# Surface crop residue effects on fungal hyphal length, soil organic carbon and soil moisture in oat (*Avena sativa*) plots.

A Thesis submitted to the Committee on Graduate Studies

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# Abstract

**Surface crop residue effects on fungal hyphal length, soil organic carbon and soil moisture in oat (*Avena sativa*) plots**

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Oats (*Avena sativa*) were grown in outdoor plots in southern Ontario in 2003 and 2004 to determine if fungal hyphae growth associated with surface residue increased soil moisture and OC through soil aggregation and if the type of residue (oat straw, mixed hay, corn stalks, compost) and plant cover (weeds, alfalfa, oats) altered fungal growth and the soil physical properties as well. OC increased significantly with dried surface residue and alfalfa ground cover over the season of the oat crop in 2003 while the oat plants had a greater effect than surface residue on OC in 2004. Soil moisture and aggregation were increased from straw and corn residue in 2004, but were reduced by the growing oat crop. Hyphal length increased only with interaction of the oat plants and residue in August, 2004 from the 2-way ANOVA analysis. The study results suggest that resistance to decomposition with high residue C/N and surface application is more important to soil OC than the amount of decomposition in moist, sandy, high OC soil conditions.



**Keywords**

Soil organic carbon, macroaggregates, mycorrhiza, fungal hyphae, surface residue, soil moisture, density fractions, oats, no-till

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Our children motivate us beyond our own comprehension. Through the eyes of my son Dan, who set a fine example of ambition and perseverance, there are no limits to what can be achieved. He was always there to make the technicalities of math and computers seem easy. It was suiting that he commented, on a previous undergraduate assignment, that my writing had the aura of a bulldozer. While I have attempted to remove the roughness from the writing process, I will remember the analogy of making a path.

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# Abbreviations

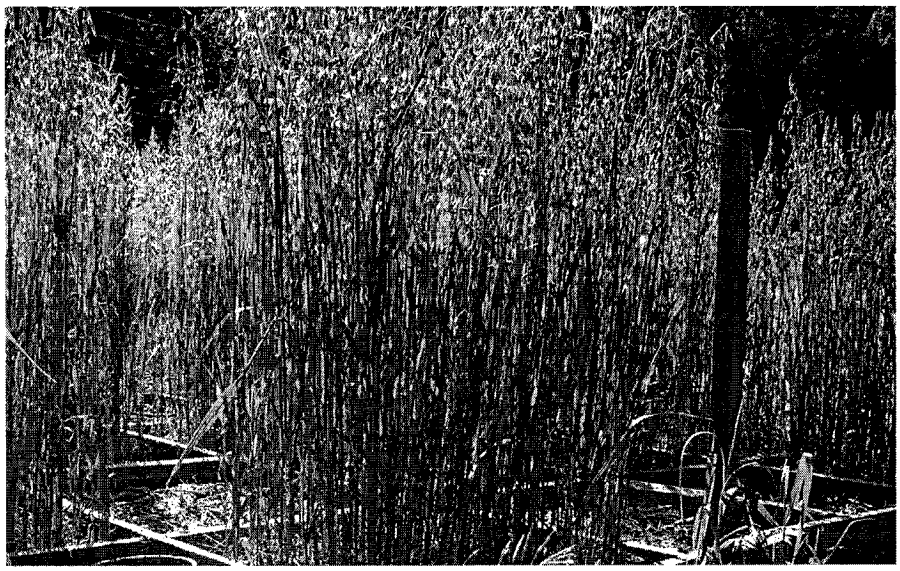
| | |
|---|---|
| AM(F) | Arbuscular mycorrhizal (fungi) |
| $C_3$ | Plants that fix $CO_2$ by the Calvin cycle (in daylight) |
| $CO_2$ | Carbon dioxide |
| C/N | Carbon to nitrogen ratio |
| CT | Conventional tillage |
| EM | Ectomycorrhiza |
| HF | Heavy fraction organic matter (>1.2 g $cm^{-3}$ specific gravity) |
| KOH | Potassium hydroxide |
| L | Litres |
| LF | Light fraction organic matter (< 1.2 g $cm^{-3}$ specific gravity) |
| LOI | Loss on Ignition |
| LSD | Least significant difference statistical post-hoc test |
| MBC | Microbial biomass carbon |
| MWD | Mean weight diameter |
| N | Nitrogen |
| $NH_4^+$ | Ammonium |
| $NO_3^-$ | Nitrate |
| $NO_2^-$ | Nitrite |
| NT | No-till |
| OC | Organic carbon |
| OM | Organic matter |
| PCA | Principle components analysis |
| POM | Particulate organic matter |
| SEM | Standard error of the mean |
| SMB | Soil microbial biomass |
| WSA | Water stable aggregates |
| %CLF | Percentage organic carbon in the light fraction organic matter |
| %CHF | Percentage organic carbon in the heavy fraction organic matter |
| w/wo | With or without |
| wt/wt | On a weight per weight basis |
| wt/v | On a weight per volume basis |
| g | Gram |
| mg | Milligram |
| mL | Millilitre |
| kg | Kilogram |
| mm | Millimeter |
| µm | Micrometer |
| ° C | Temperature in degrees celsius |
| p | Probability |
| $r^2$ | Coefficient of determination |
| g $m^{-2}$ | Grams per square metre |
| g $cm^{-3}$ | Grams per cubic centimeter |
| mg/kg | Milligrams per kilogram (conversions in Appendix G) |
| Mpa | Millipascals (unit of pressure per $m^2$) |

## Preface

This thesis is presented in manuscript format as outlined by the Watershed Ecosystems Graduate Program, Trent University, 2005. The two crop season experiments are presented as stand alone manuscripts. Methods common to both studies are detailed only in Chapter 1. The results and discussion are combined in each manuscript to reduce repetition and improve cohesion of the writing. The major issues are presented in a common general introduction, general discussion and conclusions. All references are found in a single list at the end, followed by Appendices.

Chapter 1 is a general introduction to the issues involved in altering soil organic carbon with surface crop residue and plant growth, ending with a summary of the predictions of the hypotheses that were tested. Chapter 2 is the first season experiment where the relationship of fungal hyphal length to soil aggregation, soil organic carbon and soil moisture was tested with a range of initial carbon without residue, and with different types of ground cover on high carbon soil. Chapter 3 is the second season experiment where the effects of the surface residue and the oat plants on the above variables were separated. The major findings from both seasons are summarized in a general discussion in Chapter 4, along with new analysis from combining the two years of data. The conclusions and future research in Chapter 5 summarize the overall research findings of the effects of surface crop residue on fungal hyphal length and soil organic carbon, moisture and aggregation and highlight questions arising from the thesis.

# Surface crop residue effects on fungal hyphal length, soil organic carbon and soil moisture in oat (*Avena sativa*) plots.



HIDA ROSEMARY MANNS

JUNE 9, 2006

# Chapter 1

# General Introduction

## 1.1 Rationale

The awareness of Canada to international concerns of reduction in greenhouse gas emissions through the Kyoto protocol, sustainable productivity and biodiversity, has influenced agricultural methods. Current initiatives in agriculture to turn carbon loss into gain in the soil have addressed the importance of reducing tillage and the addition of organic matter. An increase in aggregate size has been associated with increasing soil organic carbon (OC) (Hu et al., 1995; Angers et al, 1997; Beare et al., 1997), but the relationships of fungi and bacteria in this process (Hu et al., 1995) and the factors that influence OC levels in agricultural management need further study.

No-till (NT) has become popular as it reduces soil loss due to erosion, OC loss due to disturbance, and fuel cost. NT reduces the loss of OC to $CO_2$ from cultivation where mineralization is a constant force responsible for up to 50% decrease in OC in western Canadian soils (Voroney et al, 1981). With NT, crop residue is left on the surface, rather than incorporated into the soil. While decomposition has been studied intensively within the soil, or in composting heaps, there is limited study on the relationship between surface residue decomposition and soil OC content (Duiker and Lal, 1999).

Differences in soil aggregation and OC are recognized between conventional tillage (CT) and conservation tillage (ie: low till or no-till (NT)), but the understanding of mechanisms affecting soil aggregation (Sollins et al., 1996; Bronick and Lal, 2005) and OC (Beare et al., 1994) and how these factors are influenced by surface decomposition of

plant matter in association with NT are not well developed. In a long-term study on corn plant residue with tillage methods, OC increased significantly over several years with NT management where corn stalks were retained on the soil surface (Clapp et al., 2000). In another study of wheat straw decomposition which compared tillage treatments, soil OC increased linearly with mulch rate (Duiker and Lal, 1999). However, the effect of the growing crop plant on soil OC was not separated from the increase from mulch decomposition in their study and research is needed to isolate the mechanism of change on OC (Sollins et al., 1996).

1.1.2 *Surface Residue decomposition*

There are benefits to surface decomposition that are not currently taken into account in soil models. Surface decomposition of residue is not separated from overall decomposition in tilled soils in carbon models (Rickman et al., 2002). Measurements of microbial activity by $CO_2$ evolution measurements do not differentiate the role of bacteria versus fungi in the decomposition process. Saprophytic fungi were found to be more important in the decomposition of surface-applied crop residue, while bacteria were more important in decomposition of incorporated residue in experiments where the microbial community was altered with fungicide in NT and CT (Hu et al., 1995). These changes could relate to the differences in the physiology of fungi and bacteria. Surface decomposition of residue is primarily by fungi which respire aerobically (Holland and Coleman, 1987; Kendrick, 1992). Decomposition of plant matter within the soil is in lower oxygen conditions and predominated by bacteria and fungi that have more anaerobic capacity that is limited in most soil fungi (Hu et al., 1995).

Holland and Coleman (1987) tested the change in hyphal length of fungi in soil between litterbags left on the soil surface or incorporated into the soil, in the field and with laboratory incubation. Fungal abundance was greater with surface application of straw in both studies, along with increased OC retention and nitrogen immobilization, despite slower decomposition (Holland and Coleman, 1987). The reduced loss of soil OC with NT was assumed to be from the higher OC assimilation efficiency of fungi, higher biomass, and the slower decomposition of fungal cell walls that are composed of chitin and melanin (Holland and Coleman, 1987; Hu et al., 1995).

1.1.3 *Soil Aggregation*

Fungi have been associated with increased soil aggregation in grassland soils (Tisdall and Oades, 1979). The increase in fungal hyphal length that was observed with a crop of winter rye in NT was associated with increased OC in large sized aggregates (Angers, 1992; Hu et al., 1995; Angers et al., 1997; Beare et al., 1997). The differences in OC from fungal species and the relative effects of an added crop plant or crop species compared to the effect of residue itself needs additional study. Specifically, there is a lack of understanding of the factors determining soil aggregation (Sollins et al., 1996; Duiker and Lal, 1999; Bronick and Lal, 2005), and little research exists on the interaction between the factors, namely soil fauna, microbes, roots, binding agents, environmental conditions and soil structure (Six et al., 2004).

There is controversy whether the effect of fungi on soil aggregation and OC is from hyphal entanglement (Tisdall and Oades, 1982), total fungal hyphal length, (Jastrow et al., 1998), root length (Miller and Jastrow, 1990; Jastrow et al., 1998), mycorrhizal hyphae (Miller and Jastrow, 1990) or fungal exudates (Beare et al., 1997; Caesar-

TonThat and Cochran, 2000). Fungal hyphal length and secretions both contributed to the increased organic matter, moisture retention and resistance to soil erosion found with NT (Beare et al., 1997). Beare et al. (1997) concluded that an increase in extracellular polysaccharides from fungi was more important than the physical mass of fungal hyphae in NT in protecting aggregates from decomposition. However Degens et al. (1996), reported that there was little involvement of microbial polysaccharides in the stabilization of aggregates in sand, and that hyphal length from mycorrhizal fungi was the dominant factor. The results were from a greenhouse study, where measurements of aggregate formation extended over a 5-week period with ryegrass and mycorrhizal inoculation (Degens et al., 1996). The use of planting containers allowed one influence to be separated from the others in the short-term, but may not have reflected long-term effects of fungal hyphae on soil aggregation that would be seen in agriculture.

1.1.4 *Soil Organic Matter Density Fractions*

Soil organic matter is 55% OC and is comprised of decomposed plant matter and soil humic substances held together with sand, silt, clay, fungal hyphae and bacteria as soil aggregates (Kennedy, 1999). The microbial biomass (fungi, bacteria, actinomycetes and algae) is less than 10% of soil and transposes the organic matter and nutrients in the soil (Gregorich et al., 2000). The physical protection of organic matter from decomposition within aggregates (Tisdall and Oades, 1982) is based on the theory that unstable macroaggregates (particles >0.25 mm) are constantly being broken down into stable microaggregates (Six et al., 2004). A slow, but constant rate of turnover of large aggregates leads to highly stable OC in microaggregates compared to large macroaggregates (Besnard et al., 1996; Gale et al., 2000; Six et al., 2004).

Soil organic matter can be divided into particle size fractions and density fractions from 1.6 to 2.2 g $ml^{-1}$ although the critical density is not ascertained (Christensen, 1992) and depends on the density of the separating solution. The light fraction (LF) refers to partly decomposed plant material and microbial biomass that is free of minerals and floats at a lower density than heavy fraction (HF) which is organomineral complexes composed of organic matter adsorbed onto mineral surfaces within microaggregates (particles < 0.25 mm) (Christensen, 1992; Gregorich and Ellert, 1993). The LF can have from 30-40% OC from recent decomposition of plant material with a high C/N (Gregorich and Ellert, 1993). Separation of LF and HF can identify factors that control accumulation of organic matter (Strickland and Sollins, 1987). The light fraction of organic matter was correlated to the microbial respiration rate and nitrogen mineralization in studies on long-term crop rotation in a temperate climate, as an indicator of management inputs (Janzen et al., 1992).

Ros et al. (2003) found that the rate of mineralization influenced soil OC levels in a study of the effects of decomposition of fresh waste, composted waste, straw, and growing weeds in outdoor plots on a degraded soil. Composted waste increased soil OC the most, as organic matter in compost is "more stable" than fresh waste resulting in stabilization and slow mineralization of organic matter (Ros et al., 2003). Hassink (1995) divided organic matter into three densities with silica suspensions of 1.12 and 1.37 g $cm^{-3}$. The differences in the organic matter fractions of each residue were explained by their relative decomposability, measured by %OC in the light fraction (Hassink, 1995). Increased nitrogen in residue is expected to increase decomposition in soil (Bossuyt et al., 2001) where the bacterial ecosystems predominate (Hu et al., 1995). However, aerobic

fungi that would be decomposing cellulose and lignin (Jennings and Lysek, 1996) do so at low nitrogen levels as they are able to economize by recycling hyphal nitrogen (Burnett and Trinci, 1978; Hudson, 1980; Jennings and Rayner, 1984). There is little research on the relative effects of the type of residue and residue placement on OC retained from decomposition. The reasons for the differences in OC with surface application or composting have not been developed. This thesis will broach the relationship of fungi from ground cover on OC, and the relevance of decomposability of the ground cover to OC.

1.1.5 *Plant roots*

The role of living plants in soil aggregation and carbon sequestration has been debated and results are variable in literature. Agricultural cereals translocate 20-30% of OC from photosynthesis below ground for root growth and exudates (Whilhelm et al., 2004). The roots of fava bean plants stored 19% of the OC fixed by the plant in their biomass, and only 0.5% of the plant OC entered the soil directly as root secretions (Paul and Kucey, 1981). The decomposition of roots in soil depends on the lignin content of the roots and the soil OC content (Voroney et al., 1989). A greater percentage of root compared to surface residue OC was retained in the soil with isotope labelled oat (*Avena sativa* cv. Ogle) leaves vs. roots (Gale et al., 2000). Gale et al. (2000) found that surface residue OC was greatest in the > 2 mm size aggregates after 270 days which were unstable, compared to root derived OC in the < 0.250 mm size aggregates which were more stable. Simulating NT conditions, they maintained that the roots are responsible for a greater proportion of OC addition to the soil than plant shoots (Gale and Cambardella, 2000).

Plant roots can cause an increase or decrease in aggregate size, depending on the initial soil status (Kay, 1990). Angers and Caron, (1998) described an increase in soil aggregation caused by corn root mucilage, while other reports have indicated active root secretions can decrease soil aggregation (Sollins et al., 1996; Six et al., 2004). Six et al. (2004) summarized the effects of plant roots as "decreasing unstable macroaggregates" and "increasing stable microaggregates". The affect of roots on OC was found to be greater than the affect of exudates due to the slower decomposition of roots (Whilhelm et al., 2004). It is not understood how roots interact with fungi and organic matter to influence aggregate size, and the potential of cover crops to contribute to soil aggregation (Bronick and Lal, 2005).

1.1.6 *Mycorrhiza*

Soil fungi (especially Basidiomycetes) can form a symbiotic association with host plant roots called a mycorrhiza where there is mutual benefit (Allen, 1992; Carlile et al., 2001). The plant provides a supply of OC for hyphal growth in return for a supply of nutrients and moisture that the fungus can concentrate at a much steeper concentration gradients than plant roots (Carlile et al., 2001). Mycorrhizal hyphae can double the absorption zone of the plant at a lower cost (Hetrick, 1989). Ectomycorrhiza in particular, can decompose the litter layer to redistribute nutrients where availability in the environment differs spatially (Rillig and Allen, 1999; Carlile et al., 2001).

Arbuscular mycorrhizal (AM, formerly VAM) fungi form arbuscules and vesicles within the host plant, and do not have enzymes for decomposition (Findlay and Soderstrom, 1992). The action of AM hyphae is similar to that of roots in secreting soluble OC derived from the plant to maintain bacterial growth (Medina et al., 2003), and

thus mineralization of nutrients into soluble forms. AM act to concentrate nutrients in the rhizosphere, and can absorb ten times more nutrients at lower water potential with increased surface area than plant roots (Bowen, 1973). In temperate ecosystems, AM hyphae are specialized at collecting $NH_4^+$ from organic nitrogen decomposition (Azcón-Aguilar and Barea, 1992), and phosphorous in high humus conditions (Frankland et al., 1996).

Cultivation and reduction of roots in agriculture can reduce the ability of mycorrhizal fungi to colonize crop plants (Kabir et al., 1997). Spores are formed in existing roots at frost kill by the abrupt change to below freezing temperatures (Biederbeck and Campbell, 1971). The reduction in soil disturbance with NT was thought to improve AM symbiosis (McGonigle and Miller, 2000). Mycorrhizal occurrence was reduced and delayed for 50 days with tillage in a field comparison of corn roots with conservation tillage vs conventional tillage (Kabir et al., 1997). As long as minimum mycorrhizal spore inoculum density conditions were met, disturbance was more significant to reduced mycorrhizal colonization in studies on field and forest (McGonigle and Miller, 2000). A previous non-mycorrhizal crop of canola decreased subsequent colonization more than tillage, but the difference was no longer evident after 3 months growth of corn (Gavito and Miller, 1998).

AM may be more important than decomposer fungi in NT as a result of their longer retention time in soil. Mycorrhizal hyphae can remain alive from weeks to 1 year, compared to saprophytic fungi species that are rapidly recycled by soil fauna within days or weeks (Degens et al., 1996; Kabir et al., 1997). Slower growth of mycorrhizal hyphae results in a maximum quantity of hyphae, and thus effect on the soil, in September

(Biederbeck and Campbell, 1971). Jastrow et al. (1998) found the strongest single influence on the percentage of macroaggregates to be from mycorrhiza on fine roots in addition to the direct effects of the roots themselves. In $C_3$ grasses which have lower hyphal length than $C_4$ plants, the association of mycorrhizas with fine root length was more important compared to the effect of very fine roots alone (Jastrow et al., 1998). Degens et al. (1996) tested for the relative effects of mycorrhizal and/or saprophytic hyphae on soil aggregates with incubation of soil with straw residue and mycorrhizal inoculation, but no mycorrhizal effect was noted within 5 weeks in small containers. It would be beneficial to know the contribution of mycorrhizal and/or saprophytic fungal hyphae with surface residue that relates to agricultural scale and field crops. The factorial arrangement of homogeneous study plots in this thesis allowed me to separate the plant from the surface residue effect, and thus the mycorrhizal from the saprophytic fungi.

1.1.7 *Interaction of fungal species*

There are conflicting opinions as to whether saprophytic and mycorrhizal fungi are competitors for nutrients (Peterson et al., 2004), or exist in a mutually beneficial association in the plant root zone (Dighton et al., 1987; Azcón-Aguilar and Barea, 1992; Klironomos and Kendrick, 1995). Azcón-Aguilar and Barea (1992) maintain mycorrhizal colonization is increased with interaction of fungi and bacteria, but they do not compete for nutrients. Dighton et al. (1987) studied the interaction between tree seedling roots (*Pinus contorta*), mycorrhizal fungi, saprotrophic fungi and substrate decomposers in microcosms. The presence of tree seedling roots significantly enhanced the decomposition of chitin. It was deemed that a synergistic effect between mycorrhizal

decomposers and chitin decomposers increased growth in association, whereas other substrates inhibited mycorrhiza (Dighton et al., 1987). Current studies on interactions between mycorrhizas and the microbial community have seldom included the trophic levels in the food web (Wamberg et al., 2003). I did not find any published reports of the interaction of mycorrhizal fungi with decomposer species in field conditions. The factorial arrangement of treatments w/wo residue and w/wo oat plants in my thesis will test for interaction of plant mycorrhizal fungi and saprophytic fungal decomposers on hyphae length and the soil variables.

1.1.8 *Soil Moisture*

The soil surface is a zone that can impede or facilitate the movement of water into soil (Franzluebbers, 2002). It has been suggested that pore size distribution is related to the water retention property of soil (Paul and Clark, 1982; Duiker and Lal, 1999; Franzluebbers, 2002). The soil particles are separated by pores, which contribute in size and number to determining the fluid storage capacity of soil (Angers and Caron, 1998). The soil aggregates expand in size by weakening of inter-aggregate bonds (Aluko and Koolen, 2001). In a range of soil moisture content from 24-30% pore diameter increased exponentially with soil moisture (Aluko and Koolen, 2001). The size of soil aggregates thus expands and contracts rapidly in response to wetting and drying and moreso in sand than clay soil (Six et al, 2004).

An increase in water infiltration with NT was associated with an increased proportion of pores 100-500 μm (Kay and VandenBygaart, 2002) although water infiltration is also influenced by soil type (Lipiec et al., 2006). High organic matter soil was associated with increased water infiltration with tillage on clay soils (Lipiec et al.,

2006). The form of organic matter addition determines the stability and size of pores (Kay and VandenBygaart, 2002). Macropores (> 1mm diameter) tend to be stable over time when formed from soil fauna and roots (Kutilek, 2004).

Planting corn and soybean into a killed rye cover crop increased yield 44 and 30 % respectively and was attributed to increased soil moisture availability from improved infiltration and reduced evaporation (Bruce et al., 1991). Unger (1978) also found yield doubled with sorghum when straw mulch was applied in increments up to 12 t $ha^{-1}$ to clay loam soil. Water use efficiency was 2-3 times higher with mulch in their study, moreso with sufficient moisture. The increased water use efficiency was thought to have reduced plant stress and improved response of the plant to growth and grain filling from precipitation (Unger, 1978).

Soil evaporation can account for 25-50% of moisture loss in tropical climates (Ramakrishna et al., 2006). Döring et al. (2005) attributed the improvement in soil moisture from straw mulch to reduced evaporation and increased infiltration. A modeling study specifically addressed the water transfer within the plant-residue mulch layer (Gonzalez-Sousa et al., 2001). The decrease of 5-10% of annual total evaporation maintained plant transpiration longer without water stress (Gonzalez-Sousa et al., 2001).

The relative effects of soil evaporation and plant transpiration on soil moisture were measured in a Mediterranean vineyard by measuring transpiration with the stem heat balance method in comparison to the soil water balance in the soil (Trambouze and Voltz, 2001). There was a linear relationship between transpiration and soil water content; transpiration responded directly to soil moisture levels (Trambouze and Voltz, 2001). Under normal soil conditions, the loss of soil moisture was equally divided

between surface evaporation and plant transpiration (Gonzalez-Sousa et al., 2001). With mulch, the loss of soil moisture from surface evaporation was 20-30% of losses from transpiration (Gonzalez-Sousa et al., 2001).

The alteration in the soil evaporation/plant transpiration could also be in response to mycorrhizal fungal associations that are increased from surface mulch. Mycorrhiza are known to improve plant water supply when soil moisture is limited, and improve plant water-use efficiency (Rillig and Allen, 1999) that could delay the signaling within the plant to decrease transpiration. A pot study with cowpeas, showed "mycorrhizal plants" maintained stomatal conductance of water at lower soil moisture and concluded that mycorrhizal association increased available water near the plant wilting point (-0.2 to -1.5 Mpa) (Augé et al., 2001). The composition of fungal species may have an effect on soil moisture in addition to soil aggregation that can be addressed with the separation of ground cover and oat plants in this thesis.

**1.2 Objectives**

While NT appears to be beneficial for soil aggregation, moisture and OC, the changes in fungi and their relationship to these soil variables are not well understood. It is difficult to separate the effects in large scale field plots, where variance in soils and microbial measurements can limit conclusions, while greenhouse studies may not include all factors that affect fungi, particularly the persistence of mycorrhizal hyphae in soil and the soil food web. The use of study plots in this thesis is a compromise between control over variables, and inclusion of naturally occurring conditions that exist in the field that affect soil aggregation.

This study addressed the effects of surface ground covers on fungal hyphal length within the small scale of 60 cm x 60 cm plots growing oats (*Avena sativa*) to determine subsequent changes in soil aggregation, moisture and OC. The effects of the living plant cover were considered separate from the surface residue, which also separated mycorrhizal from saprophytic fungi. The effect of residue quality on soil OC changes was compared by decomposability (%CLF) and residue C/N.

The use of plots allowed for the soils to be homogenized so variance was reduced, initial OC percentages to be controlled and altered, and treatments to be replicated. The high organic matter sandy soil used in the study would contain ample nutrients for an oat crop, so no further nutrient additions were required. The soil would also contain an optimum range of spores native to the environment for fungal development. With an outdoor setting there was a complete range of trophic levels that occur in the natural ecosystem in the surrounding environment. Oat plants were selected as a crop that would germinate well in residue, respond to soil moisture conditions, and give a representative crop in the time and space provided. Oats are moderately susceptible to mycorrhizal infection which provided a relative index of comparison.

The first field season (2003) examined whether the quantity of fungal hyphae were correlated with soil aggregation, moisture and OC at all levels of initial OC without surface residue and with four different surface materials. Alfalfa (*Medicago sativa)* growing from seed, oat straw, hay, and compost were applied to the surface of high organic matter soil to impose various levels of decomposability and mycorrhizal colonization.

In the second field season (2004) the use of the plots was repeated to examine the changes in OC in a factorial experiment to separate the effect of the live oat plants from the dried surface residue on soil fungi, aggregation, moisture and OC. The effect of decomposability of the residue was differentiated between straw with low C/N and corn mulch with high C/N. The design separated the source of fungi; residue alone (saprophytic), oat plants with no residue (mycorrhizal), and the combined effect of plant and residue (mycorrhizal and saprophytic fungal interaction).

Table 1.1 Summary of Plot Treatments in 2003 and 2004

| | 2003 | 2004 |
|---|---|---|
| **Treatments** | | |
| No residue | | |
| Soil OC | 2.4, 3.8, 6.0, 6.8% | 6-7% |
| Ground cover | seeded alfalfa<br>oat straw<br>mixed hay<br>compost | oat straw<br>corn stalks |
| Soil OC | 6.4% | 6-7% |
| Plants | Oats | No plant/oats |
| **Replication** | x 3 | x 4 |
| **Design** | 2 Random Blocks | 2 x 3 factorial |

**1.3 Hypotheses**:

The primary hypothesis is that surface ground cover would increase fungal growth measured as hyphal length. The second hypothesis was that increases in hyphal

length would positively correlate with increases in soil aggregation, soil moisture and soil OC. The third hypothesis related to the role of the plant; the live oat crop would increase fungal hyphal length and the combination of added surface residue and oat plants would have an additive effect on hyphal length. The fourth hypothesis tested the affects of decomposability on fungal hyphal length and/or soil OC: a) increased %CLF would positively correlate to the decomposability of the residue and to hyphal length and b) the C/N ratio would correlate inversely to fungal hyphal length.

Surface crop residue was expected to increase soil aggregation from an increase in fungal hyphal length. It was also predicted that the living cover crops would increase hyphal length from OC allocated to root secretions, root growth and mycorrhizal fungi. In the absence of interaction between the plant and residue, I predicted an additive effect of saprophytic decomposers of the residue, and mycorrhizal fungi of the plant on hyphal length. Hyphal length would be expected to increase with residues that exert a greater positive effect on mycorrhizal hyphae development in the plant roots.

OC increases would be expected to correspond with the speed of decomposition which would increase the amount of OC input. If this was true, increased %CLF should correlate to increased fungal hyphal length and OC. A higher C/N ratio would be associated with decreased decomposition and hyphal length if growth of fungi is dependent on residue nitrogen. The decomposability should range in ascending order from low to high decomposability; a) live plants, b) dried residue with high C/N, c) dried residue with low C/N, d) compost).

# Chapter 2

## The effect of dried and live ground cover on fungi, soil moisture and soil organic carbon in oat (*Avena sativa*) plots

### 2.1 Abstract

The changes in decomposition with surface crop residue may affect soil organic carbon (OC) concentration and soil aggregation in particular. Oats (*Avena sativa*) were planted in plots in the temperate climate of southern Ontario in sandy soil with a range of OC from 2.4 to 6.8% and with four ground covers (alfalfa (*Medicago sativa)* growing from seed, dried oat straw, dried hay and compost) on top soil averaging 6.4 % initial OC. The plots tested if fungal hyphal length varied with initial OC or ground cover and corresponded to changes in soil aggregation, OC and moisture over the oat growing season.

A significant increase in soil OC (%) and moisture (%) was observed within the growing season of oat plants with all ground covers. Total hyphal length was increased in July with straw, hay and compost residues, but not at harvest. In August the number of hyphae > 5 μm (primarily mycorrhizal) correlated with % OC in ground cover plots and the number of hyphae < 5 μm (primarily saprophytic) correlated with % OC without surface cover. No treatment effects were observed for mycorrhizal occurrence on oat roots. The rate of decomposition of surface treatments measured by the % OC in the light fraction of organic matter (% CLF), correlated to soil OC over all plots and was increased with dried residue compared to live plant growth only in July. Surface applied compost resulted in greater soil moisture, OC and heavy fraction organic matter than incorporated residue, although % CLF did not change. Dried and composted surface residue was most beneficial to soil moisture and OC. Mycorrhizal hyphae correlated to

soil OC with ground cover, but total fungal hyphal length did not correlate with soil aggregation or OC.

## 2.2 Introduction

Reducing tillage and increasing soil amendments can increase soil organic matter when organic carbon (OC) gain from crop plant exudates and residue inputs surpass losses from soil respiration (Saroa and Lal, 2003). In no-till (NT) studies, the effect of reduced soil disturbance and increased surface residue on OC is often treated as a single no-till effect, whereas there may be two unique forces (Beare et al., 1997).

Efforts to sequester OC in soil may increase soil aggregation and moisture. Fungal hyphae have been associated with the increase in OC under NT through effects on soil aggregation, in a comparison of tillage systems (Hu et al., 1995). There is also a difference in the microbial community resulting from the placement of the residue; surface residue is decomposed mainly by fungi while soil incorporated residue is primarily decomposed by bacteria (Hu et al., 1995). Fungi may be more influential than bacteria in soil aggregation due to their higher OC assimilation efficiency, longer turnover time and the greater biomass of hyphae (Hu et al., 1995).

In a modeling study of the relative contributions of root secretions, root decomposition, microbial biomass and mycorrhizal fungal root colonization, Jastrow et al. (1998), found that mycorrhizal association had the greatest single effect on soil aggregation. In sandy soil, fungal hyphae were found to be the most important factor in increasing aggregate size (Degens et al., 1996). In contrast, studies on clay loam have shown clay to increase the resistance of organic matter complexes to decomposition

(Hassink, 1996) and the extracellular polysaccharides from fungi were most important for water stability of aggregates (Beare et al., 1997).

Fungi may also affect soil moisture through their contribution to soil aggregation. The amount of water held in the soil after rainfall is dependent on the pore spaces in the soil (Franzluebbers, 2002). Mulch was more influential than reduced tillage on water retention in the soil in a field study with incremental mulch rates (Duiker and Lal, 1999). Soil moisture increased up to 50% with surface applied wheat straw and was attributed to reduced evaporation and increased infiltration (Döring et al., 2005).

Substrate quality is considered a regulating factor for mineralization of OC, and can be measured by OC in light fraction organic matter (% CLF) (Conti et al., 1997). Light fraction (LF) is partially decomposed organic material that is free of mineral complexes, and therefore will float in a silica suspension at 1.2 g $cm^{-3}$ specific gravity (van den Pol-van Dasselaar and Oenema, 1999). Increases in OC from residue decomposition by fungal hyphae should be represented by the proportion of LF in organic matter similar to the relationship found in grassland soils (Hassink, 1995).

Oats (*Avena sativa*) were sown in outdoor plots to determine if changes in soil aggregate size, soil OC, and soil moisture were correlated and could be altered by the initial OC and the growth of fungal hyphae in response to residue. These relationships were tested 1) with variation in initial soil OC content with no ground cover and 2) with surface applied ground covers on soil with high OC content. The ground covers included alfalfa (*Medicago sativa*) growing from seed, dried crop residue of oat straw and mixed alfalfa/timothy hay, and composted hay/goat manure which would vary with their

decomposition rate (% CLF) and their effect on soil moisture and mycorrhizal fungal colonization on oat roots.

The primary hypothesis was that fungal hyphal length was correlated to soil aggregation, soil moisture and OC. The second hypothesis was that fungal hyphae would be increased from the addition of live/dried ground covers, and from higher initial OC. The third hypothesis was that the increase in the speed of decomposition from the type of ground cover (measured by %CLF) and the influence of the surface cover on mycorrhizal fungal development on the roots would correlate to increases in hyphal length, soil aggregation, soil moisture and OC.

## 2.3 Methods

### *2.3.1 Test plots*

Test plots were designed for the study on a farm in the southern Ontario municipality of Clarington (44° N; 78° 30'E). A wooden frame of "1x6" cedar formed 24 – 60 cm x 60 cm x 10 cm deep squares and was set in a clearing on level undisturbed sand with no vegetation. Surface soil (from 0-6 cm depth) from the nearby garden, was mixed in a large pile with hand tools. This soil had reduced soil variance compared to field scale averaging 6.4 ± 0.27 % OC and is referred to as soil. Particle size distribution was 89% sand, 11% silt and <1% clay determined by wet sieving with sodium hexametaphosphate (Appendix A, Table A.1), pH was 7.5, and bulk density was 1.15 wt/v. Soil was mixed with sand from the study site which was 87% fine sand (< 250 μm particle size) by wt (Appendix A, Table A.2) using 20 L containers to form ratios of 0.5:0.5, 0.75:0.25, and 1:0 soil:sand. Soil was also mixed with compost 0.75:0.25 (soil:compost) to increase OC. Subsequent soil analysis of each plot (see methods

2.3.2.1) revealed the combinations formed a gradient of % OC ± SEM of 2.5 ± 0.27, 3.9 ± 0.03, 5.9 ± 0.04 and 6.8 ± 0.16. The soil combination treatments, each with three replications, were allocated in random block design to 12 plots (Table 1.1, Plate Ia).

In a second block of 12 plots, four surface treatments were replicated three times and randomly allocated to plots (Table 1.1, Plate Ia). Alfalfa growing from seed, oat straw, alfalfa/timothy hay and composted hay/goat manure were the four surface treatments. The 1:0 soil combination with no ground cover was considered a control for the ground cover treatments. The straw, hay and compost were added on top of the 6.4% + 0.26 SEM OC soil base to a depth of 3-4 cm which gave 100% coverage of the soil surface. The alfalfa seed was broadcast on top the soil and lightly incorporated along with the crop seeds.

On May 14, 2003, several days following the preparation of the frame and creation of soil and surface residue treatments, oats (*Avena sativa* cv. Argyle) were sown at the rate of 400 seeds/plot by surface broadcasting on residue plots, or with light incorporation into bare soil. From 150 to175 seedlings emerged in all plots except for the surface compost that averaged 225 plants. This number of seedlings is in excess of the recommended field planting density of 240-360 plants $m^{-2}$ (Welch, 1995). Oats tiller to adjust their density according to soil conditions, so thinning was not necessary. When rainfall was insufficient 2.5 L of well water was added to each plot daily during an unusually warm and dry period in June to sustain plant growth.

Prior to mulching and seeding, soil samples were taken to determine the initial soil moisture and OC in each plot. Each plot of the soil combinations was sampled independently with 4 sub-samples to form a composite sample. The soil base used for

the surface residue plots was measured from 3 separate composite samples. In June and July, 6 to 10 randomly selected plants were gently pulled from each plot to sample the adhering soil in the root zone for moisture and OC and measure plant height. Any visible particles of surface residue were removed from the soil. In August, plant biomass and seed production were weighed and soils were sampled from the oat root zone as in previous months. July and August soil samples were also tested for fungal colony count and size, quantity of hyphal fragments, soil aggregation, water stability of aggregates and organic matter fractions. A random sampling of fine roots was removed from the plants in July and stored at 5°C for analysis of mycorrhizal colonization. Soil samples from a neighbouring oat field and the undisturbed garden where the high organic matter soil originated were tested in July for moisture and OC reference levels. Soil samples were stored at 5°C prior to analysis.

### *2.3.2 Soil Analysis*

#### *2.3.2.1 Soil Organic Carbon*

Approximately 20 g of each soil sample was oven dried at 60°C for 3 days. Soil moisture was expressed as a percentage of dry weight of soil following Nelson and Sommers (1982). Soil organic matter content was determined from 3 g of the dried soil samples from the soil moisture determination. The dried soil was sieved to pass 1 mm, and tested with two independent samples for organic matter by loss-on-ignition at 375°C for 16 h in a muffle oven (Ball, 1964). The two results were averaged. Maintaining the heat below 400°C ensured that inorganic carbon in the form of calcium carbonate was not included in the OC measurement (Ball, 1964). Using the relationship that organic matter

is approximately equal to 1.8 x OC., the percentage OC was determined from the following calculation (Nelson and Sommers, 1982):

% OC = [(mass loss after ignition (g)/dry mass before ignition (g))/1.8] x 100.

Drying soil at 105°C removes hygroscopic water that could have been included in OC loss when soil was dried at 60°C (Schulte and Hopkins, 1996). OC was reduced 22% in the soil, according to the proportion of soil:sand, following trials on the OC levels with drying sand and soil at 60°C and 105°C.

2.3.2.2 *Soil Aggregates*

The percentage of macroaggregates was calculated from the total mass of particles greater than 250 μm following shaking of 5 g air-dried soil in a nest of sieves 4, 2, 1, 0.5 and 0.25 mm sizes for 20 s (Kemper and Rosenau, 1982). Soil particles less than 250 μm in size represent microaggregates. The 1 and 2 mm size aggregates were wet-sieved after slow rewetting (Kemper and Rosenau, 1982). Each sample was allowed to absorb water for 10 min in a 1 mm sieve with the water level just reaching the aggregates. The 1 mm sieve was then raised and lowered slowly so the water went from the bottom to the top of the aggregates (5 mm) with a 5 s cycle time in 20°C water for 5 min. The aggregates were transferred to petri dishes and dried at 105°C for 24 h to determine the water stability of aggregates as a percentage of the mass after drying over the initial dry mass (Kemper and Rosenau, 1982; Angers and Mehuys, 1988). The sand particles were less than 0.5 mm in size, and were not retained on the 1mm sieve, so no correction was made for primary mineral particles in the water stability calculation (Kemper and Chepil, 1965).

2.3.2.3 *Organic Matter Density Fractions*

For each soil sample, organic matter was separated into light (LF) and heavy (HF) fractions by density fractionation with LUDOX ™ HS-30 colloidal silica (Sigma Aldrich, Oakville), specific gravity 1.20 g $cm^{-3}$ (van den Pol-van Dasselaar and Oenema 1999) following the method of Meijboom et al. (1995). The LF consists of partly decomposed plant debris with a high carbon to nitrogen ratio (C/N) while the HF is comprised of organo-mineral complexes of organic matter with lower C/N (Gregorich and Ellert, 1993). The separation of fractions into LF and HF by silica at specific gravity 1.2 g $cm^{-3}$ was confirmed by acridine orange staining of DNA on LF by Meijboom et al. (1995). The major steps in the process are outlined in the flowchart (Fig. 2.1).

The macroaggregates were dispersed by wet-sieving 5 g field moist soil through a 250 μm screen onto a 150 μm screen. The material from both sieves was collected, and the sand was separated from the organic matter by decanting off water along with material that remained in suspension 10 s after stirring. The process was repeated until no further material rose in suspension from the sand in the bottom. The sand was discarded and the organic matter was allowed to settle 2-4 hrs until the water was clear, so the excess water could be removed by decanting.

The process was repeated using LUDOX™ HS-30 colloidal silica, 1.2 g $cm^{-3}$ specific gravity, to separate the organic matter into LF that would suspend in solution from sinking HF. Sufficient silica was added to put the organic matter into suspension. After 10 minutes, the solution was stirred, and the light colored suspension was decanted from the dark colored heavy fraction that remained in the bottom of the container. This process was repeated 3 times until few additional particles separated into suspension.

**Soil Organic Matter Density Fractions**

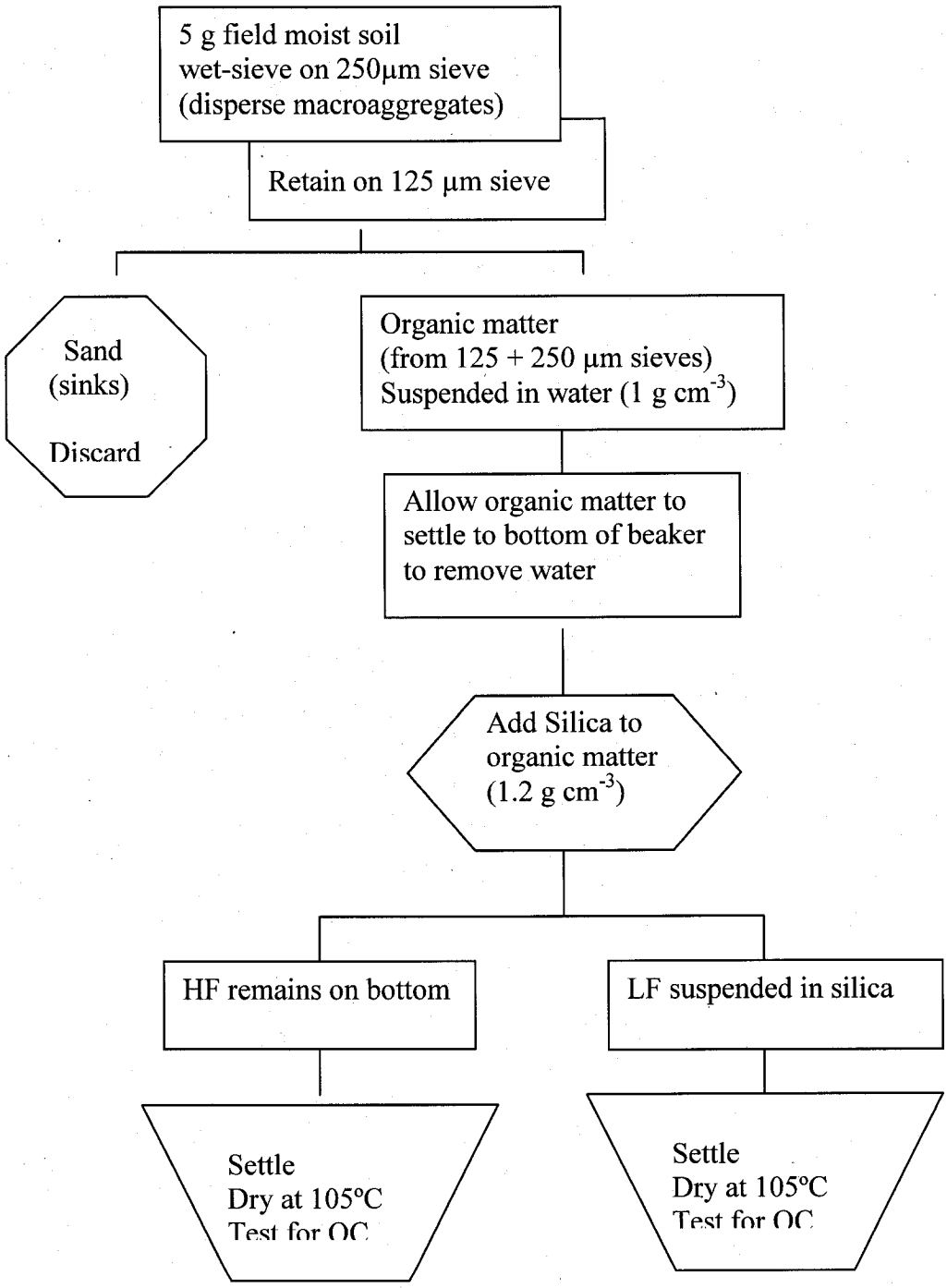



Figure 2.1. Steps in the separation of organic matter into light and heavy fractions at 1.2 g $cm^{-3}$ by LUDOX colloidal silica HS-30 (Sigma Aldrich, Oakville). The flowchart depicts the major stages in the process: dispersing the macroaggregates in the soil, separating the sand from the organic matter, and separating the light from the heavy fraction organic matter.

□

Addition of water to the containers reversed the floatation and the organic matter settled to the bottom of the container in each fraction. The samples were rinsed 3 times to remove the silica and then transferred to petri dishes to determine the mass of each fraction after drying at 105°C for 24 h. Remaining water was evaporated in the process. The % LF and % HF were calculated as the % of dried weight of each fraction over the initial mass of the soil sample (5 g) adjusted for soil moisture to dry wt. OC was measured in each fraction (% CLF and % CHF) as for soil, using loss on ignition and calculations as detailed previously (2.3.2.1).

*2.3.3 Fungal measurements*

Fungi were enumerated using the following methods: 1) by culturing from soil suspension on whey agar media 2) counting the hyphal fragments in soil suspension 3) and estimating mycorrhizal colonization on root segments. A soil suspension was made from 1 g fresh soil in 100 mL distilled water blended at high speed for 15 s and filtered with 1 mm mesh to remove heavy sediment. The filtrate was stored at 5°C. Within 24 hrs, fungi were cultured on whey agar from this soil suspension. Details are included in Appendix B, as results did not relate significantly to the thesis.

2.3.3.1 *Fungal hyphae*

Fungal hyphal growth was enumerated by counting the hyphal fragments in soil solution. A mixture of 1 mL of filtrate and two drops of 5% ink/vinegar stain was viewed in a grid-lined petri dish (12 mm squares) at 20X magnification with a stereoscopic microscope. Vierheilig et al. (1998) demonstrated that fungal structures stained black with a 5% mixture of Sheaffer skrip jet black ink (Sheaffer Pen Corp, Fort Madison, IA) obtained at a local office supply store, and 5% acetic acid (household white vinegar).

The hyphal fragments were visually distinct as > 5 μm diameter which appeared darkly stained (large hyphae), or < 5 μm diameter and lightly-stained hyphae (small hyphae) that were barely visible, often vertically floating in the suspension, and were counted separately. The distinction of diameter at 5μm was calculated from microscope pictures at 650X magnification (LuminaLeaf camera) along with a micrometer picture (Plate II).

Schreiner and Bethlenfalvay (2003) assumed hyphae > 5μm to be primarily mycorrhizal, and <5 μm to be mainly saprophytic fungi, but this was not confirmed here. Arbuscular mycorrhizal fungi (AM) were specified as having a 5-10 μm diameter (Read and Boyd, 1986), but recent research indicates they can vary in diameter within the mycelium, according to location in branching (Rillig et al., 2002b). They can be distinguished from saprophytic fungi by their characteristic branching and irregular appearance under low magnification (Rillig et al., 2002b). The count of hyphal fragments was repeated 3 times for each sample, and the average was expressed as the number per mg dry wt of soil. The total number of hyphal fragments was converted to hyphal length in cm $g^{-1}$ dry wt of soil using the observed length of an average segment of 2 mm in the 2004 data. In 2004, the fungal hyphal length was estimated for each fragment of hyphae from the known length of gridlines (12 mm) of the petri dish.

2.3.3.2 *Mycorrhizal Fungal Colonization*

To measure mycorrhizal colonization, root samples from each plot were stained with Sheaffer black ink/vinegar stain (as described for hyphal length) following an adaptation of the method by Vierheilig et al. (1998). Fine oat roots were soaked overnight in 10% KOH at room temperature, and then rinsed 3 times with tap water. The cleared roots were then stained for 1 hr with the 5% ink/vinegar stain and de-stained 48 h

with water acidified with a few drops of vinegar (5% acetic acid) per 100 mL. Samples were refrigerated (5°C) until analysis. Roots were cut into 1 cm segments and randomly selected for mounting on slides (10/slide). Vesicles in the oat roots were infrequent, so occurrence of hyphae, arbuscules or vesicles at 400X magnification within view of one pass of the microscope was counted as in Gryndler et al. (2002). The percentage occurrence of mycorrhizal structures was scored over 20-30 root segments where root details could be clearly seen. I assumed the ink stain would bond to cations in the chitin walls or lipids in cytoplasm as in other acid stains discussed in Gurr (1971). Mycorrhizal structures stained black on a background of green-gold-red plant root tissue (Plate III, D).

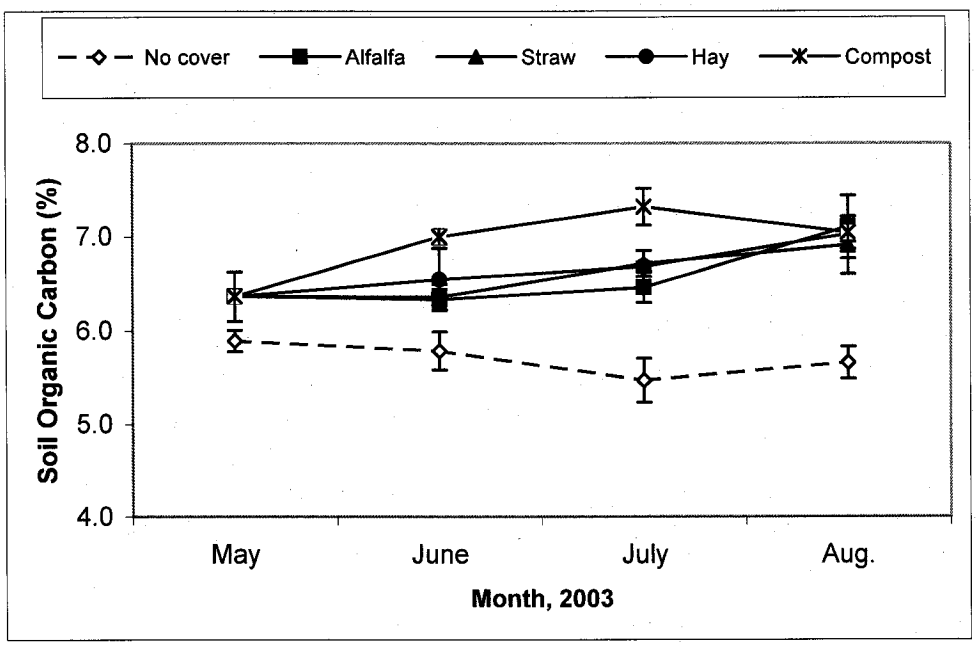
### *2.3.4 Statistical Analysis*

Data were analyzed with EXCEL (Microsoft, CA) for determining correlations and linear regressions. Statistical differences between treatments were determined from 1way fixed effects model ANOVA and post-hoc Tukey test (Statistica, Statsoft). Fungal data were not log transformed, as they were within or near normality.

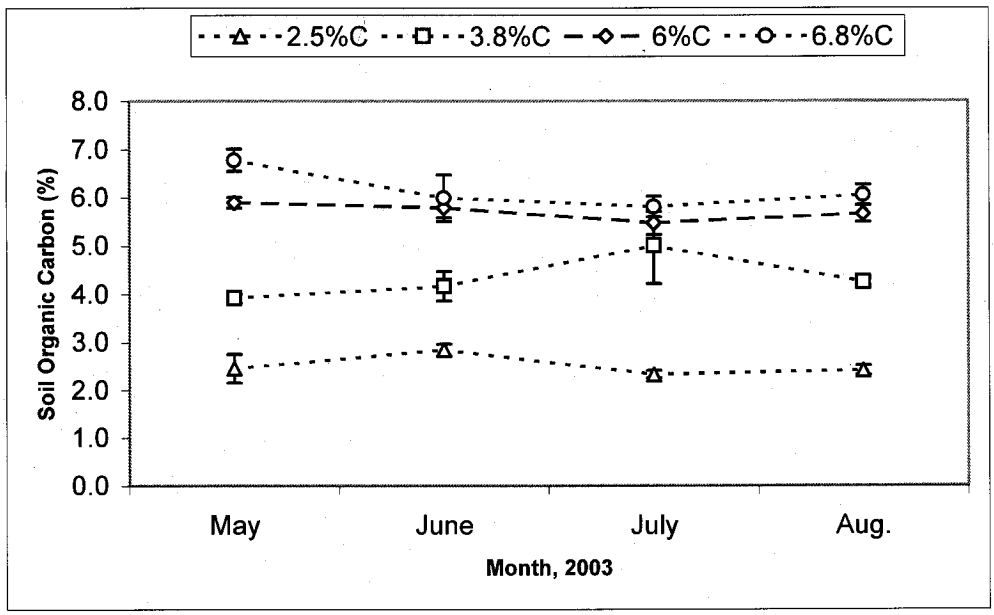
## 2.4 Results and Discussion

### *2.4.1 Soil Organic Carbon*

A significant increase in soil OC from May to August was observed only with ground cover and in all ground cover plots measured in the oat root zone. The greatest increase in OC was in the compost cover plots where measurement of soil OC (%) increased significantly by 0.95 ($p = 0.001$) in July followed by plots with alfalfa, hay, and straw surface treatments which attained significant increases of 0.74 ($p = 0.031$), 0.66 ($p = 0.003$) and 0.55 ($p = 0.003$) respectively at August sampling (Table 2:1, Fig. 2:2a).




a) Surface treatments




b) Varying initial OC from 2.5% to 6.8% with no surface treatment

Figure 2.2. Change in % OC from May to August, 2003 with a) with initial OC of 6.4% with surface treatments of seeded alfalfa, straw, hay, compost and control and b) varying initial levels of OC with no residue. Error bars represent SEM. n = 3. All surface treatments significantly increased in OC from May to August compared to the 6% OC control (a), but there were no increases in the plots without surface residue regardless of OC level.

Table 2.1. Variables where there was a significant difference between ground cover treatments compared to the control (6 % OC with no ground cover) by 1way ANOVA in July and August sampling. (Total SS = Cover SS + Sampling SS), with post-hoc comparison of individual type of compost means by Tukey HSD test. Differences significant at p = 0.05 are highlighted in **bold.** n = 3.

| | 1way ANOVA | | Tukey comparison of means to control | | | |
|---|---|---|---|---|---|---|
| | F | p | alfalfa | straw | hay | compost |
| July | | | | | | |
| Hyphae length (cm) | 11.17 | **0.001** | 0.506 | **0.002** | **0.012** | **0.004** |
| Soil moisture (%) | 3.12 | 0.066 | 0.850 | 0.210 | 0.990 | 0.820 |
| Soil OC (%) | 15.89 | **0.001** | **0.031** | **0.003** | **0.003** | **0.001** |
| Heavy fraction OC (%) | 10.18 | **0.001** | **0.003** | **0.002** | **0.016** | **0.003** |
| Light fraction (%) | 4.13 | **0.031** | 0.173 | **0.016** | 0.301 | 0.350 |
| Hyphae count (small) | 6.36 | **0.008** | 0.802 | **0.011** | 0.064 | **0.032** |
| August | | | | | | |
| Hyphae length (cm) | 1.02 | 0.445 | 0.526 | 0.970 | 0.631 | 0.533 |
| Soil moisture (%) | 6.22 | **0.009** | 0.980 | 0.440 | 0.058 | **0.012** |
| Soil OC (%) | 6.83 | **0.006** | **0.010** | **0.024** | **0.014** | **0.013** |

Without a ground cover, changes in OC were not significant; OC increased slightly from 3.9 to 4.2% and decreased slightly with initial OC of 2.5%, 5.9% and 6.8% (Fig. 2.2b).

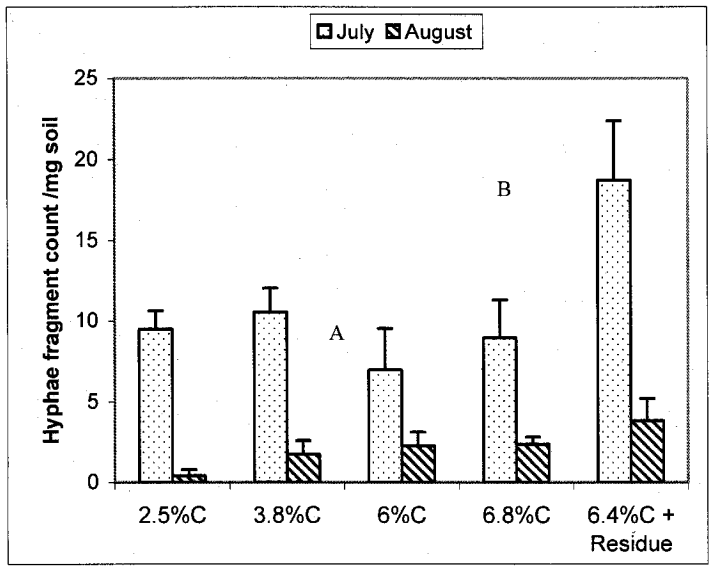
Field measurements of OC have been reported to increase slowly with significant increases from NT only observable after 4-8 years (Lal et al., 1998). Triplicate samples from a neighboring commercial oat field in July measured 2.84% OC ± 0.03 SEM, and an undisturbed sample of the same garden soil used in the experiment measured 4.5% OC in July. This is in accordance with expected levels of OC in cultivated fields around 1-3% carbon (Larson et al., 1972; Stahl et al., 1999), and in undisturbed sod from 2 to 6% OC in temperate climates (Hassink, 1994; Stahl et al., 1999). The changes in OC below levels of significance (p = 0.05) with less than 4% initial OC in the absence of ground cover in this experiment are consistent with the results of Larney et al. (1997) who found

very small changes of 2.0 mg $ha^{-1}$ with a comparison of tillage after 8 years of spring wheat in western Canada.

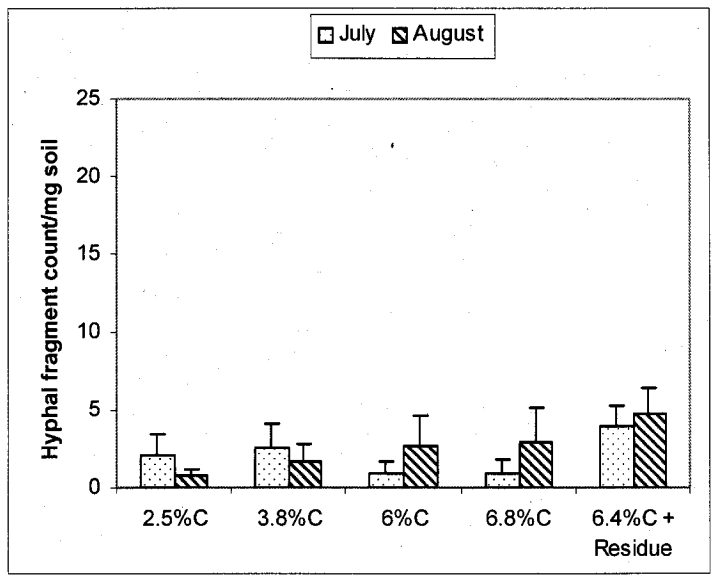
The significant increases in OC from May to August observed at high levels of initial OC in the oat root zone with ground covers would not be expected from current agricultural field studies. The homogenized soil may have reduced the variances compared to those experienced at field scale, allowing for a significant increase in a single growing season to be reported. At high levels of organic matter, OC has been found to decrease to a lower equilibrium with cultivation (Voroney et al., 1981). This trend was observed in the oat plots with incorporated residue. The decline in OC in the 6.8% OC soil with incorporated compost, suggests that the surface application was also essential for the increase in OC. Henriksen and Breland (2002) also found that although the OC mineralization rate was increased when red clover in mesh bags was mixed with soil, only with surface application was there an increase in OC.

2.4.2 *Fungal Hyphal Length*

Fungal hyphae fragments were initially counted in soil suspension. In July, the number of small fragments < 5 μm diameter, assumed to be primarily saprophytic fungi, was significantly greater than the control (6% OC) with straw ($p = 0.011$) and compost ($p = 0.032$) ground cover in the separation of significant differences by Tukey test (Fig. 2.3a). Large hyphal fragments (> 5 μm) were not significantly increased with surface residue (Fig. 2.3b). At August sampling the count of hyphae > 5 μm correlated with OC ($r^2 = 0.34$, $p = 0.05$, $n = 12$) in plots with ground cover, while the number of hyphae < 5 μm correlated to OC ($r^2 = 0.37$, $p = 0.04$, $n = 12$) without ground cover. There was no difference in the mycorrhizal occurrence on oat roots over all ground cover plots




a) Hyphae < 5 μm fragment count




(b) Hyphae > 5 μm fragment count

Figure 2.3. Count of (a) small and (b) large hyphal fragments in July (dots) and August (diagonal bars) expressed as number per mg soil from a range of initial % OC from 2.5 to 6.8%C and averaged over plots with 6.4%C soil with surface residue in July and August. Only bars marked A and B are treatment differences, significant at $p = 0.05$. Error bars represent SEM ($n = 3$).

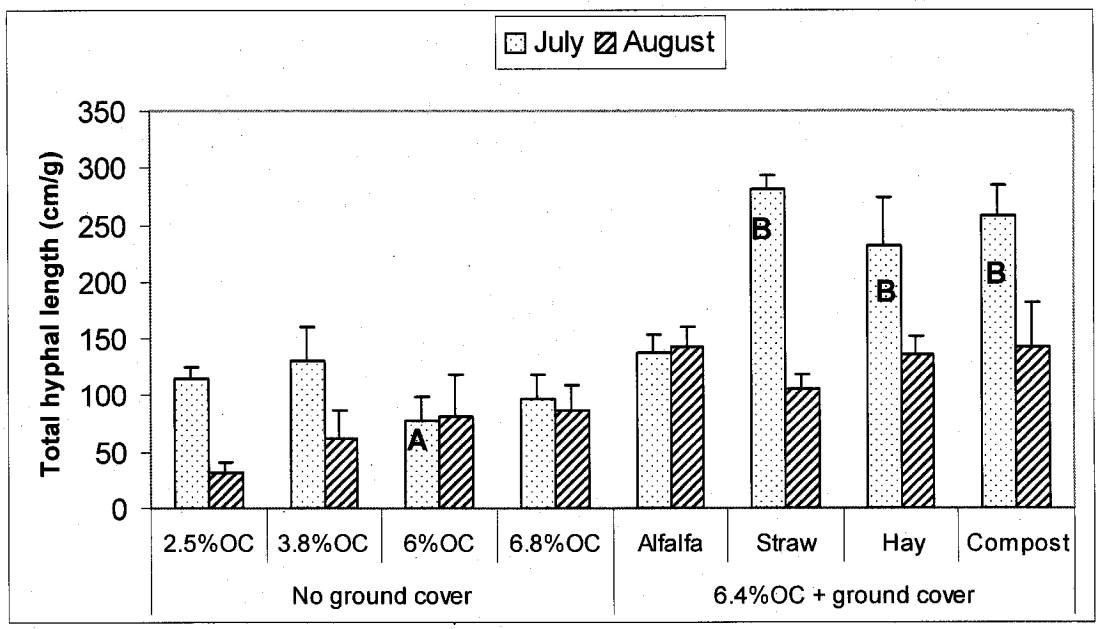



Figure 2.4. Total fungal hyphal length (cm per g soil) in July and August, 2003 over all plots with no residue at 2.5% to 6.8% OC, and with seeded alfalfa, straw, hay and compost ground cover on 6.4% OC soil. Error bars represent SEM. n = 3. Different letters are significantly different by ANOVA at p = 0.05.

compared to the control. Results are shown in Appendix C, Table C.2. Mycorrhizal hyphae count was not increased in conjunction with alfalfa growth as expected possibly due to limited growth of a few (6-10) plants less than 20 cm.

Total hyphal length was calculated from the sum of both sizes of hyphae (Fig. 2.4). Straw ($p = 0.002$), hay ($p = 0.012$) and compost ($p = 0.004$) plots had greater total hyphal length than the control plot without surface treatments in July. However, hyphal length did not correlate with the percentage of macroaggregates at the August sampling as predicted in the hypothesis. There was a larger proportion of hyphae > 5 μm and lower total hyphal length at harvest. July hyphae may have represented a rapid growth of saprophytic fungi from residue, whereas in August, the hyphae represented more mycorrhizal fungi with a slower growth rate.

### 2.4.3 *Soil Moisture*

Weather variability added a moisture effect to the study results. Initial wet weather in May was followed by hot dry weather through to the July sampling which reduced soil moisture to 15-25% in the soil combination plots and to 20-28% in ground cover plots (Fig. 2.5). After 93 mm of rain (Environment Canada, 2004) fell between the July and August sampling soil moisture increased in all plots except 1:1 sand-soil. Ground cover plots had 40% higher soil moisture ($p = 0.009$) than the plots at the same soil OC without residue. A study of wheat straw mulch with a crop of sorghum in the U.S. plains, also reported approximately 50% increase in soil moisture depending on the mulch rate with ample rainfall, but very small differences with low precipitation (Döring et al., 2005). Döring et al. (2005) attributed the improvement in soil moisture to be from reduced evaporation along with increased infiltration, but this was not evaluated here. There was no difference in yield between treatments in this thesis in ample rainfall conditions (Appendix C, Table C.1).

Soil moisture correlated to total hyphal length in July ($r^2 = 0.41$, $p = 0.026$, $n = 12$) with ground cover, which is in agreement with the highly linear relationship of fungi with soil moisture found in a wide range of soil moisture conditions from 10 to 35% (Frey et al., 1999). Soil moisture in this study ranged from 15 to 38% that would be optimal for fungal growth (Boddy, 1986).

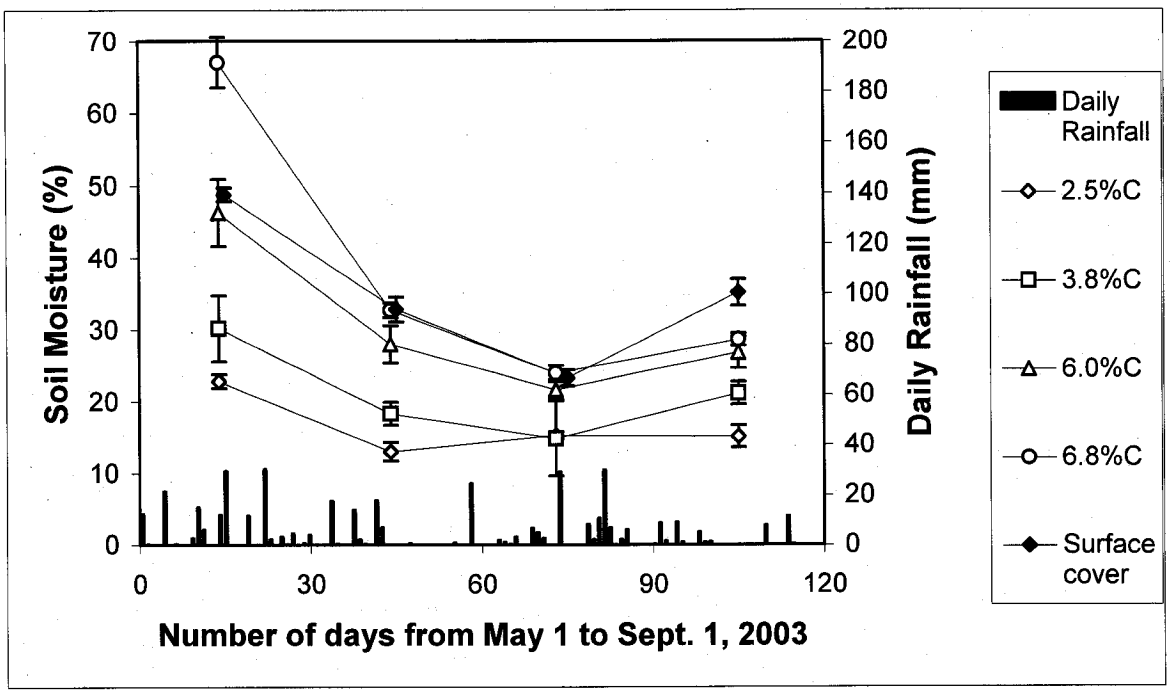
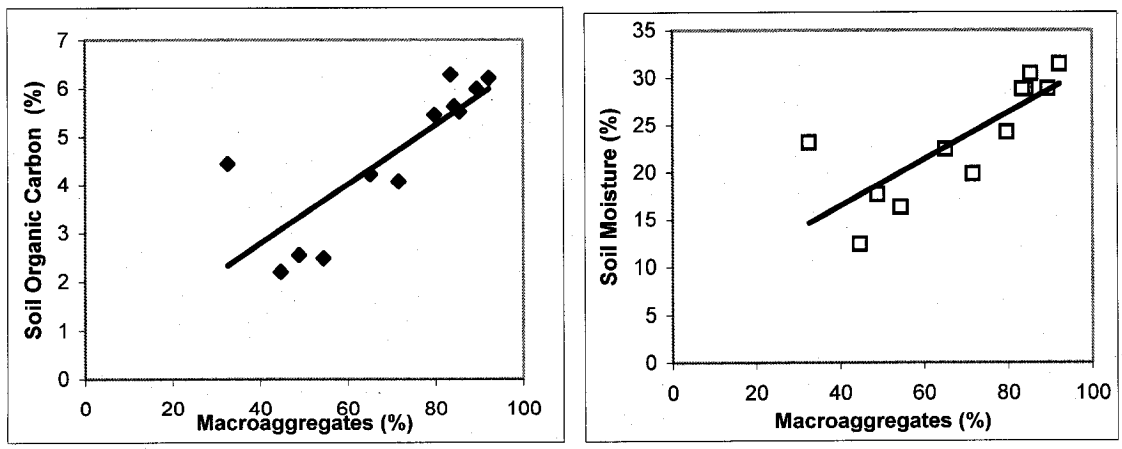



Figure 2.5. Daily rainfall (mm) comparison with soil moisture from May 15 to August 14, 2003 in oat plots with initial OC of 2.5%, 3.8%, 6.0%, 6.8% without residue, and the average soil moisture in plots at 6.4% initial OC with surface treatment. Error bars represent SEM. n = 3.




A B

Figure 2.6. Linear regression of the effect of % macroaggregates on A. soil organic carbon (%) (◆) $y = 0.06x + 0.33$, $r^2 = 0.66$, $p < 0.001$, and B. soil moisture (%) (□) $y = 0.25x + 6.59$, $r^2 = 0.630$, $p = 0.002$, over plots without ground cover (n = 12) at August, 2003 sampling.

### 2.4.4 *Soil Macroaggregates*

At August sampling the % macroaggregates averaged 79 ± 2 % in all ground cover plots but significant treatment differences were not observed (Appendix C: Fig C.1). There was a significant linear regression of soil moisture from soil % macroaggregates with variation in the initial level of OC, but not with surface treatments. The size of aggregates was important in the amount of moisture held in the soil. This is in agreement with the size of pore spaces influencing the fluid storage capacity of soil (Angers and Caron, 1998; Aon et al., 2001) and a relationship between aggregate bonds changing with soil moisture (Aluko and Koolen, 2001). Higher organic matter was consistently associated with increased infiltration whether in NT or CT, in clay or sand (Lipiec et al., 2006).

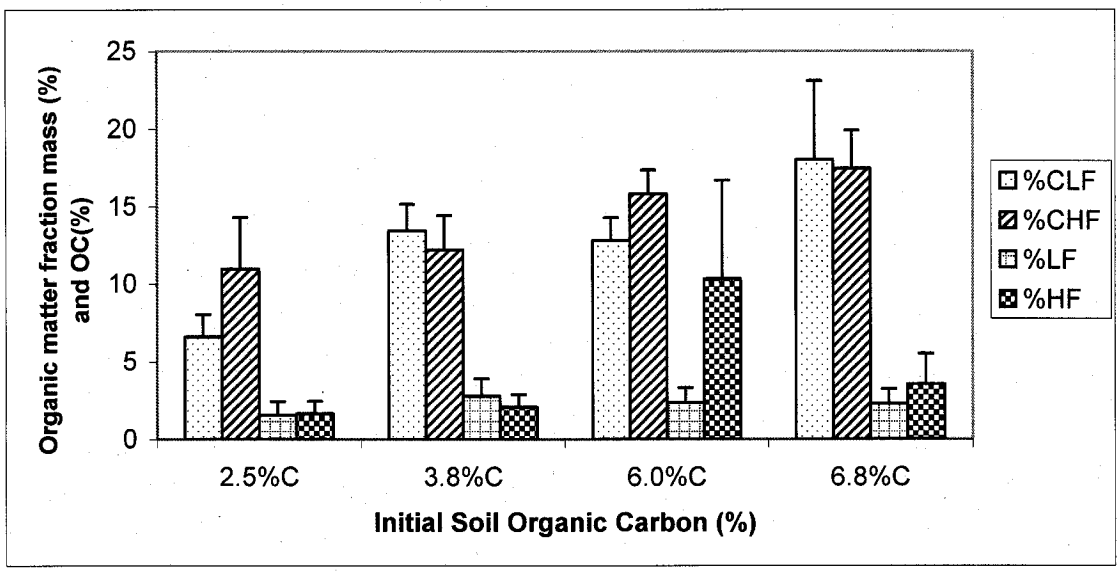
There was also a linear regression of soil OC from the % macroaggregates ($p < 0.001$) (Fig. 2.6) without ground cover and a range of initial OC at August sampling. Other studies report that increases in OC are generally related to increasing soil aggregate size (Beare et al., 1997). Macroaggregates contain higher OC (Hu et al., 1995; Saroa and Lal, 2003), but are considered less stable and thus more easily decomposed compared to microaggregates (Tisdall, 1991). Water stability of aggregates did not show significant treatment effects in this study so data are presented in Appendix C (Fig C.1). There was no further benefit from decomposing residue on increasing aggregate size. This agrees with Gale et al. (2000) who reported that aggregate size increases to a maximum and then decreases with added organic matter. Drying and wetting cycles have a large negative effect on macroaggregates, as the particle bonds are broken and the organic matter exposed for decomposition (Six et al, 2004). With ground covers this effect is reduced

(Six et al, 2004), but environmental changes did not significantly affect the size of aggregates in this study.

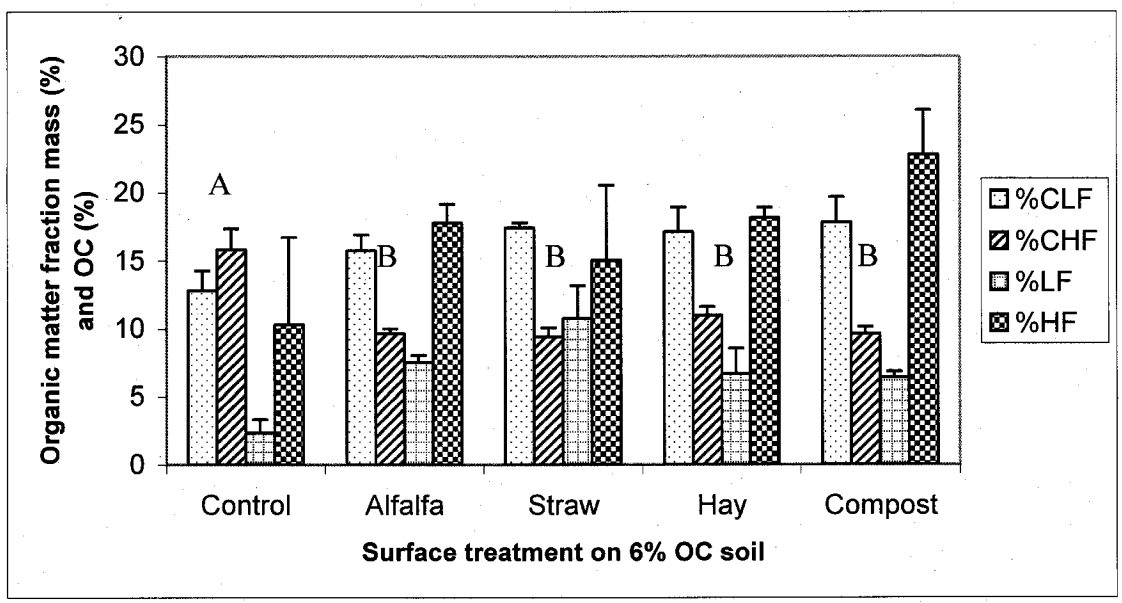
Plant growth may have contributed to the small and transient increase in OC at low initial levels of OC from root exudation and turnover of senescent roots (Warembourg and Morral, 1978). I expected an increase in OC from fungi would be more likely in the plant rhizosphere where there is a 10 fold increase in microorganisms (Lynch, 1981). In low OC soil, the OC from the plant roots would increase microbial activity in general, but soluble OC would stimulate predominantly bacterial decomposition (Elliott et al., 1979) that was not expected to correlate to OC. No increase in OC was found without ground cover, regardless of the level of OC.

2.4.5 *Density Fractions of Organic Matter*

Soil organic matter was divided into density fractions at 1.2 g $cm^{-3}$ to test if increases in LF correlated to increased OC. The % HF mass was greater than % LF mass ($p = 0.003$ t-test) in all plots at the July sampling (Fig. 2.7 a,b). There was greater % LF ($p = 0.016$) with straw residue but there was no significant difference in treatment means with % HF with surface treatment (Fig. 2.7b). There was significantly greater % CLF than % CHF with all ground covers ($p < 0.001$, $n = 12$). The LF is characterized by a high C/N with up to 30 – 40 % OC compared to HF with a lower C/N ratio and a low % OC (Gregorich and Ellert, 1993). The % CHF was significantly decreased with ground cover ($p = 0.001$) and in all covers compared to the plots at 6% OC with no surface treatment (Fig. 2.7b), while the % CLF increased, but not significantly, with ground cover (Table 2.1). My % CLF from 10-20% was a little lower than figures observed by




a) Variation in initial OC with no surface treatment




b) Variation in surface treatment at 6% OC

Figure 2.7. Comparison of density fractions into light fraction organic matter mass and heavy fraction as a % of initial wt of soil sample and corresponding % OC in light and heavy fractions in (a) soil combinations 2.5% to 6.8% OC and (b) in 6.4% initial OC soil with four ground covers of alfalfa, straw, hay and compost in comparison with 6.0% OC soil with no cover. Error bars represent SEM (n = 3). Significant difference at $p < 0.05$ between A and B.

Janzen et al. (1992) in a wide range of cropping systems where % CLF was 20-30% and highest with perennial forages or continuous cropping.

Soil OC was determined from linear regression with % CLF by $y = 2.88x + 28.85$, $r^2 = 0.42$, $p = 0.009$, $n = 15$ over all plots with ground cover (Fig. 2.8). The correlation of % CLF to soil OC with surface residue, supported the statement by Janzen et al. (1992) that % CLF is a useful indicator of recent decomposition for residue management.

2.4.6 *Residue comparison*

Living ground cover had lower % CLF ($p = 0.049$), total hyphal length ($p < 0.001$) and OC ($p = 0.02$) compared to dried cover in July at the peak of oat growth (Table 2.2), but there were no significant differences among ground covers on soil variables at August sampling. Living mulches have been cited to increase soil moisture from reduced evaporation and increased infiltration of water in return for slightly lower yield of corn (Martin et al., 1999) and potatoes (Boyd et al., 2001) but there was no difference in yield measured by seed weight or plant biomass in my results (Appendix C, Table C.1).

Changes in LF and HF during decomposition related to the quality of the residue. When treatments were grouped according to living, dried or composted residue, % CLF was significantly greater by t-test with dried and composted residue than living ground cover, referring to higher decomposability of the dried and composted residue. Incorporated compost had significantly less moisture ($p < 0.001$), OC ($p = 0.02$) and HF ($p = 0.01$), than surface applied compost in August, while % CLF did not vary between the two treatments by t-test in this study. Lynch (2002) found that compost alone altered the soil fractions, with increased LF in field tillage comparisons. The increase in LF and

not HF from compost may relate to the higher density used to separate the fractions (1.7 g $cm^{-3}$) in their study (Lynch, 2002).

Hassink (1995) found that difference in the type of residue incorporated into the soil resulted in different allocation of decomposed residue to the soil fractions; straw increased LF, lucerne increased an intermediate fraction between specific gravity of 1.12 and 1.37 g $cm^{-3}$, and compost increased HF. Ros at al. (2003), also found composted residue to have the highest effect on microbial activity and OC, and attributed this to improved stabilization and slow mineralization of organic matter. The association of compost with HF and OC and not % CLF observed when compost was surface applied but not incorporated, suggests that the formation of organic matter complexes in HF may influence soil OC more than the amount of decomposition.

**2.5 Conclusions**

Soil OC increased significantly in the plant rhizosphere during the oat growing season with ground cover addition, but decreased in control plot treatments without surface treatment. In July, total hyphal length was increased with ground cover with a high proportion of saprophytic fungi. In August, the large (> 5 μm) hyphae were more numerous, but hyphal length was not increased with living/dried surface treatment. The number of hyphae > 5 μm correlated to OC with ground cover, but total hyphal length did not correlate to soil aggregation or OC over all plots at harvest. Mycorrhizal colonization on oat roots did not vary significantly with/without ground cover or with the type of ground cover. The percentage of macroaggregates correlated to soil moisture and OC with variation in initial OC content from 2 to 6.8%, but not with ground cover treatments.

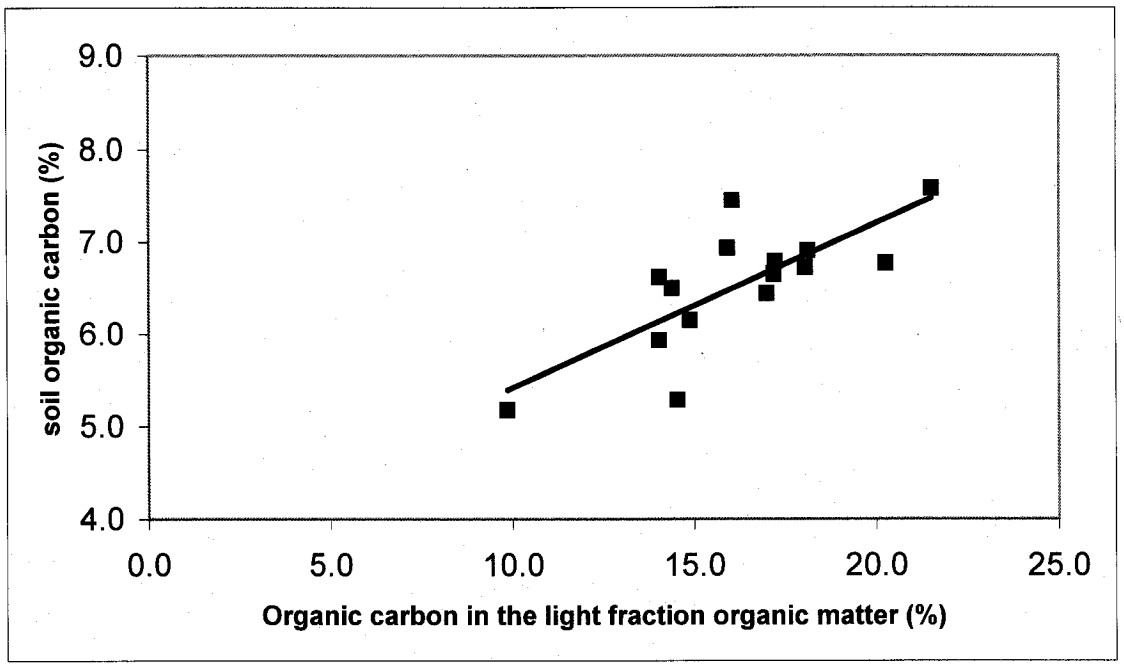
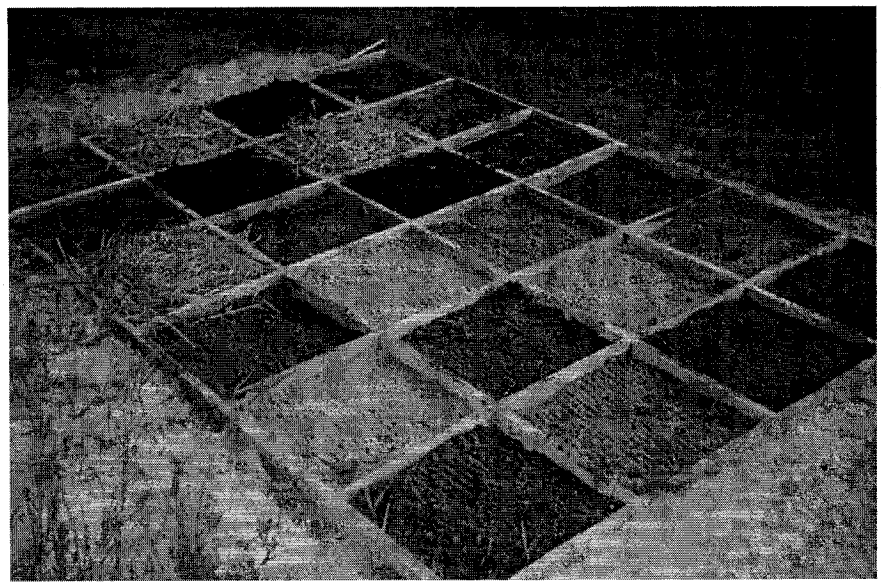



Figure 2.8. Linear Regression of % CLF on soil OC (■) $y = 0.178x + 3.640$, $r^2 = 0.565$, $p = 0.001$ in oat plots with ground cover of weeds, alfalfa, straw, hay or compost at 6.4% OC soil (n =15).

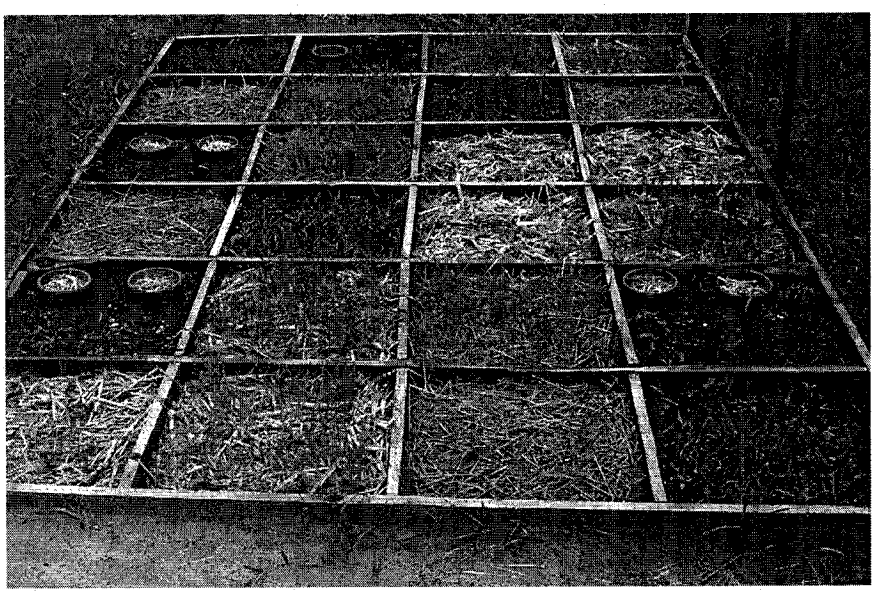
Table 2.2. July comparison of means and SEM of % CLF, hyphal length (cm/g), moisture, OC, % mycorrhizal occurrence, % HF between live ground cover (weeds and alfalfa) (n = 6), dried residue (hay and straw) (n = 6) and compost surface applied and incorporated into soil (n = 3) by 1way ANOVA model. Unlike letters denote significant differences.

| Variable | Type of Ground Cover | | | | Compost | | | |
|---|---|---|---|---|---|---|---|---|
| | Living cover | | Dried cover | | Incorporated | | Surface | |
| | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM |
| Light fraction OC (%) | 14.3 | 1.1 a | 17.3 | 0.8 b | 18.0 | 5.1 b | 17.8 | 1.9 b |
| Hyphae Length (cm) | 108.1 | 17.8 a | 256.6 | 22.8 b | 98.0 | 20.7 a | 258.1 | 26.4 b |
| Soil moisture (%) | 21.3 | 1.3 a | 25.7 | 1.5 b | 24.8 | 1.1 a | 25.0 | 1.1 a |
| Soil OC (%) | 6.0 | 0.3 a | 6.7 | 0.1 b | 5.8 | 0.2 a | 7.3 | 0.2 b |
| Mycorrhiza (%) | 64.6 | 6.4 a | 55.1 | 7.7 a | 81.9 | 9.1 b | 70.4 | 6.7 b |
| Heavy fraction (% ) | 14.0 | 3.4 a | 16.6 | 2.6 a | 3.6 | 2.0 b | 22.8 | 3.2 a |

The % CLF was a measure of residue decomposition and correlated with OC over all ground cover treatments. The % CLF, hyphal length, and OC were increased with dried residue compared to live plant growth in July, but did not vary with the type of ground cover at harvest sampling. Surface treatment had a greater effect compared to incorporation apart from OC input, as the compost used as a ground cover increased soil moisture, OC and HF, compared with incorporated compost, although there was no difference in decomposability (% CLF). The use of dried ground cover was most beneficial in preserving soil moisture and OC while maintaining plant productivity.



a) 2003



b) 2004

Plate I: Experimental treatments in the oat plots a) 2003 and b) 2004

a) 2003. Soil combinations in the foreground 12 squares with variation in soil color according to the %soil/sand (3 replications). Ground cover treatments at the opposite end of the frame with soil added to all the plots and hay and compost treatments added. Straw was not yet present at the time of the photograph.

b) 2004. Random assignment of treatments with 4 replication with no plant/oat plants x no residue/oat straw/corn stalks. The picture was taken June 4, 2004 with oats germinated.

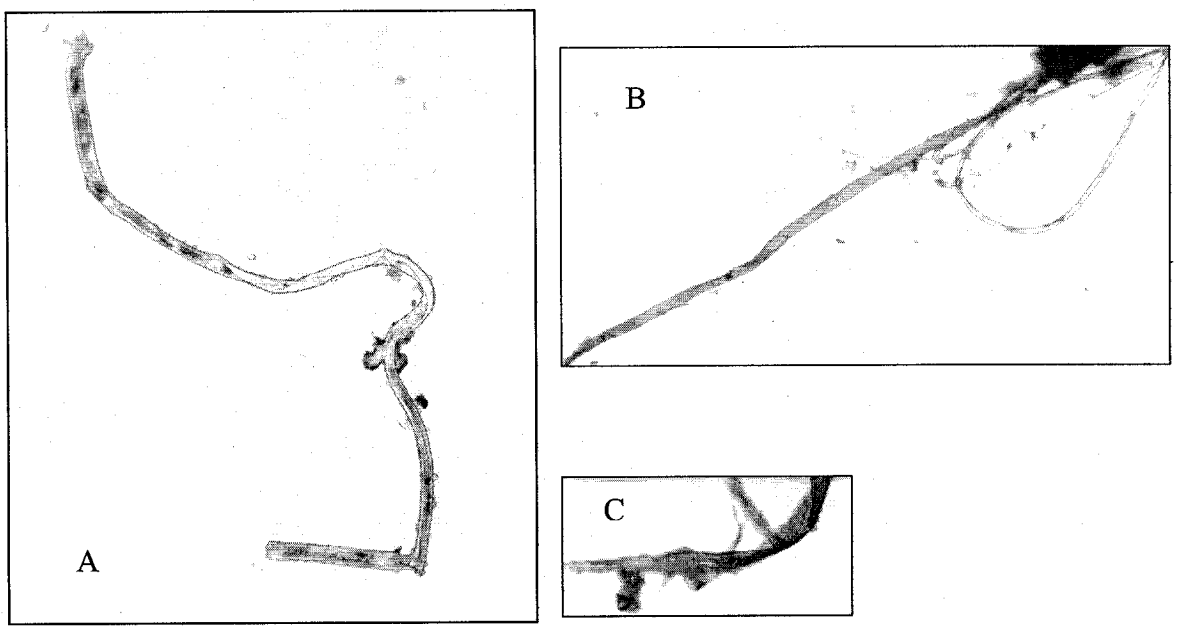
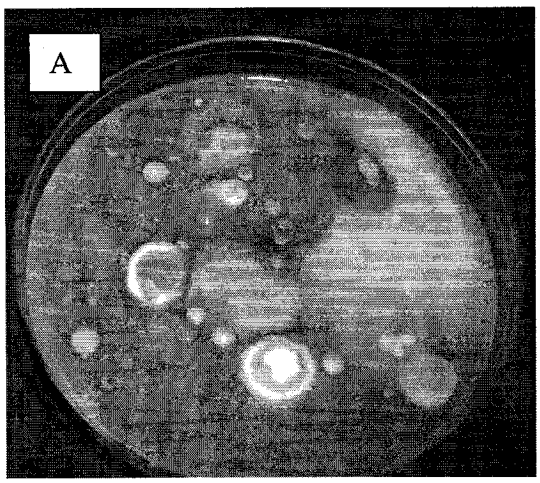
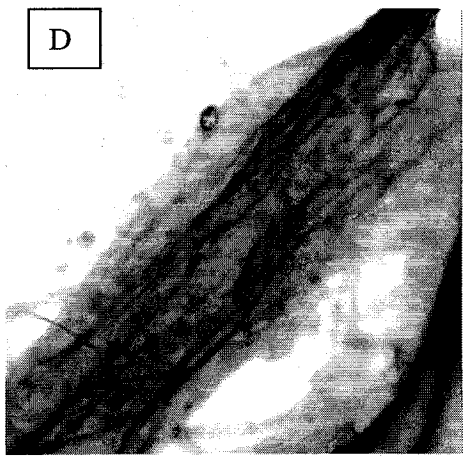
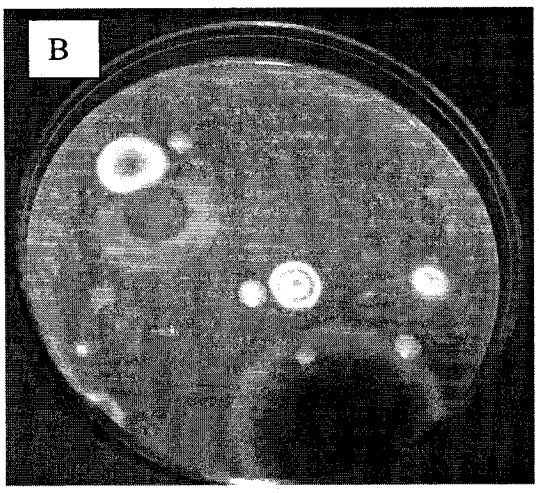


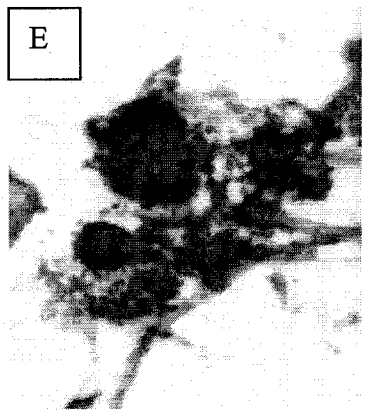


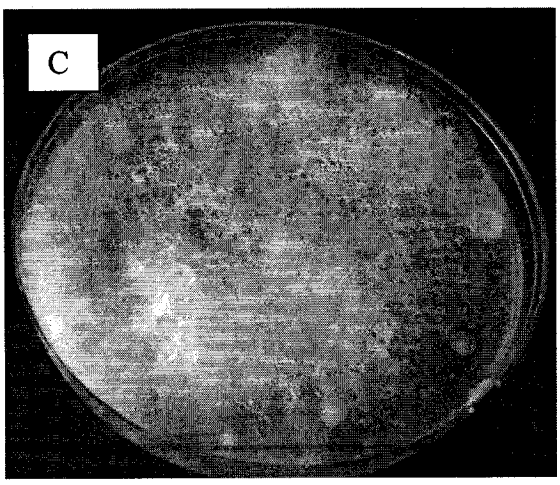
Plate II. Fungal Hyphae photographed at 625X magnification by a Microlumina Leaf Camera on an Olympus Microscope. Photographing a micrometer at the same magnification provided a size estimate of hyphal length and diameter. A. hyphal length 1.5 mm and diameter 20 μm, B. large hyphae: length 1 mm, diameter 7 μm small hyphae: length 1 mm, diameter 1 μm. C. hyphal diameter 20 μm, D. hyphal length 1.5 mm; hyphal diameter 2 μm. Fungal species unidentified.



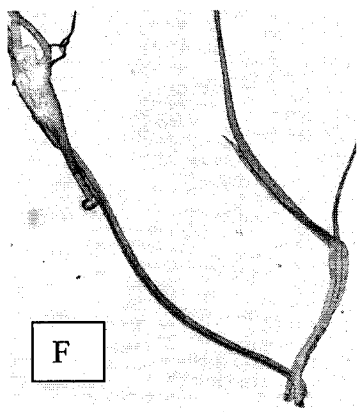
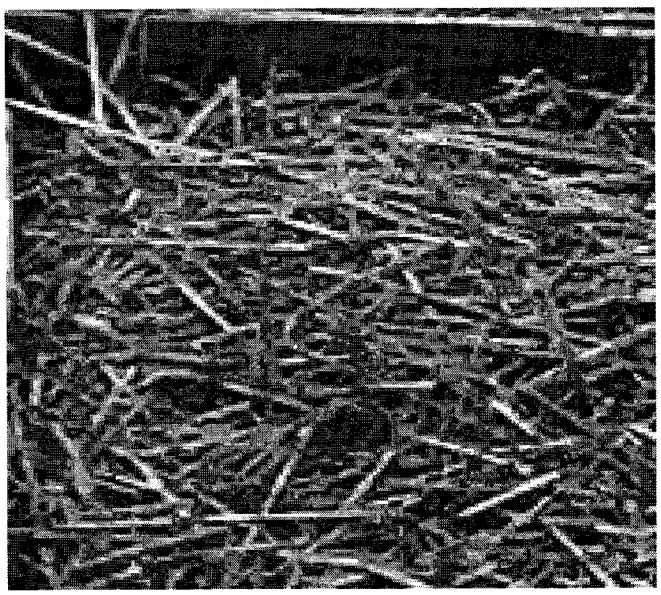




Plate III Digital photographs (actual size) of fungi cultured on Petri dishes showing small colonies (A, B) and filamentous growth C). Mycorrhizal hyphae and vesicles D) on an oat root stained with ink/vinegar at 400 X magnification. Fungi from Petri dishes at 400X magnification E) *Rhizopus*, F) Basidiomycete unidentified



a) Oat Straw

b) Corn stalks

Plate IV Chopped Oat straw (a) and corn stalks (b) used as surface residue in 2004 plots.

# Chapter 3

## Effects of oat (*Avena sativa)* plants and surface residue (oat straw and corn stalks) on fungal hyphal length, and soil moisture, aggregation and carbon.

### 3.1 Abstract

No-till (NT) agriculture is seen as a method to reduce soil and organic carbon (OC) loss, but there are few studies about surface decomposition of crop residue and the changes in fungi that may affect soil aggregation, moisture and OC. To test the relative contribution of the growing plant and dried surface residue to increases in OC observed in Chapter 2, plant and residue treatments were assigned in a factorial arrangement; with/without oat (*Avena sativa*) plants and with no residue, oat straw or corn stalks applied to the soil surface. The changes in the carbon to nitrogen ratio (C/N) of residue and the % OC in the light fraction organic matter in soil were compared between the oat straw and corn stalk residue. The growing oat plants in July decreased soil moisture and aggregate size. The percentage of macroaggregates (> 250 μm diameter particles) was subsequently increased by residue in August. Soil OC was increased by surface residue application in July. At harvest sampling in August, only the oat plants increased soil OC compared to the soil control.

Interaction between plant and residue increased total fungal hyphal length in the treatment combination of oat plants plus residue more than the effect of the oat plants or corn residue alone. Corn residue with a high C/N resulted in increased soil OC compared to straw, although the amount of OC loss (g) and % OC in the light fraction in soil were similar with both residues. In all corn residue plots, the percentage of heavy fraction organic matter correlated to hyphal length and OC. The oat crop plant was essential for

the increase in hyphal length. The resistance to decomposition, with high C/N of residue, was more important to OC increases than the amount of residue decomposed and %CLF.

### 3.2 Introduction

No-Till (NT) agriculture can reduce $CO_2$ loss from mechanical disturbance of soil where cultivation is the primary cause. Whilhelm et al. (2004) maintained that if all other factors in the environment were constant, the amount of residue returned to the soil determined increases in soil organic matter (and thus OC) content. The type of tillage also determines residue placement so surface residue remaining from NT may have a unique effect on OC and soil moisture in addition to benefits from reduced disturbance of aggregates.

Clapp et al. (2000) found that only with NT and residue returned to the surface, OC increased substantially in long-term field experiments of the effects of tillage, residue and fertilization on continuous corn cropping. This may relate to the earlier findings of Holland and Coleman (1987) that a greater portion of $^{14}C$ was retained in the soil when straw was surface applied rather than incorporated into the soil. In the previous season of this thesis study, OC increased significantly ($p < 0.001$) averaging a 0.65 ($\pm$ 0.1 SEM) increase in soil OC % from May to August only when ground cover was applied (see Chapter 2).

NT agriculture would allow fungi to become dominant in surface decomposition (Hu et al., 1995). Surface decomposition by fungi is also much slower than bacterial decomposition beneath the soil surface (Parton et al., 1987). It has been shown that the transfer of OC into stable microaggregates (< 250 μm) is highest with slowest turnover time (Tisdall and Oades, 1982). Only slow turnover of macroaggregates (> 250 μm)

promotes stable OC (Besnard et al., 1996; Six et al., 2004). The relationship of slow decomposition to OC is contrary to the expectation that rapid turnover time is necessary to increase OC (Lovelock et al., 2004).

The rate of decomposition of residue is reflected in the amount of OC in the light fraction of organic matter (% CLF) that is partly decomposed (Janzen et al., 1992). The carbon to nitrogen ratio (C/N) is thought to control the speed of decomposition where the amount of nitrogen available limits microbial growth. A study by Conti et al. (1997) reported an increased decomposition rate with low C/N and low OC soil with maize stubble residue, although no growing crop was included in the analysis.

The role of mycorrhizal fungi in soil aggregation has been established in grassland soils (Tisdall and Oades, 1979). Fungal hyphae were believed to provide transient binding along with roots in the formation of aggregates (Tisdall and Oades, 1982). In tall-grass prairie, Jastrow et al. (1998) found that mycorrhizal root colonization had the largest single effect on soil aggregation, followed by root length, and microbial biomass. Agricultural crops may have different relationships to the soil critical variables, as mycorrhizal occurrence varies with the plant species, and agricultural practices as well. The influence of surface crop residue on the volume of mycorrhizal hyphae has not been addressed in agriculture, and the relative contribution of saprophytic and mycorrhizal hyphae to soil aggregation and OC is unknown.

The purpose of this study was to test whether; 1) decomposition of dried straw and corn surface residue increased total fungal hyphal length with and without oat plants, and 2) if interaction of the residue and plant affected hyphal length. The effect of the crop plant on fungal hyphal length, soil aggregation, moisture and OC was separated

from the effect of residue by the factorial design. The effect of high and low C/N on % CLF was compared with straw and corn residue. The hypotheses were 1) plants and residue would both increase fungal hyphal length and the effects would be additive, 2) increased hyphal length would also increase soil aggregation, moisture and OC, and 3) a lower C/N ratio of straw compared to corn residue would have increased hyphal length, % CLF, and soil OC in residue treatments.

## 3.3 Methods

### *3.3.1 Study Plots*

Outdoor plots in the southern Ontario municipality of Clarington (44° N, 78° 30'E) described in Chapter 2, were used with a random factorial experimental design. A perforated plastic lining was added to the bottom of the plots to prevent upward movement of water and weed growth. Each of the 24 sections of the wooden frame was filled with a 12 cm depth of soil using a volume measure to ensure all squares had the same amount of soil. The soil was formed from 10 years of continuously decomposing a mulch layer on top of sand. The soil tested 89% sand (fine and very fine), 11% silt, <1% clay, pH of 7.5 and bulk density 1.15 wt/v and was mixed to reduce variance in soil conditions. Initial OC averaged 6.9% ± 0.63% SEM as detailed in OC sampling (3.3.3)

Randomly selected plots in the factorial set received the mulch; the factors were: no plants or oats x no residue, straw residue or corn stalk residue (Table 1.1, Plate I b). Oat straw from a neighboring farm and corn stalks that had stood the winter in a nearby field were mulched coarsely in a chipper-shredder (screen was removed) to a suitable size to be applied to the plots (Photo Plate IV). Mulch was placed on the soil surface on top of a plastic netting with 2 cm mesh that allowed the mulch to be easily lifted up and

weighed at harvest. The initial weight of straw and corn mulch was 264-270 g/plot and 192-200 g/plot, respectively representing a 3-4 cm depth as in Chapter 2. This rate of addition represents 4.3 tons/ha for straw and 6.3 tons/ha for corn residue which is about three times the mulch rate specified to maintain soil organic matter when incorporated into field soils of 2.6 – 5.3 tons/acre (1.1 – 2.17 tons/ha) (Balandreau and Knowles, 1978; Bruce et al., 1991). I estimated decomposition to be less than 50% per year from observation of a mulched cornfield in proximity to my study. At less than 50% per year decomposition, the corn mulch in the oat plots in this study would represent a depth that would accumulate on the surface after 2 seasons in NT.

Several days following preparation of the plots, oats (*Avena sativa* cv. Ida) were seeded May 6, 2004 by broadcasting seed over the mulch, or lightly raking the seeds into bare soil. Plots were kept weed free by hand weeding. Oats were thinned to 120 plants/plot that represented a field density of 333 plants $m^{-2}$ that is within the normal planting density for oat crops (Welch, 1995). Although dry weather delayed germination, a higher than normal amount of rain fell during the rest of the season so supplemental watering was not needed. Rainfall was measured after each weather event and compared with Environment Canada statistics.

*3.3.2 Sampling*

Soil samples were collected during creation of the plots before the mulch and seed were applied. A composite sample of approximately 250 g was formed from 4 independent samples through a 10 cm depth for each of the 24 plots. Sampling was repeated monthly until harvest, August 9, 2004. For subsequent soil samples, the top 2 cm was removed before sampling in bare soil plots, and the mulch layer was moved aside

on surface residue plots. In plots with oat plants, the soil sample was taken close to the oat roots without disturbing the plants. Any visible residue was removed. Half of each sample was promptly stored at 4°C and the other half frozen at -20°C. Plant height was measured using a 15 cm grid to select plants for sampling. At harvest the plants were cut 2 cm above the ground, dried 24 h at 80°C and weighed. The lifted mulch was washed to remove adhering soil and dried for 24 h at 80°C prior to weighing. Plant roots were loosened gently and extracted from the soil, and the mass recorded following washing and air-drying. A small sample of fine roots (about 20) was removed for mycorrhizal analysis.

*3.3.3 Soil Testing*

Soil moisture was determined by loss of mass after oven drying at 105°C and expressed as a percentage of dry weight (Nelson and Sommers, 1982). The oven dried soil samples were sieved to pass a 0.5 mm sieve and soil OC was determined from duplicate samples by loss on ignition at 375°C for 16 h in a muffle oven (Ball, 1964). The percentage OC was calculated from the proportion of the mass loss after ignition over the dry mass prior to ignition divided by 1.8, the proportion of OC to organic matter (Nelson and Sommers, 1982).

The aggregate size distribution was calculated by shaking 5 g of air-dried soil through a nest of sieves, sizes 4, 2, 1, and 0.25 mm, for 15 min as described by Kemper and Rosenau (1982). Each sieve size mass was recorded. The percentage macroaggregates was calculated from the mass of aggregates greater than 0.25 mm divided by total initial dry wt (5 g) x 100.

The 1-2 mm size aggregates were further analyzed for wet aggregate stability as described in Chapter 2 (2.3.2.2). The mean weight diameter index was calculated from the aggregate size distribution as the sum of the mean diameter of each weight class x the percentage mass of each size class of air-dried aggregates for all size classes (Kemper and Rosenau, 1982).

*3.3.4 Fungal hyphal length and Mycorrhizal colonization*

A soil suspension was made by combining 1g of thawed soil with 100 mL autoclaved water at high speed in a blender for 15 s. The soil solution with suspended organic matter was decanted off from sand grains that remained in the bottom of the beaker 10 seconds after stirring. The decant was stored refrigerated during analysis, up to 4 weeks, and hyphal length did not appear to change over time. Culture on whey media proceeded within 1-2 days of solution preparation. The fungal colony count and size analysis was not significant to the study, so results are reported along with the Chapter 1 culture analysis in Appendix B. Final figures were adjusted for the moisture percentage in the soil where fresh/frozen soil was used for testing.

Fungal hyphal length was estimated from observation of the soil suspension in a dish with 1.2 cm gridlines under 20 X magnification with a stereoscopic microscope. Hyphal length is a measure of the density of fungal growth in the soil (Kabir et al, 1997) and Lactophenol aniline blue stain was used to differentiate live hyphae (Berg et al., 1998) and thus measure recent fungal growth. Aniline blue (also known as cotton blue) is adsorbed to the β-glucan linkages in the cell walls (Paul and Clark, 1982). The length of each piece of blue hyphae in 10 mL of suspension with 3 drops of Aniline blue stain, was estimated in comparison with the known length of grid lines. The hyphal lengths

were summed in each dish, repeated 3 times, and averaged to give the total length in cm $g^{-1}$ soil dry wt. The length estimation of individual hyphae was confirmed by microscope photos (Plate II).

Mycorrhizal colonization on oat roots was enumerated with root staining by ink/vinegar stain following an adaptation of the method of Vierheilig et al. (1998) as detailed in Chapter 2. Evidence of hyphae, vesicles, or arbuscules that occurred on each 1 cm piece on a single view of the microscope at 400X magnification was scored as a percentage occurrence of the total number of inspected pieces (approximately 40) (Gryndler et al., 2002).

*3.3.5 Density fractionation of organic matter*

Light fraction organic matter (LF) is described as plant matter in the early stages of decomposition which is high in carbon, lipids and lignin (Lavelle and Spain, 2001). The heavy fraction (HF) is humic and mineral material or complexes that have a much higher density, and thus can be separated from LF by specific gravity (Lavelle and Spain, 2001). As detailed in Chapter 2 (2.3.2.3), a 5 g sample from each plot was wet-sieved to separate organic matter from sand. The organic matter was further separated into LF and HF with LUDOX $^{TM}$ HS-30 colloidal silica (Sigma Aldrich, Oakville), 1.2 g $cm^{-3}$ specific gravity (van den Pol-van Dasselaar and Oenema, 1999), following the method of Meijboom et al. (1995). The % LF and HF were calculated as the % dried mass of the fraction over the initial 5 g of whole soil adjusted for moisture content to dry weight. The discarded sand plus wt of LF and HF should total the initial dry mass of the sample. The LF and HF sediment were further tested for OC content (% CLF and % CHF), as discussed for soil (3.3.3).

### *3.3.6 Residue Organic Carbon and Nitrogen*

The straw and corn residue were assessed for OC and organic nitrogen at planting and harvest sampling. The residue was contained in 0.5 mm netting for washing, dried at 80°C for 24 h and ground in a Wiley mill prior to analysis. OC was determined as per soil. Organic nitrogen was determined with a LECO FP428 autoanalyser using the Dumas method (McGill and Figueiredo, 1993). The soil C/N was also calculated at harvest. The soil C/N should have been uniform in the homogenized soil in May and nitrogen concentrations were not varied specifically in the treatment design. There were no significant treatment differences in the soil nitrogen results, so methods and results are reported in Appendix C.

### *3.3.7 Statistics*

Results were examined with 2-way ANOVA model (Statistica, Statsoft): F = (Residue MS/error MS) + (Plant MS/error MS) + (Residue x plant interaction MS/error MS). Significant differences between the three residue treatments (control, straw and corn) were further tested with Fishers Least Significant Difference post-hoc test. Straw and corn residue results were combined for analysis as "residue" where there were no significant differences among the individual treatments. Normality was ascertained with the Ryan-Joiner test. All t-test probabilities reported from a comparison of two means were two-tailed. The study variables were separated into unrelated factors using Principal Components Analysis.

## 3.4 Results/Discussion

### *3.4.1 2-way model ANOVA (Oats x Residue)*

The ANOVA table summarizes the significant main effects of surface residue and the oat plants (Table 3:1). Surface residue increased soil moisture, macroaggregates and OC while the oat plant effects on variables were dependent on the plant growth stage. The live oat plants decreased soil moisture and aggregation at the July sampling, while the dried oat plants at seed set increased OC at August sampling.

### 3.4.2 *Soil Moisture*

There was an increase in soil moisture from residue ($p < 0.001$) of both straw and corn, and a corresponding decrease from the oat plants ($p < 0.001$) in July that caused interaction of the residue on the plant (Fig. 3:1). There was a much smaller, yet still significant reduction in soil moisture from the oat plant in August.

Table 3.1. 2 way model ANOVA (oats x residue) results of main effects of the oat plant and residue along with interaction term on soil variables. P values significant at 0.05 are highlighted in BOLD. Post-hoc comparison of residue means by LSD test.

| | *2-way ANOVA* | | | *LSD comparison of means* | | |
|---|---|---|---|---|---|---|
| July | Plant | Residue | Interaction | Straw-soil | Corn-soil | Straw-Corn |
| Moisture (%) | **<0.001** | **<0.001** | **<0.001** | **<0.001** | **<0.001** | 0.465 |
| Macroaggregates (%) | **-0.022** | 0.173 | 0.365 | 0.069 | 0.518 | 0.218 |
| Organic Carbon (%) | 0.974 | 0.062 | 0.853 | **0.040** | **0.040** | 1.000 |
| Fungal hyphal length (cm/g) | 0.248 | 0.139 | **0.035** | 0.250 | 0.051 | 0.376 |
| August | | | | | | |
| Moisture (%) | **-0.033** | 0.090 | 0.514 | 0.181 | **0.031** | 0.358 |
| Macroaggregates (%) | 0.187 | **0.042** | 0.714 | **0.031** | **0.025** | 0.920 |
| Organic Carbon (%) | **0.014** | **0.045** | 0.858 | 0.110 | 0.330 | **0.015** |
| Fungal hyphal length (cm/g) | **0.007** | 0.454 | **0.040** | 0.371 | 0.232 | 0.753 |

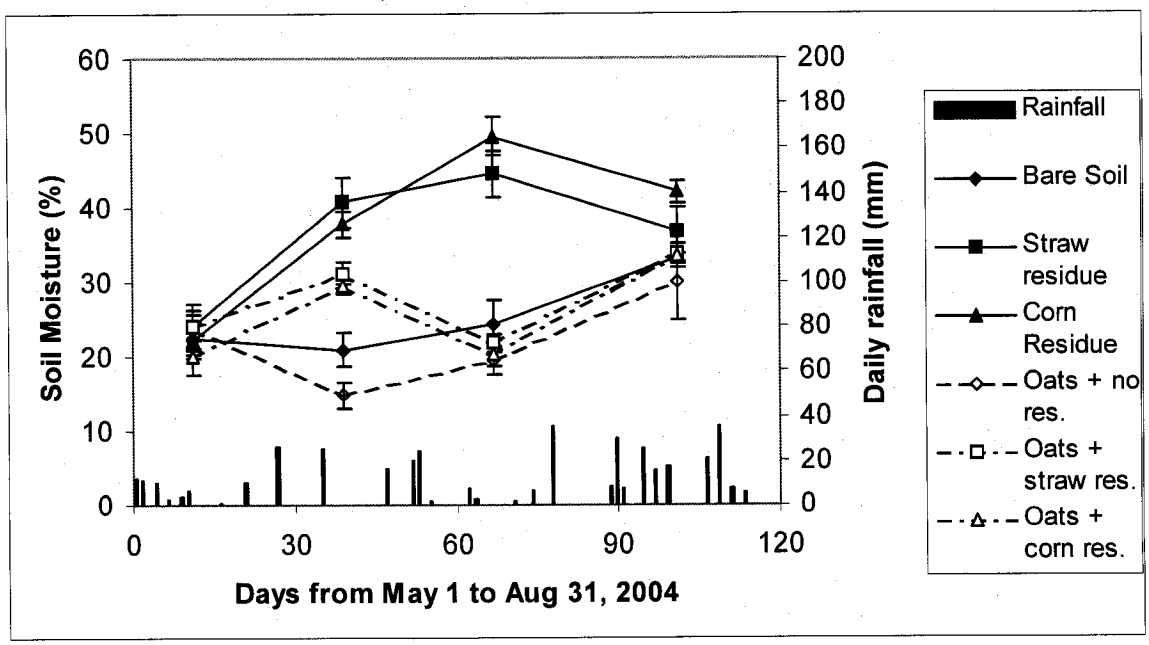



Figure 3.1. Soil moisture (%) at monthly sampling without plants in bare soil, straw residue, corn residue and with oat plants with; bare soil, straw residue, corn residue. Error bars represent SEM. (n = 4). Rainfall (mm) bar graph recorded at the study site from May 1st to August 15, 2005.

The soil moisture was approximately twice as high with surface mulch alone compared to plots with bare soil without plants. The difference between the residue alone treatments, and oat plants plus residue, represents increased uptake of water by the oat plants. This would enable the growing plants to maintain transpiration longer without restriction from soil moisture (Gonzalez-Sousa et al., 2001). Additional transpiration from surface residue would sustain photosynthesis in the plant for a longer time. There was no difference observed in plant growth or yield with residue (Appendix C, Table C.1) from increased transpiration that would have been expected in drought stressed conditions.

The straw mulch rate in my plots of 4.3 t/ha is greater than the rate of 3.4 t/ha quoted in Döring et al. (2005) as a minimum straw mulch rate necessary for significant evaporation control. The improvement in soil moisture from straw mulch was attributed to reduced evaporation and increased infiltration (Döring et al., 2005). The decrease in evaporation from surface residue and an increase in plant transpiration associated with oats grown in surface residue are in agreement with changes found in a modeling study (Gonzalez-Sousa et al., 2001) and in a field study with sorghum (Zaongo et al., 1997).

*3.4.3 Fungal Hyphal Length*

Fungal hyphae were differentiated only as "live" by the Aniline Blue stain to indicate recent activity that would correlate with changes in soil aggregation, LF and OC. Fungal hyphal length increased from 45 cm $g^{-1}$ in May to 60 cm $g^{-1}$ soil through June and July in all plots except bare soil. Hyphal length then decreased in August, to levels observed for bare soil in May, in all plots except with the combination of oats and residue where hyphal length increased (Fig. 3.2). There was significant interaction ($p = 0.035$) on the increased hyphal length from corn residue (Table 3.1) in July. In August there was increased hyphal length from the plant ($p = 0.007$) along with interaction ($p = 0.040$) where the hyphal length increased with the oat plant and residue together, but decreased with residue alone.

Both surface residue (oat straw or corn stalks) and oat plants were essential for the increase in hyphal length, as plots with residue alone or oat plants alone decreased hyphal length to the base level of bare soil over the season. The species of fungi that were evaluated as live hyphae were not identified, although it is possible they are a mix of mycorrhizal and saprophytic fungi as in the previous season study in plots with

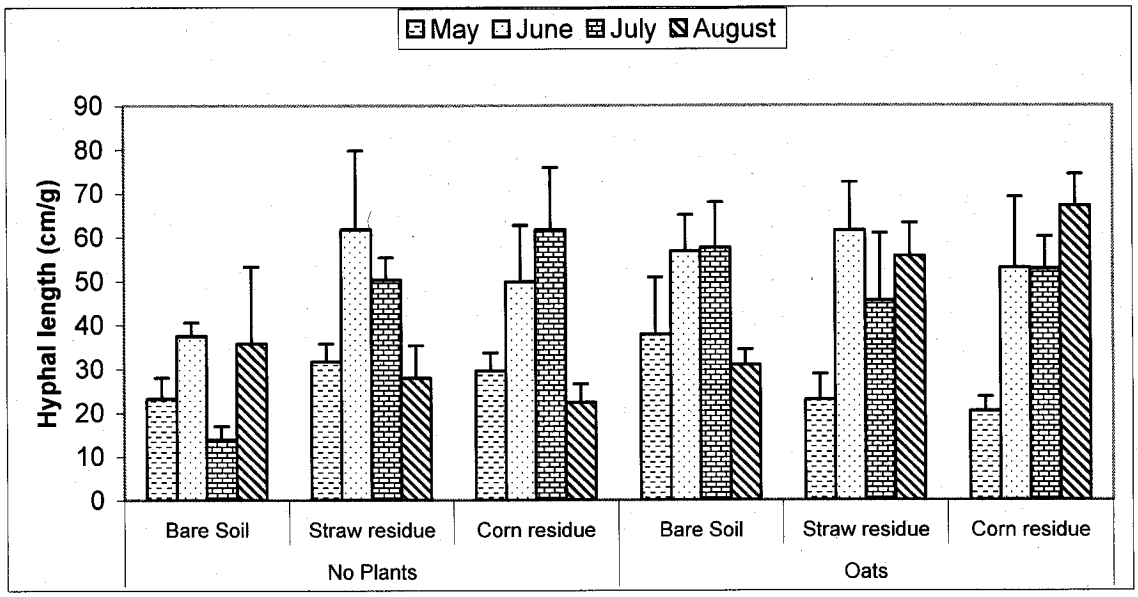



Figure 3.2. Fungal hyphal length (cm/g soil) measured in plots with no plant/oat plants and bare soil, straw residue, or corn residue at May, June, July, and August sampling. Error bars represent SEM. n = 4.

oats plus residue (Fig.2.3). There was also a division of fungi from the plot treatments with residue alone (saprophytic) and with oat plants alone (primarily mycorrhizal). Plant plots with residue, particularly with corn residue, showed a slow and steady increase in hyphal length from May to August, 2004. This could correspond to a peak of mycorrhizal growth in September, which has been previously observed (Biederbeck and Campbell, 1971; Kabir et al., 1997). Saprophytic fungal growth is maximum with warm temperature in July where fungal hyphal length was highest.

If mycorrhizal hyphae were dominant in August, the increase in hyphal length only with interaction of residue and plant implies a synergy of mycorrhizal and saprophytic fungi. This interaction resulted in much greater growth than the addition of

hyphal length from corn residue alone, or oat plants with no residue. My results agree with other reports on the interaction of microbial species. Singh and Kapoor (1999) found decomposer fungi had the greatest single effect on plant yield and soil nitrogen and phosphorous, all of which were 20% greater with mycorrhizal fungi, bacteria and saprophytic fungi together, than with any single or pair of the factors. Dighton et al. (1987) also found a synergy with mycorrhizal fungi of seedling tree roots that increased the decomposition rate of chitin in a microcosm study. Klironomos and Kendrick, (1995) found increased mycorrhizal hyphae in the presence of saprophytic decomposers of surface litter in simulated forest microcosms resulted from reduced predation by soil microarthropods on mycorrhizal hyphae. Mycorrhizal hyphae (Read et al., 1989) and plant roots (Balandreau and Knowles, 1978), both act as sinks for ammonium that is released from saprophytic fungi after decomposition of the residue. This would contribute to the increased growth of mycorrhizal and saprophytic fungi in concert.

*3.4.4 Soil Macroaggregates*

The oat plants significantly decreased aggregation ($p = 0.022$) in July (Fig 3.3). The % macroaggregates was subsequently increased by both straw ($p = 0.031$) and corn ($p = 0.025$) residue in August, over the no residue control with and without oats. The significant linear regression ($y = 0.66x - 8.56$, $r^2 = 0.67$, $p < 0.001$, $df = 23$) of soil aggregation with soil moisture in all plots, (Fig. 3.4), was strongest in residue plots. This agrees with previous studies on soil aggregation and water storage. In an extensive comparison of mulch rates/soil depth over NT, plow, and ridge till in 2 $m^2$ plots, residue determined the water retention of the soil, not tillage (Duiker and Lal, 1999). Franzluebbers (2002) studied water infiltration in NT and CT with specially constructed

drainage tubes in a greenhouse. The infiltration of water was 3 x greater under NT in their study and the infiltration rate was linearly related to the mean-weight diameter of soil aggregates and to soil porosity resulting in increased moisture holding capacity. The increased water holding capacity of increased organic matter was attributed to the increase in water content and reduction in water leaching to lower levels (Franzluebbers, 2002).

The water stability of the aggregates declined from May to June ($p < 0.001$ t-test) from 82.4% ± 3.5 SEM to 55.3% ± 1.4 SEM from the mixing of the soils in the creation of the plots. From June to August the water stability of the aggregates increased to an average of 76.9% ± 1.3 SEM in all plots which approached the May value. Plots with oat plants had higher wet aggregate stability than plots without plants in July and August, but this was not a significant effect, possibly due to the small differences over all treatments, so data are shown in Appendix C (Fig. C.2). Over all the plots, hyphal length was correlated to water stability of aggregates ($r^2 = 0.24$, $p = 0.015$, $n = 24$) (Fig. 3.5), with the strongest relationship observed in residue plots.

The weighted distribution of the aggregate size classes (mean weight diameter), calculated from the August distribution of soil aggregates (Appendix C, Fig. C.3), was correlated to OC ($r^2 = 0.41$, $p = 0.023$) and hyphal length ($r^2 = 0.38$, $p = 0.03$) over all plant plots ($n = 12$). This was stronger than the relationship of the water stability index to OC at any time in this study.

Highly significant linear relationships have been reported between OC concentrations and water stability, mean weight diameter or wet sieve index (Unger, 1997). This would be most common with NT where soil is not disturbed. In 113 soil

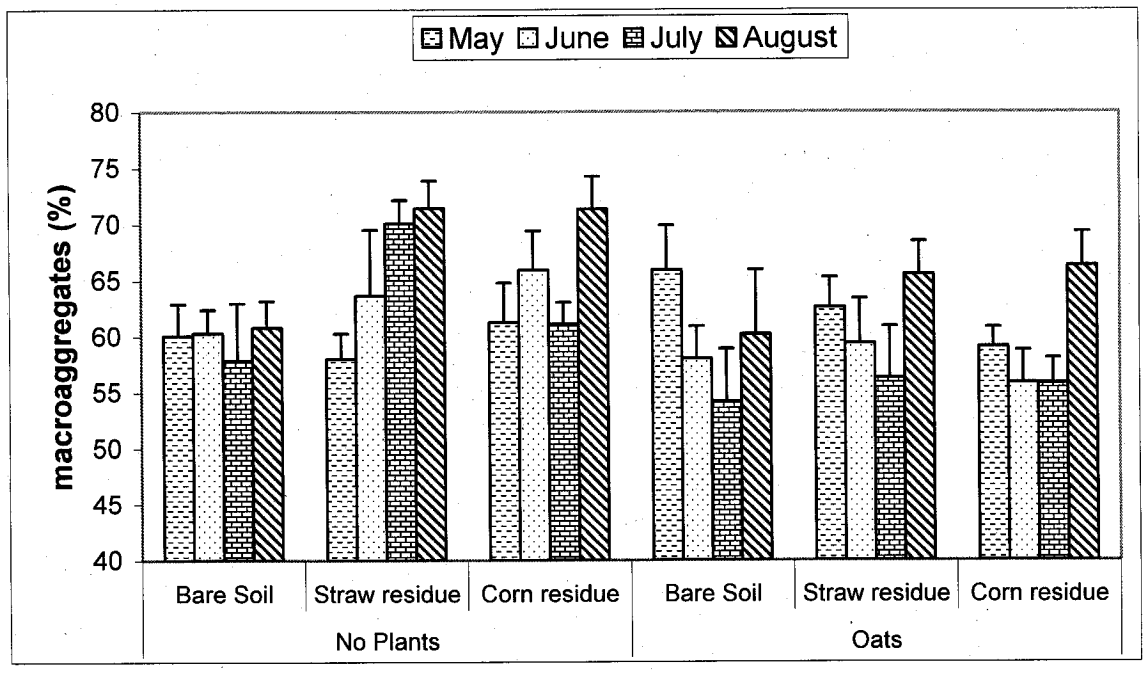



Figure 3.3. Percentage of soil macroaggregates measured in plots with no plants/oats with bare soil, straw residue, corn residue at May, June, July and August sampling. Error bars represent SEM, n = 4. There were no significant differences between treatments at any sampling time.

samples, Unger (1997) found an overall positive relationship between OC and aggregate stability. Angers and Mehuys (1988) found the water stability index of 1-2 mm aggregates were not as sensitive as the mean weight diameter to the effects of cropping treatments in field plots growing barley, alfalfa or corn using minimum disturbance after tillage. The mean weight diameter of aggregates only partly correlated with OC content ($r = 0.74$) reported in their study (Angers and Mehuys, 1988).

An increase in aggregate size from increased fungal hyphae was expected to correlate to changes in OC in my hypothesis but fungal hyphal length did not have significant correlation to the % macroaggregates except with the small sample treatment of corn residue plus oats ($n = 4$). Fungi have been associated with increased soil

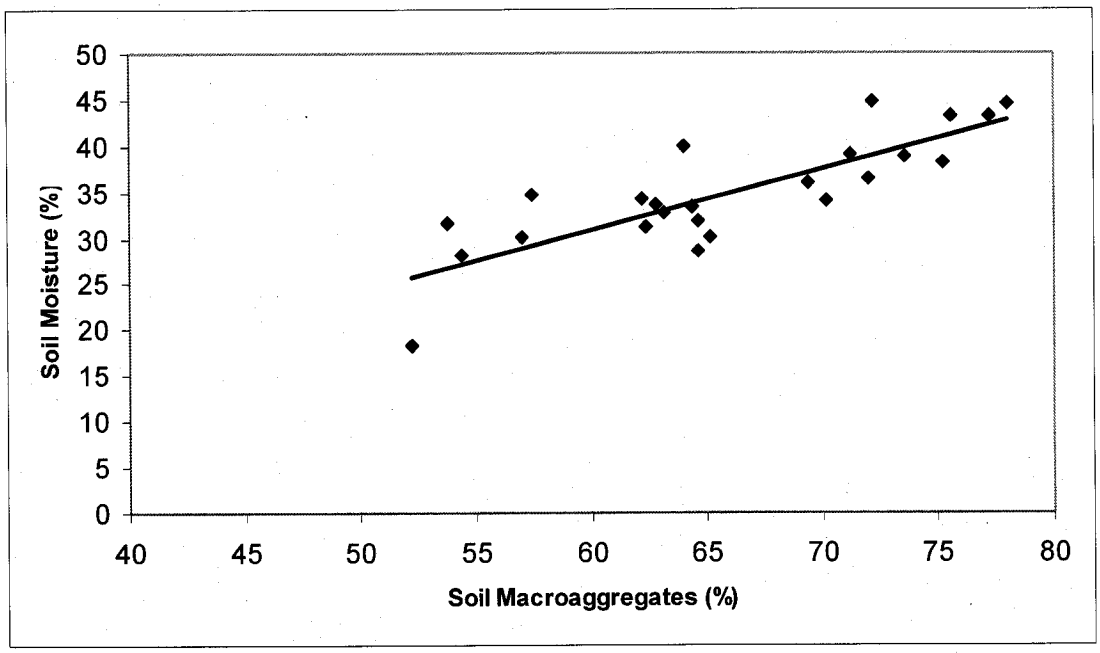



Figure 3.4. Linear regression of the effect of % macroaggregates on soil moisture (%) in August for all samples plots, $y = 0.66x - 8.56$, $r^2 = 0.67$, $p < 0.001$, $n = 24$.

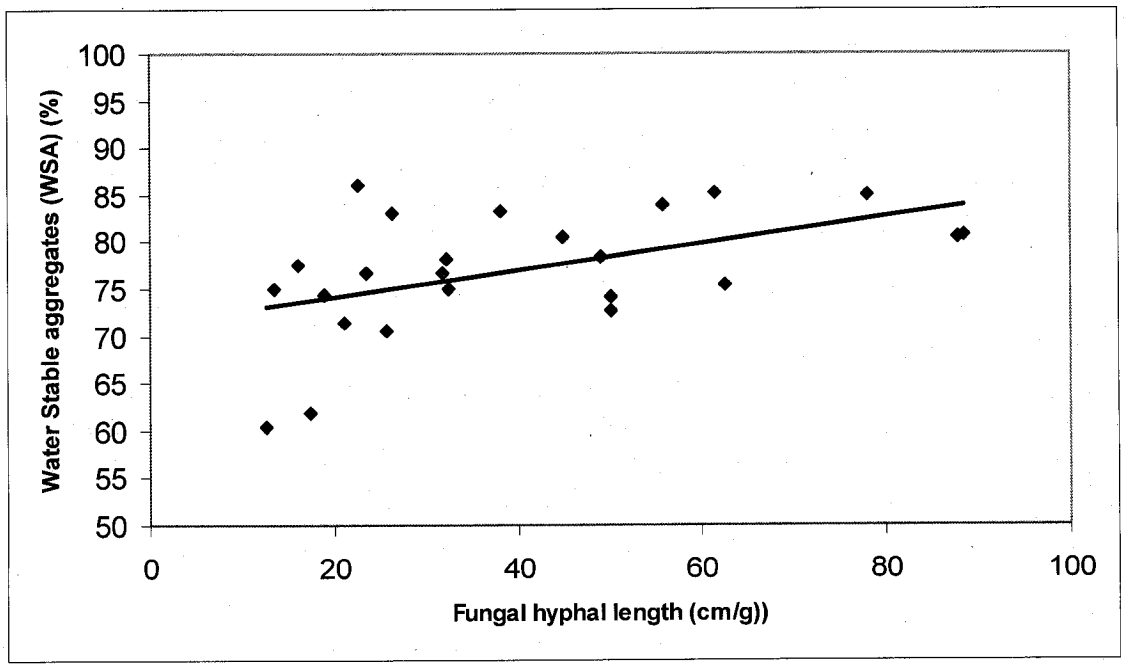



Figure 3.5. Linear regression of fungal hyphal length (cm/g) on water stable aggregates over all plots ($n = 24$) at August sampling; $y = 0.14x + 71.27$, $r^2 = 0.24$, $p = 0.015$.

aggregation in grasslands (Tisdall and Oades, 1979) and with winter rye planted into sorghum residue in NT (Beare et al., 1997) where there is high abundance of mycorrhizal fungi. Saprophytic fungal biomass from wheat straw decomposition was found essential to development of macroaggregates in 14 days in a microcosm study (Bossuyt et al., 2001), and the authors also suggested that only a threshold amount was necessary for aggregate formation. There may be differences in the effect of decomposer fungal species and mycorrhizal fungal hyphae on soil aggregates and OC. Oats have a lower amount of mycorrhizal association compared to grass, winter rye or corn, which may have contributed to the lack of correlation between hyphal length and % soil macroaggregates in this study compared to reported literature.

The hyphothesis predicted an increase in hyphal length to indirectly correlate to OC from reports where increased OC has been associated with increased aggregate size (Hu et al., 1995; Angers et al., 1997; Beare et al., 1997. Hyphal length did not correlate to OC in any treatment in this study. In a field study comparing soil aggregation in cropping systems, there was no significant relationship found between aggregate stability and OC in wet conditions (Hermawan and Bomke, 1997). The linear regression of mean weight diameter to OC was highest ($r^2 = 0.705$) when aggregate water content was between 20-30% in their study (Hermawan and Bomke, 1997). This would indicate the high soil moisture of 35% in my study that negatively affected aggregate size could have reduced the linear relationship of hyphal length to soil aggregation and between soil aggregation and OC. Macroaggregates contain a higher, yet more labile fraction of soil OC (Tripathy and Singh, 2004). The influencing factors appear paradoxical; while moisture is necessary to maintain fungal growth and to increase soil aggregation, the

binding power of organic matter is reduced in wet weather (Wilson, 1988). It is recognized that due to the supply of air and moisture, aggregates are constantly decomposing and must be continually maintained (Holland and Coleman, 1987).

The decreased % macroaggregates with oat plants in July was contrary to the increase in macroaggregates from oat plants predicted in the hypothesis. The decrease in macroaggregates from the oat plant in July and not in August, is consistent with high exudation rates early in the growing season that diminish when the plant dries (Whilhelm et al., 2004). There is debate over the effect of polysaccharides in the binding process of macroaggregates and their contribution to stable OC (Angers and Caron, 1998; Six et al., 2004). The negative effect of plants on soil aggregates in July, indicate root secretions do not increase soil aggregation or OC at high levels of OC, as may be expected when soil aggregation and OC are at low levels (Hale et al., 1978).

*3.4.5 Organic Carbon (OC)*

Soil OC in plots with residue (with and without oat plants) was increased significantly with straw ($p = 0.04$) and corn ($p = 0.04$) residue by LSD comparison of means compared to the "no residue plots" in July (Fig. 3.6). The soil OC increased again from July to August, ($p = 0.001$ t-test) over all plots. There was a significant effect of the oat plants ($p = 0.014$) at August sampling. Plots with corn residue plus oats had the highest OC, but due to a large increase in bare soil alone, corn residue had significantly greater OC than straw residue ($p = 0.045$) with and without oats, and the no residue plots were intermediate in OC. The large increase of approximately 1% of soil OC in plots with soil alone in August as well, indicated other factors in the environment (such as

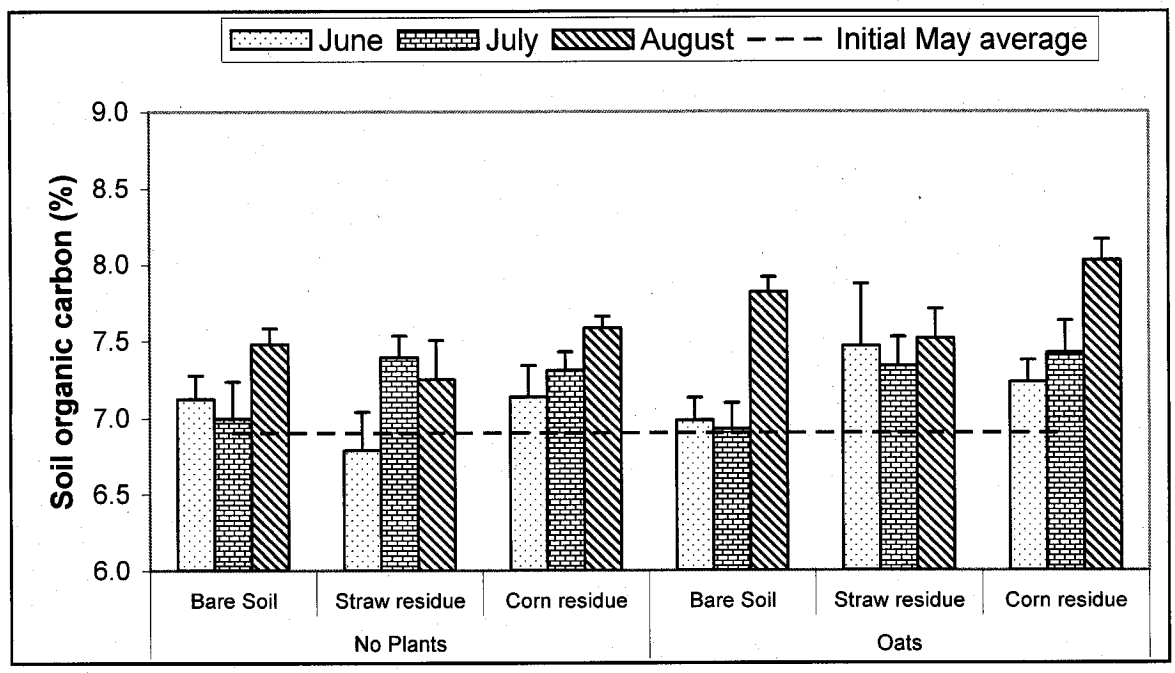



Figure 3.6. Soil OC (%) comparison in plots with no plants/oats with no residue, straw residue or corn residue at each month sampling of May average of 6.9%, June, July and August 2004. Error bars represent SEM, n = 4.

algae, cyanobacteria, soil fauna, or insects) may have increased OC in all the plots in the warm and constantly moist conditions of the preceding four weeks.

The increased effect of the oat plants on soil OC may be related to high root mass stimulated by the heavy rain. In a pot study under growth chamber conditions, Gale and Cambardella (2000) found a greater amount of $^{14}C$ was retained from oat roots in the soil (42%) compared to oat leaves left to decompose on the surface (16%). The authors suggested that a portion of root OC is rapidly released into the soil after plant senescence, followed by increases in stabilized, protected, slow decomposition forms (Gale and Cambardella, 2000). The initial decomposition over 90 days in their study is consistent

with the length of time for lignin to become stabilized in soil (Parton et al., 1987). It would be possible for a large volume of very fine roots to have an effect on soil OC within 30 days (Jastrow et al, 1998). Balesdent and Balabane (1996) also calculated that corn roots provided a larger amount of OC than corn leaves and stalks left on the soil over a year (57 g $cm^{-2}$ compared to 36 g $cm^{-2}$ respectively).

Mycorrhizal fungal colonization on the oat roots did not show significant treatment effects (Appendix C, Table C.2). The colonization of mycorrhiza on oat plant roots does not correspond directly to hyphal length (Schenck, 1982) as hyphae development varies with species and soil conditions (Johnson and Pfleger, 1992). Mycorrhizal hyphae have been found to contain a protein glomalin which is water insoluble and heat stable (Rillig et al., 2002a) and contains 60% OC (Wright and Upadhyaya, 1998). Glomalin can be linked to soil OC storage from its association with increased aggregation (Rillig et al., 2002a), and through slow decomposition to a direct correlation with OC (Rillig et al., 2003). Glomalin concentration decreased by only 25% after 5 months in a prairie soil (Steinberg and Rillig, 2003). With high rainfall conditions, the slower decomposition of mycorrhizal hyphae compared to saprophytic fungal hyphae could have contributed to the increased hyphal length, soil OC and water stability of aggregates observed with oat plants. Hyphal length may have been reduced in plots with plants without residue from predation by soil microarthropods as suggested by Klironomos and Kendrick (1995).

*3.4.6 Residue Decomposition and Soil OC Mass Balance*

The initial C/N ratios for straw and corn were 31 and 74, respectively. There was no difference in the total residue mass loss (g) or residue OC loss (g) between straw or

corn residue (Table 3.2). However by percentage mass, corn residue had less residue loss ($p < 0.001\%$), OC loss ($p = 0.001$) and % nitrogen loss ($p < 0.001$) than straw reflecting the higher weight of corn residue and slower decomposition. The OC loss from residue calculated in the Mass balance analysis ranged from 32 to 66 g in individual plots or from 89 to 183 g $m^{-2}$ (Table E.2, Table 3.4). See Appendix E for mass balance calculation details.

Assuming that 50% of the OC would be retained in the soil after decomposition, this averages around 60 g $m^{-2}$ OC that could increase soil OC. This is less than 1/10 of the increase in soil OC that was recorded over the 4 month growing season (Table 3.4). Plant root exudates and mycorrhizal fungal biomass were estimated to contribute about the same OC as from residue decomposition. However, plant root biomass decomposition would have contributed 5 times that amount, or about 50% of the increase in soil OC recorded assuming about 50% of the roots decomposed into soil OC within that time. The large increases in plots without plants are unexplained.

*3.4.7 Organic Matter Density Fractions*

The % CLF did not differ significantly among treatments (Table 3.3) or correlate to OC. The average % CLF of 18.83 ± 0.39 SEM was greater than the average % CHF (15.6 ± 0.52 SE) ($p < 0.001$) and % HF (29.62 ± 1.37 SEM average) was greater than % LF (7.17 ± .51 SE average) ($p < 0.001$) in all plots (Fig. 3.7). The %OC in each fraction is similar to results of other studies. Lynch (2002) reported 2.62% OC in LF and 23.71% OC in HF of 3-4% OC soil in the 0-10 cm depth in field studies. Janzen et al. (1992), also found %CLF from 20-30% on various field locations in the prairies.

Table 3.2. Comparison of means ± SEM of study variables in % and mass loss, w/wo oats with straw and corn residue decomposition. Significant differences between straw and corn by 2-way model ANOVA are highlighted in bold where $p < 0.05$, $n = 16$.

| Residue | | Straw | | Corn | | Straw | | Corn | | Straw/corn |
|---|---|---|---|---|---|---|---|---|---|---|
| Plant | | - | | - | | Oats | | Oats | | ANOVA |
| | | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM | p value |
| Mass | | | | | | | | | | |
| Residue loss | g | 76.86 | 3.68 | 77.07 | 6.94 | 85.13 | 2.08 | 80.86 | 8.43 | 0.734 |
| OC loss | g | 41.48 | 3.06 | 41.85 | 2.27 | 43.92 | 2.11 | 50.62 | 6.78 | 0.398 |
| Nitrogen loss | g | 1.44 | 0.07 | 0.28 | 0.03 | 1.41 | 0.10 | 0.33 | 0.05 | <0.001 |
| Percentage | | | | | | | | | | |
| Residue loss | % | 49.47 | 2.12 | 33.98 | 3.09 | 53.92 | 0.95 | 35.41 | 3.59 | <0.001 |
| OC loss | % | 58.40 | 4.03 | 38.23 | 2.07 | 60.89 | 2.85 | 45.89 | 5.84 | 0.001 |
| Nitrogen loss | % | 64.63 | 2.90 | 19.56 | 2.41 | 62.10 | 3.83 | 22.08 | 3.82 | <0.001 |
| Residue CN | | 37.39 | 2.08 | 56.95 | 2.67 | 33.24 | 2.31 | 51.95 | 6.37 | <0.001 |

Table 3.3. Comparison of means ± SE of %CLF and %CHF with no residue, straw residue, and corn residue, w/wo oat plants. Treatments were not significantly different. $n = 4$.

| | | %CLF | | %CHF | |
|---|---|---|---|---|---|
| Plant/no Plant | Residue | Mean | SEM | Mean | SEM |
| No plant | No residue | 20.07 | 2.24 | 15.45 | 1.97 |
| | Straw residue | 18.37 | 2.14 | 14.37 | 1.90 |
| | Corn residue | 19.24 | 2.19 | 17.44 | 2.09 |
| Oats | No residue | 17.69 | 2.10 | 15.60 | 1.97 |
| | Straw residue | 18.37 | 2.14 | 15.01 | 1.94 |
| | Corn residue | 19.24 | 2.19 | 15.77 | 1.99 |

Table 3.4. Mass balance calculations of the OC ± and SEM existing in the soil in May, July and August, the difference in OC between each time interval, and the possible input of OC from surface residue and oat plants.

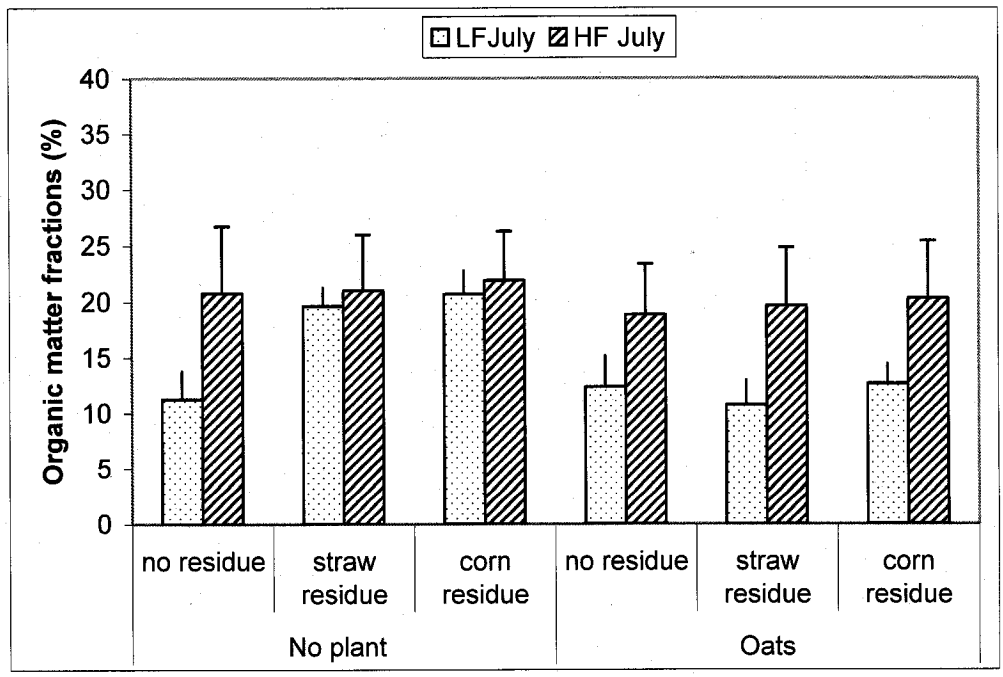
| **OC Mass Balance in g $m^{-2}$** | | | | | | |
|---|---|---|---|---|---|---|
| g OC in 50 Kg/0.36 $m^2$ | | | Plot treatments (Mean figures) | | | |
| over 12 cm depth | **No Plants** | | | **Oat Plants** | | |
| Residue | No | Straw | Corn | No | Straw | Corn |
| May, 2004 Soil OC | 9208 | 9028 | 9694 | 9236 | 9833 | 10208 |
| SEM | 551 | 106 | 119 | 51 | 268 | 99 |
| July, 2004 Soil OC | 9722 | 10278 | 10153 | 9625 | 10194 | 10306 |
| SEM | 328 | 195 | 170 | 234 | 265 | 291 |
| Aug, 2004 Soil OC | 10389 | 10069 | 10528 | 10861 | 10444 | 11153 |
| SEM | 143 | 359 | 109 | 140 | 266 | 190 |
| | | | | | | |
| May to July change in OC | 514 | 1250 | 458 | 389 | 361 | 97 |
| SEM | 680 | 192 | 164 | 208 | 494 | 385 |
| July to Aug change in OC | 667 | -208 | 375 | 1236 | 250 | 847 |
| SEM | 379 | 448 | 189 | 323 | 303 | 401 |
| May to Aug change in OC | 1181 | 1042 | 833 | 1625 | 611 | 944 |
| SEM | 574 | 410 | 210 | 184 | 305 | 205 |
| | | | | | | |
| May to August | | | | | | |
| Residue OC loss g $m^{-2}$ | | 115 | 116 | | 122 | 141 |
| SEM | | 8.5 | 6.3 | | 5.9 | 18.8 |
| 50% OC assimilated g $m^{-2}$ | | 58 | 58 | | 61 | 71 |
| | | | | | | |
| Plant contributions* | | | | | | |
| Root biomass (19%) ^ | | | | 1018 | 790 | 887 |
| Root exudates 0.5% | | | | 27 | 21 | 23 |
| Mycorrhizal biomass 1% | | | | 54 | 42 | 47 |
| Total OC from plant Ps to soil | | | | 1099 | 852 | 957 |

*Carbon fixation and allocation rates by Paul and Kucey, 1981
Fixation rate: 7.6 mg of OC per gram shoot per hour
Root allocation: 19% biomass, 28% respiration, 0.5% exudates

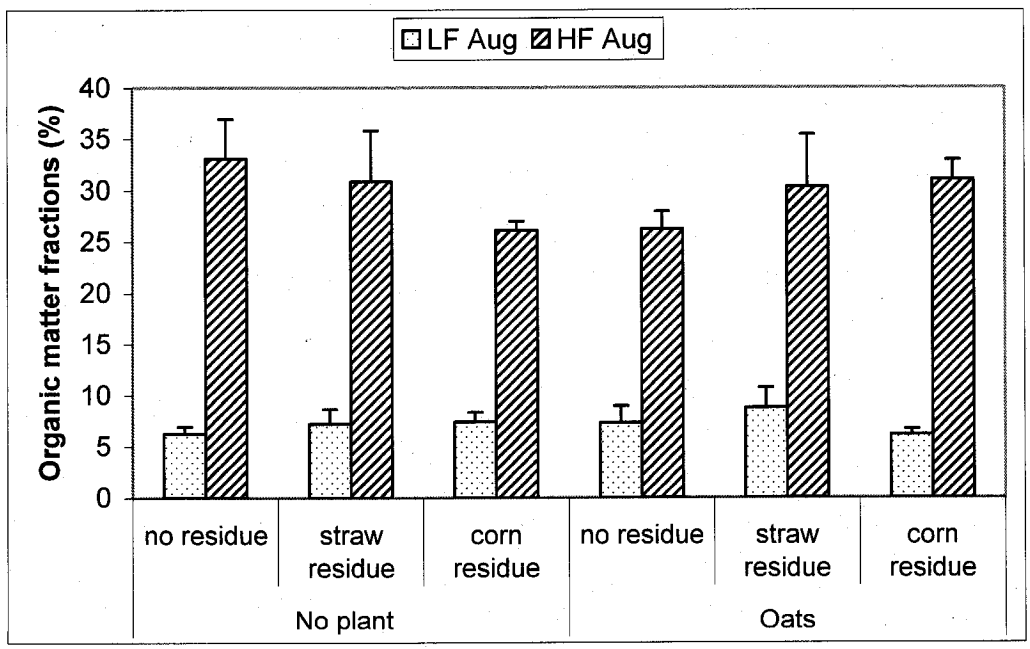
^ Available for decomposition

The high %CLF in my study would indicate the presence of newly formed and highly labile OC. Bending et al. (2000) found LF to increase with mulching on sandy loam with the measurement of microbial populations by substrate profile. The %CLF was related to biochemical composition of the additions of various residues decomposing within soil (Bending et al., 2000). Hassink (1995) reported the effect of residue was greatest on LF, and was greater on sand than clay. My study differed from this general trend. From July to August, LF decreased and HF increased in all plot treatments (Fig. 3.7) that may be related to their relative protective capacity in high rainfall and relationship to soil aggregate size. There was a correlation of LF to macroaggregates in July ($r^2 = 0.28$, $p = 0.007$) and August ($r^2 = 0.16$, $p = 0.05$) over all plots ($n = 24$). In plots with oats in corn residue ($n = 4$), LF and HF appeared as opposites; hyphal length correlated negatively to LF ($r^2 = 0.94$, $p = 0.03$), and positively to HF ($r^2 = 0.89$, $p = 0.05$). At August sampling, HF correlated to hyphal length ($r^2 = 0.78$, $p = 0.004$) and OC ($r^2 = 0.63$, $p = 0.019$) in all treatments with corn residue ($n = 8$).

The increase in HF in August may be associated with mycorrhizal hyphae, and slower decomposition of corn residue into more stable forms. In August, there was an increase in OC with the corn residue and oats where hyphal length correlated to HF, compared to lower OC with straw residue and oats where hyphae length correlated to LF. Corn residue also has a higher lignin content and C/N than straw. Lignin is primarily decomposed by fungi (Basidomycetes) for which lignin is the sole source of OC, and nutrient nitrogen is repressive (Kirk et al., 1980). High lignin content allows direct stabilization of plant material into slowly decomposing organic matter pools (Carter and Stewart, 1996) that are represented by HF in this study. There would be decomposition




a) July




b) August

Figure 3.7. Light fraction (LF) and heavy fraction (HF) of soil organic matter, separated at density of 1.2 g $cm^{-3}$ in a) July and b) August in plots with no plants/oats and with no residue, straw residue or corn residue. Error bars represent SEM. n = 4. In August, LF and HF are significantly different, $p<0.05$.

of lignin in the 4 month study time, although a direct estimate of lignin loss from residue, existence of lignin in soil aggregates and evidence of lignin decomposer species would be needed to confirm this. The higher recalcitrance of corn residue could explain the correlation of HF with MWD ($r^2 = 0.95$, $p = 0.025$, $n = 4$) only with corn and oats where the decomposed material increased the HF rather than the LF fraction and had different effects on soil aggregation and OC. Plots with straw residue alone did not maintain OC in August but plots with oat plants plus residue had 2 x the OC of straw or corn residue alone (Table 3.4).

*3.4.8 Principal Components Analysis*

Principal components analysis replaces original correlated variables with unique representative factors. The major variables in this study were separated into three factors; moisture, macroaggregates, and LF represented labile OC that accounted for most of the 29% variance explained by PC1, water stable aggregates, soil OC and hyphal length represented a second factor of stable OC that accounted for about 26% of the variance explained by PC2, and HF primarily accounted for the 3$^{rd}$ PC that explained 15% of the total variance (Table 3.5).

HF is visually close to stable OC while LF is correlated to labile OC in the plot of the 1$^{st}$ two principal components (Fig. 3.8). Dinesh (2004) also reported metabolic activity to represent the 1$^{st}$ factor of the PCA while fungal decomposition represented a second factor with a leguminous cover crop tilled into the soil for 10 years in a tropical climate.

Table 3.5. Principal components factor score coefficients of principal soil variables from August, 2004 sampling of all plots (n = 24) along with the %variance explained by each PC.

| Variable | PC 1 | PC 2 | PC 3 | |
|---|---|---|---|---|
| % soil moisture | 0.412 | -0.150 | 0.191 | |
| % macroaggregates | 0.462 | 0.034 | 0.080 | |
| % WSA | -0.083 | 0.349 | -0.009 | |
| Hyphae length (cm/g) | 0.053 | 0.368 | 0.252 | |
| % organic carbon | 0.084 | 0.446 | -0.160 | |
| % light fraction | 0.310 | 0.309 | -0.378 | |
| % heavy fraction | 0.076 | -0.023 | 0.832 | |
| % Variance explained | 0.295 | 0.264 | 0.156 | 0.715 |

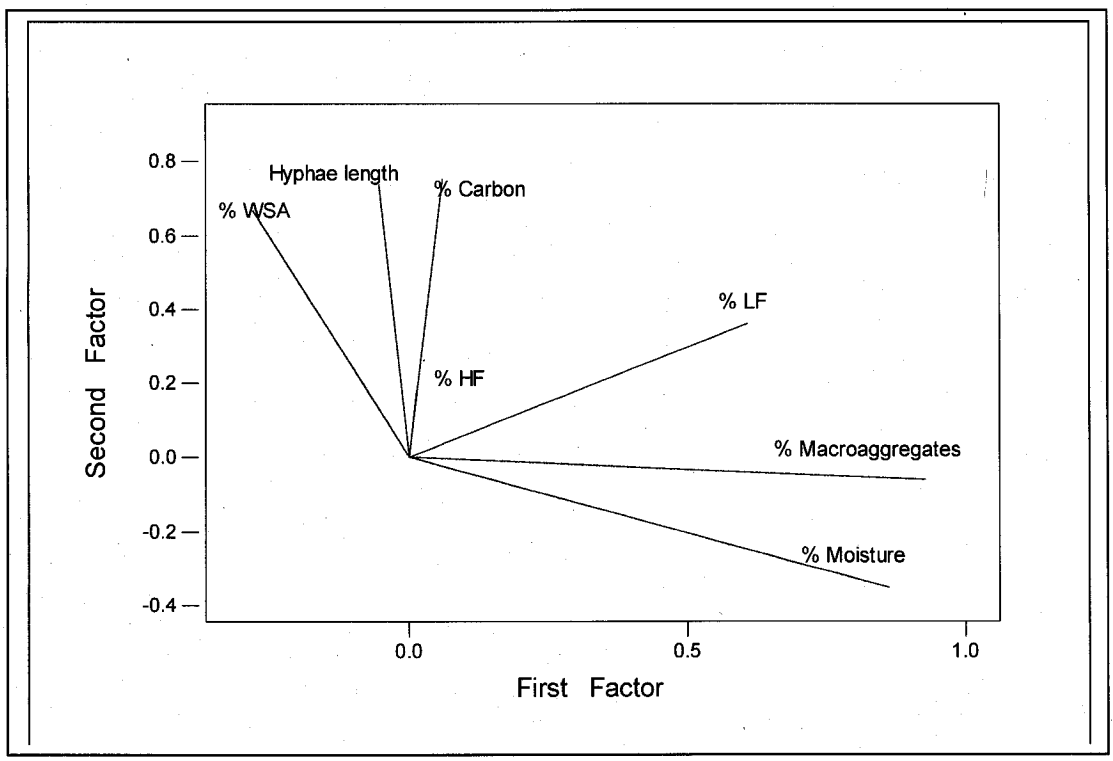



Figure 3.8. Principal components loading plot of first two PC's of selected soil variables at August sampling over all plots treatments, n = 24. PC1 includes moisture, macroaggregates and LF, PC2 includes WSA, soil OC and hyphal length, and PC3 is HF.

A very high level of organic matter (which would have larger size aggregates) is thought to be associated with high LF, greater C/N, and external hyphae (Six et al., 2004). In my results, the % macroaggregates, but not soil OC, correlated to LF in July and August over all plots, but was not generally correlated to the soil C/N or hyphal length. The lack of correlation may have been from the high level of aggregation and moisture in the study, or effects of different fungal species from the surface residue treatments. The resistance to decomposition measured in HF and hyphal length was more important than the amount and speed of decomposition (%CLF) in maintaining soil moisture, aggregation and OC in my results. The large unexplained increase with soil alone from July to August limits the conclusiveness of the study.

### 3.5 Conclusions

Surface applied straw and corn residue increased soil aggregation, moisture and OC in the study plots. The live oat plants in July decreased soil moisture and % macroaggregates while hyphal length and OC increased with the dried oat crop in August. High soil moisture or small variances at high OC may have reduced the predicted relationships of fungal hyphal length to macroaggregates and OC. Oat plants alone had a significant effect on soil OC in August. In plots with oats and corn residue together the hyphal length was greater than the length found with the addition of hyphal length in oat plants alone or residue plots separately implying a synergy of the plant mycorrhizae with residue.

Although the decomposition of residue by mass loss (g), OC loss (g) and % CLF was similar for straw and corn residue, corn residue had significantly greater C/N and soil OC than straw. Heavy fraction organic matter was associated with stable increases in OC

while the light fraction was associated with labile OC increases correlated to soil moisture and aggregate size in principal components analysis. The storage of OC in HF was more important than the amount of residue measured as % CLF to accumulating soil OC. Corn residue is an abundant resource that would have maximum benefit to improve soil moisture and OC in subsequent crops in No-till agriculture, when left to decompose slowly on the soil surface.

# Chapter 4

# General Discussion

*4.1 Soil Organic Carbon*

An increase in soil OC from surface ground covers and oat plants was observed in both crop seasons. Soil OC increased over the three month growing season in all ground cover treatments in 2003 but not in plots without surface treatment regardless of initial OC. In the factorial experiment in 2004, there was a significant effect of the straw and corn residue on increasing soil OC in July, but only the effect of the oat plant was greater than the control plots of bare soil at August harvest by Fishers LSD test. Plots growing oats in corn residue had the highest soil OC at each sampling, and plots with corn residue (w/wo oats) had significantly higher soil OC than straw residue. Significant soil OC increases in the plots with soil alone could not be explained, and may have affected results in other treatments as well.

Significant increases in soil OC within the growing season have not been reported in field studies. Most increases with NT are measured over 4-8 years in long-term studies, and are estimated to average 0.05% a year (Lal et al., 1998). In this study we reduced analytical variance by homogenizing the soil prior to setting out experimental treatments. High rates of residue addition and high soil moisture available for plant growth may have contributed to the average of 0.5% increase in OC observed in two seasons. My results support the theory of the litterbag study by Holland and Coleman (1987), that soil OC is increased in the short term only with surface application of residue.

Although there was not strong correlation of fungal hyphal length to soil OC in my study as hypothesized, there was some evidence that mycorrhizal hyphae were involved in the OC increases observed. Fungal hyphal length was significantly increased in treatments with plant/residue interaction of straw and corn residues in 2004. In the month of August soil OC increased with corn residue significantly more than straw (Table 3.2, Chapter 3). The change in soil OC between straw and corn residue may have been related to changes in the soil organic matter density fractions (LF and HF) from decomposition. HF was correlated to hyphal length and OC with corn residue, but not with straw residue. Soil OC associated with straw decomposition resulted in increased LF in the soil, which could be rapidly recycled without increasing OC. In contrast, soil OC associated with corn stalk decomposition correlated to HF which represents formation of organic matter complexes more resistant to decomposition. Thus, the highest soil OC was in the treatments receiving corn residue together with growing oat plants.

A comparison of residue quality was reported by Hassink, (1995), who separated the density fractions of soil organic matter derived from incorporated residue with silica. Straw increased OC in LF, while farmyard manure increased OC in HF, owing to differences in the rate of decomposition. In my study in both season, HF increased with all ground cover plots compared to no residue plots and %CLF did not vary significantly between ground covers. Compost was both surface applied and incorporated in my 2003 study. OC and HF were significantly greater with surface application compared to incorporated compost, while %CLF did not vary.

The mass loss of residue (g) over the 4 months season in my oat plots was less than 0.1 of the increase in soil OC. The mass balance figures (Table 3.4, Chapter 3) show that OC can significantly increase in soil, although we do not understand all the factors. Taking into consideration that my soil OC of 6-8% is about 2-3 times higher than field scale, I have reported soil OC of 9000-11000 g $m^{-2}$. A comparison of soil OC in No-till and conventional till in various field sites in Eastern Canada found soil OC in the surface 10 cm increased from 2400 to 3000 g $m^{-2}$ in response to 11 years of management although no differences existed when including historic OC to 60 cm (Angers et al., 1997). The highest OC figures reported in field studies are with clay soils (Angers et al., 1997; Wanniarachchi et al., 1999). A comparison of cropping systems with corn on two study sites reported 2474 ± 208 g OC $m^{-2}$ on clay and 828 ± 150 g OC $m^{-2}$ on sandy soil in 0-10 cm depth (Wanniarachchi et al., 1999).

The high rainfall for 4 weeks prior to harvest may have related to increases in OC. Plant root mass and mycorrhizal occurrence on roots were greatest with oat plants in no residue in 2004 (Appendix C) which could have contributed to the increased OC from plants alone that was not observed with 2003 results. The high rainfall in 2004 could also be associated with added nitrogen, algae, or soil fauna that acted on all plots. This would account for increased soil OC in plots with no residue with and without plants that represented a background level of factors beyond the study treatments occurring in all plots which are not explainable.

*4.2 Soil Moisture*

Without adequate soil moisture little microbial activity and decomposition can take place as soil moisture is the driving variable in decomposition (Boddy, 1986). While

soil moisture may affect fungal hyphal growth and fungal species, water input was not controlled in this study. Moisture retained in the soil was treated as an independent variable. There was similar total rainfall during the 2003 and 2004 study seasons; however, in 2004 there was a much drier start and a much wetter finish prior to harvest (Fig. 4.1). In 2003, soil moisture was 40% greater in oat plots with surface residue compared to plots without residue, with the same initial OC. This increase was only seen in residue plots without oat plants in July 2004, as the high rainfall prior to harvest recorded high moisture in all plots.

In tropical climates, straw mulch increased soil moisture with small effects in high rainfall (Ramakrishna et al., 2006) and with larger effects with drier conditions (Acharya et al., 1998; Ramakrishna et al., 2006). A modeling study on the effects of a plant-residue layer found evaporation was reduced by 10% with mulch when conditions were wet (Gonzalez-Sousa et al., 2001). Reduced evaporation would subsequently prolong higher transpiration by the plant before leaves adjust stomatal conductance for reduced available soil moisture (Trambouze and Voltz, 2001). This adjustment by the plant resulted in the interaction term of the residue and oat plant and the large difference in soil moisture between residue alone, and residue plus oat plants in July, 2004. In the high moisture conditions no difference in plant growth was evident, but this would be a consideration with moisture restriction.

Fungal hyphal length had higher correlation to soil moisture than other soil variables over all treatments in 2003 and 2004. Frey et al. (1999), also found the highest correlation of fungal growth to be with moisture with wide variation in test sites. In general, field moisture of 25-37% is optimum for fungal growth (Boddy, 1986),

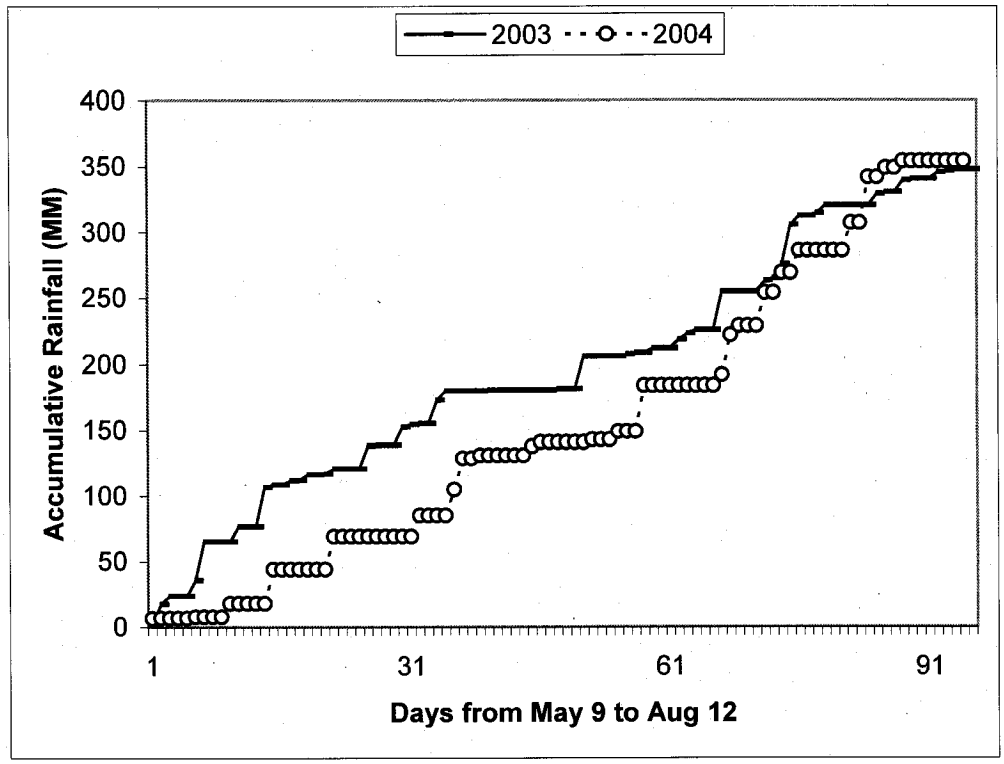



Figure 4.1. Accumulated rainfall (mm) over the 90 days of the study period from May 9 to Aug 12 in 2003 and 2004.

although Frey et al. (1999) found fungi to be active at higher and lower soil moisture than bacteria. While there is an effect of soil moisture on the selection of fungal species, I was examining the effect of fungal hyphal length on soil moisture from water transport and increased soil aggregation. Mycorrhizal associations are known to increase water retention in cowpeas studied in pots that was attributed to mycorrhizal effects on soil structure through soil aggregation (Augé et al., 2001).

The majority of Ectomycorrhiza are Basidiomycetes and secrete insoluble compounds that act as binding agents of aggregates (Caesar-TonThat and Cochran, 2000). Basidiomycetes are the primary decomposers of lignin (Caesar-TonThat and

Cochran, 2000). In a microcosm study, where soil aggregates were formed with and without added straw only with the added straw (basidiomycetes) was soil aggregation increased (Caesar-TonThat and Cochran, 2000). Basidiomycetes can develop strands of multiple hyphae called rhizomorphs which carry water as well as nutrients (George et al., 1992; Jennings and Lysek, 1996). The correlation of water stability of aggregates to fungal hyphal length in the 2004 plots may reflect the contribution of fungal hyphae and fungal exudates to soil aggregation. In addition, there may be a small contribution of fungal hyphae directly to soil moisture that would be more noticeable in dry conditions, and would need to be specifically tested for.

*4.3 Soil aggregation*

Among plots that varied in initial OC without residue, the % macroaggregates increased from July to August, 2003 and was correlated to %OC. The % macroaggregates increased with straw and corn residue amendments with oats in 2004 only after the oat plants had matured and dried. In August, 2004, there was an effect of residue on increasing soil aggregation following an effect of the oat plants on decreasing aggregation in July. The % macroaggregates was not correlated to an increase in OC with surface residue as expected. Increases in OC have been found primarily with increases in aggregates of greater than 2 mm size (Angers, 1992; Angers et al., 1997; Six et al., 2004). My description of macroaggregates was > 0.25 mm compared to 2 mm which also may have contributed to the lack of correlation with OC. Only with recent decomposition from ground cover was the %CLF correlated to OC and macroaggregates in 2003 (Chapter 2). The high rainfall that could decrease soil aggregation, and the small

change in the % macroaggregates in the high OC soil, may also have contributed to the lack of correlation of % macroaggregates with OC in this study.

The % macroaggregates was highly correlated to soil moisture over both seasons, and supported the theory that the amount of moisture held in the soil is dependent on the pore space (Franzluebbers, 2002). The maximum water holding capacity of soil should be a function of total pore volume from 0.5 μm to 50 μm for water infiltration and pore sizes greater than 50 μm that correspond to water drainage (Tarawally et al., 2004). The size of pores is defined by the structural stability of soil aggregates (Tarawally et al., 2004). Residue application was associated with an increased amount of large macropores which were related to OC content (Duiker and Lal, 1999) and stability from soil fauna and roots (Kutilek, 2004). The factors that increase OC from residue are associated with moisture retention, although I did not find published reports on the relationship in current literature.

There was no correlation of fungal hyphal length to soil aggregation or OC in oat plots with or without surface residue in 2003. In plots with oat plants growing in corn residue in 2004, where there was highest hyphal length, there was a positive correlation of hyphal length to soil aggregation, although with a sample size of four the relationship is not conclusive. The lab study by Frey et al. (2003) showed that fungal biomass produced from surface decomposition of straw was related to increased OC in macroaggregates. In contrast, my study with surface residue in variable moisture conditions did not find a correlation between hyphal length and soil aggregation over all plots in 2004.

Field studies relating hyphal length to soil aggregation have been in grassland soils, where mycorrhizal occurrence is very high (Tisdall and Oades, 1979), in fields with rye grass which is highly mycorrhizal (Tisdall and Oades, 1979; Hu et al., 1995), or at low levels of OC (Beare et al., 1997). Total hyphal length of 20 m $g^{-1}$ has been measured in grassland soils and up to 2500 m $g^{-1}$ in forest soil (Hu et al., 1997). Arbuscular mycorrhizal hyphae are greatly reduced in agriculture (< 1 m $g^{-1}$) compared to up to 50 m $g^{-1}$ in grassland soil and from 2-14 m $g^{-1}$ in forest soil (Read and Boyd, 1986). My hyphae measurements of 0.1-3.0 m $g^{-1}$ were very small in comparison to grassland soil. I suggest that the soil aggregation in this study may have been reduced by high mineralization from bacteria growth at high moisture, which negated the positive effect of a relatively small amount of fungal hyphae.

In the separation of the soil variables by principal components analysis, macroaggregates were a labile factor correlated with soil moisture and LF, while hyphal length correlated with stable soil indices of water stable aggregates and OC. Macroaggregates were correlated to LF in my study, similar to the report by Hassink (1995), who found aggregate size to increase in the short-term in response to increased LF. LF is a significant part of soil organic matter particularly in connection with sand and with constant input of residue as in perennial crops (Christensen, 1992). On sand, the LF contributed to organic matter as partly decomposed, particulate plant and animal remains and the HF comprised organic coatings of mineral quartz, observed in water repellent sands (Christensen, 1992).

In sandy soil, fungal hyphae play an important role in the formation of organic matter complexes (Hassink, 1995; Degens et al., 1996). According to Degens et al.

(1996), only hyphae can provide the physical binding for large aggregates with sand. Hyphal growth is slower on clay soil (Kabir et al., 1997) but the clay improves the binding of microaggregates (Caesar-TonThat and Cochran, 2000; Bronick and Lal, 2005) and is associated with increased OC (Christensen, 1992). The use of soil with a high sand content, may have resulted in optimum fungal growth but at the same time reduced the % macroaggregates in this study compared to reports obtained in clay loam soil. While aggregates increase in size with recent decomposition (LF), they also decrease readily (Angers et al., 1997) as they are less stable (Tisdall, 1991). In the high moisture conditions in my study, macroaggregates may have been reduced resulting in a more meaningful correlation of fungal hyphal length to water stable aggregates in 2004.

The species of plant may also affect the amount of fungal hyphae as crops can vary in the degree to which they form mycorrhizal associations, and the characteristics of the specific mycorrhizal species (Johnson and Pfleger, 1992). Haynes and Beare, (1997) reported that the root mass of the species was correlated to soil aggregation in a pot study of grains, grasses and legumes while in field studies aggregate stability followed the order of mycorrhizal colonization; ryegrass >wheat > lupin (non mycorrhizal). Rye grass, with high root mass and mycorrhizal fungi, resulted in high correlation of fungal hyphal length with macroaggregates and OC in a till/NT study (Hu et al., 1997). Oats are not highly mycorrhizal, and may not have provided an optimal plant host for mycorrhizal associations. The measures of fungal hyphal length and soil aggregation and their correlation may have been affected by; high soil moisture, high % macroaggregates, high proportion of sand:clay, and low % mycorrhizal colonization. All

these factors may have reduced the correlations between hyphal length and soil aggregation compared to studies with ryegrass.

*4.4 Plant Roots*

While plant growth itself is a source of OC in foliage, seeds and roots, the effects of the plant on the soil were variable. At the peak of growth in July 2004, the oat plants decreased soil moisture from water uptake and decreased soil aggregation presumably from increased mineralization associated with root activity. In August, there was an increase in OC in plots with senescent oat plants, and a decrease in soil moisture as well. At the high levels of soil aggregation in this study, active oat plant roots decreased soil aggregation, which is in agreement with Kay (1990), who stated the direction of change depends on the initial soil aggregation status. Six et al. (2004) summarized that roots "decrease unstable macroaggregates" which would describe the high organic matter soil with 60-80% of macroaggregates in this study.

The secretion of exudates from roots and root decomposition may have contributed to the correlation of soil aggregation to OC in the gradient of initial OC without residue. However, with surface residue, soil aggregation decreased with root growth in high OC soil. According to Jastrow et al. (1998), the action of an abundance of roots limits aggregation, as the disturbance and bacterial action stimulate decomposition. Root exudates are primarily a source of OC for bacteria, not fungi (Elliott et al., 1979). At plant senescence, root exudation stops and is replaced by saprophytic fungal decomposers of root material (Wamberg et al., 2003). The change from root exudation to decomposition from July to August may explain the changing response of soil aggregation and OC from the oat plants over the crop season.

According to the mass balance figures (Table 3.4 in Chapter 3), it is possible that the oat plant roots in our study plots could have accumulated in biomass 1000 g OC $m^{-2}$. The calculations were based on harvested shoot weights in the study and the OC fixation rate of 7.6 mg OC per g of shoot per hour and root allocation percentages of 19% to biomass, 28% to respiration and 0.5% to exudates described in Paul and Kucey (1981) (Table E.5). If all the roots decomposed within the growing season as well, the plant roots could have contributed most of the increase in soil OC. If approximately ½ of the OC allocated to root biomass is recycled during the plant growing time from very fine roots, that could account for up to 500 g $m^{-2}$ OC or 36%, 75% and 54% of the OC increase from oat plants with no residue, straw residue, and corn residue respectively. Along with OC from residue estimated at 61 and 71 g $m^{-2}$ for straw and corn, the known inputs can account for a substantial amount of the OC increase. However, there is no explanation for the increases in soil OC without oat plants of up to 1000 g $m^{-2}$.

*4.5 Mycorrhiza*

In the 2004 factorial set fungal hyphal length was significantly higher with surface straw and corn than with either residue alone or no residue with and without oats. Only with the interaction of the residue and mycorrhizal hyphae from the plant was hyphal length increased in August, 2004. The increase was assumed to be from predominantly mycorrhizal hyphae of the plants that were increased with interaction of the residue. The separation of hyphal number at a diameter of 5 μm in 2003, suggested that the fungal hyphae in July were primarily saprophytic, and in August, slightly more mycorrhizal hyphae than saprophytic fungal hyphae were counted. The data by Kabir et al. (1997) following hyphal density and mycorrhizal colonization over two seasons,

recorded a low level of hyphae in the soil throughout the year (assumed saprophytic), with a peak in August corresponding to mycorrhizal colonization from plants. These observations would correspond to my calculations and assumptions.

The increase in hyphal length from interaction between the plant and residue supports several studies that found synergy within the microbial community (Dighton et al., 1987; Klironomos and Kendrik, 1995; Singh and Kapoor, 1999). Klironomos and Kendrik (1995) found a mutual relationship of decomposer and mycorrhizal fungi species in microcosms and attributed the increase in total fungi to reduced microarthropod predation on mycorrhizas. If that is true, increased predation could have decreased hyphal length of saprophytic hyphae in plots without plants, and also decreased hyphal length of mycorrhizal hyphae in plots with plants alone in this study. There was no significant difference in the mycorrhizal fungal colonization observed on the oat plant roots with or without surface residue.

Mycorrhizal occurrence formed a linear regression with HF ($r^2 = 0.32$, $p = 0.04$) over all plots with oat plants plus dried surface residue in 2003, and 2004 ($n = 13$) (Fig. 4.2). The 2004 figures were adjusted for the difference in soil moisture between the two years, resulting in no significant difference in means or variance between the two data sets. The linear regression of mycorrhizal occurrence to HF implies that mycorrhizal fungi (hyphae, vesicles, or secretions) are associated with the formation of organic matter complexes.

The protein glomalin, secreted from AM hyphae, is water insoluble and heat stable (Rillig et al., 2002a) and composed of 60% OC (Wright and Upadhyaya, 1998). Glomalin has been correlated with aggregate stability (Wright and Upadhyaya, 1998;

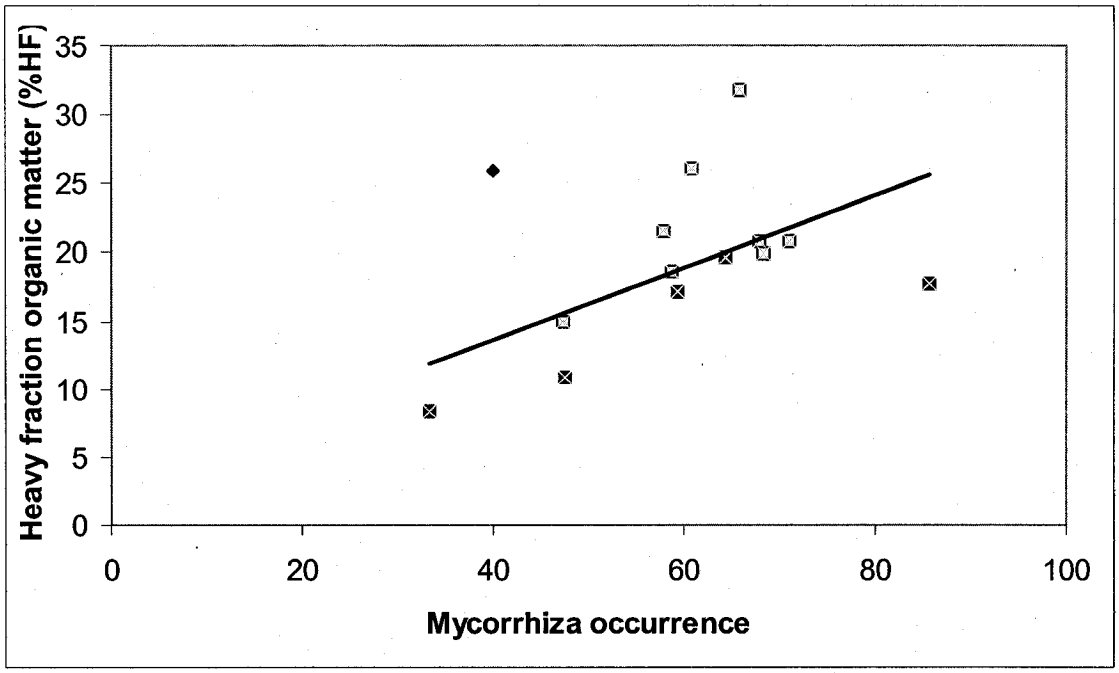



Figure 4.2. Linear regression of % HF on % Mycorrhizal fungal occurrence on oat roots over all dried surface residue plots in 2003 (■) and 2004 (□). $y = 0.264x + 3.085$, $r^2 = 0.324$, $p = 0.04$, $n = 13$.

Augé et al., 2001; Rillig et al., 2002a) and is potentially important in the slowly decomposing OC pool (Rillig et al., 2003). There was a significant correlation of glomalin to soil OC and nitrogen ($p < 0.001$) in forest and field soils (Rillig et al., 2003). The hyphae of basidiomycete fungi which form ectomycorrhizal (EM) associations are water stable too (Caesar-TonThat, 2002) and secrete insoluble compounds (Caesar-TonThat and Cochran, 2000). Mycorrhizal hyphae would therefore be resistant to decomposition in moist conditions compared to other fungal hyphae and increase water stability of aggregates. The retention of OC in soil must be increased by the type of fungal hyphae involved in response to the characteristics of the residue. With corn residue, which has a higher lignin content than other residue, a higher number of basidiomycete decomposers and higher ectomycorrhiza may explain the correlation of

HF to hyphal length and OC. Basidiomycetes and ectomycorrhiza would have to be separated from other fungi in the study design, or by test to ascertain their specific effect on OC.

*4.6 Surface residue; %CLF, C/N, and Mycorrhiza*

Mineralization of OC in the soil is related to microbial activity in general and %CLF (Conti et al., 1997). The %CLF was correlated to macroaggregates ($r^2 = 0.42$, $p = 0.009$, $n = 15$) in all plots with surface residue/live ground cover in 2003 and to OC ($r^2 = 0.60$, $p < 0.001$, $n = 20$) in all plots with surface residue in both seasons. Growing weeds and alfalfa had the lowest %CLF, compost residue the highest, and dried straw, hay and corn residue measurements were intermediate.

The narrow range of decomposability in the dried residue, did not give significant differences in hyphal length, macroaggregates or OC between straw/hay, and straw/corn. The measurement of decomposition by mass, OC and nitrogen with straw/corn in 2004, revealed no significant difference in residue mass or OC loss by weight. The same amount of material was decomposed regardless of the residue quality or nitrogen level. There was no correlation between the residue C/N and %CLF in straw or corn decomposition. This does not support the assumption that nitrogen increases decomposition or the observance that a low C/N was associated with a higher decomposition rate with maize residue (Conti et al., 1997). Decomposability would only be an issue if lack of nitrogen inhibited decomposition. Although mycorrhizal occurrence on oat roots was significantly decreased in straw residue compared to hay residue in this study in 2003, there was no difference in soil OC between residue treatments. In

contrast, in 2004, there was no difference in mycorrhizal occurrence on oat roots grown in straw and corn residue, but soil OC was significantly increased with corn residue.

My study found dried and composted residue to have significantly greater hyphal length, soil moisture, and soil OC than the live surface cover. Intercropping has been discussed as a means to reduce soil erosion while reducing evaporation and increasing water infiltration in cropping systems (Boyd et al., 2001). While the use of live covers may be advantageous for overall productivity, the soil acquires more benefits from dried plant matter according to results in this study on soil aggregation, moisture and OC.

*4.7 Summary*

%CLF was correlated to OC in 2003 as a measure of recent decomposition from surface residue. Contrary to reports in NT studies that found LF closely related to OC, the correlation of %HF with hyphal length and soil OC with all corn residue plots (w/wo plants) would indicate HF is more closely related to stable OC accumulation in soil. The higher occurrence of mycorrhizal hyphae at August sampling and the linear regression of % occurrence of mycorrhiza on HF over all plots with surface residue, suggest that the effect of surface residue on mycorrhizal hyphae growth may have contributed to the significant increase in OC recorded in the plots. The type of fungi in response to the type of substrate and environmental conditions may be more important to the form of decomposition and organic matter retention than the amount of decomposition. The contribution of oat plant roots and increases in bare soil alone were not isolated in the study and limit the decisiveness of conclusions regarding the direct influence of surface residue on soil OC.

# Chapter 5

## Conclusions and Further Research

Only with a range of initial soil OC from 2.4 to 6.8% in July, 2003, was the percentage of macroaggregates correlated with soil OC as hypothesized from literature reports. Hyphal length was correlated to water stability of aggregates but not to soil aggregation and OC as has been found in grassland and in agriculture at lower levels of soil OC. This could be due to the high level of soil OC, and the high rainfall that affected the water stability of the aggregates. The significant linear regression of % macroaggregates to soil moisture over all plots in both seasons, supported an association of soil aggregate size in the water retention property of soil. Further study is needed to separate the effect of reduced evaporation from the mulch from the contribution of fungal hyphae which can contribute to soil moisture directly, or through soil aggregation. It is not clear in the literature if there are limits on the size of aggregates from the quantity of fungal hyphae and the amount of rainfall. I am interested in determining if there is a difference in soil moisture and soil aggregation depending on the specific species or mix of species in response to surface residue.

Surface residue increased fungal hyphal length and soil OC in both seasons. Only with the oat plants and residue together were soil OC and hyphal length observed to increase. The significant interaction of oat plants and residue in 2004 indicated a synergy between mycorrhizal and saprophytic fungi that results in increased hyphal length greater than the individual treatment sums with corn residue. The seasonal trend of mycorrhizal growth may have contributed to increased hyphal length with residue and oat plants in August. The linear regression of mycorrhizal occurrence to HF over ground cover plots

with oats in 2003 and 2004, suggests that the mycorrhizal hyphae may contribute to the retention of OC in a more stable form that had greater resistance to decomposition in the high rainfall conditions. This may relate to the contribution of mycorrhizal hyphae in binding of aggregates, or from the protein glomalin secreted by mycorrhiza. Further study on the contribution of decomposer species, ectomycorrhiza and arbuscular mycorrhiza would bring out the relative effects of the fungal species involved on changes in soil aggregation and OC.

HF correlated to hyphal length and soil OC with corn residue but not with straw residue. Plots (w/wo oats) with corn residue with a high C/N had increased soil OC compared to straw residue. The association of corn residue with HF may be more influential in conserving soil OC than the speed of decomposition promoted from the higher nitrogen concentration in straw. The %CLF correlated to soil OC in all surface residue plots, as the closest indicator of soil OC transformations. Further research on the role of nitrogen in surface decomposition is needed to determine if nitrogen fixation in the plant rhizosphere is increased from surface residue and if additional nitrogen is beneficial to decomposition and retention of OC in soil.

The soil OC mass balance over the 2004 study period highlighted the fact that the level of soil OC was very high compared to amounts reported in other tillage studies, and increases in soil OC were greater than the obvious inputs of residue decomposition and plant root deposition. This leaves a question; "where is the increase in soil OC coming from"? Soil algae are capable of fixing up to 1.4 t $ha^{-1}$ (Metting, 1981) or 127.4 g $m^{-2}$. Soil algae are stimulated by high levels of phosphorous and organic matter (Lavelle and Spain, 2001) and high soil moisture that promotes anaerobic conditions (Metting, 1981).

These conditions that allow maximum growth of soil algae were present in the plots in constantly warm and humid weather conditions from July to August 2004. There are no reports of the effect of surface residue on algal growth. Soil fauna and above ground insects may also be factors that increase soil OC by concentrating nutrients (nitrogen and OC) into more stable forms. In order to determine where specific/or relative contributions to soil OC originate, I would continue to work with the homogenized high organic matter soil to minimize variance and separate the factors with studies repeated outdoors and under controlled conditions indoors. Tests should be designed to isolate the effects of each element on soil OC.

This study lent insight into the relationship of fungi with soil aggregation and OC that change with the addition of surface residue with oat plants. It is important to understand the interactions that affect plants in the natural environment. The sandy soil was advantageous, as there were no clay bonding effects that could inhibit mineralization and affect OM decomposition (Hassink, 1995). The effects of fungi may be more evident in sandy soils compared to loam soils and with high organic matter, so the changes in fungi were most pronounced. It needs to be studied further if a higher percentage of clay would improve OC retained from surface residue by its ability to improve the bonding of organic matter complexes (HF) (Tisdall and Oades, 1982; Franzluebbers et al., 1996) thus increasing the resistance of aggregates to decomposition.

Soil OC increases from surface residue amendments in this study were an order of magnitude greater than predicted in current literature regarding agricultural systems. The increases of 0.5 to 1% OC represented increases of up to 10 g $kg^{-1}$ compared to increases anticipated from NT alone, which were estimated at 0.05% (Lal et al., 1998) or around

500 mg $kg^{-1}$ $yr^{-1}$. Corn residue may provide an optimum scenario, where existing residue can be mulched to improve soil conditions. Considering the 3.2 million acres of corn produced in Canada (Statistics Canada, 2002), leaving the crop residue on the surface could reduce atmospheric carbon by 28,500 tons per yr. This is similar to the figures generated by a carbon model which estimated increases from 0.6 to 1.2% OC per year in agriculture would be possible to mitigate 0.1% of the planned $CO_2$ reduction by 2012 (Paul et al., 1997).

Mulching is a very successful management technique for the benefit of soil OC and soil moisture. The nutrient cycling, and microbial and insect food webs may also be significantly different in surface mulch, compared to existing data on cultivation systems and need to be researched. Understanding the actions and interactions of the microbial community is important for sustainable agricultural management but requires study (Medina et al., 2003). The development of mycorrhizal associations in agriculture could bring cropping systems closer to natural ecosystems where nutrients are more efficiently conserved (Tiquia et al., 2002). Foremost, aerobic fungi that decompose surface residue may be a very efficient system that will benefit many aspects of agriculture and ecology. Further study is needed to find the best ways to manage crop residue when considering soil nutrients, moisture, soil and $CO_2$ loss, weed control, insect balances and pathogens along with yield.

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# Appendix A

## Particle Size Distribution

Table A.1. Proportion of sand/silt and clay determined by dispersion of soil aggregates with sodium hexametaphosphate (5 g $L^{-1}$) from 20 g soil in 250 ml solution and wet-sieved through a 250 µm screen onto a 63 µm screen. Organic matter was removed from sand in the > 63 µm fraction by decanting material that remained in suspension 5 seconds after stirring. Sand and silt was dried at 105° C for 24 hr. n = 3. The remaining 44% of sample not sand/silt may represent some sand remaining in microaggregates not dispersed, or sand lost in the wet-sieving/decanting process, as SOM determined by LOI is < 13%, leaving 31% unaccounted for.

| Measurement | Mean | ±SEM |
|---|---|---|
| Sample dry wt. (g) | 16.5 | 0.1 |
| Sand (>63 µm) dry wt (g) | 8.0 | 0.3 |
| Silt (<63 µm) dry wt (g) | 1.0 | 0.1 |
| Sand % of total sample | 48.4 | 1.6 |
| Silt % of total sample | 5.9 | 0.6 |
| Sand % of Sand/Silt | 89.2 | 0.9 |
| Silt % of Sand/Silt | 10.8 | 0.9 |

Table A.2. Particle Size distribution by weight of 50 g samples of sand (n = 3) for 15 s in a shaker with sieve sizes 500 µm, 250 µm, 125 µm, 63µm.

| Particle Size Distribution | | | |
|---|---|---|---|
| Sand | Sieve size* mm | Mass g | Mass % |
| Coarse | 0.500 | | |
| Medium | 0.250 | 0.85 | 2 |
| Fine | 0.125 | 29.36 | 61 |
| V Fine | 0.630 | 13.08 | 27 |
| Silt | <0.630 | 5.18 | 11 |
| Total | | 48.46 | 101 |
| | | | |
| %Sand | | | 89 |
| %Silt | | | 11 |

*CSSC (Canadian System of Soil Classification) sizes (Sheldrick and Wang, 1986).

# Appendix B

## Fungal Culture on Whey Agar

### B.1 Method

Fungal species were cultured on whey agar using the soil suspension prepared for the hyphal length measurements. Fresh whey produced from goat milk cheddar cheese, available at the study farm site, was used as a medium to culture fungi. The casein hydrolysates in whey are molecular complexes of casein and proteins (Goel et al., 1999) that represent complex nitrogen sources known to maximize growth of lignin degrading fungi on whey (Boyle, 1998). Non-sporulating species which grow from hyphal fragments could be represented in fungal populations on whey (Burnett and Trinci, 1978). The whey was non-selective to fungi species, while being inhibitory to bacterial growth because of its pH of 4.5 (Warcup, 1960).

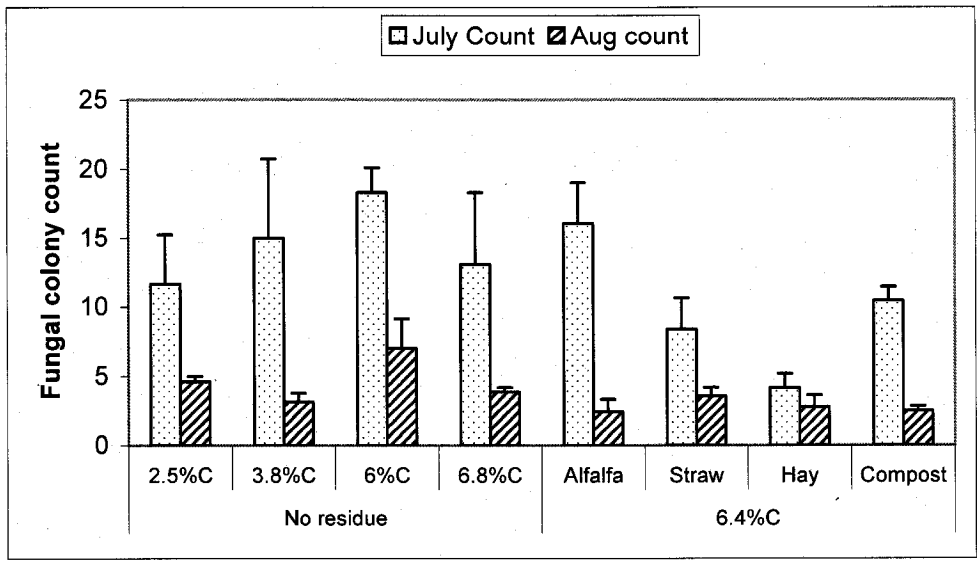
Following heating of the whey to 100°C for 20 min and straining through fineweave cotton cloth to remove milk solids, the clarified whey was autoclaved with 2% agar at 115 psi for 15 min. After the plates had cooled, the surface of the whey agar plates was spread with 0.4 mL of the filtrate (from Chapter 1) for all 24 samples, with three replicates each. This process was the same in 2003 and 2004, except that additional dilutions were formed in 2004. The soil suspension was combined with sterile water to form dilutions of 1/100, 1/1000, 1/10,000. The inoculated plates were incubated in the dark at room temperature (20°C) for 5 days. Control samples of air, water, and whey media alone ensured that fungal cultures were derived from the soil samples. Only the 1/100 dilution had a suitable number and size of colonies for analysis. Small, circular colonies were counted to evaluate the volume of fungi in the soil represented as spores.

The area of mycelia from large fast growing colonies was estimated as a percentage of the area of the dish covered to enumerate the amount of fungal hyphae related to fast growing zygomycetes. The colony growth was identified by morphology (Domsch and Gams, 1970; Kendrick, 1992; Esser and Lemke, 2001; Watanabe, 2002).

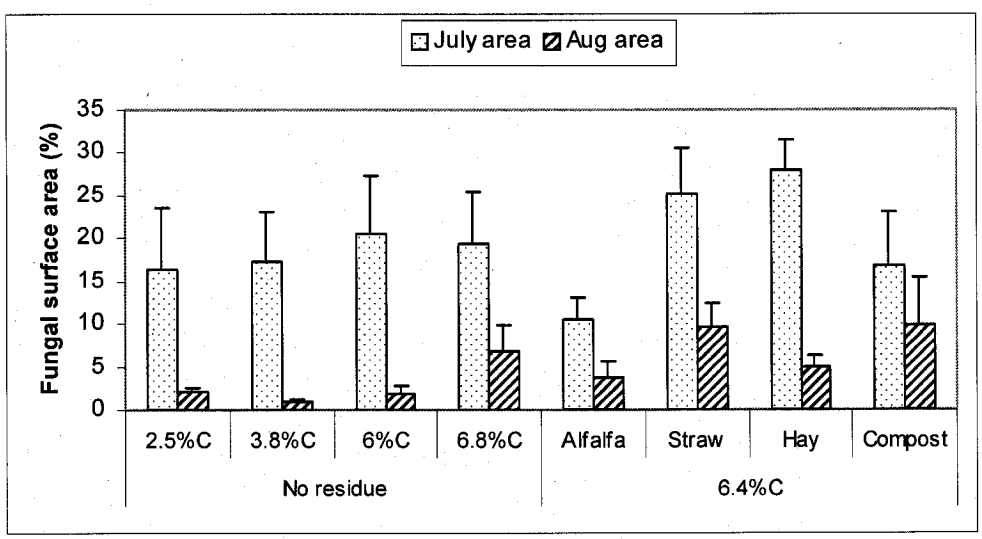
**B.2 Results and Discussion**

The colony count (Fig. B.1a, Fig. B.2a) and the percentage of surface area (Fig. B.1b, Fig. B.2b) of the whey agar covered by fungal mycelia were not significantly different with added ground cover in 2003 or 2004. A negative correlation of colony count with mycelia area ($r^2 = 0.64$, $p = 0.002$, $n = 12$) was observed with ground cover plots in July, 2003 consistent with an inhibition of small slow growing colonies by fast growing filamentous species. The increased size of colonies and decreased colony count with surface residue is consistent with a change in fungal species. Henriksen and Breland (2002) had found the species of bacteria and fungi changed with the type of substrate, surface application or incorporation, and decomposition time. The increased mycelia area of fungi, or total hyphal length with ground cover did not correspond to % macroaggregates or OC in 2003 or 2004. Increases in aggregate size and OC were not correlated to the growth of filamentous fungi from residue decomposition as hypothesized.

In 2004, the fungal species evaluated for mycelia area were identified as species of Zygomycetes (*Mucor* and *Rhizopus*). Zygomycetes are rapidly growing fungi that feed on easily metabolized sugars (Lacey, 1986). However, these species are rapidly recycled within days up to a week, and would not remain at harvest sampling. At August sampling, in plots with oats growing in corn residue, fungal mycelia area correlated

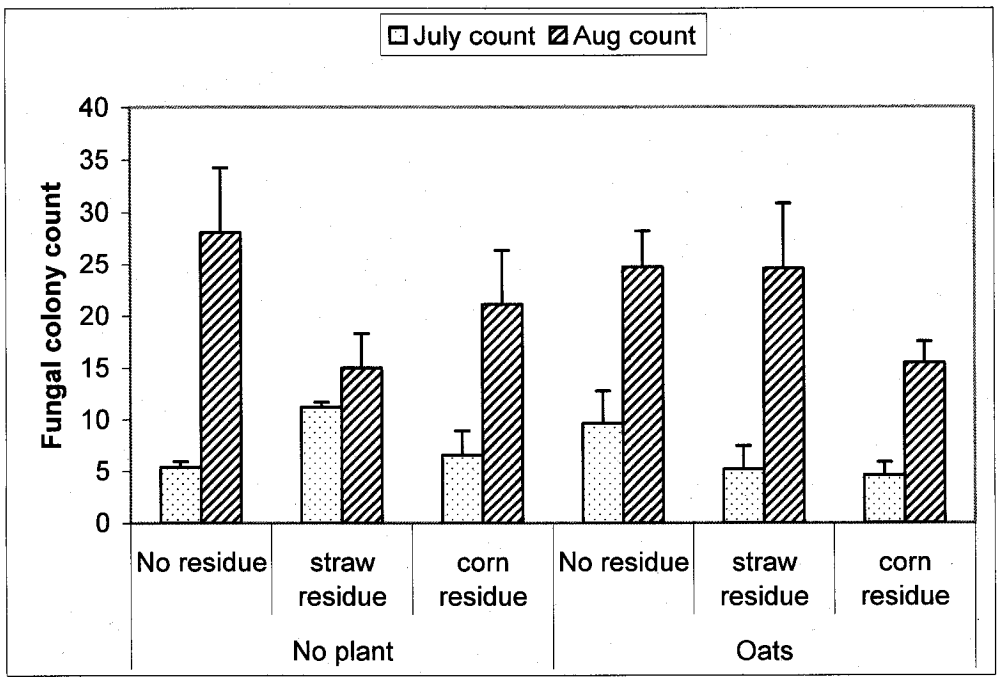



a) Fungal colony count

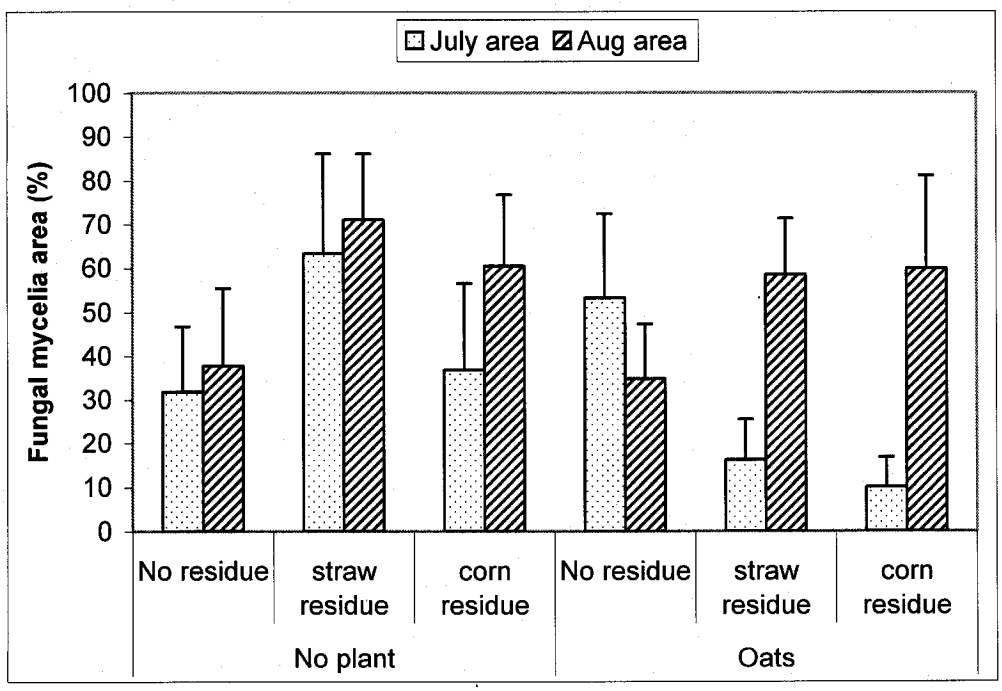



**b)** Fungal mycelia growth measured by surface area

Figure B.1: Fungal growth on whey agar measured by the a) number of colonies and b) mycelia area in July and August, 2003 with no ground cover at 4 levels of OC (2.5%, 3.8%, 6% and 6.8%) and at 6.4% OC with four different surface covers (alfalfa, straw, hay and compost). Error bars represent SEM, (n = 3). Treatments did not vary significantly.




a) Fungal colony count




b) Fungal mycelia area

Figure B.2. Fungal growth on whey agar measured by means of a) colony count and b) % surface area of mycelia in July and August. Error bars represent SEM. n = 4. There are no significant differences between treatment means.

positively to %LF ($r^2 = 0.98$, $p = 0.008$, $n = 4$) and negatively to %HF ($r^2 = 0.97$, $p = 0.015$, $n = 4$). The more easily decomposed residue measured by the fungi mycelia area increased the LF, but was not associated with organic matter complexes represented by HF, and subsequent changes in more stable OC.

# Appendix C

**Oat plant yield and mycorrhizal occurrence on oat roots**

Table C.1: Mean ± SEM of oat seed mass (g $m^{-2}$) and oat plant biomass (g $m^{-2}$) in plots with and without surface residue in 2003 and 2004.

| | Oat seed wt. | SEM | Oat biomass | SEM |
|---|---|---|---|---|
| **No ground cover 2003** | -- | g $m^{-2}$ | -- | |
| **2.5%OC** | 46.94 | 13.01 | 564.81 | 69.47 |
| **3.8%OC** | 48.89 | 1.41 | 617.59 | 12.96 |
| **6%OC** | 42.42 | 9.14 | 669.44 | 38.89 |
| **6.8%OC** | 53.75 | 17.38 | 813.89 | 245.49 |
| **Ground cover 2003** | | | | |
| **alfalfa** | 81.69 | 35.06 | 825.93 | 138.42 |
| **straw** | 31.38 | 15.15 | 433.33 | 113.87 |
| **hay** | 47.02 | 21.33 | 603.70 | 69.47 |
| **compost** | 57.02 | 20.35 | 1010.19 | 47.64 |
| **2004** | | | | |
| **No residue** | 217.03 | 23.82 | 294.91 | 38.44 |
| **Straw residue** | 195.64 | 19.90 | 282.27 | 9.35 |
| **Corn residue** | 229.92 | 32.08 | 301.60 | 43.67 |

Table C.2: Mean ± SEM of occurrence of mycorrhizal structures on oat roots (%) including occurrence of vesicles (%) in plots in 2003 and 2004 with and without surface residue.

| | %Mycorrhiza | SEM | Vesicles (%) | SEM |
|---|---|---|---|---|
| **No ground cover 2003** | | | | |
| **2.5%OC** | 75.77 | 2.77 | 0.33 | 0.33 |
| **3.8%OC** | 78.13 | 6.09 | 0.33 | 0.33 |
| **6%OC** | 70.73 | 11.63 | 2.33 | 1.20 |
| **6.8%OC** | 81.93 | 9.11 | 0.00 | 0.00 |
| **Ground cover 2003** | | | | |
| **alfalfa** | 58.46 | 5.51 | 0.00 | 0.00 |
| **straw** | 40.31 | 4.14 | 2.00 | 1.15 |
| **Hay** | 69.79 | 8.08 | 3.67 | 1.45 |
| **compost** | 70.36 | 6.65 | 1.67 | 0.88 |
| **2004** | | | | |
| **No residue** | 88.33 | 1.60 | 4.82 | 1.79 |
| **Straw residue** | 85.53 | 7.23 | 5.95 | 2.42 |
| **Corn residue** | 89.75 | 3.66 | 12.24 | 3.12 |

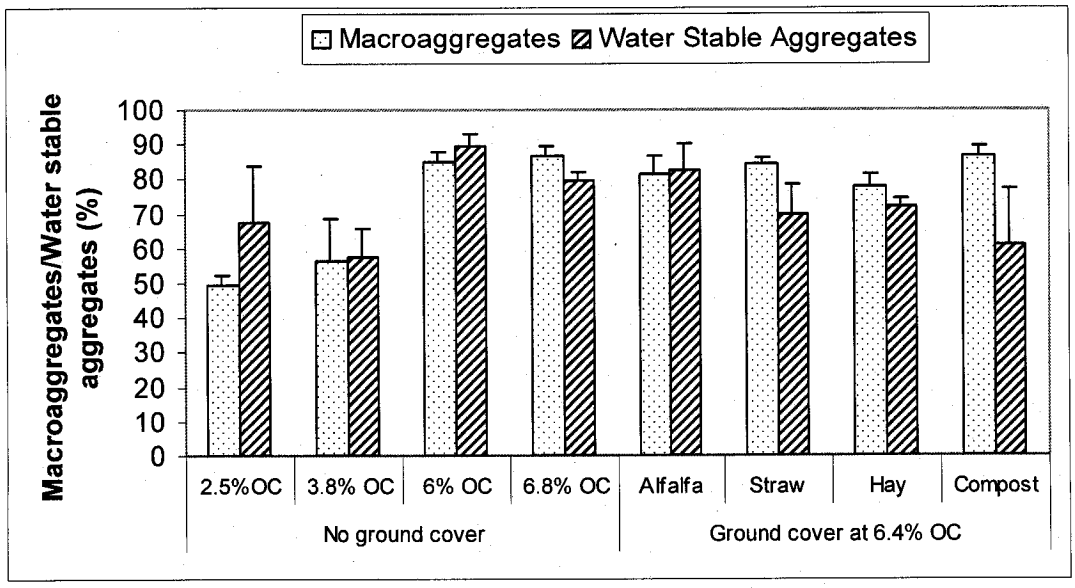



Figure C.1. Water stable aggregates (%) measurement in July, 2003 and macroaggregate (%) in August, 2003 in plots from 2.5% to 6.8% OC with no ground cover, and in plots with average 6.4% OC with ground covers of growing alfalfa, dried straw, dried hay, or compost. Error bars represent SEM. n = 3. There were no significant differences between treatments at p = 0.05.

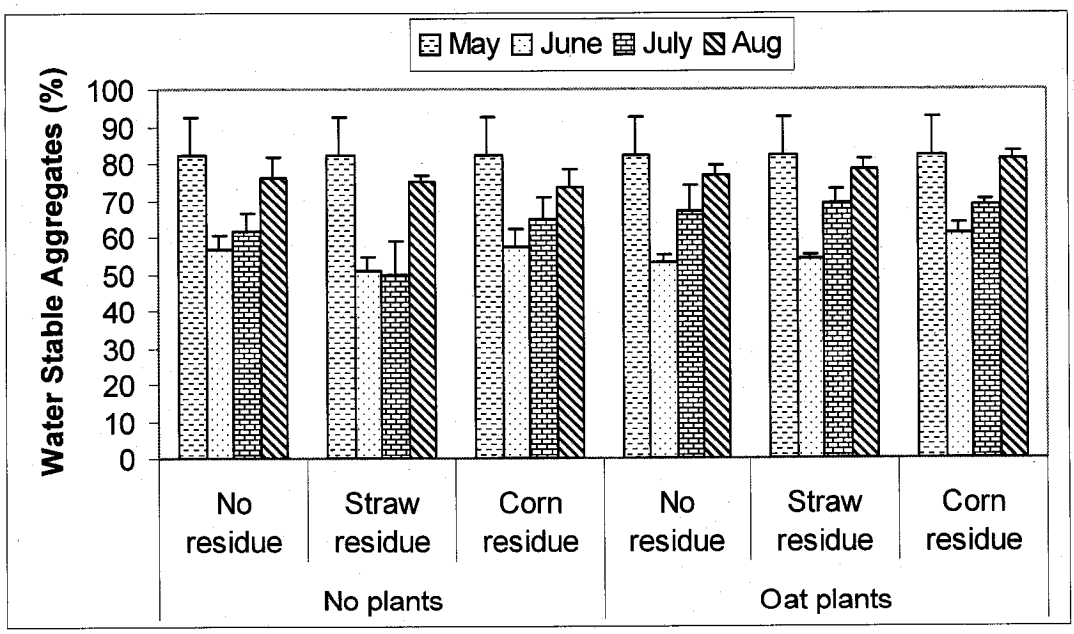



Figure C.2. Water stable aggregates (%) from May to August, 2004 over plot treatments with and without oat plants, and with no residue, straw residue and corn residue. Error bars represent SEM. n = 4. June figures were significantly decreased from May over all treatments, but there were no significant treatment differences at p = 0.05.

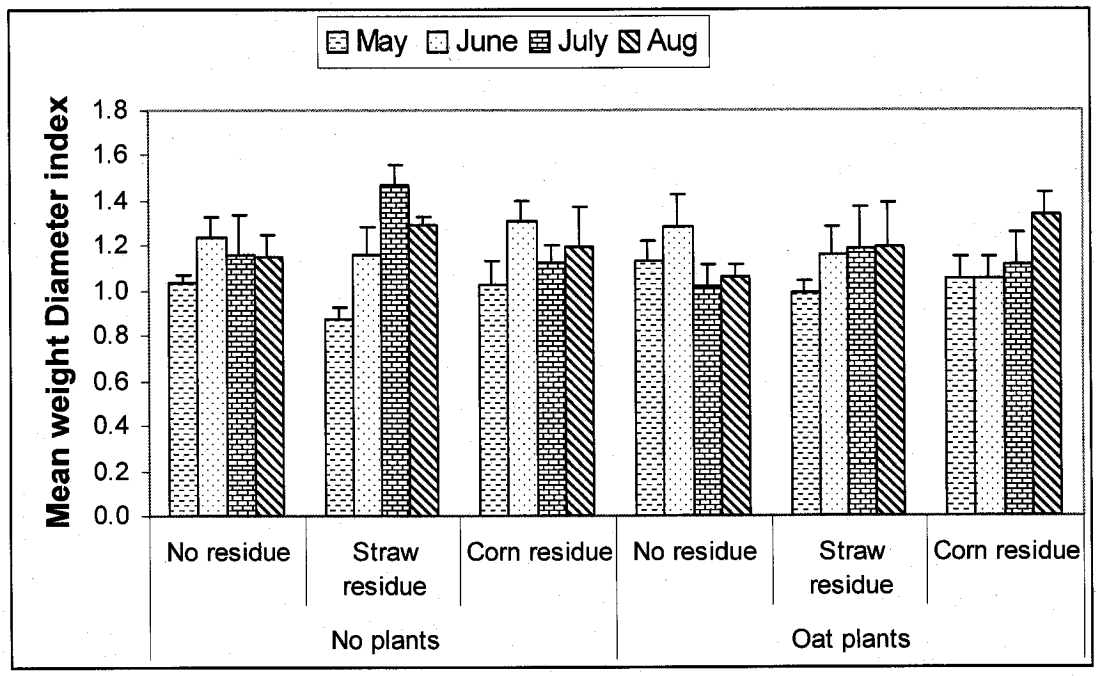



Figure C.5. Mean weight diameter index from May to August, 2004 over plot treatments with and without oat plants, and with no residue, straw residue and corn residue. Error bars represent SEM. n = 3. There were no significant treatment effects of oat plants, or straw or corn residue at p = 0.05.

# Appendix D

## Soil Nitrogen

### D.1 Methods

The soil C/N was calculated from OC and total nitrogen and expressed in mg $kg^{-1}$. Soil samples were measured for nitrate ($NO_3^-$), ammonium ($NH_4^+$) and organic nitrogen that together add up to total nitrogen. Soil samples dried at 105° C were tested for inorganic soil nitrogen by extraction of 3 g samples with 20 mL 1M KCL and measurements were recorded with colorimetric technique, as per the method of Keeney and Nelson (1982).

### D.2 Results and Discussion

$NH_4^+$ decreased slightly from May levels, to an equilibrium level around 20 mg $kg^{-1}$ for all plots through July and August. The slowest decrease was in plots with corn residue. There was interaction between residue and the plant in July on $NH_4^+$, but no other significant effects. $NO_3^-$ values (Fig. D.1) ranged from ½ -2 times $NH_4^+$, and determined the trend of total inorganic nitrogen which decreased due to residue ($p = 0.022$) and plant ($p < 0.001$) along with interaction ($p = 0.017$) in July (Table D.1). In August there was an increase in $NO_3^-$ ($p = 0.012$) and total inorganic nitrogen ($p = 0.03$) from residue. Inorganic nitrogen correlated to moisture in August, in agreement with a study on water relations and mineral nitrogen that found inorganic nitrogen to relate to soil moisture in sandy soil in the range from 20% to 60% water filled pore space, and this relationship improved with high soil OC (Drury et al., 2003) which would be within our study conditions.

Organic nitrogen was the major form of total nitrogen, with inorganic nitrogen representing less than 1%. While organic nitrogen decreased with residue alone, there was no significant residue or plant effect over all treatments (Fig. D.2). Over all data (n = 24) inorganic nitrogen correlated to macroaggregates ($r^2 = 0.42$, $p < 0.001$) and moisture ($r^2 = 0.46$, $p < 0.001$) in July, relating to significant decreases in $NO_3^-$ from the plant. Over all residue plots the soil C/N correlated negatively to the changes in residue C/N ($r^2 = -0.28$, $p = 0.04$, $n = 16$) and % nitrogen loss from residue ($r^2 = -0.24$, $p = 0.05$), $n = 16$). This suggests that there was interchange of nitrogen from the residue to the soil, and nitrogen was not been taken from the soil for decomposition, as is commonly expressed.

Over all residue plots, total soil nitrogen correlated to mean weight diameter ($r^2 = 0.26$, $p = 0.042$, $n = 16$). Angers et al. (1997) found the soil C/N ratio to relate to increased aggregation. Havlin et al. (1990), found soil OC and N accumulation to be correlated $r = 0.98$ in long-term studies on tillage rotation and nitrogen in sorghum, soybeans and corn. This correlation was not established here, possibly from the high soil OC and moisture levels.

Table D.1. 2-way ANOVA model (oat plant x residue) probabilities of main effects of oat plant and residue on soil nitrogen variables. P significant at < 0.05 highlighted in **Bold**. Negative probabilities indicate decrease effect.

| | *2-way ANOVA* | | | *LSD comparison of means* | | |
|---|---|---|---|---|---|---|
| | Plant effect | Residue effect | Interaction | Straw-soil | Corn-soil | Straw-Corn |
| July | | | | | | |
| $NH4^+$ | **0.040** | 0.210 | **<0.001** | 0.720 | 0.097 | 0.193 |
| $NO3^-$ | **-<0.001** | **-0.022** | **0.017** | **0.022** | **0.011** | 0.752 |
| Inorganic N | **-<0.001** | **-0.046** | **0.026** | **0.036** | **0.027** | 0.894 |
| August | | | | | | |
| $NH4^+$ | 0.850 | 0.594 | 0.500 | | | |
| $NO3^-$ | 0.970 | **0.012** | 0.245 | 0.067 | **0.003** | 0.170 |
| Inorganic N | 0.965 | **0.031** | 0.550 | 0.073 | **0.010** | 0.350 |
| Organic N | 0.461 | -0.161 | 0.553 | 0.213 | 0.063 | 0.498 |
| Soil C/N | 0.850 | 0.080 | 0.575 | 0.610 | **0.033** | 0.090 |

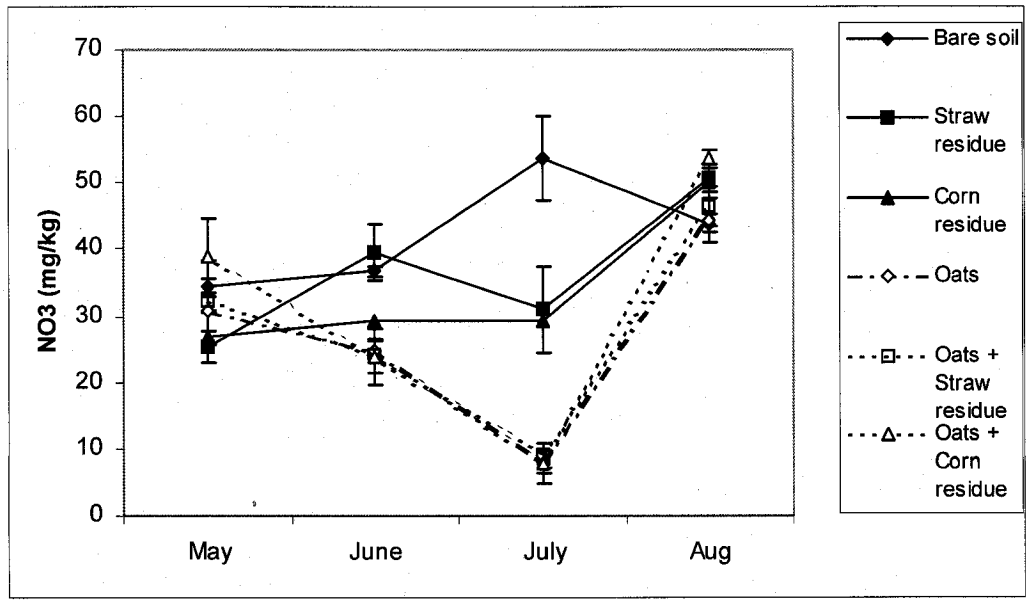



Figure D.1. Soil $NO_3^-$ in mg/kg at each sampling time from May to August, 2004, in plots with no plants ; bare soil, straw residue, corn residue and with oat plants with; bare soil, straw residue, corn residue. Error bars represent SEM, n = 4.

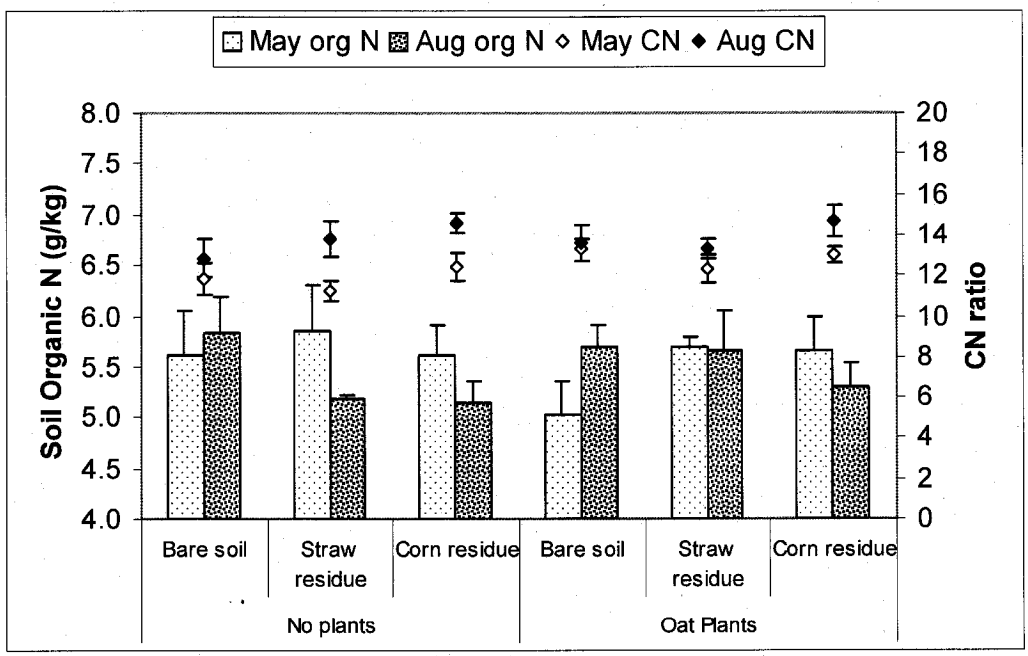



Figure D.2. Organic nitrogen (N) compared from May (Initial average of 5500 mg/kg) to August, 2004 measurement and the CN ratio compared from initial C/N which averaged 13 to August C/N over plot treatments with no plant/oat plants and with bare soil, straw or corn residue. Error bars represent SEM, n = 4.

# Appendix E

## Mass Balance Calculations (2004)

Table E.1. Residue mass loss in g and % from May to August in each plot with residue of Oat straw of Corn stalks with and without oat plant growth.

| Plot treatment | replicate | | Initial (g) | Harvest (g) | loss (g) | %Mass loss |
|---|---|---|---|---|---|---|
| Straw residue | 1 | | 153.59 | 77.20 | 76.39 | 49.74 |
| | 2 | | 157.60 | 71.80 | 85.80 | 54.44 |
| | 3 | | 153.82 | 86.00 | 67.82 | 44.09 |
| | 4 | | 156.04 | 78.60 | 77.44 | 49.63 |
| Corn residue | 1 | | 226.86 | 169.90 | 56.96 | 25.11 |
| | 2 | | 230.43 | 147.70 | 82.73 | 35.90 |
| | 3 | | 225.78 | 145.80 | 79.98 | 35.42 |
| | 4 | | 224.42 | 135.80 | 88.62 | 39.49 |
| Straw residue and oats | 1 | | 160.37 | 72.60 | 87.77 | 54.73 |
| | 2 | | 156.52 | 75.20 | 81.32 | 51.96 |
| | 3 | | 154.95 | 73.10 | 81.85 | 52.82 |
| | 4 | | 159.46 | 69.90 | 89.56 | 56.16 |
| Corn residue and oats | 1 | | 225.34 | 165.70 | 59.64 | 26.47 |
| | 2 | | 229.57 | 154.30 | 75.27 | 32.79 |
| | 3 | | 232.04 | 135.30 | 96.74 | 41.69 |
| | 4 | | 225.49 | 133.70 | 91.79 | 40.71 |

Table E.2. Calculation of OC loss in g and % wt. from residue in each plot with surface residue of Oat straw or corn stalks with/without oat plant growth.

| Plot treatment | | Initial (%) | Initial (g) | Harvest (%) | Harvest (g) | loss (g) | %OCloss |
|---|---|---|---|---|---|---|---|
| Straw residue | 1 | 45.70 | 70.19 | 40.20 | 31.03 | 39.16 | 55.79 |
| | 2 | 45.70 | 72.02 | 36.60 | 26.28 | 45.74 | 63.51 |
| | 3 | 45.70 | 70.30 | 42.30 | 36.38 | 33.92 | 48.25 |
| | 4 | 45.70 | 71.31 | 30.80 | 24.21 | 47.10 | 66.05 |
| Corn residue | 1 | 48.25 | 109.46 | 43.70 | 74.25 | 35.21 | 32.17 |
| | 2 | 48.25 | 111.18 | 44.90 | 66.32 | 44.87 | 40.35 |
| | 3 | 48.25 | 108.94 | 45.50 | 66.34 | 42.60 | 39.10 |
| | 4 | 48.25 | 108.28 | 46.80 | 63.55 | 44.73 | 41.31 |
| Straw residue and oats | 1 | 45.70 | 73.29 | 34.90 | 25.34 | 47.95 | 65.43 |
| | 2 | 45.70 | 71.53 | 44.10 | 33.16 | 38.37 | 53.64 |
| | 3 | 45.70 | 70.81 | 33.50 | 24.49 | 46.32 | 65.42 |
| | 4 | 45.70 | 72.87 | 42.65 | 29.81 | 43.06 | 59.09 |
| Corn residue and oats | 1 | 48.25 | 108.73 | 43.00 | 71.25 | 37.48 | 34.47 |
| | 2 | 48.25 | 110.77 | 41.30 | 63.73 | 47.04 | 42.47 |
| | 3 | 48.25 | 111.96 | 31.30 | 42.35 | 69.61 | 62.17 |
| | 4 | 48.25 | 108.80 | 45.20 | 60.43 | 48.37 | 44.45 |

Table E.3. Calculation of Nitrogen (N) loss in g and % wt. of residue in each plot with surface residue of oat straw of corn stalks with and without oat plant growth from May to August, 2004. The carbon to nitrogen ratio (C/N) is also given.

| | | Residue nitrogen loss | | | | | | C/N ratio | |
|---|---|---|---|---|---|---|---|---|---|
| Plot treatment | | Initial N% | Initial N g | Harvest N% | Harvest N g | N loss % | N loss g | Initial C/N | Final C/N |
| **Straw residue** | 1 | 1.44 | 2.21 | 0.99 | 0.76 | 65.44 | 1.45 | 31.70 | 40.61 |
| | 2 | 1.44 | 2.27 | 1.17 | 0.84 | 62.98 | 1.43 | 31.70 | 31.28 |
| | 3 | 1.44 | 2.22 | 1.08 | 0.93 | 58.07 | 1.29 | 31.70 | 39.17 |
| | 4 | 1.44 | 2.25 | 0.80 | 0.63 | 72.02 | 1.62 | 31.70 | 38.50 |
| **Corn residue** | 1 | 0.65 | 1.47 | 0.68 | 1.16 | 21.65 | 0.32 | 74.23 | 64.26 |
| | 2 | 0.65 | 1.50 | 0.82 | 1.21 | 19.14 | 0.29 | 74.23 | 54.76 |
| | 3 | 0.65 | 1.47 | 0.88 | 1.28 | 12.57 | 0.18 | 74.23 | 51.70 |
| | 4 | 0.65 | 1.46 | 0.82 | 1.11 | 23.66 | 0.35 | 74.23 | 57.07 |
| **Straw residue and oats** | 1 | 1.44 | 2.31 | 0.94 | 0.68 | 70.45 | 1.63 | 31.70 | 37.13 |
| | 2 | 1.44 | 2.25 | 1.43 | 1.08 | 52.29 | 1.18 | 31.70 | 30.84 |
| | 3 | 1.44 | 2.23 | 1.20 | 0.88 | 60.69 | 1.35 | 31.70 | 27.92 |
| | 4 | 1.44 | 2.30 | 1.15 | 0.80 | 64.99 | 1.49 | 31.70 | 37.09 |
| **Corn residue and oats** | 1 | 0.65 | 1.46 | 0.65 | 1.08 | 26.47 | 0.39 | 74.23 | 66.15 |
| | 2 | 0.65 | 1.49 | 0.86 | 1.33 | 11.07 | 0.17 | 74.23 | 48.02 |
| | 3 | 0.65 | 1.51 | 0.86 | 1.16 | 22.85 | 0.34 | 74.23 | 36.40 |
| | 4 | 0.65 | 1.47 | 0.79 | 1.06 | 27.94 | 0.41 | 74.23 | 57.22 |

Table E.4. Calculation of OC loss in g /plot from the mean of the surface residue mass loss and the % OC in May and August, in comparison to mean of soil OC for each treatment: oat straw/corn stalk residue x no plants/oat plants. Each plot =0.36m$^2$.

| **May to August 2004** | | **Plot treatments** | | | | |
|---|---|---|---|---|---|---|
| | | No plants | | Oat Plants | | plot |
| | Straw residue | Corn residue | Straw residue | Corn residue | =0.36m$^2$ |
| Initial %OC (May) | 6.50 | 6.98 | 7.08 | 7.35 | %OC |
| Soil OC (g) (50 kg x %OC) | 3250.00 | 3490.00 | 3540.00 | 3675.00 | g OC |
| | | | | | |
| Surface residue initial mass (g) | 155.26 | 226.87 | 157.82 | 228.11 | g |
| Harvest residue mass | 78.40 | 149.80 | 72.70 | 147.25 | g |
| Residue mass loss (g) | 76.86 | 77.07 | 85.12 | 80.86 | g |
| | | | | | |
| Initial OC (%) | 45.70 | 48.25 | 45.70 | 48.25 | % |
| Initial OC (g) | 70.95 | 109.46 | 72.12 | 110.06 | g OC |
| Harvest OC % | 37.48 | 45.23 | 38.79 | 40.20 | % |
| Harvest OC (g) | 29.38 | 67.75 | 28.20 | 59.19 | g OC |
| OC loss (g) (Initial - harvest) | 41.57 | 41.71 | 43.92 | 50.87 | g OC |
| | | | | | |
| Soil OC (August) (%) | 7.25 | 7.58 | 7.52 | 8.03 | %OC |
| Soil OC (g) /50 kg soil mass | 3625.00 | 3790.00 | 3760.00 | 4015.00 | g OC |

Table E.5. Calculation of plant OC fixation from photosynthesis based on carbon fixation and allocation to root rates given in Paul and Kucey (1981). The OC input from the oat plants with no residue, straw residue and corn residue was calculated per plot per season, and converted to g $m^{-2}$ based on harvest shoot biomass and OC allocations to root biomass, root exudates, and mycorrhizal biomass.

| Plant carbon fixation | | | | |
|---|---|---|---|---|
| | Oat Plant Plots | | | |
| | No residue | straw residue | Corn residue | |
| Mean plant biomass/plot | 705 | 547 | 614 | Harvest fresh wt. |
| SEM | 74 | 17 | 114 | |
| OC fixation rate (7.6 mg/g shoot/hr) | 5 | 4 | 5 | g OC per h |
| 12 hr day | 64 | 50 | 56 | g OC per day |
| 30 days | 1930 | 1496 | 1681 | g OC per season |
| Root Biomass(19%) | 367 | 284 | 319 | g OC per plot |
| Root Secretions 0.5% | 10 | 7 | 8 | g OC per plot |
| Mycorrhizal Biomass | 19 | 15 | 17 | g OC per plot |
| To soil | 396 | 307 | 345 | g OC per season |
| Root Biomass(19%) | 1018 | 790 | 887 | g OC per $m^2$ |
| Root Secretions 0.5% | 27 | 21 | 23 | g OC $m^2$ |
| Mycorrhizal Biomass | 54 | 42 | 47 | g OC $m^2$ |
| To soil | 1099 | 852 | 957 | g OC $m^2$ |
| Carbon fixation and allocation rates by Paul and Kucey (1981) | | | | |

# Appendix F

Comparison of soil OC using Fresh or Frozen/Thawed soil samples

## F.1 Fresh/Frozen Test

The undisturbed plots were sampled in April 2005 to test soil OC levels remaining after the winter, and to determine why a second sampling of 2004 soil OC resulted in lower OC figures only in August. The soil was subjected to the same freezing and thawing that the August 2004 samples received during the testing process. The freezing and thawing lowered the soil OC (Fig. F.1) and accounted for the difference. One interesting note is the large decrease in "plant only" plots over the winter between the two fresh sampling times. This would be indicative of greater soluble OC from increased secretions by the plant roots in the high moisture conditions.

Freezing and thawing is known to decrease aggregate size. The formation of ice crystals in the spoil pores expands the inter-aggregate bonds, followed by collapse of the bonds on thawing (Aluko and Koolen, 2001; Six et al, 2004). Macroaggregates are most affected, and moreso with sand than clay (Six et al, 2004). A moisture content of 24-30% increased the pore diameter exponentially (Aluko and Koolen, 2001). The high sand content in our macroaggregates, along with soil moisture greater than 30% could have contributed to reduction in aggregate size and thus mineralization of OC by bacteria in warm air with freezing and thawing of the August 2004 soil samples.

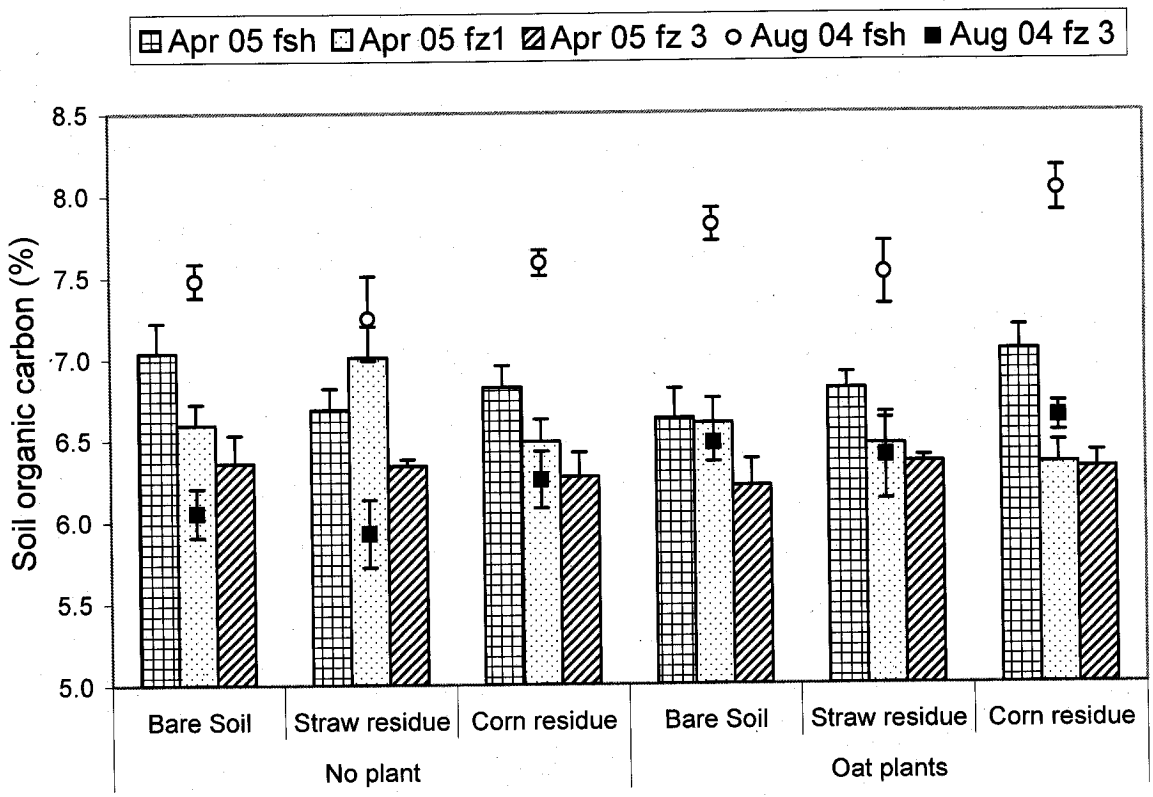



Figure F.1. Soil organic carbon (%) calculated for each treatment (with/without oat plants and with bare soil, straw residue or corn residue) from soil in August 2004 that was a) fresh (dried within 24 h), b) frozen and thawed to 20°C 3 times prior to drying, c) sampled the following April (2005) in the plots and dried within 24 h, d) sampled in April 2005 and frozen and thawed once prior to drying, 3) sampled in April 2005 and frozen and thawed to 20°C 3 times prior to drying. n = 4. Error bars represent SEM. There are only significant differences in the August 2004 fresh samples that are discussed in 2004 results. There is increased soil OC in the plant plots compared to no plant plots, and with corn residue w/wo plants compared to straw residue.

# Appendix G

**Table G.1. Carbon conversions**

| | Carbon Conversion from mass OC per soil volume | | | | | | |
|---|---|---|---|---|---|---|---|
| To Convert | g $m^{-2}$ | kg $m^{-2}$ | g/ha | kg ha | g kg | kg/kg (g/g) | % |
| $m^{-2}$ / 1000 = kg $m^{-2}$ | 1000 | 1 | | | | | |
| kg $m^{-2}$ X 10 = g $ha^{-1}$* | | 1 | 10 | | | | |
| g $ha^{-1}$ / 1000 = kg $ha^{-1}$ | | | 1000 | 1 | | | |
| kg $ha^{-1}$ / 4 = g $kg^{-1}$ | | | | 1 | 0.25 | | |
| g kg / 1000 = kg $kg^{-1}$ | | | | | 1000 | 1 | 100% |
| kg/kg or (g/g) / 100 = % | | | | | 10 | 0.01 | 1% |
| * 1 kg/ha = 0.89 lbs/acre | | | | | | | |
| 1 ton/ha = 0.405 ton/acre | | | | | | | |

Table G.2. Calculation of soil OC in corn acreage in Canada

Atmospheric reduction @ 0.5%/yr = 5 g/kg/yr = 20 kg/ha/yr = 17.8 lbs OC/acre/yr
Canadian crop Acreage = 100 Million Acres*
Carbon increase/Acre = 17.8 lbs OC/Acre
Carbon increase/Canada = 1780 M lbs OC = 0.9 M tons OC = 900,000 Tons OC
Corn in Canada = 17.8 lbs OC/Acre x 3.2 M acres*
Carbon increase/corn = 28,500 Tons OC

*Gov't of Canada, 2002, Canada Census