conventional plots have been managed as such continuously since 1992, while the current four year rotations have been in place since 2004. The annual grain rotation consists of wheat – flax – oats – legume and the perennial forage and grain rotation is made up of wheat-flax-alfalfa-alfalfa. In the annual grain rotation the legume is soybean under conventional management and hairy vetch/barley (*Hordeum vulgare* L.) green manure in organic. In the perennial forage rotation, the alfalfa is broadcast seeded with a mix of timothy or orchardgrass and clover. Since 2007, composted cattle manure has been added once every four years to half of each main plot in the organic perennial rotation only. The manure is applied based on a combination of amount of phosphorous removed in crop product and the amount of phosphorus recommended by soil tests and is approximately 80 kg P/ha every four years. The conventional plots receive inorganic N and P annually based on soil test results. All treatments were rotovated in the fall, usually once but sometimes twice, and rotovated then harrowed prior to seeding in the spring. The rotovator, while not an ideal tool for maintaining soil health, was selected to reduce the risk of moving soil out of plots (David Lobb, U of M, pers. comm.). Briefly, crop management consisted of seeding in springtime, fertilization at time of seeding (conventional plots only), harvesting alfalfa two times during the season, and harvesting grain crops with a plot combine, leaving straw in the field. In the conventional plots, weeds were controlled with post-emergence herbicides applied several weeks after crop emergence. In the organic grain crops, a wheel hoe was used to control weeds growing between the rows. Replicated restored grassland plots at the site serve as an ecological benchmark. They were planted in mixed native grasses in 1993 and plant residue is burned every four to five years. Details of land management and soil and climate data are given in Bell et al. (2012) and Braman et al. (2016). For biomass production of these systems over time see appendix C, and for yield see Carkner et al. (2019).

3.3.2 Soil Sampling Procedures

Sampling for aggregate stability occurred in the spring, just prior to tillage and seeding (May 3-6 and 9-11, 2017). Grassland plots were sampled later (June 9 and 11) because the heavy clay soil was too wet to sample in May that year. Soil cores were taken from both plots that had grown wheat or flax the previous year and were sampled at depths of 0-10cm and 10-20cm. Four samples were taken per 4x28m plot, using a 6cm diameter hollow soil corer. As the soil was very hard, a sledge hammer was used to push the core into the ground, and it was dug out with a shovel. Samples were carefully removed from the corer and placed in large flat bottomed paper bags which were transported in a single layer on trays. This was done to avoid disturbing the aggregates. Subsamples were carefully broken up by hand, passed through a 6.3mm sieve, and then air dried before being stored. Bulk density samples were taken at the middle of 0-10cm and 10-20cm depths using hollow rings (core volume 50.64 cm^3). These samples were also used to determine water content of the soil.

3.3.3 Soil Analysis

Wet aggregate stability was determined by wet sieving using a Yoder type wet sieve shaker (Yoder, 1936). Stacks of five sieves with mesh diameters of 0.25mm, 0.5mm, 1mm, 2mm, and 4.75mm were used following the methods in Angers and Mehuys (1993) for size distribution of water-stable aggregates. These are all considered macroaggregates, as they are greater than 0.25mm in diameter. In this study small macroaggregates are those with diameters between 0.25 and 1mm and large macroaggregates are those greater than 1mm. Forty grams of soil were placed on the top sieve which was under about 0.5cm of water, and once the soil was fully wetted sieving was initiated. The sieves moved vertically a distance of 10cm at a rate of 32 oscillations per minute for ten minutes. After sieving, samples were washed into pre-weighed jars, dried at 105^{o} C for 48 hours, and then weighed. Mean weight diameter (MWD), a measure of the size distribution of water-stable aggregates, was calculated using the following formula:

$$\mathrm{MWD} = \sum_{i=1}^{n} x_i \, \mathrm{WSA}_i$$

Where x_i is the mean diameter of size fraction $_i$ and WSA$_i$ is the proportion of aggregates retained on sieve $_i$ (Angers and Mehuys 1993).

Bulk density samples were weighed immediately after collection for soil water content, oven dried at 60^{o}C until they reached constant weight, and then weighed to determine the mass for bulk density and gravimetric water content calculations.

3.3.4 Statistical Analysis

Analysis of variance for aggregate stability data was performed using PROC MIXED in SAS 9.4 (SAS Institute Inc., 2016), with the study as a completely randomized design. The univariate procedure was used to test normal distribution of data and residuals. The repeated / group= statement was used to account for heterogeneity of variance of the residuals, with group being the factor with the lowest AIC value. If assumptions of the ANOVA were not met, data were log10 transformed. Treatment was a fixed factor and there were no random factors. The treatments were annual organic, annual conventional, perennial conventional, perennial organic, perennial organic with manure, and grassland. Each crop and depth combination was analysed separately because the intent of the study was to compare the effects of the treatments on AS within each crop type and depth, not to see the effects of crop type and depth on AS. The Tukey-Kramer post-hoc test was used for means separation with an alpha value of 0.05 as a significance level.

As an additional test, to increase the number of experimental units in the analysis to get a better estimate of experimental error, and to confirm the previous analysis, an ANOVA which combined the two crop types and depths was also done. Methods were the same as the original ANOVA except the fixed factors crop (two levels) and depth (two levels) were added to the model and the grassland treatment was removed.

Three single degree of freedom a priori contrasts were done, with treatments and crop type as fixed factors, and the two depths analyzed separately. The contrasts corresponded to the first three hypotheses of the present study, and compared the annual organic and perennial organic treatments, the perennial organic and perennial organic manure treatments, and the perennial organic manure and annual conventional treatments. For the final contrast, the perennial organic manure treatment was chosen as the best organic treatment in terms of agronomic performance, and the annual conventional was chosen as the treatment most similar to standard practices in the region.

Analysis of variance for proportion data was done using GLIMMIX with a beta distribution. Treatment was the fixed effect and there were no random effects. The treatments were the same as above, and the two crop types and depths were analyzed separately so that the grassland treatment could be included in the model. The laplace method was used to run the model with the ccll link function because it gave the chi square value closest to 1. Again, the Tukey-Kramer procedure was used for means separation with an alpha value of 0.05.

3.4 Results

First MWD results will be discussed, followed by the amounts of stable aggregates within individual size classes.

MWD of water stable aggregates was similar among arable treatments in the wheat phase of the rotation to a depth of 20cm and in the top 10cm of soil in the flax phase (Figure 1). The grassland plots had much higher MWD than all of the arable treatments except in the 10-20cm depth of the flax phase, where MWD of the three perennial treatments (conventional, organic, and organic + manure) did not differ from the grassland. Also at the 10-20cm depth in the flax phase, the two annual treatments (conventional and organic) had lower MWD than the grassland, and the annual organic treatment was lower than two of the perennial treatments (conventional and organic + manure). The three perennial treatments in the flax phase at 10-20cm depth were the only treatments with a similar MWD to the grassland.

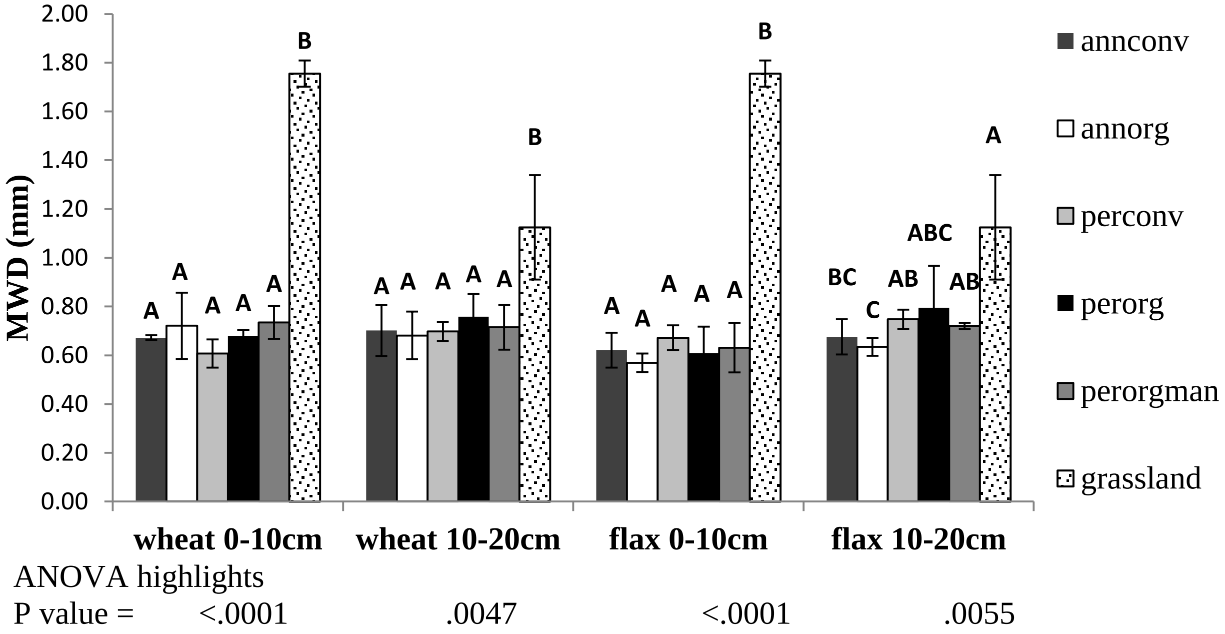


Figure 1. Mean weight diameter (MWD) of stable aggregates in treatments at Glenlea Long-term Rotation in the wheat and flax phases of the rotation at two depths. Annconv stands for the annual conventional grain rotation, annorg for the organic annual grain rotation, perconv for the conventional forage-grain rotation, perorg for the organic forage-grain rotation, perorgman for the organic forage-grain rotation with manure added, and grassland for the grassland/prairie plots. Letters indicate significance at alpha = 0.05. The Tukey-Kramer post hoc test was used, and error bars represent one standard deviation on either side of the treatment mean.

Aggregate size classes were also analyzed individually to better understand what fractions of the soil were being affected by management treatments (Figures 2 and 3). It is possible that the MWD formula could mask changes in individual size categories, as different combinations of numbers could give the same MWD. Analysis of the size fractions individually did reveal a little more variation in certain size fractions of stable aggregates. For example, the general trend within the aggregate class sizes (Figures 2 and 3) was that the grassland had fewer smaller aggregates (0.25-0.5mm) and more large aggregates (1-2mm and 2-6.3mm) than the other treatments. In the intermediate (0.5-1mm) class size, at 10-20cm depth, all treatments and the grassland were similar, but at 0-10cm there was more variation among treatments. In both wheat and flax phases, at 0-10cm, the annual conventional plots had the most 0.5-1mm aggregates, the grassland treatment had the least, and the two perennial organic treatments were intermediate, in some cases significantly fewer than the annual systems. In the flax phase, both conventional treatments (annual and perennial) had significantly more 0.5-1mm stable aggregates than the two organic perennial plots (with and without manure), which again had significantly more 0.5-1 mm stable aggregates than the grassland plots.

At 10-20cm, the arable treatments and the grassland were more similar to each other in the largest aggregate size class (2-6.3mm). In fact, for flax all treatments including the grassland were similar to each other. This contrasted with results for wheat where all treatments were similar to the grassland except for annual conventional, which had fewer stable aggregates than the grassland but was similar to the other treatments. Therefore, it was observed that where there were differences between arable treatments, the perennial treatments were more similar to the grassland than the annual treatments.

When data from the two crop years and depths were analyzed together, and the prairie omitted from the ANOVA model, no effect of treatment was seen on MWD. When there was an effect of the treatments on individual size classes in this model, it was very similar to the results when the crops and depths were analyzed separately. This showed that analyzing the data from both crop years and depths together, and thereby increasing the number of experimental units, did not change the results.

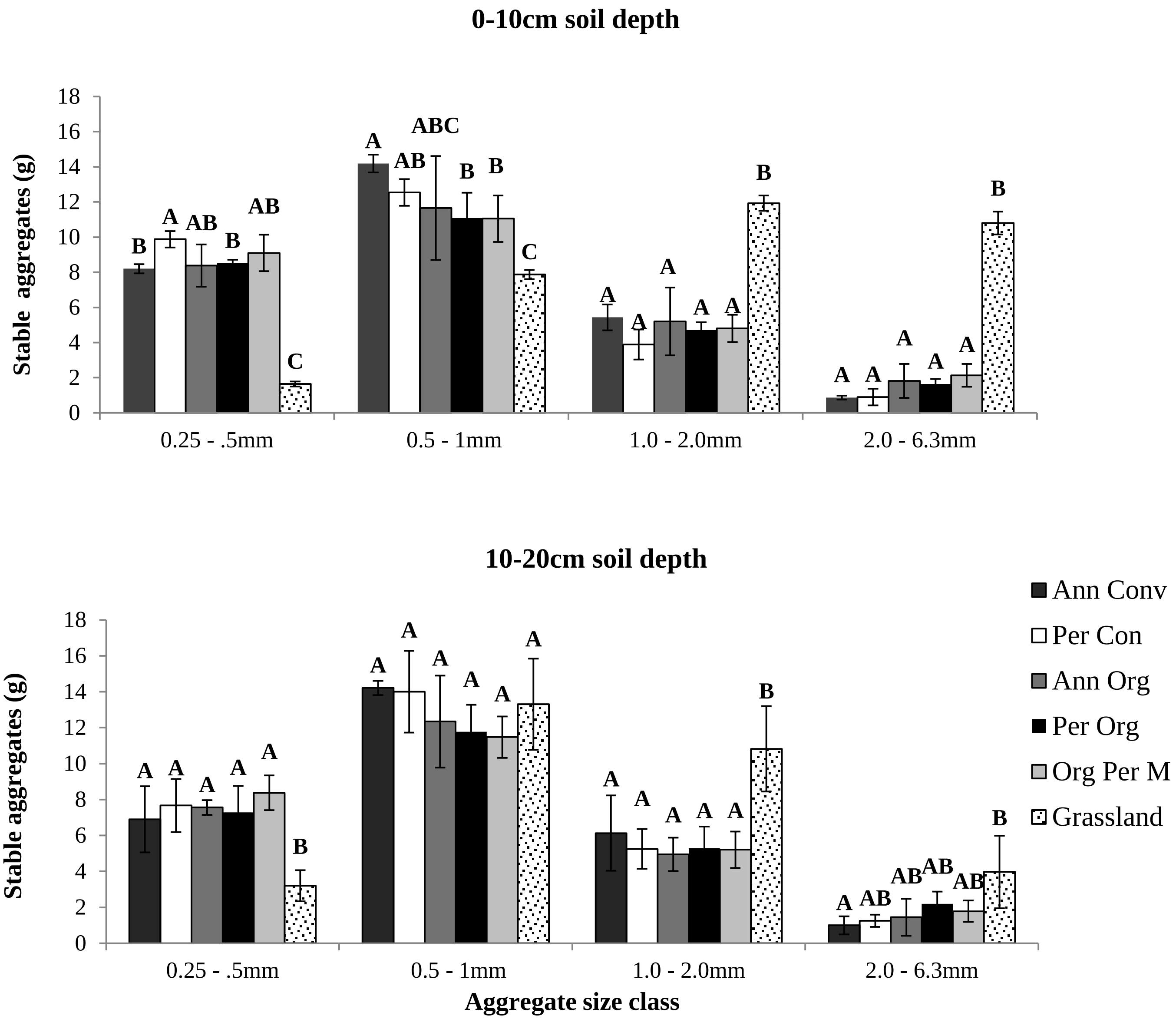
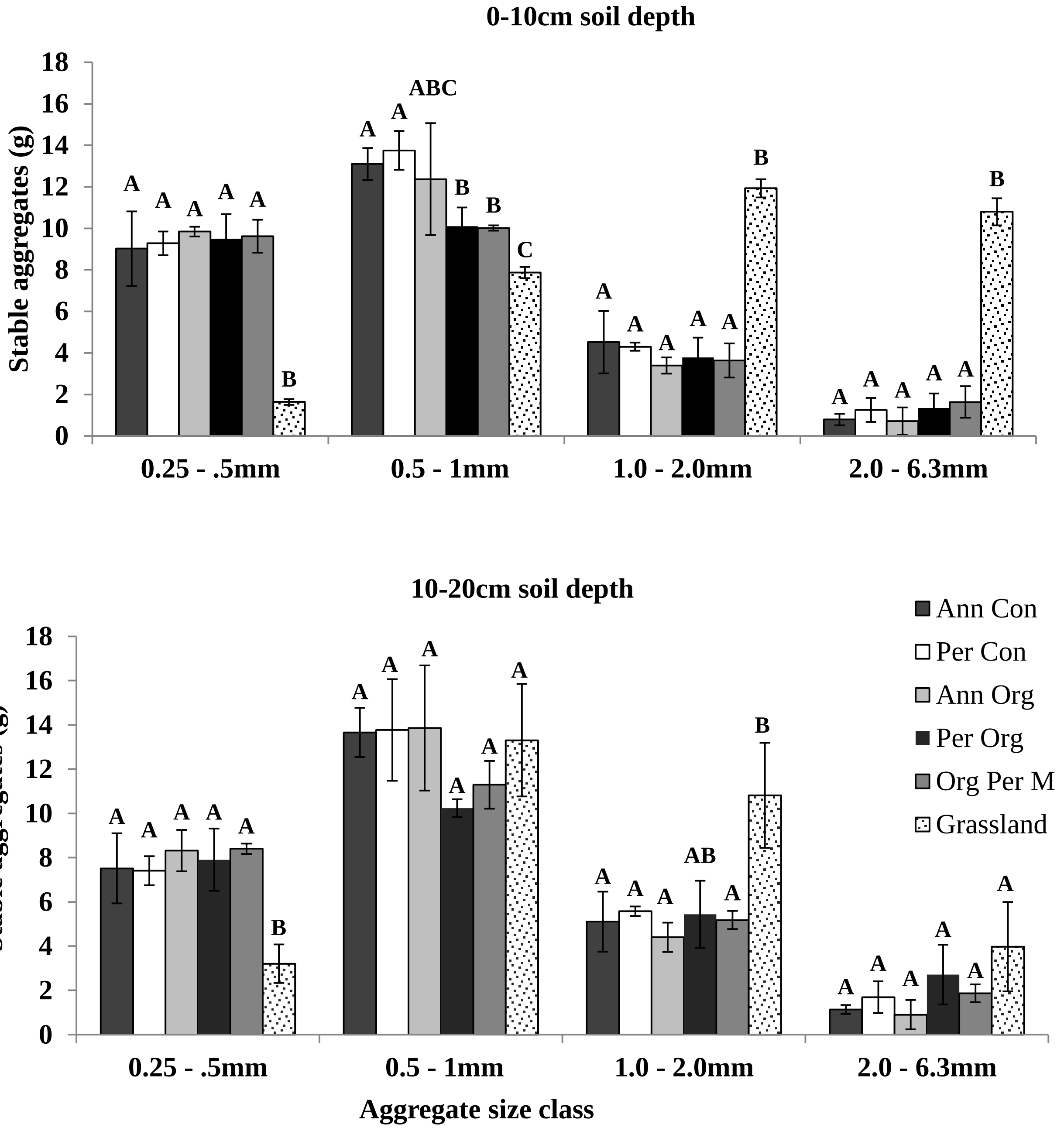


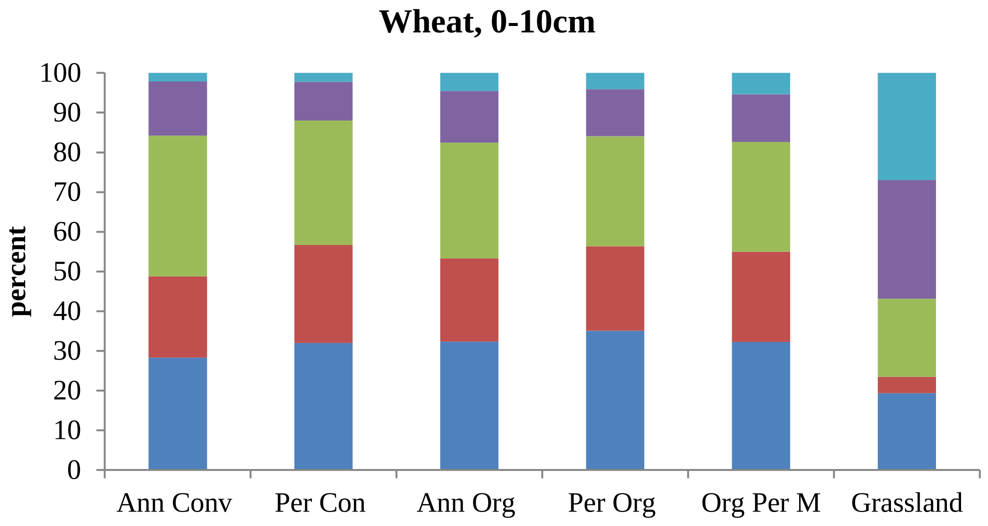
Figure 2. Stable aggregates (g) in individual size classes in treatments at two depths at Glenlea in wheat plots. Ann conv stands for the annual conventional grain rotation, per conv for the conventional forage-grain rotation, ann org for the organic annual grain rotation, per org for the organic forage-grain rotation, per org M for the organic forage-grain rotation with manure added, and grassland for the grassland/prairie plots. P value = 0.05. Error bars represent one standard deviation on each side of the mean.



0-10cm soil depth

10-20cm soil depth

Figure 3. Stable aggregates (g) in individual size classes in treatments at two depths at Glenlea in flax plots. Ann con stands for the annual conventional grain rotation, per con for the conventional forage-grain rotation, ann org for the organic annual grain rotation, per org for the organic forage-grain rotation, per org M for the organic forage-grain rotation with manure added, and grassland for the grassland/prairie plots. Error bars represent one standard deviation on each side of the mean.


Wheat, 0-10cm
percent
100
90
80
70
60
50
40
30
20
10
0
Ann Conv
Per Con
Ann Org
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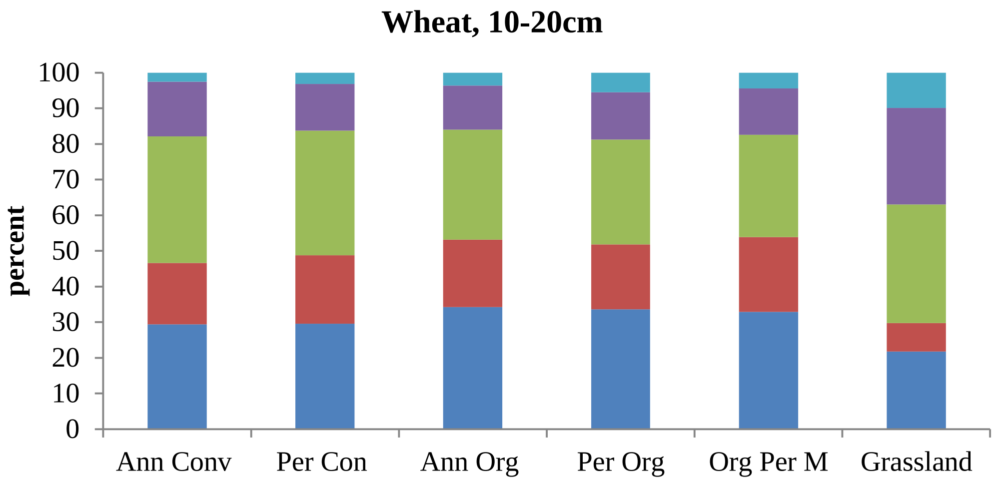
Org Per M
Grassland


Wheat, 10-20cm
percent
100
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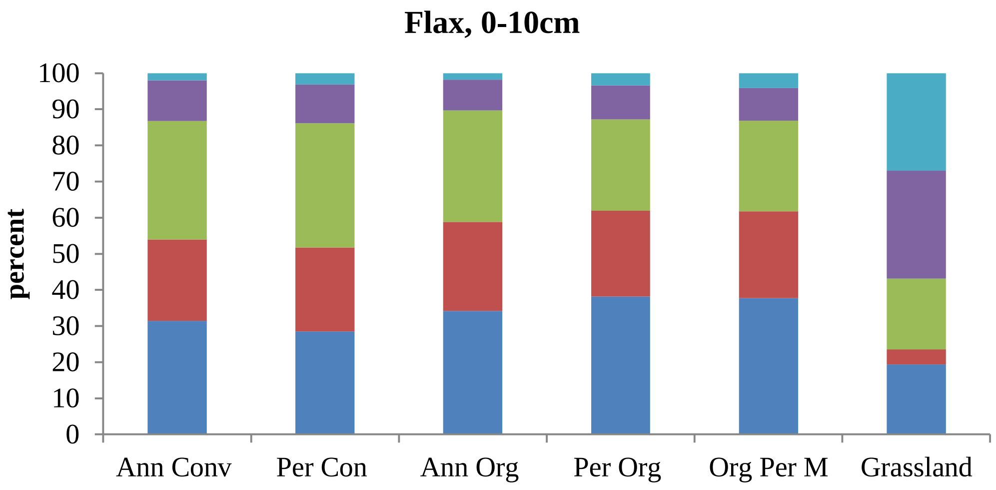
Flax, 0-10cm
percent
100
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80
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60
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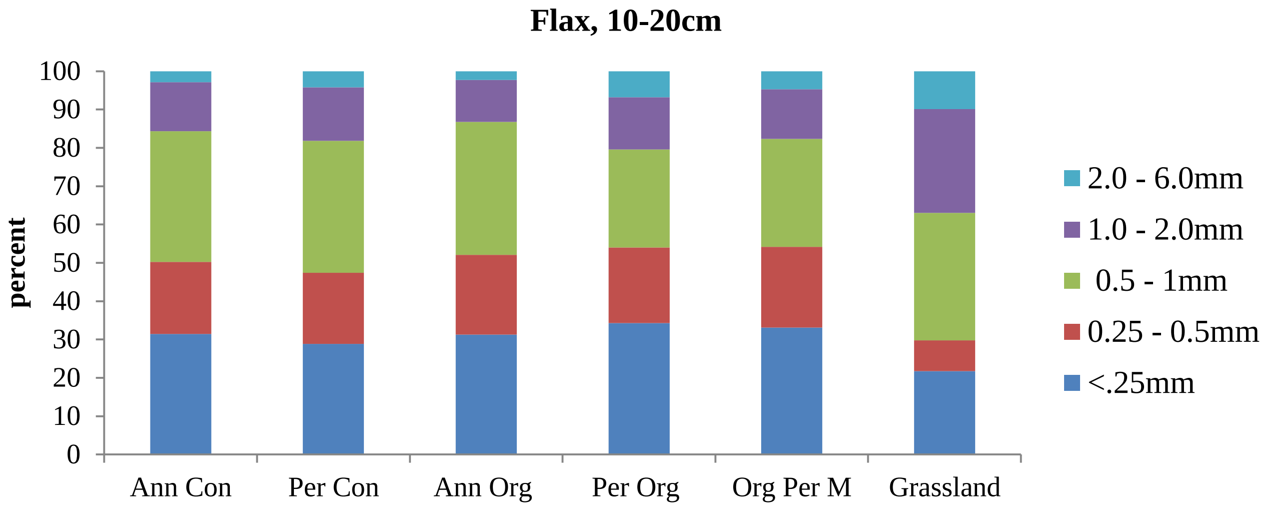


Figure 4. Percent of total soil in aggregates of different size classes in the treatments at Glenlea Long-term Rotation at two depths and phases of the rotation. Ann con stands for the annual conventional grain rotation, per con for the conventional forage-grain rotation, ann org for the organic annual grain rotation, per org for the organic forage-grain rotation, per org M for the organic forage-grain rotation with manure added, and grassland for the grassland/prairie plots.

Table 1. Proportion data for the 2-6.3mm aggregate size class. Percent of total soil in aggregates in the 2-6.3mm size class in treatments at Glenlea Long-term Rotation at two depths and phases of the rotation. Letters indicate statistical significance using the Tukey-Kramer test for means separation with alpha = 0.05. Ann con stands for the annual conventional grain rotation, per con for the conventional forage-grain rotation, ann org for the organic annual grain rotation, per org for the organic forage-grain rotation, per org M for the organic forage-grain rotation with manure added, and grassland for the grassland/prairie plots.

	Wheat				**Flax**			
	0-10cm		**10-20cm**		**0-10cm**		**10-20cm**	
Ann Conv	2.17	C	2.50	B	1.98	B	2.85	B
Per Con	2.26	C	3.13	B	3.14	B	4.22	AB
Ann Org	4.54	BC	3.63	B	1.80	B	2.25	B
Per Org	4.12	BC	5.50	AB	3.38	B	6.78	AB
Org Per M	5.34	B	4.45	AB	4.09	B	4.68	AB
Grassland	27.00	A	9.93	A	27.00	A	9.93	A

The proportion analysis of 2-6.3mm aggregates in the wheat phase of the rotation at 0-10cm (Figure 1) showed that the perennial organic treatment with manure had a greater proportion of large aggregates than the two conventional treatments.

The a priori contrasts showed no significant effects at 0-10cm depth, but at 10-20cm depth the perennial organic treatment had higher MWD than the annual organic treatment (p = 0.0260), showing an increase in MWD with perennials under organic management. At both depths there was no effect of manure in the organic perennial rotation on MWD, and there was no difference in MWD between the agronomically best organic rotation (perennial organic with manure) and the standard conventional rotation (annual conventional).

The grassland had higher bulk density (BD) than the perennial conventional and perennial organic treatments in the wheat phase at 0-10cm depth (Table 2). Though they were not significantly different, the three perennial treatments had notably lower BD than the annual treatments, ranging from 10% to 24% lower. In the flax phase of the rotation, at 0-10cm depth, there were no significant differences in BD, though again the perennial treatments had notably lower BD than the annuals. At 10-20cm depth in both crop phases the grassland had lower BD than the annual conventional treatment.

Table 2. Bulk density (g/cm^3) in treatments at Glenlea Long-term rotation in the wheat and flax phases of the rotation at two depths. Letters indicate statistical significance using the Tukey test for means separation with alpha = 0.05. Ann con stands for the annual conventional grain rotation, per con for the conventional forage-grain rotation, ann org for the organic annual grain rotation, per org for the organic forage-grain rotation, per org M for the organic forage-grain rotation with manure added, and grassland for the grassland/prairie plots.

	Wheat		**Flax**	
	0-10cm	**10-20cm**	**0-10cm**	**10-20cm**
Ann Con	0.96 ab	1.17 a	0.88 a	1.22 a
Ann Org	0.88 ab	1.11 ab	1.02 a	1.17 ab
Per Con	0.79 b	1.04 ab	0.78 a	1.07 ab
Per Org	0.76 b	1.07 ab	0.76 a	1.06 ab
Per Org M	0.73 ab	1.04 ab	0.79 a	0.99 ab
Grassland	1.07 a	0.93 b	1.07 a	0.93 b

3.5 Discussion

The grassland in this study serves as a bench mark for soil health. As would be expected, it had a much higher MWD than the arable treatments. Figure 2 and 3 show a trend of the grassland plots having fewer small aggregates and more large aggregates relative to arable treatments. If similarity to the grassland signifies better soil health in this soil type, then fewer small macroaggregates (0.25-1mm) and more large macroaggregates (1-6.3mm) are indicative of higher AS/soil health, and the transition between these two phases is somewhere around the .5-1 mm size class. Other studies have also shown increases in large macroaggregates (>2mm) (Hermawan and Bomke 1997; Angers 1992), similar to this study, as aggregate stability (MWD) increases, whereas others have found increases in all macroaggregates (Guo et al. 2010). In this study, 62% to 72% of total soil was contained within the macroaggregate fraction (>0.25mm) in the arable treatments and 78% to 80% in the grasslands (Figure 4). Large macroaggregates (>2mm), however, made up only 1.8% to 6.8% of the total soil in arable treatments and up to

27% in the grassland. Soils in other studies had much higher proportions of large macroaggregates, for example Angers (1992) found that >2mm aggregates increased from 25% to 45% after alfalfa on a clay soil (47% clay).

A few observations in this study showed similarity in AS between perennial organic systems and the grassland treatment. For example, in the flax phase of the rotation at 10-20cm depth, the MWDs of the three perennial systems (perennial conventional, perennial organic, and perennial organic + manure) were similar to the grassland. An a priori contrast found the perennial phase to increase MWD at 10-20cm depth under organic management. Also, in the cases where there was some variation among arable treatments within size classes of aggregates, the perennial treatments tended to be similar to the grassland and the annual treatments different. Thus in some cases, particularly in the deeper depth, the perennial treatments had a more positive effect on AS compared to the annual treatments. These results mirror those of Braman et al. (2016) who observed that, unlike the annual and conventional treatments, the two organic perennial systems at Glenlea were similar to the grassland in terms of microbial biomass carbon (MBC), soil respiration and qCO. Perhaps the microbial activity in the organic perennial plots is related to the increased AS in some cases. Others have observed positive correlation between AS and MBC (Sparling et al. 1992; Hurisso et al. 2013). The perennial plots have plants growing for a greater portion of the year and a break from tillage, which could lead to more OM input to the soil and less disturbance and this may be one reason for greater AS compared to the annual plots. The organic alfalfa plots at GLTR were also observed to have more of the weed quackgrass (*Elymus repens* L.) than other plots, due to absence of herbicide use in the organic rotation. The dense roots systems of the quackgrass could possibly contribute to increased aggregation.

However, while some examples showed superior AS in the perennial rotation at Glenlea, in most cases, particularly at 0-10cm depth, AS was similar between perennial and annual rotations. An explanation for the lack of differences may be related to the choice of alfalfa as the perennial phase species. Monocots are reported to form aggregates better than dicots (Oades 1993). Monocot root systems form particularly well aggregated soils because they have more fine roots which dry the soil more evenly, with many small cracks in many directions that form and stabilize aggregates (Oades, 1993). Rilig et al. (2002) found that grasses increased AS more than a legume and forb species while another study found that forb species increased AS more than grasses and legumes in the top 15cm of a serpentine soil (Eviner and Chapin 2002). In terms of crop plants, Materechera et al. (1992) found that ryegrass (*Lolium rigidum* Gaud.) had the greatest effect on AS followed by wheat and then pea. Thus the minimal effect of the perennial rotation could be partly that alfalfa, being a legume, has lower potential for improving soil aggregation than other species, particularly grasses. Several studies, however, have found that two to five years of alfalfa improved AS (Williams et al. 2017; Guo et al. 2010; Su et al. 2009; Angers 1992) and Li et al. (2019) found that alfalfa had higher SOC stocks and root biomass (both of which relate to AS) down to 150cm than a perennial grass (*Leymus chinensis* (Trin.) Tzvelev). Whether or not alfalfa improves soil structure also depends on the initial soil conditions, for instance, Guidi et al. (2017) found that one year of alfalfa improved AS in degraded soils but not in less degraded soils in a ploughed organic rotation. On silt loam soils, Pena-Yewtukhiw et al. (2018) found AS increased during the first two years of grass-legume sod, with little additional change found after the third year. Other reasons for lack of effect of alfalfa, especially compared to the grasslands, could be that the above ground alfalfa biomass was removed, and extra tillage was required to terminate the alfalfa compared to other treatments.

In this study adding composted manure to organic perennial plots that received no other nutrient inputs had no effect on AS. Generally, manure additions have been found to improve AS in cropping systems, including in organic and tilled systems (Romano et al. 2017; Williams et al. 2017) and when compared to inorganic fertilizer (Ozlu and Kumar 2018, Pulleman et al. 2017). Wortmann and Shapiro (2007) found that manure increased stable macroaggregates (>0.25mm) and decreased microaggregates (<0.25mm), but also that its effect on AS was not detectable four years after manure application. This is only notable because the manure at GLTR is added once every four years, and the phases in the present study were sampled two and three years after the most recent manure application. Because of this, some effects of the manure may no longer have been detectable, but the main mechanism by which the manure was hypothesized to increase aggregation was through improved crop growth and thus plant biomass and SOC throughout the rotation due to improved plant nutrition in an otherwise nutrient poor (particularly P) organic system. Although it did not affect AS, the composted manure did increase wheat yield and alfalfa biomass, though not flax yield, in the organic forage rotation at GLTR (Carkner et al. 2019)

One potential explanation for the lack of improvement in AS in arable treatments in this study could be the relative importance of physical processes on aggregate formation and stabilization in heavy clay soils (Oades 1993). In soils with a high clay content, especially shrink-swell clays, drying and wetting cycles and consequent cracking of the soils are responsible for much of the aggregate formation with biotic factors being less important, whereas biotic factors are very important in loams and sands (Oades, 1993). As the soil at Glenlea is a vertisol, this could explain the lack of response in the surface soil layers to the different cropping practices. It could also explain why the grasslands had a much higher AS than all other treatments, despite interventions in some of the treatments such as perennials in rotation and manure which have improved AS in many other studies. In heavy clay soils, the properties of the clays are important and cracking due to drying and shrink swell cycles causes aggregate formation. Plant roots dry out the soil immediately surrounding them more quickly than areas without plant roots, causing uneven drying of the soil. When plants are growing in rows, a stronger drying effect within the rows than between the rows causes cracking between and parallel to the rows. Whereas in a densely broadcasted stand of pasture, such as the Glenlea grassland plots, the plants and roots are more evenly spread out, and as they dry they cause smaller cracks in many directions, which gives the soil a more consistent coarse granular soil structure (Oades 1993). Thus the arrangement of plants can affect aggregation and this would at least partly explain the better aggregation in the grasslands compared to all of the arable treatments. Similar to the mostly small stable aggregates found in the arable treatments in this study, Materechera et al. (1992) observed more small stable aggregates in a heavy clay soil (‘black earth soil’, 67% clay) than a 19% clay ‘red-brown earth’ soil, though the small aggregates in the clay soil were more stable. Wet-dry cycles decreased the amount of large aggregates and increased the amount of small aggregates. They attributed this to the cracking and shrink-swell activity of the heavy clay. Novelli et al. (2013) found that cropping intensity (fraction of the year with plant cover) was important in increasing AS in a mollisol but had no effect in a vertisol, despite higher SOC stocks and concentration in the vertisol. They suggested that, in the vertisols in their study, SOC was important in increasing aggregation only when soils had more than 35 g C / kg, and that at lower levels SOC was not an important aggregating agent (Novelli et al. 2013). This again could explain why plant cover increased AS in the grasslands but not the arable systems, as C stocks were higher under the grasslands than other treatments at Glenlea in 2009 (Bell et al. 2012). The organic forage grain plots at Glenlea had an organic carbon concentration of 24.3 g / kg and the organic annual plots an organic carbon concentration of 29.7 g / kg at 0-15cm depth in 2009 (Bell et al. 2012).

The finding that long-term grasslands had much higher AS than arable systems is consistent with the literature. The same grasslands at Glenlea also have higher SOC stocks than the arable treatments (Bell et al. 2012). Older grasslands have been found to have more root biomass than younger grasslands or annually cropped soils (Acharya et al. 2012). Root carbon is known to have a longer residence time in the soil (Kong and Six 2010; Rasse et al. 2005) and organic compounds from roots, both residues and exudates, are important in aggregate formation and stabilization (Six et al. 2004). Roots and associated hyphae are also important in forming larger aggregates through physical entanglement (Six et al. 2004). The high plant density and random orientation of grassland plants means aggregation in the grasslands should also be occurring through physical processes (Oades 1993). Thus it makes sense that soils covered in dense grasses that have not been disturbed for 25 years show high aggregate stability. Many studies have found higher AS under long-term perennial grasslands than under annual crops (Sparling et al. 1992; Wright and Anderson 2000; Dominy and Haynes 2002; Vezzani et al. 2018). As well, AS has been found to be higher in native grasslands than planted pastures (Hebb et al. 2017).

Organic and conventionally-managed plots behaved similarly in terms of AS. Where differences were observed they tended to be between annual and perennial treatments rather than organic and conventional. The contrasts and ANOVA found no difference in MWD between the agronomically best organic management system, the perennial rotation with manure, and the standard annual conventional rotation. The only exception was that there were fewer small aggregates (0.5-1mm) in the perennial organic with manure plots than conventional, making them more similar to the grassland.

That an effect of the perennials on MWD was seen only at the lower depth could be related to tillage being concentrated in the upper 10cm of the soil at GLTR. The grassland plots were the only untilled treatment, and they were more similar to arable treatments at 10-20cm.The lower depth is only minimally disturbed by tillage, perhaps this allows the effects of the management practices to persist for longer. Tillage in the fall after harvest may mask some of the effects of the management practices, by disrupting aggregates that formed over the growing season, and prevent these effects from being detectable in the following spring. Given that these treatments have been in place for 25 years though, it seems likely that at least some effect of aggregation would be detectable post tillage. Hermawan and Bomke (1996) found that an effect of winter cover crops on AS was still seen in soil sampled after spring tillage. They also found that spring tillage increased 2-6mm aggregates and MWD in some treatments, which was attributed to the drying effect of tillage on the soil. Kabir and Koide (2000) also detected an effect of cover crops on AS after spring tillage and 54 days of crop growth. Nine months after cover crop termination however, Blanco-Canqui et al. (2013) found no residual effect of cover crops.

3.6 Conclusion

At Glenlea, the long-term grasslands had the highest MWD and more large aggregates than arable treatments. The perennial treatments in some cases were similar to the grassland, or were intermediary between annual treatments and the grassland. In the wheat phase of the rotation at 0-10cm depth, the perennial organic system with manure had a greater proportion of >2mm aggregates than the two conventional rotations. As well, the inclusion of perennials in rotation in the organic system increased AS at 10-20cm depth. The increased AS due to inclusion of perennials in the rotation was likely due to having roots growing in the soil for a greater portion of the year and less frequent soil disturbance. The lack of difference seen in many cases with the perennials could be related to the use of alfalfa rather than grasses as the perennial crop. Neither manure nor management system (organic or conventional) had an effect on AS. This may be due to both regular tilling of the plots disrupting the aggregates and to the vertisolic nature of the clay soils, which may require higher levels of SOC before an effect of additional OM is seen on aggregation. The perennial rotation had a perennial crop growing fifty percent of the time, yet in most cases it was more similar in AS to the annual rotations than the grasslands. These findings underscore the importance of long-term mixed native grasses in improving soil quality, relative to changes in arable cropping practices.

4. EFFECT OF FOUR YEARS OF ALFALFA ON AGGREGATE STABILITY IN AN ORGANIC ANNUAL GRAIN ROTATION

4.1 Abstract

No-input organic annual grain rotations can perform poorly agronomically and can degrade the soil. Perennial forages can be used in organic rotations to rehabilitate the soil, improving soil quality as well as having other benefits. Aggregate stability is an important measure of soil quality that pertains to soil structure and organic carbon cycling and is sensitive to cropping practices. The objective of the present study was to see if one to four years of perennial forages inserted into an organic annual grain rotation improved aggregate stability. This study took place at a long-term experiment where an organic annual grain rotation (wheat-flax-oat-hairy vetch) was being rehabilitated by converting it to alfalfa forage for four years. The rotation was fully phased, allowing sampling when the alfalfa had been growing from zero to three years in 2017, and one to four years in 2018. Aggregate stability was measured using wet sieving to determine mean weight diameter (MWD). The amount of stable aggregates in different size classes was also compared. The alfalfa improved MWD in the top 10cm compared to non-alfalfa plots, but the number of years that the soil had been under alfalfa did not affect MWD. Small differences within some aggregate class sizes were seen in both cases.

4.2 Introduction

Organic grain rotations with no external nutrient inputs and insufficient soil building phases in rotation have been found to suffer large yield losses compared to conventional rotations (Carkner et al. 2019). Perennial forage/pasture phases have been found to improve yield in subsequent crops as well as have other benefits, such as improving soil quality (Hoyt 1990; Grace et al. 1995; Hebb et al. 2017), and they are often used in organic rotations (Nelson et al. 2010). Alfalfa is commonly used in this capacity in this region and it is a strong nitrogen supplier for subsequent crops and has a deep root system. Periods of alfalfa can increase yield on subsequent crops (Hoyt 1990) but it has also been used to remediate soil (Guidi et al. 2017; Guo et al. 2010) and as a regular part of rotations to improve soil quality and fix nitrogen (Williams et al. 2017). Soil health is of great interest to organic farmers and thus there is interest in how such a strategy, designed to increase yields, would affect soil health. As a measure of soil health, this study focused specifically on aggregate stability, as it affects many different processes in the soil, particularly concerning soil structural properties, soil organisms, and carbon cycling.

The present study investigated the insertion of a four year alfalfa phase as a one-time intervention into an organically managed annual grain rotation for the purpose of system rehabilitation. This study took place at a long-term rotation trial in southern Manitoba, the Glenlea Long-term Rotation (GLTR). At GLTR, there is a four year organic annual grain rotation, which has no external nutrient inputs and includes one year of leguminous green manure (GM) to provide nitrogen. The one year of GM out of four does not supply sufficient N for the rest of the rotation (Carkner et al. 2019). This has led to nitrogen deficiency and poor agronomic performance. The first objective of this study was to see if inserting an alfalfa forage phase into an organic annual grain rotation would increase soil aggregate stability compared to when there was no alfalfa. The second objective was to see if aggregate stability increased over the four years of the alfalfa phase. The hypotheses were that aggregate stability would be higher where alfalfa forage had been grown, and, secondly, that aggregate stability would increase over the four consecutive years of alfalfa. This is because alfalfa, as a perennial with a large root system, has potential to increase organic carbon inputs to the soil, support the soil biotic community, increase soil surface cover, and reduce tillage, all of which have been linked to increased aggregate stability.

4.3 Methods

4.3.1 Study Site

An experiment has been conducted within the greater Glenlea rotation experiment for the past several years. This experiment is testing the effect of inserting a multi-year alfalfa crop into the annual organic rotation, a rotation which has suffered from both low yields (Carkner et al. 2019) and lower microbial biomass activity (Braman et al. 2016). The annual organic grain only plots were split to compare the base rotation (hairy vetch/barley green manure-wheat-flax-oats) with a system where only alfalfa was grown for one full rotation cycle. The alfalfa was broadcast seeded with a grass and legume. It was mowed two or three times a season, but no biomass was removed, to simulate grazing. The rotation has received no external inputs except for seed since its establishment in 1992. For details of the rotation site and management see chapter 3 and Bell et al. (2012).

The four year period of alfalfa has been phased into the rotation starting with the hairy vetch phase since 2014. In 2016 there were plots that had been in the alfalfa phase for zero to three years, and in 2017 from one through four years. Therefore, this provided an opportunity to see if alfalfa had an effect on AS compared to where there was no alfalfa as well as to compare the effect of one, two, three and four year alfalfa stands on soil aggregate stability.

4.3.2 Soil Sampling

Each year of the alfalfa phase was sampled for aggregate stability, bulk density, and water content between May 3 and 11th, 2017 when plots had been in alfalfa from zero to three years, and again on May 8, 10 and 11, 2018, after the first plots had completed the four years in alfalfa. The same sampling procedures for aggregate stability were used as in chapter 3. The only exception was in 2018 after wind erosion had deposited soil in some of the plots. In these cases the wind-deposited material was removed to expose the original soil surface so the sample could be taken from the original soil surface downwards, as judged by the alfalfa stubble.

4.3.3 Soil Analysis

Soil sub samples were crumbled by hand and passed through sieves (mesh diameter of 6.3mm in 2017 and 8mm in 2018) before being air dried and stored. Aggregate stability and bulk density were determined using the same methods as in chapter 3.

4.3.4 Statistical Analysis

Analysis of variance of AS data was performed using PROC MIXED in SAS 9.4 (SAS Institute Inc., 2016), with number of years in alfalfa as the fixed factor and no random factors. Each year and depth was analyzed separately. The univariate procedure was used to test for the normal distribution of the data and residuals. The repeated / group= statement was adjusted to account for heterogeneity of variance. Least significant difference (LSD) was used as a means separation test with an alpha value of 0.05. This test was chosen because this is an exploratory study and AS is a highly variable measurement. It is appropriate because there are only four treatments being compared. A linear regression with combined data from both years was also done in SAS.

4.4 Results

Mean weight diameter results for both 2017 and 2018 will be discussed first followed by individual size class data for both years.

In 2017, MWD in the top 10cm was higher where alfalfa had been growing for one and three years than where no alfalfa had been grown (Figure 5). The MWD in year two of alfalfa was not significantly different from the other years. At 10-20cm depth the MWD of all years was similar.

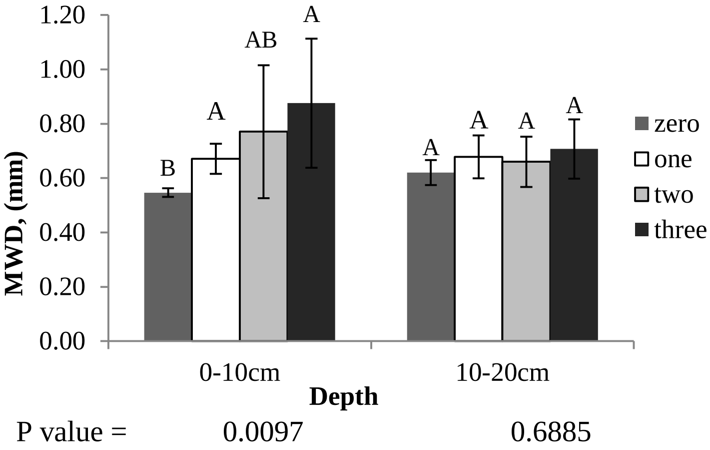


Figure 5. Mean weight diameter (MWD) of stable aggregates with zero to three years in alfalfa, at two depths, in 2017. Letters indicate significance at alpha = 0.05, LSD post hoc test. Error bars represent one standard deviation on either side of the mean.

In 2018, when alfalfa had been growing for one through four years, MWD was similar between years in both depths (Figure 6). No comparison with a ‘zero’ alfalfa year was possible in 2018.

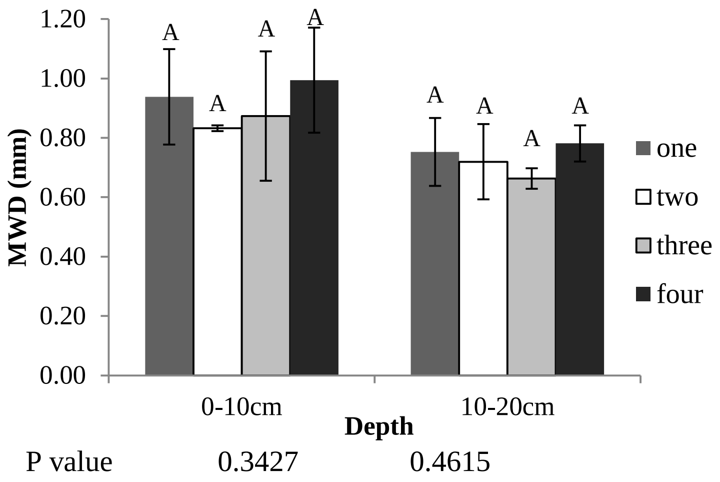


Figure 6. Mean weight diameter (MWD) of stable aggregates with one to four years in alfalfa, at two depths, in 2018. Letters indicate significance at alpha = 0.05, LSD post hoc test. Error bars represent standard deviation.

The class sizes of stable aggregates were analyzed individually to see if there were any differences that were not detected by the MWD formula. In 2017 at the 0-10cm depth, and in 2018 at 10-20cm depth, there were differences between years in the 0.25-0.5mm and 1-2mm size classes (Figures 7 and 8). In 2017 at 10-20cm and 2018 at 0-10cm there were no significant differences between treatments within aggregate size classes.

In 2017 (Figure 7), at 0-10cm depth, there were more stable 0.25-0.5mm aggregates in year zero than in years one and three. There were more 1-2mm stable aggregates in years one and three than year zero. In both cases year two was similar to all other years.

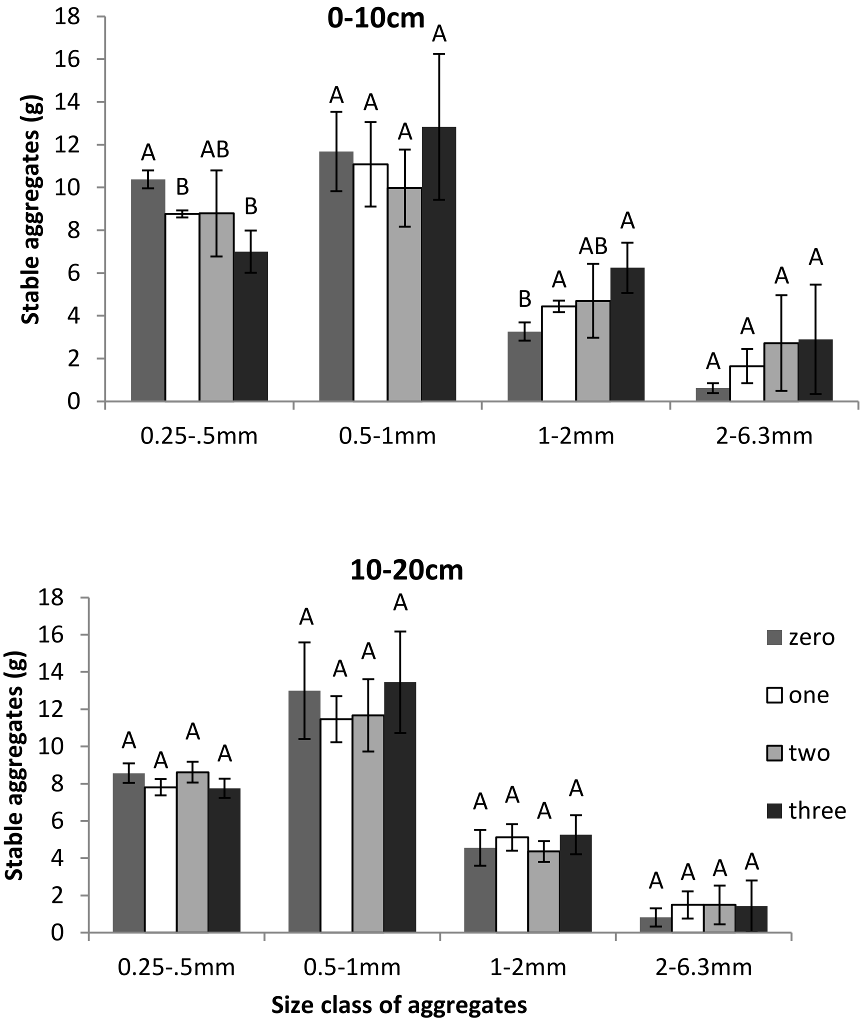


Figure 7. Stable aggregates (g) in individual size classes in plots in alfalfa for zero to three years at two depths in 2017. Letters indicate significance at alpha = 0.05, LSD post hoc test.

In 2018 (Figure 8), at 10-20cm depth, there were more stable aggregates of 0.25-0.5mm in year three than year four, with years one and two being similar to both. There were more 1-2mm size stable aggregates in year four than three, again with years one and two being similar to both.

In both years and depths there were no differences in the 0.5-1mm and >2mm size classes.

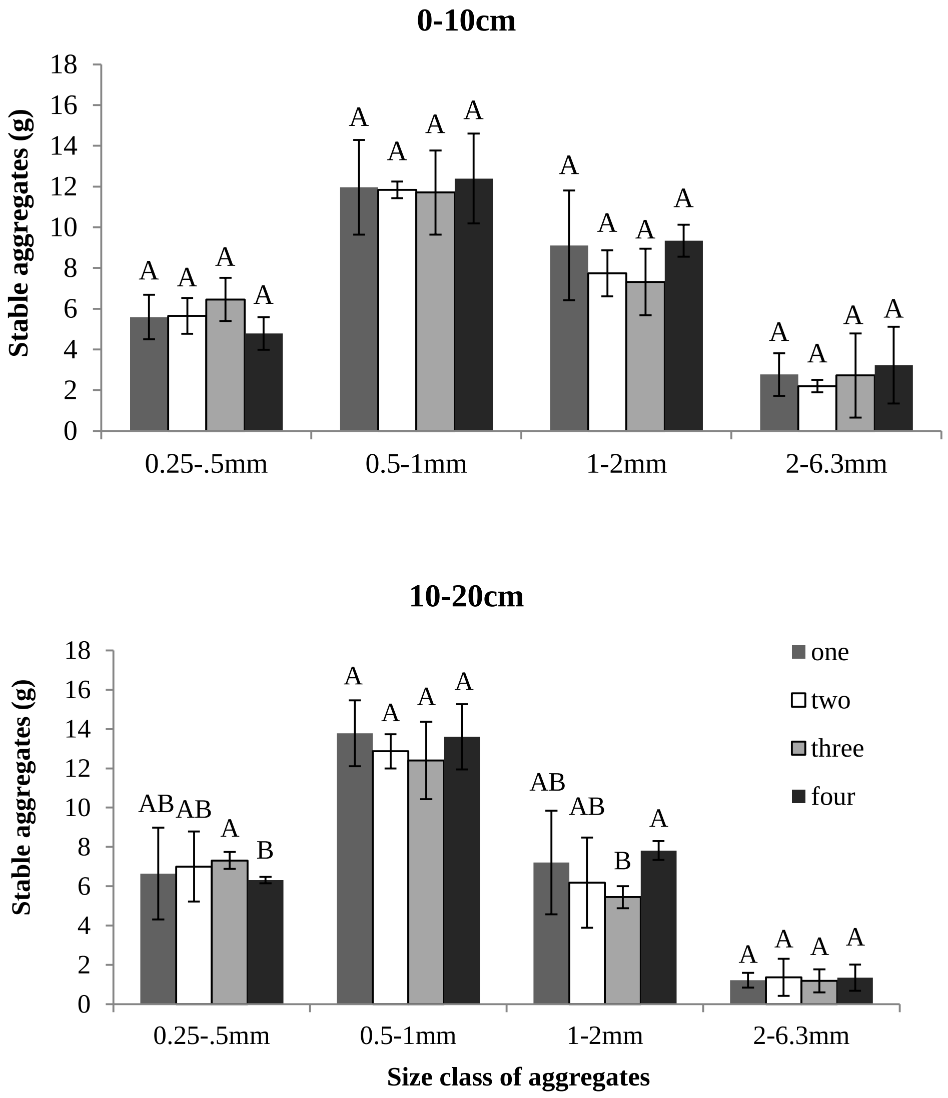


Figure 8. Stable aggregates (g) in individual size classes in plots in alfalfa for one through four years at two depths in 2018. Letters indicate significance at alpha = 0.05, LSD post hoc test. Error bars represent one SD on either side of the mean.

A linear regression of the data from both 2017 and 2018 combined showed that AS increased from plots with no alfalfa to those that had been in alfalfa for one to four years in the top 10cm of soil ($y = 0.6400 + 0.0867 x$, $r^2 = 0.2994$, $p = 0.0057$). However, when the zero alfalfa treatment was removed from the same dataset, the regression was no longer significant ($y = 0.7190 +$

0.0580x, $r^2 = 0.1136$, $p = 0.1352$). A linear regression with all the treatments at 10-20cm depth was also not significant ($y = 0.6536 + 0.0219x$, $r^2 = 0.0960$, $p = 0.1407$). This supports the results from the ANOVA which showed an increase in MWD with the presence of alfalfa but no change with the number of years in alfalfa.

BD was not statistically different between treatments at either depth in either year (Tables 3 and 4).

Table 3. Bulk density (g/cm^3) with zero to three years of alfalfa at the Glenlea Long-term Rotation in 2017. Letters indicate statistical significance using the Tukey test for means separation with alpha = 0.05.

Years in alfalfa	0-10cm		10-20cm	
zero	0.99	a	1.22	a
one	0.92	a	1.16	a
two	1.06	a	1.19	a
three	1.07	a	1.17	a

Table 4. Bulk density (g/cm^3) at Glenlea Long-term Rotatio in plots with one to four years of alfalfa in 2018. Letters indicate statistical significance using the Tukey test for means separation with alpha = 0.05.

years in alfalfa	0-10cm		10-20cm	
one	1.04	a	1.21	a
two	1.06	a	1.15	a
three	1.06	a	1.21	a
four	1.05	a	1.19	a

Precipitation in October, April and May leading up to spring sampling was higher in 2017 than 2018 (Government of Canada, n.d.). Soil gravimetric water content (% soil water) was also higher in 2017 (Table 5). A wind erosion event also occurred in the spring of 2018, prior to sampling in May.

Table 5. Fall and spring precipitation from the year leading up to sampling and soil gravimetric water content (% soil water) at time of sampling at Glenlea in 2017 and 2018. Soil water content is an average value for all plots sampled, at 0-10cm and 10-20cm depth. Precipitation data is for Winnipeg (Government of Canada, n.d.).

	% soil water		**precipitation**		
sampling year	**0-10cm**	**10-20cm**	**October**	**April**	**May**
2017	39.68	36.57	64.1mm	31.7mm	24.6mm
2018	34.40	33.09	20.9mm	1.7mm	13.5mm

4.5 Discussion

In 2017, at the surface depth, the presence of alfalfa increased MWD after one and three years. In 2017 MWD also appeared to increase with number of years in alfalfa, but the differences were not significant. This was partly the result of the high variability of the AS data. Within size classes, the pattern of fewer small aggregates (0.25-0.5mm) and more large aggregates (1-2mm) corresponding to higher MWD in treatments with alfalfa compared to those without, was seen in 2017 at 0-10cm depth. In 2018 at 10-20cm there was a similar pattern of fewer small and more large aggregates in the fourth year of alfalfa than the third, showing a pattern of increasing AS that was not seen in the MWD. This is consistent with another study at the same site (chapter 3), which found that soils under long-term perennials had more large aggregates and fewer small aggregates than arable treatments. This suggests that where slight differences were seen in the present study, the trend was one of AS increasing with alfalfa and length of time under alfalfa. Therefore, where there were differences in class sizes they were consistent with our hypotheses of improved aggregation in soils with alfalfa as well as with time in alfalfa. However, this improvement was only seen in one depth per year, in a different depth each year, and never in the largest (2-6.3mm) size fraction. Also, in 2017, improvements were only seen in years one and three compared to year zero, and in 2018 improvements were seen in year three compared to four. In 2017, the year zero plots were fallow since the previous fall (during the period after grain crop harvest that year), whereas the other three plots had alfalfa stubble and residue cover over the winter. Lack of plant cover increases the soils exposure to aggregate disrupting forces over the winter and could partly explain the lower aggregation in year zero compared to years one and three in 2017. There is seasonal variation in the stability of aggregates in annual cropping systems, with aggregate stability increasing over the crop growing season and decreasing over winter (Grover 2008) and winter cover reduces the seasonal destruction of aggregates, thus maintaining higher AS (Grover 2008; Hermawan and Bomke 1997).

An effect of alfalfa on MWD and individual size classes of aggregates was only seen in the top 10cm of soil in 2017 whereas in 2018 slight differences due to number of years in alfalfa were only found at the deeper depth. Prior to the first year of the alfalfa phase the plots were tilled regularly, so changes may be partly the result of one to four years break from tillage. Since the soil was tilled down to about 10cm it makes sense that more of a change in MWD was seen in the higher depth because this depth would have been tilled up until the first year of alfalfa was seeded, whereas the lower depth would have been mostly unaffected by tillage both before and after the alfalfa was planted. Many studies have reported increased AS in the surface soil with no-till compared to tillage (Six et al. 1998; Helgason et al. 2010; Du et al. 2015), though this difference is often restricted to about the top 5cm, with little difference in AS between tilled and untilled soils at greater depths (Beare et al. 1994; Eynard et al. 2004). AS of soils in long-term perennials though tends to be higher than both tilled and no-tilled annual systems throughout the root zone (Six et al. 1998; Eynard et al. 2004), so an increase in AS at depth would be expected eventually under long-term alfalfa.

Alfalfa in rotation affected MWD in the spring of 2017 but not 2018. In 2018 there was no zero alfalfa treatment, which may explain the lack of treatment effect that year. The results of the regression analysis support this. One other difference between the two years was precipitation. The spring of 2018 was much drier than 2017 and soil gravimetric water content was lower in 2018 than 2017 (Table 5). The lower precipitation and soil water content leading up to sampling in 2018 could have affected aggregate formation and cycling at that time. Since, in this study, samples were air dried before wet sieving, the water content at sampling time should not affect the aggregate stability measuring process, but it would affect aggregate formation prior to sampling. A review by Amézketa (2010) found that the water content of the soil at time of sampling influenced measurements of AS, but that the nature of the relationship was not clear because in some studies higher soil water content increased AS whereas in others AS was decreased with higher soil water content. In terms of the effect of water content on the stability of aggregates in the field, aggregates with higher water content tend to be more resistant to dispersion due to slaking, but more susceptible to being disrupted by impacts such as rain drops as wet aggregates have lower shear strength (Amezketa 2010). Kay et al. (1994) found that AS decreased with the number of wetting (rain) events prior to sampling, and that long-term management practices affected the responsiveness of the aggregates to wetting events. The effect of wetting events was strongest in soils with lower stability, and smaller aggregates were less likely to fragment (Kay et al. 1994). In the present study, precipitation having a greater fragmenting effect on larger and weaker aggregates could perhaps be one reason why there was more differentiation between treatments in 2017, the wetter year. Campbell et al. (1993a) found higher wet AS in drier growing seasons. Kay et al. (1994) found that AS was highly variable between years and that the variability due to weather was frequently greater than that due to the effects of management practices.

Another point of interest is that a strong wind erosion event occurred at Glenlea in April 2018. This could have affected the surface soil of some of the plots. Since these plots were covered in alfalfa stubble they likely would have accumulated rather than lost soil. This would have meant more fine soil particles on the surface of the soil, and should have affected only a small fraction of the 0-10cm cores. Where there was wind deposited soil it was moved aside in order to sample from the original soil surface downwards, as judged by the alfalfa stubble.

In this study an effect of alfalfa on MWD was seen in in the top 10cm of soil in 2017. Other studies have found short periods of alfalfa and other forages to increase AS. In soils that had previously been regularly ploughed, Angers et al. (1992) found that MWD increased during the first three to four years of alfalfa and then stopped increasing in the fifth year. Pena-Yewtukhiw et al. (2018) found that during a three year grass-legume sod intervention aggregation was improved during just the first 2 years, while the third year had little effect. Guo et al. (2010) found that three different perennial treatments, alfalfa, sweet clover, and natural regeneration, grown after many years of annual grains, all resulted in higher wet AS in the soils after five years. Some studies show more mixed results in terms of the impact of perennial forages on AS. After just one year of alfalfa intervention, in a ploughed organic system, Guidi et al. (2017) observed an increase in large macroaggregates in the most degraded soils but not in the less degraded soils. Similarly to the present study, where AS was variable between replicates and years, Sparling et al. (1992), in a study surveying several farms, found that the effects of short periods of pasture (1-4 years) on AS were highly variable. AS also appeared to depend more on the cropping history prior to the pasture phase than the length of the pasture phase, and, after four years of pasture, AS was still lower than in soils under long-term pasture at all of the sites (Sparling et al. 1992).

There are two main mechanisms by which the alfalfa rehabilitation phase could affect AS. One is due to the perennial nature of alfalfa, which increases the time that living roots are in the soil, plant cover is on the surface, and increases root biomass with consequent effects on SOC and the rhizosphere microbial community. The other is reducing tillage frequency, which was discussed previously. Grover (2008) found that the proportion of the year with living roots in the soil was the most important predictor of AS (percent water stable aggregates >.25mm), compared to tillage frequency, MBC, SOC, or soil water content. Alfalfa plants have deep root systems, and root exudates and decomposing tissues feed the soil microbial community, which is important in forming and stabilizing aggregates. Bacteria are particularly important in the formation and stabilization of microaggregates, which are more resistant to cropping practices than larger aggregates (Tisdall 1994). After adding alfalfa to a grass pasture mixture, Hurisso et al. (2013) observed an increase in MWD which was correlated with increases in MBC but not SOC, demonstrating the importance of soil microbes. Roots and hyphae particularly form large macroaggregates which are sensitive to destruction by agricultural cropping practices (Tisdall 1994). Alfalfa forms associations with arbuscular mycorrhizal fungi (AMF) which could further increase its potential to form aggregates. In pot studies, alfalfa and other forage legumes (lupine [*Lupinus augustifolius* L.] and white clover [*Trifolium repens* L.]) have been found to have a strong positive effect on AS relative to other crops including wheat, maize, tomato and several grasses (Reid and Goss 1981; Haynes and Beare 1997). In both cases, the legumes had lower root mass and length compared to the other crops but were associated with more fungal hyphae (mycorrhizal in the case of alfalfa and likely saprotrophic fungi in the case of the aggregates associated with lupine) and thought to have a different microbial community in the rhizosphere, which could be related to higher nitrogen content in the legume root exudates. Continuous alfalfa can also increase OC and N in aggregates compared to wheat only and legume grain rotations (Chu et al. 2016).

Although this study demonstrated that the alfalfa improved aggregate stability compared to having no alfalfa (Figure 5), it did not show a change in MWD with the number of years in alfalfa when there was no zero alfalfa treatment. It is possible that it could take more than three or four years of continuous alfalfa to have a large impact on AS at GLTR. As seen in chapter three, the perennial grasslands at the GLTR had much higher AS compared to the arable plots, but the grasslands had been in place for 25 years, much longer than the alfalfa plots. As well as being established for a much longer time, the grassland plots may also have increased AS more than the alfalfa because they were composed of a mix of mostly grasses and some forbs, which some studies have found to have a stronger aggregating effect than legumes (Eviner and Chapin 2002; Rilig et al. 2002). That AS did not increase with number of years in alfalfa could be because the soils at Glenlea are vertisols and so aggregate formation is likely largely dependent on physical processes rather than biotic aggregating agents (Oades 1993). In a study also on vertisols, Novelli et al. (2012) found that organic carbon inputs only increased AS after SOC reached 35 g C / kg in the soil. Below that level, SOC was not important in increasing AS. So it may take more than four years of alfalfa to reach the threshold level of SOC needed to see a consistent increase in AS in the soils at GLTR. In 2009, the organic annual plots at GLTR contained 29.7 g C / kg at 0-15cm depth (Bell et al. 2012).

4.6 Conclusion

The presence of an alfalfa stand for the purpose of soil rehabilitation in an organic annual grain rotation increased MWD in the top 10cm of soil, but MWD was not affected by the number of years the alfalfa stand had been in place. In 2018 there were small changes in some size fractions of aggregates but not others between years three and four in alfalfa. The limited effect of the alfalfa phase could be due to the vertisolic soils at GLTR having a low response to OM inputs in terms of aggregate formation. That the alfalfa phase was only four years long could compound this, since in this soil type it may take longer to increase AS through biotic means. It could also be that alfalfa is not the best species for forming aggregates, perhaps more success would be found with a mixture of grasses and forbs. However, the other functions of alfalfa in terms of rehabilitating an organic annual grain rotation, such as nitrogen fixation and organic matter production, must be balanced with its potential to improve soil structure. Given the variation in AS between treatments and years, maybe partly due to precipitation, it is possible that more clear conclusions could be drawn from studying additional seasons. It would also be interesting to measure AS in the first annual crop following the alfalfa intervention and compare this to the corresponding plot that had no alfalfa intervention.

5. EFFECT OF SELF-REGENERATING BLACK MEDIC COVER CROP ON AGGREGATE STABILITY AT A LONG-TERM STUDY IN SOUTHERN SASKATCHEWAN

5.1 Abstract

Cover crops are an important strategy to improve annual grain rotations. Black medic has been used as a self-regenerating cover crop because it produces large amounts of seed and regrows from the seed bank each year, eliminating the need to re-seed a cover crop every year. Little is known about how this cover crop affects the soil, especially physical properties including aggregate stability, an important measure of soil health. This study looked at the effect a self-regenerating black medic cover crop on AS in a no-till system. Wet aggregate stability (AS) was measured in 2017 at a long term experiment in Indian Head, Saskatchewan. This experiment consisted of a no-till grain rotation (wheat-flax-canaryseed) with and without self-regenerating black medic cover crops grown under two nitrogen fertilizer levels. Soil from a nearby fence line that had been under long-term grass was also sampled. Wet AS was measured with a wet sieve shaker with stacked sieves to determine mean weight diameter (MWD) of stable aggregates. The medic cover crop increased MWD by 21% in the low N treatment of the wheat phase of the rotation. The high N treatment also increased MWD in the wheat phase. In the flax phase, high N fertilizer inputs and the medic cover crop did not increase AS.

5.2 Introduction

Cover cropping is an important strategy to improve annual grain systems. In the northern prairies they function to have growing plants in the soil during the fall, winter and spring seasons, between crops of annual grains (e.g., Thiessen Martens et al. 2001; Cicek et al. 2015). This keeps living plants in the soil during the portion of the year that it would otherwise be bare, replacing winter fallow. The many benefits of cover crops include protecting soil from erosion, adding OM to the soil, adding nitrogen if they are legumes, retaining nutrients, and supporting the soil biotic community (Schipanski et al. 2014). Cover crops bring some of the benefits of perennials to annual systems and have long-term soil building and ecological benefits which may be best seen in long-term studies where benefits that take many years to accumulate can be detected.

One barrier to adoption of cover crops by farmers is the cost of seed and seeding every year. A system that addresses this is self-regenerating black medic (*Medicago lupulina* L.) which produces large amounts of seed from which it regrows each spring, or when used in a wheat fallow system where medic germinates in autumn then overwinters to form a self-regenerating cover crop the following year (Sims, 1988). Black medic is a low growing legume naturalized to North America that grows under the crop canopy during the growing season and then keeps growing in the fall after harvest. It provides soil cover during the fall and winter and also fixes nitrogen. It has been found to increase crop yield when nitrogen fertilizer inputs are low (May and Entz 2016) and thus is of interest in low input and organic agriculture. Less is known about how such a cover crop affects soil health. Aggregate stability (AS) is an important measurement of soil health as it is relates to soil structure, water infiltration, soil erosion susceptibility, and carbon cycling in the soil (Six et al. 2004). The effects of other cover crops on aggregate stability have been mixed and vary with cover crop species and management factors (e.g., García-González et al. 2018; Blanco-Canqui et al. 2011; Kabir and Koide 2000; Sainju et al. 2003). Thus, there is interest in how this unique self-regenerating cover crop system influences aggregate stability in a no-till grain rotation.

This study took place at a self-regenerating black medic cover crop experiment which was established at the Agriculture and Agri-food Canada experimental farm in Indian Head, Saskatchewan in 2002. The objective was to see if fifteen years of the black medic cover crop influenced aggregate stability in a no-till site at two nitrogen fertilizer levels. The hypothesis was that the black medic cover crop would increase aggregate stability. The mechanism was postulated to involve increased nitrogen and carbon inputs to the soil with medics, an increase in living roots that support the soil microbial community for a greater portion of the year, and increased surface cover.

5.3 Methods

5.3.1 Study Site

This study took place at the Indian Head Long-term Black Medic Rotation (principle investigator Bill May), at the Agriculture and Agri-food Canada Research Farm in Indian Head, Saskatchewan. The soil is a Rego Black Chernozem, of the soil type Indian Head heavy clay, with 16.3% sand, 20.6% silt, and 63.1% clay, pH of 7.5 and organic carbon concentration of 24.5 g C / kg (Campbell et al. 1993b). The field study was established in 2002, and has a split plot design with presence of black medic (*Medicago lupulina*, cv. ‘George’) cover crop as the main effect, crop type as the sub plot, and nitrogen fertilizer rate as the sub-sub plot. It is a fully phased three year rotation of wheat-flax-canary seed, with three replicates, all under no-till conventional management. The split plots are 10.67m x 4m in size. The wheat cultivar used was Unity (seeded at 250 pl/m2) and the flax cultivar was CDC Bethune (seeding rate 63 kg/ha). The three nitrogen fertilizer rates were 20%, 60%, and 100% of required N based on soil test recommendation. Medic was controlled with Lontrel in non-medic plots. For further details of rotation management, see Lupwayi et al. (2017) and Turmel et al. (2011).

5.3.2 Soil Sampling Procedures

On May 18 and 19, 2017, soil samples for AS and BD were collected from the wheat and flax stages of the rotation, at two N fertilizer application rates (20% and 100% of soil test recommended N), in both the medic and no medic blocks. Soil samples from a fence line near the plots that had been under long term grass cover were also collected as a long-term perennial comparison (four soil cores were taken and bulked in the field). All samples were taken at a depth of 0-10cm in the stubble row from the previous year’s crop, as the plots were seeded with a no-till drill just prior to sampling and these areas had experienced the least soil disturbance. Medic plants had been terminated with herbicide prior to seeding and were not present in the plots at time of sampling. Sampling followed the protocol described in chapter 3. One plot was missing due to a seeding error, so only two replicates were sampled from the medic low N treatment in the flax phase of the rotation.

5.3.3 Soil Analysis

Aggregate stability and bulk density were determined using the same methods as in chapter 3. Due to the presence of coarse sand particles in the Indian Head soils, a correction for coarse primary particles was made after wet sieving. For this correction, the aggregates were mixed with sodium hexametaphosphate and shaken for 45 minutes to disperse aggregates (Angers and Mehuys 1993) after each fraction of stable aggregates was dried and weighed. Samples were then passed through the sieve corresponding to their size fraction to separate out the coarse particles. The mass of the coarse particles was subtracted from the associated stable aggregate sae. This followed the methods in Angers and Mehuys (1993).

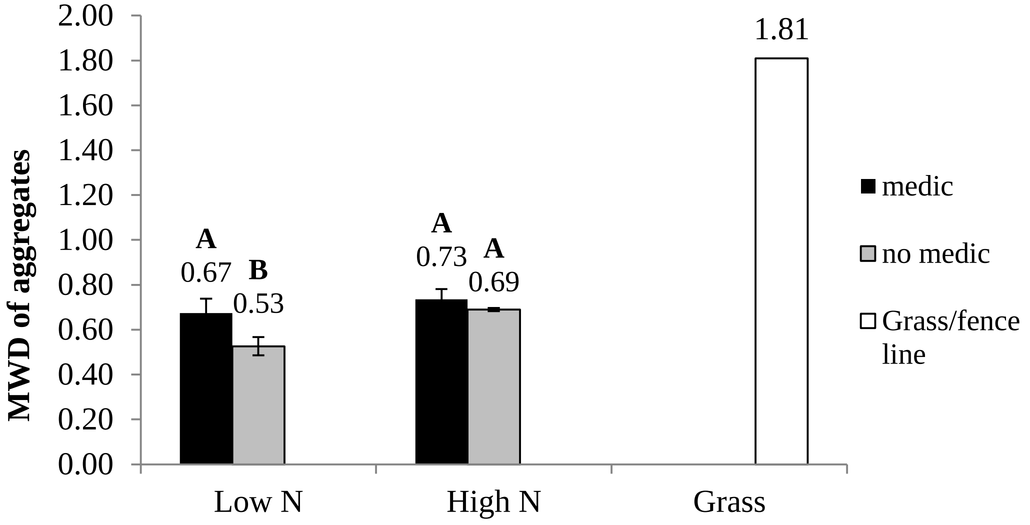
5.3.4 Statistical Analysis

Analysis of variance was performed using PROC MIXED in SAS 9.4 (SAS Institute Inc., 2016) with the experiment as a split plot design. Medic was the main plot effect and N fertilizer rate was the split plot effect. Medic and N fertilizer rate were fixed effects and block and block*medic were the random factors. The two crop phases of the rotation were analyzed separately. Assumptions of the ANOVA were tested for as in chapter 3.

5.4 Results

5.4.1 MWD

A significant medic by fertilizer N rate interaction was observed for MWD in the wheat phase. The basis of this interaction was an increase in MWD with medic in the low N but not the high N fertilizer treatment (Figure 9). Individual main effects were also significant. For example, the cover crop increased MWD by 21% in low N conditions, and the high N rate increased MWD where there was no cover crop. Averaged across N rates, the medic presence increased MWD as evidenced by a significant medic effect (Figure 9).



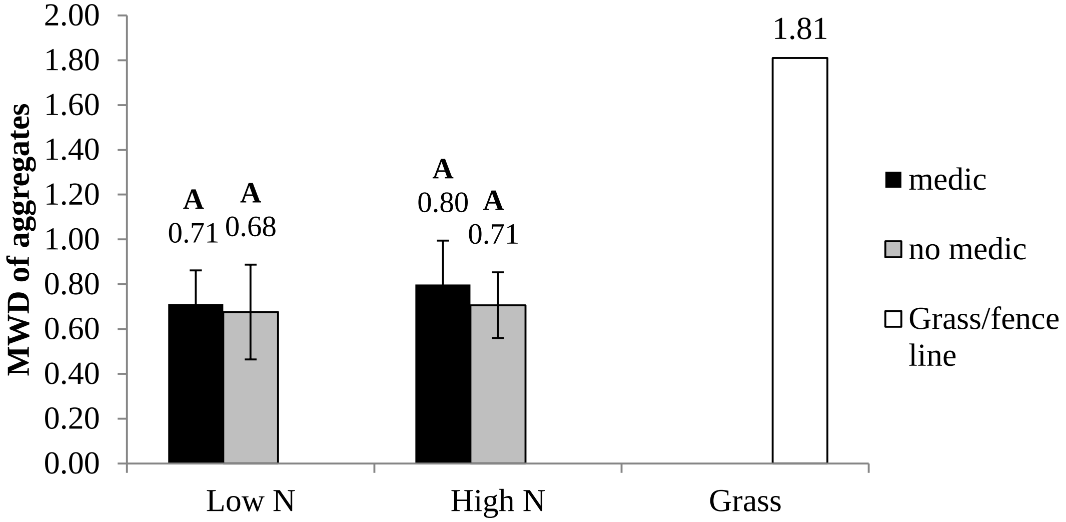
ANOVA highlights SE = 0.02614

Factor	**p-value**
Medic	0.0295
Fert	0.0026
Medic x Fert	0.0421

Figure 9. Mean weight diameter (MWD) of stable aggregates with and without black medic cover crop at two nitrogen fertilizer levels, following the wheat phase of the rotation, at 0-10cm depth. The fence line is included for interest; it was not included in statistical analysis. Tukey-Kramer test used for means separation with alpha value of 0.05. Error bars represent 1 SD on either side of the mean.

No significant medic or N rate effect was observed for MWD in the flax phase (Figure 10).

Soils under long-term grass by a fence line near the plots appear to have much higher MWD than all other treatments, in both the wheat and flax phases (Figures 9 and 10) though this was not statistically analyzed.



ANOVA highlights

Factor	p-value	SE	
Medic	0.6248	Medic low N	0.1294
Fert	0.6341	Medic high N	0.1057
Medic x Fert	0.8325	No medic low N	0.1057
		No medic high N	0.1057

Figure 10. Mean weight diameter (MWD) of stable aggregates with and without black medic cover crop and at two nitrogen fertilizer rates, in the flax phase of rotation, at 0-10cm depth. The fence line is included for interest, it was not part of the statistical analysis. Tukey-Kramer test used for means separation with alpha value of 0.05. Error bars represent one SD on each side of the mean.

5.4.2 Individual Aggregate Size Classes

The size classes of stable aggregates were analyzed individually. In general, in the wheat phase, results showed that high N and medic cover crop increased the proportion of large aggregates and resulted in fewer smaller aggregates. This was especially true when the low N no medic treatment was compared with all others. For example, in the wheat phase there were more stable aggregates of the 0.25-0.5mm class size in the no medic low N treatment than the no medic high N (Table 6). In the 1-2mm size class the no medic low N treatment had fewer stable aggregates than the other treatments and in the 2-6.3mm size class, both high N treatments had more stable aggregates than the no medic low N treatment. In terms of microaggregates and other materials less than .25mm in diameter (includes organic materials and primary particles: everything <0.25mm) the no medic low N plots had more <0.25mm materials than the no medic high N plots.

In the flax phase there was no variation between treatments in any class size (Table 7).

Table 6. Grams of aggregates per size class with and without black medic cover crop and at two nitrogen fertilizer rates following the wheat phase, at 0-10cm depth. Though it was not included in the statistical analysis, the fence line/grassland numbers were included for interest. Alpha = 0.05. Tukey-Kramer post-hoc test.

		<.25mm*		0.25-.5mm		0.5-1mm		1-2mm**		2-6.3mm***	
Low N	**medic**	15.29	bd	7.60	ab	10.30	a	5.20	a	1.61	ab
	no medic	17.73	ab	8.79	a	9.54	a	3.00	b	0.94	b
High N	**medic**	15.53	ac	7.28	ab	9.75	a	5.08	a	2.36	a
	no medic	15.92	cd	7.71	b	9.53	a	4.83	a	2.00	a
Fence line		8.66		2.18		6.82		10.26		12.08	

*sig effect of N fertilizer and interaction but not medic
**sig effect of medic, N fertilizer, and interaction
***sig effect of medic and N fertilizer, not interaction

Table 7. Grams of aggregates per size class with and without black medic cover crop and at two nitrogen fertilizer rates following the flax phase of rotation, at 0-10cm depth. Though it was not included in the statistical analysis, the fence line/grassland numbers were included for interest Alpha=0.05.

		<.25mm		0.25-.5mm		0.5-1mm		1-2mm		2-6.3mm	
Low N	**medic**	14.91	a	8.02	a	9.66	a	5.44	a	1.97	a
	no medic	13.91	a	9.81	a	8.46	a	6.49	a	1.34	a
High N	**medic**	15.13	a	7.54	a	9.04	a	5.25	a	3.03	a
	no medic	14.85	a	8.54	a	9.57	a	4.98	a	2.06	a
Fence line		8.66		2.18		6.82		10.26		12.08	

There were no significant differences in bulk density (Table 8).

Table 8. Bulk density (g/cm^3) with and without black medic cover crops at two nitrogen fertilizer levels after wheat and flax stages of the rotation at Indian Head. Letters indicate statistical significance using the Tukey test for means separation with alpha = 0.05.

		Wheat		**Flax**	
Low N	**medic**	1.20	a	1.28	a
	no medic	1.27	a	1.23	a
High N	**medic**	1.15	a	1.20	a
	no medic	1.25	a	1.12	a

In summary, both a medic cover crop and a high rate of N fertilizer increased MWD in the wheat phase. This was similar to the results within different class sizes, where, in the wheat phase, treatments with medic cover crop and / or high N had more 1-2mm and 2-6.3mm aggregates and fewer 0.25-0.5mm aggregates than the treatment with no medic and low N. The fence line, which had a much higher MWD, had fewer small aggregates and more large aggregates (1-6.3mm) than the arable treatments. This is the same trend seen in the Glenlea grassland (chapter 3).The pattern of non-cover cropped soils having more small aggregates and fewer large aggregates was also found by Nouri et al. (2019).

5.5 Discussion

After 15 years, differences between management practices were only seen in one of the two rotation phases measured, that is, in the wheat but not the flax phase. One of the reasons no changes were observed in the flax phase could be that the flax plots were more variable within treatments and had higher SE than the wheat phase (Figures 1 and 2). There was one particular outlier in rep 1 flax with no medic and low N, in which there were 13.04g of stable aggregates in the 1-2mm size class and only 3.86 and 2.58g each in the other two replicates. The largest amount of 1-2mm aggregates in any of the other treatments was 6.92g (excluding the fence line which had 10.26g). The lack of change seen in the flax phase could also be due to short-term seasonal effects on aggregation, with the difference being related to the crop species effect on AS. Grasses have been reported to have a strong influence on aggregation (Oades 1993) and Materechera et al. (1992) found more large aggregates under ryegrass and wheat compared to pea. Additionally, the crops with higher AS had higher root length density (Materechera et al. 1992). The lower root and straw biomass production of flax relative to wheat (Gan et al. 2009) suggests it could be less effective in forming and stabilizing aggregates, as root growth is very important in this process, particularly in forming macroaggregates (Reid and Goss 1981; Jastrow et al. 1998; Jung et al. 2011). This would mean that the crop year effect was strong relative to the long-term management effects (cover crop and N rate), which were significant in the wheat phase. Some studies have shown that it can take a long time, even decades, before management effects on soil properties are consistent across all phases of a rotation (Janzen et al. 1992; Kiani et al. 2017). However, Campbell et al. (1993b) found that wet AS results reflected long-term management more than very recent management/treatments in the last year. Interestingly, Campbell et al. (1993b) also found that the rapid wetting technique (prior to wet sieving), which is the same method used in the present study, was better at discriminating between treatments, including between arable plots and native grasslands. This was in comparison to a slow wetting technique, also commonly used, which found little difference even between grasslands and arable plots. This, and other studies, supports the methods used in this study.

The interaction between medic cover crop and nitrogen rate was significant in this study. The black medic cover crop increased AS in low nitrogen but not high nitrogen conditions in the wheat phase. Medic biomass was higher in the low N treatment (879 kg/ha) compared to the high N treatment (474kg/ha) averaged over years of the study and the three rotation phases (May and Entz 2016). This was likely partly due to decreased competition with the crop plants, which had higher grain yield in the high N treatment (May and Entz 2016). It makes sense that medic would have more of an effect under low N conditions since the medic had higher biomass, which would mean it probably fixed more N, which would have a greater effect in the low N treatments than in high N treatments which already have an adequate supply of N for crop growth. Additionally, N level only had an effect on AS where there was no medic cover crop. N rate affects the growth of both the crop plants and the cover crop and medic also affects N supply and the growth of the crop plants. Both factors increase nitrogen which can increase biomass, but the high N rate didn't increase AS when medic was present. This is supported by the findings of May and Entz (2016) which found that the medic cover crop increased grain yield with the 20% N rate but not at 60% and 100% N rates. It makes sense because at higher N rates the crop biomass outcompetes the medic and at low N rates the medic supports crop growth with biological N fixation. Other studies have found interactions between cover crops and nitrogen rates. For example, King and Blesh (2018) found that the effect of cover crops on SOC was slightly increased with low rates of N application (<75 kg N / ha/ year) compared to higher rates (>75kg/ha).

Cover crops have been found to increase AS in other studies (Blanco-Canqui et al. 2011; Hermawan and Bomke 1997; Kabir and Koide 2000; Villamil et al. 2006) and can do so through several mechanisms. They increase the proportion of the year that has vegetative soil cover and living roots. Roots and associated hyphae are important in the formation of macroaggregates (Reid and Goss 1981; Tisdall 1994) and provide organic matter inputs to the soil. Cover crops have been found in a meta-analysis to increase SOC (King and Blesh 2018) and they also influence soil organisms which produce organic binding compound compounds, for example polysaccharides (Lal 2015). At the black medic experiment site at Indian Head, the black medic cover crop increased bacterial diversity and changed the soil bacteria community structure (Lupwayi et al. 2017), but had no effect on AMF colonization of roots, which was high throughout (Turmel et al. 2011). Legume cover crops such as black medic increase soil nitrogen via biological fixation. This can increase crop biomass which could further increase AS through increasing plant residue and exudates. Legumes have been found to increase AS, particularly in soils with lower OM (Campbell et al. 1993 b). As well as initial soil conditions, the effect of cover crops on AS varies with the plant species that make up the cover crop. Five years of winter and spring triticale, spring lentil and pea cover crops were observed to increase wet AS while winter lentil was not (Blanco-Canqui et al. 2013). García-gonzález et al. (2018) found more large water stable aggregates with a barley cover crop than vetch after 10 years. In some cases cover crops have had no effect on AS (Nunes et al. 2018; Mendes et al. 1999; Sainju et al. 2003; Subbian et al. 2000). In two of these studies cover crops were being grown in tilled vegetable systems, which generally require more soil disturbance than grain crops (Mendes et al. 1999; Sainju et al. 2003), and in several cases only dry AS was measured (Mendes et al. 1999; Sainju et al. 2003; Subbian et al. 2000), which can be less destructive of aggregates than wet sieving (Sainju et al. 2003). However, Nouri et al. (2019) found an increase in dry AS but not wet AS after 34 years of cover crops.

In this study, a higher rate of N fertilizer increased AS where there were no cover crops but not where there were cover crops. A higher N fertilizer rate could increase AS by increasing biomass production of the crop plants. This in turn would affect the amount of residue and organic materials entering the soil, which can be important binding agents for stable aggregates. Nitrogen fertilizer rate also affects the soil bacterial community (Lupwayi et al. 2017) which could affect aggregation. However, results in the literature are mixed. Guo et al. (2019) found that NPK fertilizer tended to increase MWD and Subbian et al. (2000) found a very slight increase in dry MWD but no change in percent dry stable aggregates with nitrogen fertilizer. Others found no effect of NPK fertilizer on AS (Eviner and Chapin 2002; Mi et al. 2018), whereas manure and straw did increase MWD (Mi et al. 2018). Blanco-Canqui et al. (2011) also found no effect of four different levels of N application rate on AS.

The effect of fertilizer on AS can depend on the amount of fertilizer and initial soil fertility. High levels of N fertilizer (202 kg N / ha / y) have been observed to decrease AS (proportion of macroaggregates) and lower levels of N fertilizer (67 kg N / ha / y) to have no effect in soils where switchgrass was being grown for biomass (Jung et al. 2011). In addition to lowering AS, high levels of N fertilizer decreased root weight and length and increased SOC, whereas the lower N rate had no effect on root weight or length but still increased SOC. This suggested that root growth was more important than SOC in forming stable aggregates in those soils (silt loam), and that high N rates decreased root growth (Jung et al. 2011). Contrarily, Sainju et al. (2003) found higher MWD with full as opposed to half N rate. Campbell et al. (1993b) found that fertilizer increased wet AS at a less fertile site but not at a more fertile site. AS was generally increased by any practices that increased organic residue input to the soil, suggesting that organic binding materials were important in forming aggregates in those soils (Campbell et al. 1993b). The soils in the present study are fairly low in organic carbon (24.5 g C / kg) and they are responding to full rates of N fertilizer, so increased AS is likely associated with increased organic matter production and organic binding agents.

The soils under long term grass measured in this study were not part of the experimental design and as such not replicated or included in the statistical analysis. Perennial grasslands, as the native ecosystem of the study region, have an important role as benchmarks for soil health. Cover crops are a tool to improve an annual system with the goal of making it more similar to a perennial system in terms of ecological functioning, while retaining an acceptable yield. Thus it is of interest to compare soils in a no-till cover cropped system to those under long-term grasses.

It is interesting that after fifteen years the arable plots had much lower AS than the fence line despite being managed with no-till. This is supported in the literature, as studies show that soils under long-term grasslands or native sod have greater AS than those under no-till management (Six et al. 1998; Eynard et al. 2004, Vezzani et al. 2018) and this has been attributed to the greater root development of perennials compared to annuals (Eynard et al. 2004). Part of the increase in AS at the fence line could be due to higher OM from accumulation of wind-blown soil (May, pers. comm.), but it is also likely related to the perennial nature of the grasses near the fence line. Perennial grasslands ability to increase AS compared to annual cropping systems is supported in the literature (Wright and Anderson, 2000; Vezzani et al. 2018; Miller and Jastrow 1990), as well as in chapter 3 of this thesis. Grover (2008) found that the proportion of time with roots in the soil had a greater effect on wet AS than the frequency of tillage events. The same study also found that having living roots in the soil for a greater period of time was more strongly related to AS than SOC content (Grover 2008) which supports the function of perennials, not just the higher OM, in increasing AS in the fence line. The medic cover crop also increases the proportion of the year with living roots in the soil, and it did increase AS in the wheat phase of the rotation. However the medic is terminated and regrows from the seed bank every spring so it still functions as an annual, growing a new root system each year, so it makes sense that the fence line soils had higher AS than those under the cover crop.

5.6 Conclusion

The medic cover crop increased AS with low nitrogen application in the wheat phase of the rotation but not the flax phase. High nitrogen rate also increased AS where there was no medic cover crop in the wheat phase. A common mechanism whereby the medic cover crop and higher nitrogen fertilizer rate may have increased AS was through increased soil N, though mechanisms of N enrichment are different. The medic may also have contributed to higher AS through increased root growth throughout the year and by providing winter soil cover. The lack of response to treatments in the flax phase could be due to higher variation and crop specific effects. The fence line soils had much higher AS than the arable plots, likely due to greater root development and higher soil OM. This study shows that a self-regenerating black medic cover crop can improve AS in low N no-till conditions.

6. GENERAL DISCUSSION AND CONCLUSIONS

6.1 General Discussion

The much higher AS of the grasslands at both study sites demonstrates the importance of long-term perennials compared to annuals in improving soil health. It also shows the much greater magnitude of difference between the arable and long-term grassland plots than between arable treatments. Additionally, the cover crop study showed the difference between arable and grassland soils in an untilled environment, suggesting the greater importance of roots and perenniality than tillage practices. This evidence supports the role of native perennial plantings in agroecosystems.

At GLTR, the perennial rotations (two years of alfalfa and two years of annual grains) had a perennial crop growing fifty percent of the time, yet in most cases the perennial rotations had similar AS to the annual rotations, and much lower than the grasslands. While these plots would have been in alfalfa for approximately 12 of the past 25 years, the perennial phase was discontinuous, and had a minimal effect. It may be that the two year annual grain phases and tillage countered much of the potential positive effect of the alfalfa/grass forage phases. The four year stand of alfalfa in the alfalfa intervention study allowed for measurement of AS after one to four years of alfalfa forage that had not been disturbed by tillage. In the first year of sampling one to three years of alfalfa increased AS compared to soils that had no alfalfa. However, no difference was seen with increasing number of years in alfalfa. This evidence suggests that though the first year of alfalfa increased AS (and it was a small increase), perhaps alfalfa stands of longer than four years could be beneficial. In the Northern Great Plains, Entz et al. (1995) reported that the average stand length of alfalfa and alfalfa/grasses was six and a half years in Manitoba and Saskatchewan. Development of perennial grains and grass-fed ruminant production systems offer new potential avenues to increase perennial plantings and perhaps also perennial stand length.

Part of the reason for including a cover crop in annual rotations is to ‘perennialize’ the rotation; i.e., to bring some benefits of perennials to an annual crop system. Cover crops in grain rotations could act as an intermediary between annual grain cropping and perennials. At the cover crop rotation in IH, the black medic cover crop did indeed increase AS with low N inputs in one phase of the rotation. These results show the benefits of cover crops for AS in low input systems. This supports an important role for cover crops in organic systems.

The AS results in these studies were variable, with changes due to treatments seen in only one out of two years (experiment 2) or in only one of two phases of a rotation (experiment 3) or very little change from treatments after 25 years (experiment 1). What does this say about the value of this measurement? It could be that the limited number of replicates (three in all cases) was not enough to distinguish treatment effects from the natural variability of the soil, as AS is known to be quite a variable soil property. The other important factor is soil type at GLTR, which may have been responsible for the very minimal effects seen in the two experiments at that site (1 and 2). If organic aggregating factors were not important in these soils then it makes sense that not much change was seen due to the cropping practices used. Perhaps other soil health parameters would be better suited to detecting changes in the Glenlea soils. For example, since aggregation may not be strongly linked to SOC inputs in vertisols (at least at low levels), fractions of SOM, particularly those which respond more rapidly to management changes, such as light fraction SOM, could be directly measured instead. Other measures of soil structure such as infiltration rate could also be useful, and have been found to be particularly responsive to management changes (Stewart et al. 2018). Biotic indicators, such as enzyme assays, MBC and MBN may also be useful, and showed responsiveness to management changes in a meta-analysis of soil health indicators (Stewart et al. 2018).

Another point to resolve is to what extent AS was influenced by short-term seasonal factors, as opposed to the long-term rotation treatment effects. What was the relative influence of each in these studies? Seasonal factors that could affect AS are crop species and weather. In terms of weather, soil moisture, number of wetting and drying events, and wind erosion could all influence AS. Can crop species have such a strong effect that it can mask long-term rotation effects? The use of long-term experiments is important in these cases.

6.2 Future Research

It would be interesting to measure other variables in the soils of the same treatments at the two study sites to determine what factors were important in aggregate formation and stabilization in those soils. Measurements of interest would include SOC, MBC, and light-fraction carbon to see which if any carbon pools were related to AS. It would also be interesting to measure root biomass and length to see how important living roots were to AS, and relate this to the relevant carbon pools. Pot studies could also be done with the Glenlea soils to compare the effects of physical (relating to wetting and drying cycles of the clays) and chemical/biotic (sugars, residues, plants) factors on aggregation on GLTR soils.

It would also be interesting to learn more about how the treatments in these studies affected soil organisms, particularly mycorrhizal fungi and bacteria, and how soil biota relate to AS. An initial goal of this project was to look at AMF diversity in the treatments at GLTR. AMF are important in macroaggregate formation (the type of aggregate most influenced by agricultural management in terms of both formation and destruction) and are known to be affected by agricultural practices. In fact, higher AMF colonization (Entz et al. 2004) and spore abundance (Welsh, 2007) were found in the organic treatments at GLTR than in conventional, but species diversity and community structure have not been studied. Flax root samples were taken from the treatments at GLTR as part of this MSc. study. DNA was extracted from the roots and evidence of AMF was found using PCR. However, sequencing of a number of samples showed very little AMF DNA.

The treatments at Glenlea had notable, though insignificant, differences in bulk density. These were not taken into account in the study because the AS measurements were done on a mass basis. What are the implications of having similar AS between, for example, the annual and perennial rotations, but lower bulk density in the perennial rotations? It may be interesting in the future to try measuring AS on a volume basis.

This study found small or no effects of one to four years of alfalfa on AS in studies one and two. It would be interesting to determine how many years it would take to see a strong effect of alfalfa on AS in the soils at Glenlea. It would also be interesting to see how many years of perennials would be needed in an annual grain rotation to improve soil health/AS, and then to see how many years of annual crops could be grown between perennial phases to maintain soil quality. In the shrink swell clays at GLTR many years may be required before high enough SOC is achieved. It would also be interesting to compare the aggregating effects of different forage species and mixtures in the field. Would alfalfa ever improve AS at GLTR? The organic alfalfa plots had lower SOC concentration than the annual and conventional rotations in the top 15cm (Bell et al. 2012), though SOC is not always correlated to AS.

It could be interesting to sample the alfalfa rehabilitation study again in subsequent years, as the conditions were different between the two years. Also, it was too early in the sequence to do so, but measuring the effect of the alfalfa phase throughout the four years of grains following it would be interesting. First to see the difference between them and the controls that had no alfalfa phase, to see if there was any effect at all, and then if there was to see how many seasons it lasts.

6.3 Conclusions

At GLTR the grasslands had much higher AS than any of the arable treatments. The perennial phase in the organic system increased MWD at 10-20cm depth. In a few cases some size classes of aggregates from the perennial treatments were similar to the grassland but there was very little difference between arable treatments. The second study at GLTR looked at one to four consecutive years of alfalfa without tillage, and found that AS did improve in the top 10cm of soil compared with the system of ‘zero alfalfa’, but not with additional years in alfalfa. Therefore, some AS improvement with alfalfa was detected.

At the second study site, in SK, a self-regenerating black medic cover crop increased AS in low nitrogen input conditions in one phase of the rotation but not the other. High N rate increased AS with no cover crop but not with a cover crop. This site, unlike GLTR, was managed using no-till. Despite this, grass covered areas near the plots had much higher AS than the grain plots with or without cover crops. At GLTR the benefit of the grassland was seen as well. The strongest point to be drawn from this work is the huge gap in AS between all of the arable treatments, including those with best management practices that should in theory improve soil quality, and the grasslands. It shows that, at least in the heavy clay soils of these two long-term rotations, short perennial phases, manure inputs, and cover crops, though they can show significant though slight improvements in some cases, do not improve AS nearly as well as long-term perennial grasses.

7. REFERENCE MATTER

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7.2 Appendices

7.2.1 Appendix A. List of Abbreviations

AMF	arbuscular mycorrhizal fungi
AS	aggregate stability
CT	conventional till
GLTR	Glenlea Long-term Rotation
GMD	geometric weight diameter
GRSP	glomalin related soil protein
MBC	microbial biomass carbon
MBN	microbial biomass nitrogen
MWD	mean weight diameter
NT	no till
OM	organic matter
SOC	soil organic carbon

7.2.2 Appendix B. Crop Biomass at Glenlea Long-term Rotation 2008-2018

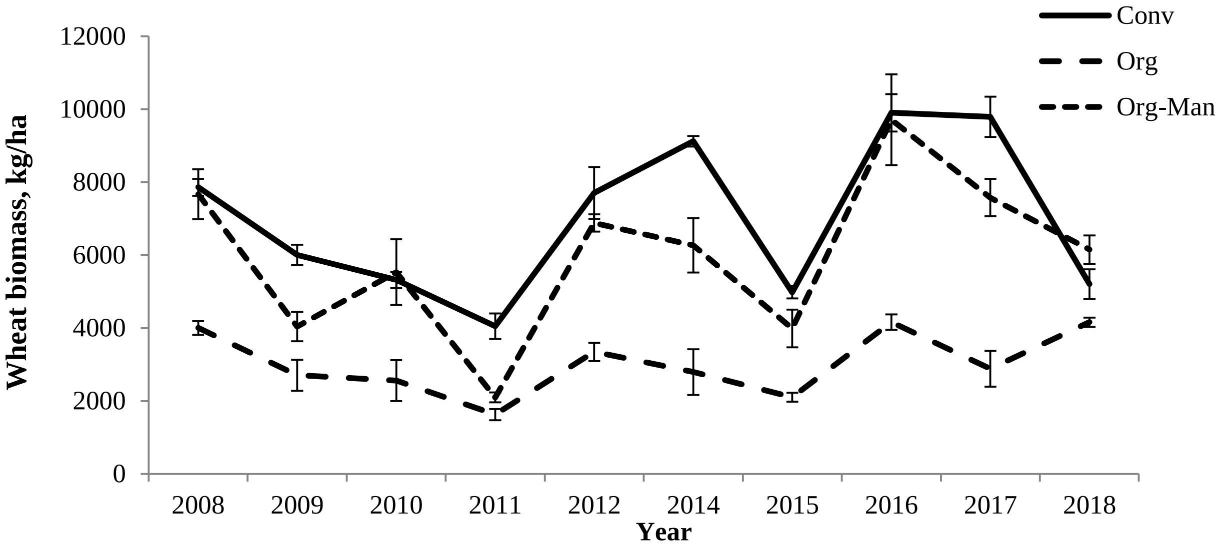

Figure 11. Biomass (kg/ha) of wheat crop at Glenlea Long-term Rotation from 2008-2018. The three treatments are conventional management, organic management, and organic management with composted manure additions.

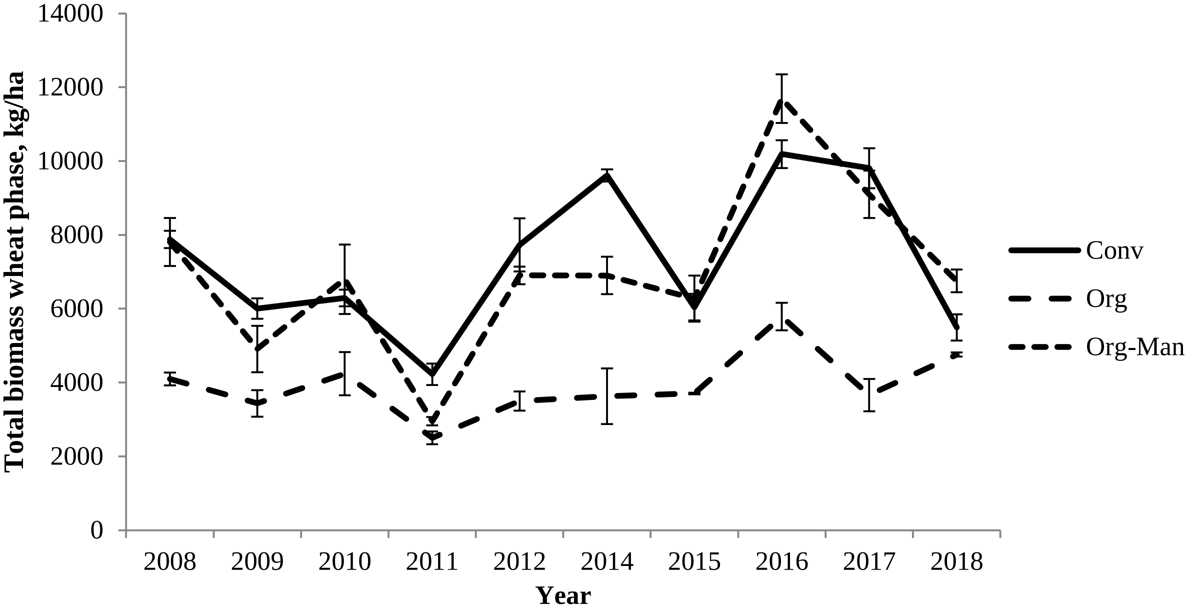

Figure 12. Total biomass (kg/ha) of wheat crop plus weeds at Glenlea Long-term Rotation from 2008-2018. The three treatments are conventional management, organic management, and organic management with composted manure additions.

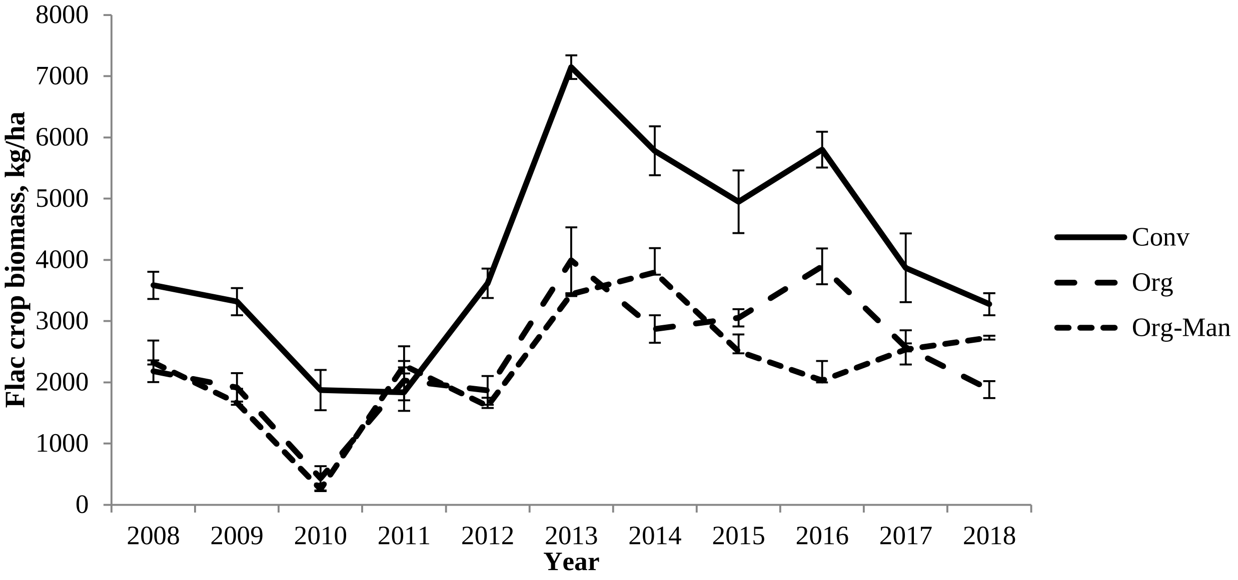

Figure 13. Biomass (kg/ha) of flax crop at Glenlea Long-term Rotation from 2008-2018. The three treatments are conventional management, organic management, and organic management with composted manure additions.

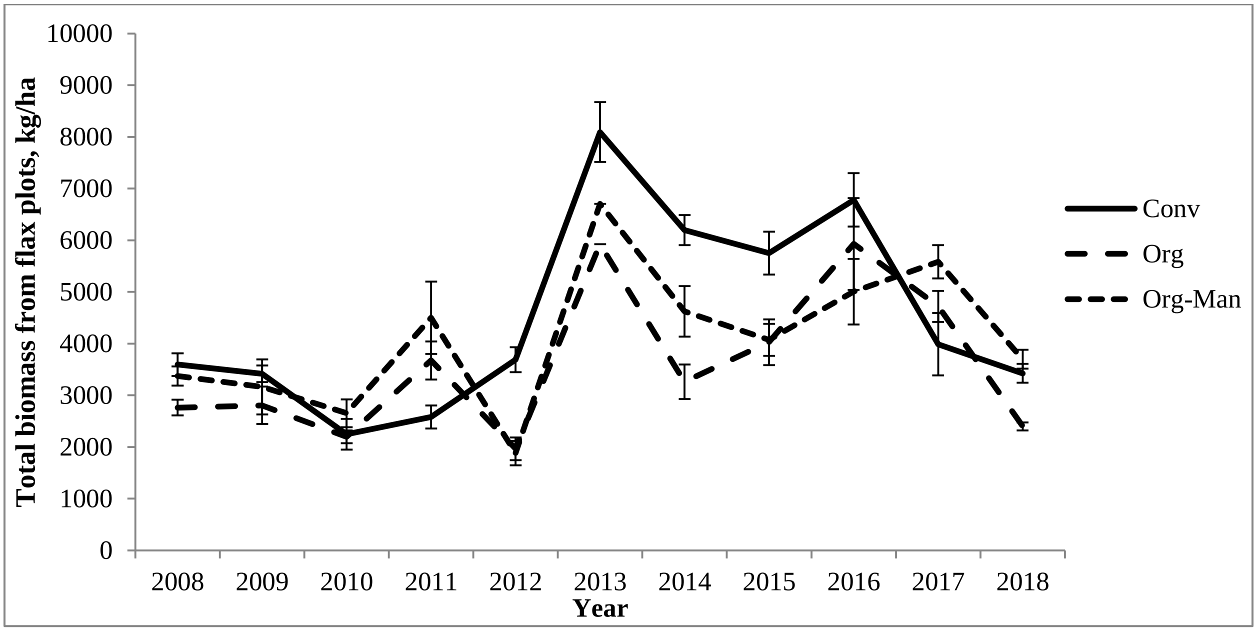

Figure 14. Biomass (kg/ha) of flax crop plus weeds at Glenlea Long-term Rotation from 2008-2018. The three treatments are conventional management, organic management, and organic management with composted manure additions.

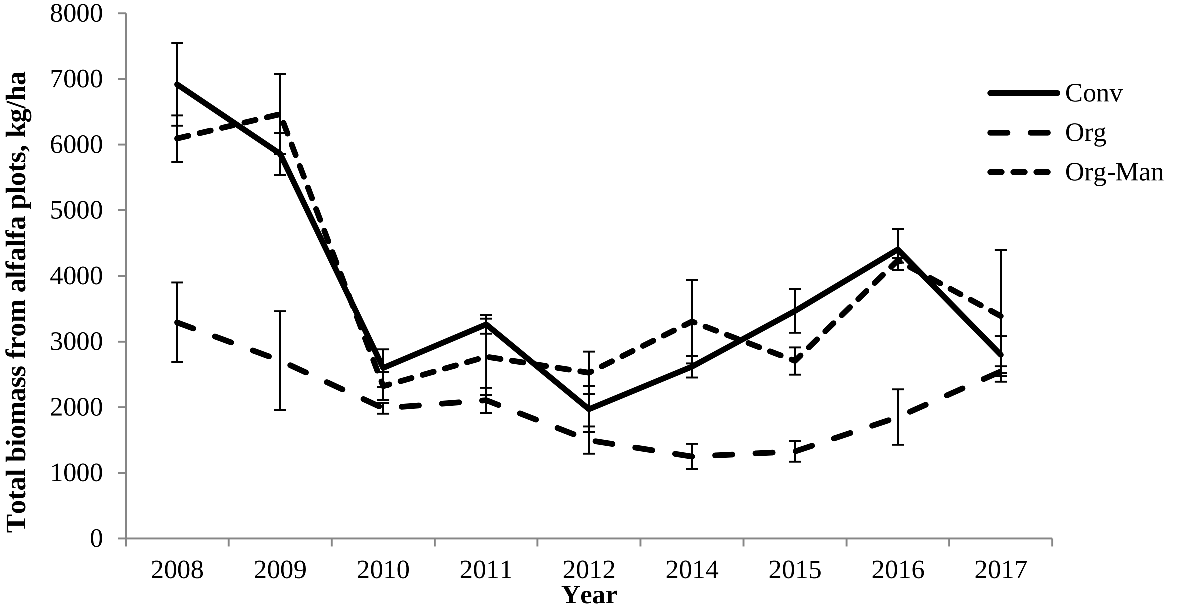

Figure 15. Total biomass (kg/ha) of year one alfalfa plots (includes other forages planted with alfalfa and weeds) at Glenlea Long-term Rotation from 2008-2018. The three treatments are conventional management, organic management, and organic management with composted manure additions.

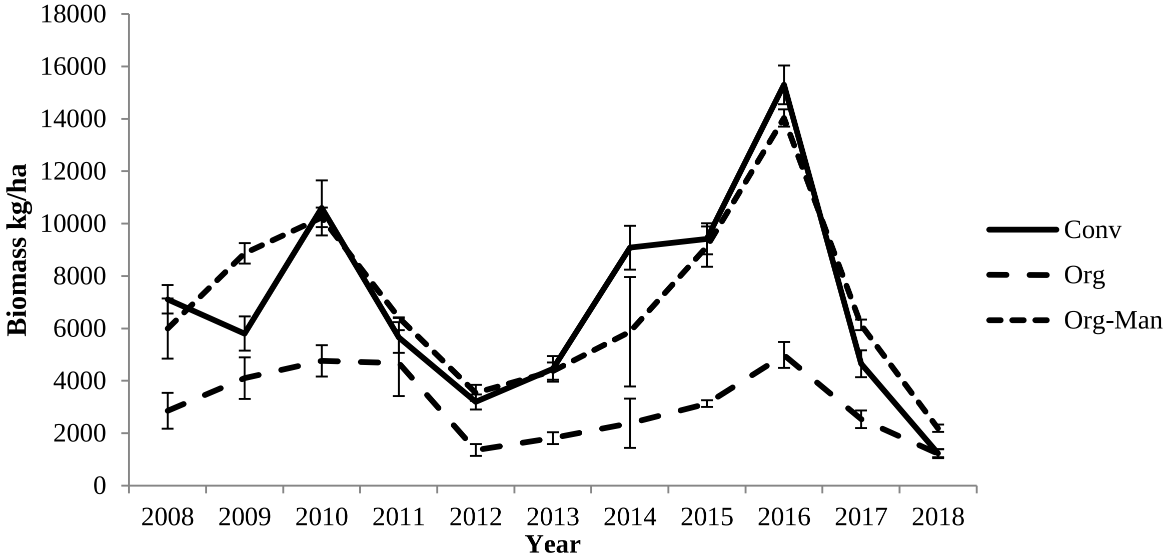

Figure 16. Total biomass (kg/ha) of year two alfalfa plots (includes other forages planted with alfalfa and weeds) at Glenlea Long-term Rotation from 2008-2018. The three treatments are conventional management, organic management, and organic management with composted manure additions.