

Laboratory-Measured Soil Organic Carbon Mineralization in Soil Samples from Six Long-Term  
Crop Rotations in Alberta as a Function of Sample Disturbance

by

Kyle William Kipps

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Department of Renewable Resources  
University of Alberta

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## **Abstract**

Soil organic carbon (SOC) plays a dual role in soil; as a key compound determining the function and performance of agricultural soils and as the main terrestrial pool of carbon. The carbon content of cultivated soils has been found to depend on management practices. This laboratory incubation serves to quantify the effect of different long-term crop rotations and type of physical disturbance on the amount of potentially mineralizable carbon. Potentially mineralizable carbon was measured by the amount of CO<sub>2</sub> respired from the soil under laboratory conditions for a defined period, in this case 182 days. Soil samples were collected from the University of Alberta Breton Plots and Agriculture and Agri-Food Canada's Lethbridge Research Centre. Headspace samples from the incubation chambers were taken on a decreasing frequency over the course of 182 days and analyzed using gas chromatography. Mineralized carbon proved to be dependent on the interaction of the sample location (Breton or Lethbridge), crop rotation and disturbance treatment applied. Generally, crop rotations with higher light fraction (LF) contents resulted in higher potentially mineralizable carbon. Increasing physical disturbance was found to decrease the LF content and increase the C:N ratio of the remaining LF both of which reduced the potentially mineralizable carbon. The total SOC content was not found to decrease with physical disturbance suggesting that the carbon formerly in the LF, which was mineralizable, was transferred within the soil and retained. The results suggest that in addition to crop rotation, particle size of crop residue returned to the soil may offer management options for control of SOC dynamics in tilled soils. Understanding the mechanisms controlling SOC dynamics of these soils is beneficial for improved management of SOC and greenhouse gas emissions in cultivated soils.

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## List of Abbreviations

<b>C</b>	Carbon
<b>C:N</b>	Carbon to nitrogen ratio
<b>DRP</b>	Dish, ring and puck mill
<b>GWC</b>	Gravimetric water content
<b>iPOM</b>	Intra-aggregate particulate organic matter
<b>LF</b>	Light fraction
<b>MRT</b>	Mean residence time
<b>N</b>	Nitrogen
<b>OM</b>	Organic matter
<b>POM</b>	Particulate organic matter
<b>SOC</b>	Soil organic carbon
<b>WFPS</b>	Water filled pore space

## **1. Chapter 1 – General Introduction**

### **1.1. Soil-Carbon Interactions**

Carbon dioxide from the loss of soil organic carbon (SOC) is one of the greenhouse gases emitted by agriculture (Pretty et al., 2000). Agriculture interacts with as much as 10.6% of all of the earth's biomes, by area, and at present is responsible for the management of approximately 128 Gt of the global carbon stocks (Watson, 2000). The majority of SOC loss attributed to agriculture occurs during the initial cultivation of grasslands. This deficit in SOC storage creates an opportunity to return carbon to the soil (Tisdall and Oades, 1982; Huggins et al., 1998; Janzen, 2006) Hence agriculture provides an opportunity to mitigate anthropogenic carbon emissions through “carbon sink expansions” (McCarl and Schneider, 2001). The SOC content of a cultivated soil is highly dependent on its management with the chosen nutrient, crop rotation and tillage regime as possible determining factors in the carbon balance (Grant et al., 2001; Campbell et al., 1996). Soil organic carbon is also beneficial to agriculture; providing and enhancing many soil processes and contributing to enhanced productivity (Dyck et al., 2012). The management of cultivated ecosystems differs from that of grasslands and forests in terms of decomposer communities and as such, they require their own unique study and consideration (Hendrix et al. 1986).

A great deal of research in soil science has been dedicated to the investigation of practices intended to maximize carbon sequestration in cultivated soils and the mechanisms responsible for this sequestration (Six et al., 2002; Hassink 1997). However, as identified by Janzen (2006), maximizing carbon sequestration at the expense of nutrient turnover and other soil processes may not be desirable in all situations. Tillage and fallow practices might be necessary in certain production systems where conventional pesticides are not available or outside of allowable practice such as in organic agriculture (Standards Council of Canada, 2011). Tillage may also be necessary in areas prone to late seeding conditions to achieve optimal soil temperatures for germination and early seedling development (Johnson and Lowery, 1985). Furthermore, occasional tillage events might be necessary to redistribute SOC and nutrients that become stratified through long-term no-tillage (Duiker and Beegle, 2006). Retention and accumulation as well as loss of SOC through mineralization are both part of health soil functioning and, therefore, necessitate the consideration and further study of at least portions of the SOC pool as transient in the soil (Dyck et al., 2012; Janzen 2006).

#### **1.1. Soil Organic Carbon Stabilization**

It has been observed in long-term experiments that changes to the SOC content will eventually reach a new equilibrium for a given management practice (Janzen, 1998). The remaining SOC exhibits a much

slower turnover rate than the fraction lost or accumulated during land use or management changes (Huggins et al., 1998). There have been a number of mechanisms, both biotic and abiotic, proposed for the long-term stabilization of SOC (Six et al. 2002). Three popular classifications for the possible mechanisms of SOC stabilization proposed by Six et al. (2002) include physical stabilization in microaggregates, adsorption to silt and clay particles, and biochemically transformed SOC to recalcitrant forms. Recently, the concentration of carbon in the soil available for microorganisms has been suggested as a factor in determining the stability of SOC (Don et al., 2013). A great deal of work has been done to explore these different mechanisms of stabilization often with much conflicting evidence depending on the environmental circumstances. Certain soil systems may have only one mechanism regulating SOC dynamics, while another could have several working in unison (von Lutzow et al., 2007). The main challenge when evaluating the relevance of stabilization mechanisms, as identified in von Lutzow et al. (2007), is the inability to fraction “homogeneous or functional OM pools” for analysis. Table 1.1 provides a list of common fractions discussed in subsequent sections, their definitions and methods of determination. Hence the continued debate and refinement of methodology in this area. Understanding the various mechanisms and their role in specific soil types could aid in land use designation and assist in management decisions to conserve soil types at risk of degradation through SOC loss.

#### **1.1.1. Chemical Recalcitrance**

Chemical recalcitrance is the “selective preservation of certain recalcitrant organic compounds, due to their molecular-level characteristics” (Marschner et al., 2008). Compounds with chemical recalcitrant properties would be stable in the soil by their nature and accumulate over time. Comparing the turnover rates of various chemical compound classes to the bulk SOC pool did not yield any results suggesting differences attributed to their chemical nature (Marschner et al., 2008). They also observed evidence for the priming effect and attributed the appearance of recalcitrant SOC to substrate limitations. Although, two-year incubation experiments did demonstrate a residence time of around a century for black carbon, it still showed degradation over time, especially in the presence of additional substrates. They did observe that free SOC, without soil mineral particle association, had the highest turnover rates compared to the bulk SOC pool. While they concluded that SOC without mineral association did not contribute to the stable SOC pool, they did caution that advancements in fractionation schemes and analytical methods could provide additional information. A review of SOC recalcitrance by Dungait et al. (2012) concluded that while the theory has “empirical meaning” there is a “lack of an adequate molecular and mechanistic definition”. It is suggested that the appearance of recalcitrant SOC could be attributed to the lack of “biological,

physical and chemical” conditions conducive to decomposition (Dungait et al., 2012). An investigation into the molecular structure of stable SOC (MRT 680 years), which was the mineral associated fraction, did not find a material composition consistent with “highly aromatic humic-like organic compounds” thought to be chemically recalcitrant (Kleber et al., 2011). Rather, compounds with a “chemically labile molecular form” like proteins and polysaccharides were found in mineral association to make up the stable pool. A review into methods of measuring SOC pools suggested that the composition of compounds comprising stable SOC pools does not significantly change over time (Paul et al., 2006).

Clarification of chemical recalcitrance and its implications were eloquently presented in a paper by Baldock and Skjemstad (2000). They noted that there are different fractions within SOC that are preferentially utilized, but all fractions are utilizable. In organic soils, the composition of the SOC determines its rate of decomposition; however, in mineral soils there are additional mechanisms. The preservation of SOC or its derivatives and rates of decomposition are determined by interactions with the soil matrix. The mechanisms they proposed included the soil chemistry, mineralogy and structure of the matrix.

### **1.1.2. Aggregation**

Adoption of conservation tillage practices have been shown to increase SOC compared to conventional tillage methods (Alvarez, 2005; Campbell et al., 1996). This increase in SOC content has been attributed to physical stabilization of SOC in soil aggregates (Six et al., 1999). The reduced tillage frequency increases the stability of the aggregates. It has been observed that crushing of macroaggregates from no-till systems resulted in increased mineralization of C and N in incubation experiments whereas the same treatment in conventional tillage systems yielded no change in mineralization rates (Beare et al., 1994). Another study comparing carbon mineralization in undisturbed and laboratory sieved soils (< 4 mm) from a range of management histories did not find any effect on potentially mineralizable C (Curtin et al., 2014). Microbial community composition has been found to differ between conventional and conservation tillage with the relative importance of bacteria over fungi increasing in the former, which is associated with a higher metabolic rate (Gupta and Germida, 1988; Hendrix et al., 1986). It has been suggested that the increases in soil carbon contents attributed to improved management practices are dependent on the continuation of these practices and preservation of aggregates (Janzen et al., 1998; Six et al., 2000). Conventional tillage systems have been found to decrease quantities of macroaggregates and increased quantities of degraded microaggregates (Six et al., 2000). Carbon associated with larger particle size fractions is less stable than carbon in association with primary silt and clay sized particles (Hassink, 1997). Physical protection of SOC

in macroaggregates is not likely to be responsible for the length of turnover times (> 500 years) observed in the stabilized SOC pool due to the high frequency of turnover even under zero till systems in this time scale (AARD, 1999).

### **1.1.3. Organomineral Association**

Organomineral association refers to the adsorption of SOC to soil mineral particles. A study by Skjemstad et al. (1986) using nuclear magnetic resonance to study the effect of cultivation on SOC concluded that the SOC pool with the longest retention time was “stabilized by physical associations or made inaccessible to degradative enzymes rather than” by chemical recalcitrance. Protection of SOC by silt and clay was observed by Hassink (1997) where the loss of carbon from primary soil particle fractions  $\leq 20 \mu\text{m}$  was less than that of fractions  $\geq 20 \mu\text{m}$  for tilled soils. They found that there was a maximum amount of SOC that could be protected in association with clay and that it was correlated with the clay and silt content of the soil. They suggested that any further organic matter additions beyond the protective capacity of the silt and clay would become associated with larger soil fractions (aggregates) in grasslands. Incubation experiments investigating the influence of soil structure on SOC decomposition led to the suggestion that “the control soil structure has on carbon dynamics takes place at spatial scales below those modified in this experiment (<13 mm).” This supports the findings of Ladd et al. (1985) where an eight-year field experiment showed greater retention of isotope labelled residue in soils of heavier texture. In the same study light textured soils showed mineralization of SOC while heavy clay soils did not show any significant loss of SOC. Mechanisms of SOC stabilization offered by soil texture can be by organomineral association or soil structure, both of which are dependent on soil texture (Dungait et al., 2012; Juarez et al., 2013). Plante et al. (2006) found the silt-sized physical fraction of a soil with low SOC “to be an important location for stabilization”. The protection offered by organomineral association relies on the adsorption of SOC onto mineral particle exchange sites by bonds that are stronger than that of the enzyme active sites (Dungait et al., 2012). Although organomineral SOC stabilization is very stable and not as likely affected by management practice, Bremer et al. (1994) found continuous cropping to increase both free and complexed pools of SOC over wheat-fallow systems. However, a soils capacity for organomineral SOC stabilization is limited as demonstrated by Hassink (1997). Soil pore characteristics determine the quantity of sites appropriate for microbial activity through pore geometry and “spatial distribution of water filled pores” (Juarez et al., 2013).

#### **1.1.4. Biologically Relevant Pore Space**

Soil porosity refers to the space between physical particles, both mineral and organic, within soil (Hao et al., 2008). Porosity can be quantified in a number of ways such as total porosity, size of pores or proportion of pores within a size class. The pore characteristics of a soil are determined by, but not limited to its SOC content, texture and structure. Porosity can also be quantified as structural or matrix porosity (Dexter et al., 2008a). Structural porosity refers to pore space between aggregates (Dexter et al., 2008b) Matrix pores are those not affected by aggregation but are those inherent to soil texture.

In the most succinct explanation of biologically relevant pore space, Baldock and Skjemstad (2000) note that all mineralization processes take place in soil pores and that “the distribution of pore sizes can influence the biological stability of organic materials in the soil.” Pore size distribution regulates the availability of water and oxygen and controls the types of mineralization mechanisms by limiting decomposer accessibility to the substrate. Porosity in itself can regulate the amount of mineralization in a soil, simply by determining the total available habitat. By compressing a soil and reducing its porosity, Franzluebbers (1999) observed a decrease in cumulative soil respiration in a range of soils with different clay contents. The decrease was observed for a range of soil moisture contents for each soil type. The mechanisms increase in complexity when pore size distribution, soil moisture content and microbial community interactions are taken into consideration.

Yoo et al. (2006) demonstrated the controlling effect soil pore size distribution and aggregation have on stabilization of SOC mineralization rates. Their work showed the significance of “biologically relevant pores”, those with a pore diameter  $\leq 30 \mu\text{m}$ , where differences in pore size distribution affected by tillage “altered the physical availability of substrates and optimal water conditions for biological activity”. When these pores were saturated with water they observed a decrease of SOC mineralization rates. The stabilizing effects were more strongly observed in a grassland soil than a cultivated soil, both with the same texture, due to the smaller total volume of micropores as a result of greater aggregation. The total volume of micropores in the grassland soil became saturated before the cultivated soil, expressed as a reduction in SOC mineralization at a lower total soil percent saturation. The decrease in SOC mineralization rates was attributed to anaerobic conditions induced by the saturated pores (Yoo et al., 2006; Zausig and Horn, 1992). Inversely, the grassland soil was also found to have a lower SOC mineralization rate than the cultivated soil at reduced soil moisture contents due to enhanced desiccation provided by a larger proportion of macropores (Yoo et al., 2006). Desiccation in the large pores prevented microbial access to substrates and decomposition as microbial activity has been found to be greatest at

the “gas-water interface” (Yoo et al., 2006; Strong et al., 2004). Anaerobic conditions induced in micropores provide a likely mechanism to stabilize SOC in disturbed systems due to the larger proportion of small pores. It was noted in Chen et al. (2015) that “soil pore-size distribution could play a more important role than soil bulk density (or soil total porosity) in C decomposition processes.”

Another study by Killham et al. (1993) investigating carbon turnover as influenced by the effect of substrate location and pore water regime found micropore saturation to enhance decomposition. When two micro pore classes (< 6 µm and 6 - 30 µm) were compared it was found that the larger pores within the range had higher rates of turnover especially when the larger pores were saturated. They suggested that the larger pores, especially when saturated, allowed “access of protozoal grazers to primary decomposers”. This was shown by the larger pores having lost a greater percentage of the added C as CO<sub>2</sub> and having a lower percentage retained in the microbial biomass, suggesting increased predation of the primary decomposers. After only three days in all treatments, nearly the entire added C had been consumed by the microbial biomass - the difference in respiration between the two pore classes was only observed when those larger pores were saturated. When the larger pores were dry, the amount of C mineralized in larger pores was very similar to that of smaller pores.

#### **1.1.5. Substrate Limitation**

It has been proposed that SOC concentration is responsible for regulating SOC mineralization at low concentrations of carbon substrate (Don et al., 2013). Substrate-concentration-regulation shares similarities with physical-protection-stabilization mechanisms in that they both relate to the physical separation of the decomposer and substrate. A logarithmic relationship between the concentration of carbon substrate and CO<sub>2</sub> production was demonstrated for grassland soils (Don et al., 2013). They proposed that this relationship could also be described as a decreasing likelihood of substrate decomposition with decreasing substrate concentration. They attributed this relationship to the development of net negative energy conditions for microorganisms at low substrate concentrations, causing the organisms to go dormant until conditions improve. This mode of carbon stabilization appears to play a role in SOC stabilization in grasslands, however, in cultivated soils it seems less likely as organisms and substrates are repeatedly moved by tillage or seeding operations and the probability for interaction increases over time. This mechanism might share responsibility with the physical stabilization mechanism for the observed flush of CO<sub>2</sub> immediately after disruption of soil (Plante and McGill, 2002; Juarez et al., 2013).

### **1.1.6. Soil Nitrogen**

Addition of nutrients such as nitrogen has generally been found to increase SOC through the subsequent increase in plant productivity and additions of plant litter to the soil system (Grant et al., 2001; Izaurre et al., 2001). The effect of nitrogen on SOC content is less clear and seems to vary depending on plant residue additions to the soil (Fog 1988; Clapp et al., 2000). A review of the effect of nitrogen additions on mineralization of a wide range of substrates, including SOC, noted that there is often no effect; however, when significant effects were found, nitrogen caused the preferential decomposition of “easily degradable organic matter with low C/N ratio” (Fog, 1988). A field experiment manipulating the retention or removal of corn stover found similar results when nitrate additions accelerated fresh corn residue decomposition, but suppressed the mineralization of SOC (Green et al, 1995). Another field experiment using corn also found that mineralization of native SOC was reduced with nitrogen additions and retention of corn stover, but found that nitrogen additions, with stover removal, resulted in increased SOC mineralization (Clapp et al., 2000). Suppression of mineralization for certain types of organic residues with addition of nitrogen is attributed to alterations to the balance of composition between decomposer groups and the prevention of certain enzymes (Fog 1988). A long-term research experiment from Illinois showed increasing loss of SOC in the upper 0.46 m of a fertilized continuous corn rotation profile compared to the control plot even with the return of residue (Khan et al., 2007). However, in a long-term research experiment from Lethbridge, Alberta there was no significant effect of fertilizer on mineralized carbon in a ten-week incubation study (Bremer et al., 1994). Due to inconclusive results of the effect of nitrogen on SOC mineralization rates it is unlikely that nitrogen plays a dominant role in SOC stabilization, but it warrants consideration when investigating other mechanisms of SOC stabilization.

### **1.2. Long-Term Sites**

The term “long-term sites” is ambiguous and depends on the questions being asked in the research (Janzen 1995). Bremer et al. (1995) considered studies > 10 years to satisfy the criteria to be considered a long-term site for research into organic carbon dynamics. They found that, following management changes, SOC was close to reaching a new equilibrium within 10 – 20 years. Once an equilibrium is established for a given management practice, SOC contents are stable for a significant amount of time. An experiment at Indian Head, Saskatchewan that was initiated on soils that had been under fallow-wheat or fallow-wheat-wheat for 50 years did not observe any changes in the SOC content over the course of an additional 30 years (Lemke et al., 2012). Some management changes such as imposing different fallow periods on an already cultivated soil appear to take longer, with a new equilibrium being reached around

30 years (Janzen et al., 1998). Application of treatments that proceed at different rates can result in very large differences until equilibrium is reached. After the establishment of the research plots at Agriculture and Agri-Food Canada's Lethbridge Research Centre, the wheat plots with fallow phases in the rotation reached equilibrium within 22 years, while the continuous wheat plots did not until 67 years (Monreal and Janzen, 1993). However, at the long term Breton Plots a stable SOC content has not been demonstrated for any of the fertility treatments of the wheat-fallow or wheat-oat-barley-hay-hay rotations after 70 years (Grant et al., 2001). As pointed out in Janzen et al. (1998) errors can occur when using historical data or archived soil samples due to differences in techniques between researchers and interpretation of historical records. Trends in the SOC measurements and modeling show the rate of SOC loss from the rotation to be decreasing and approaching a steady state (Grant et al., 2001). Although, recent research on the Breton Plots' wheat-fallow rotation under full fertilization (NPKS) has shown an increase in SOC levels from 1980 to 2008, 50 to 78 years after the rotation was established (Giweta et al., 2014).

Following conversion from native grassland to cropping, SOC losses of 17, 21 and 23% under continuous wheat, fallow-wheat-wheat and fallow-wheat, respectively were observed after 80 years at Lethbridge (Monreal and Janzen, 1993). A long-term study at the University of Missouri's Sanborn Field found that following the conversion of native grassland to continuous wheat the SOC losses were approximately 72% until reaching a new equilibrium in 27 years (Balesdent et al., 1988). Native SOC at the Morrow Plots in Illinois declined 45 – 60% over 28 years (Huggins et al., 1998). They attributed the losses to the labile carbon fraction, which has a much higher rate of turnover and is more sensitive to changes in management. They estimated that the labile pool would be slightly larger than the stable SOC pool at the Sanborn Field and these pools would have turnover times of 20 and 556 years, respectively (Huggins et al., 1998). Although the proportions of change may vary between experiments depending on soil and climatic conditions, the majority of SOC loss consistently occurs within 20 – 30 years. Seemingly, the remaining total SOC is much more stable and less prone to rapid loss. For the purposes of studying SOC dynamics the application of management treatments for a minimum of 20 – 30 years seems appropriate.

### **1.3. Mineralizable Carbon**

Mineralizable carbon refers to SOC, which through a metabolic pathway within the soil microbial community can be respired as CO<sub>2</sub> within a specified period. In incubation experiments mineralized C is commonly used to estimate the labile SOC pool (Bremer et al., 1994). However, since mineralizable C is determined under laboratory conditions, which are not the same as field conditions, the estimated pool

may not equal the true pool in the field (Janzen, 1992). Subsequently, mineralizable C is sometimes referred to as potentially mineralizable C or cumulative mineralized C to indicate that the values are limited to the unique conditions of the method of determination (McDaniel et al., 2014; Franzleubbers, 1999). Furthermore, it is important to consider that a wide range of time periods have been used to quantify mineralizable carbon, ranging from 14 to 360 days (McDaniel et al., 2014; Franzleubbers, 1999; Bremer et al., 1994; Collins et al., 1992, Campbell et al., 1991a; Campbell et al., 1991b).

### **1.3.1. Crop Rotation Influences on Mineralizable Carbon**

An incubation study utilizing soils from the Cropping Biodiversity Gradient Experiment at the W.K. Kellogg Biological Station Long Term Ecological Research site showed the effect of crop rotation on cumulative C respiration and potentially mineralizable C (McDaniel et al., 2014). The experiment had been in operation for 11 years at the time of soil sampling for the incubation experiment. They evaluated five different rotations: corn, corn-soy, corn-soy-wheat, corn – soy – wheat + red clover cover crop and corn – soy – wheat + red clover & rye cover crop which were either one, two or three year rotations. The soil samples were obtained following corn harvest of each rotation. Initial soil characteristics including C, N,  $\text{NO}_3^-$ , and  $\text{NH}_4^+$  were not statistically different between rotations, but the C:N ratio of the corn-soy and corn-soy-wheat-red clover cover crop were significantly higher than the other rotations. The cumulative C mineralization results after 360 days were higher for rotations with increased complexity in the rotation. They suggested that the low potential C mineralization rates in the simple rotations were due to substrate limitation as indicated by the corresponding low  $\beta$ -glucosidase-to-phenol oxidase ( $\beta\text{G:PO}$ ) ratio. The ratio provides the proportions of active enzymes targeted to two different chemical compounds, cellulose in the case of  $\beta$ -glucosidase and lignin for phenol oxidase (Waldrop et al., 2004). A decrease in the presence of  $\beta$ -glucosidase is assumed to occur due to the scarcity or depletion of chemically labile cellulose, while an increase in the presence of phenol oxidase occurs as the microbial community increases its utilization of the less desirable and chemically more recalcitrant lignin. They also note that while their sampling did not show rotation influences on total carbon content, studies currently in review show large increases in the sand-corrected C contents of the complex crop rotations used in the McDaniel et al. (2014) study. In either case, they suggest that “enhanced microbial activity and [an] increase of microbial-available SOM” are all benefits of complex crop rotations.

A short-term incubation experiment from a long-term crop rotation experiment in Washington also found a strong rotation effect on cumulative carbon mineralization (Collins et al., 1992). The rotations wheat-pea, continuous wheat, and wheat-fallow had been established for 56 years at the time of soil sampling

and were compared to a long-term grass pasture. Samples were taken during the wheat phase of the cereal rotations. The lowest cumulative mineralization and carbon content were found for the wheat-fallow soil. Continuous wheat and wheat-pea were not significantly different from each other for either parameter, but were intermediary between wheat-fallow and the long-term pasture. The long-term pasture had the greatest cumulative mineralization as well as carbon content. Similar trends were found for the soluble-C content of the soils, which was used to approximate the labile fraction. Collins et al. (1992) also found that decreases in SOC observed in the crop rotations were correlated with proportionate reductions in the size of the microbial biomass, similarly to McDaniel et al. (2014).

An additional rotation experiment, named “Rotation 120”, at Agriculture and Agri-Food Canada’s Lethbridge Research Centre was established in 1951 to evaluate the sustainability of crop rotations currently in use in the area (Smith et al., 2012). Rotations included fallow-wheat, fallow-wheat-wheat, continuous wheat, and fallow-wheat-wheat-hay-hay-hay with some variation to include fertility treatments. A study by Bremer et al. (1994) evaluated the effect the rotation treatments had on light fraction (LF) soil organic matter (SOM), total and mineralizable carbon. At the time of soil sampling, the rotations had been established for 41 years. A ten-week incubation study was used to determine mineralizable carbon. Mineralized C was strongly influenced by the phase of rotation, but also showed it to be cyclical in nature. The fallow phases reduced the amount of mineralized carbon, but after a few years of repeated cereal, such as in fallow-wheat-wheat, the amount was comparable to that of continuous cereal. The greatest mineralized C was observed in phases toward the end of the fallow-wheat-wheat-hay-hay-hay rotation. They found a significant correlation between mineralized C and LF carbon; however, it only explained 31% of the variability. They noted that there was a large amount of variability in both mineralized C and LF carbon, which may have added to the variability of the relationship. The variability in mineralized C was not observed in LF carbon or total SOC with rotation phase. General trends reported showed mineralized C to increase with increasing SOC measured to 30 cm depth. In a review of 11 long-term agricultural experiments across Canada it was found that conversion of annual crops to perennials increased SOC content (VandenBygaart et al., 2010).

### **1.3.2. Physically Uncomplexed SOC**

Soil organic carbon can be conceptualized and studied in pools such as physically uncomplexed SOC (Gregorich and Beare, 2008). Physically uncomplexed SOC is commonly separated as LF using a dense liquid with specific gravity of commonly around  $1.7 \text{ kg}\cdot\text{L}^{-1}$  in agricultural soils (Malhi et al., 2011; Janzen et al., 1992). A comparison of SOC content from an agricultural tillage experiment in different soil fractions

including whole soil, light fraction, intra-aggregate particulate organic matter and mineral size classes was conducted by Six et al. (2001). Carbon and nitrogen concentrations in the light fraction were found to be at least three times the concentration in the next highest fraction. The residence time of LF was found to be longer than intra-aggregate particulate organic matter, but less than the mineral-associated classes. The authors suggest this shows that LF originates from the biologically recalcitrant, intra-aggregate particulate organic matter (iPOM). Evaluating a variety of soil types from agriculture, pasture and forest land use showed the light fraction had a higher C:N ratio than the heavy fraction (Baldock et al., 1992). They postulate that the change in C:N ratio is due to the less decomposed nature of the organic materials in the light fraction, which is congruent with the results of Six et al. (2001). Results of a study conducted on soil from a California grassland showed that LF was composed of more than 90% SOC that had a more rapid rate of turnover than SOM in organomineral associations (Baisden et al., 2002).

The light fraction is of particular interest because of its lack of association with mineral particles, which changes its behavior in the soil carbon cycle (Gregorich and Beare, 2008). The responsiveness of LF to different long-term crop rotations was demonstrated with greater LF carbon increases in response to increases in carbon inputs than the bulk SOC pool (Plante et al., 2006; Janzen et al., 1998; Bremer et al., 1994). Furthermore, LF carbon was found to be twice as high in a continuous spring wheat rotation compared to a spring wheat-fallow rotation in both conventional and zero-tillage systems (Larney et al., 1997). It has also been found to increase under reduced tillage (Malhi et al., 2001). The LF pool is also sensitive to land use changes, exhibiting the greatest decrease compared to other SOC pools following conversion of a grassland to a cultivated ecosystem after 35 years (Skjemstad et al., 1986). Microbial biomass N has also been found to be positively correlated with LF suggesting its importance as a readily available substrate for microorganisms (Janzen et al., 1992). While LF C has been found significantly correlated with mineralized C, turnover times suggest that LF C includes SOC that is not part of the mineralizable C pool (Bremer et al., 1994). Subsequently caution must be exercised when LF carbon is used to approximate the size of the labile carbon pool.

#### **1.4. Soil Porosity**

##### **1.4.1. Soil Porosity and Soil Organic Carbon**

Increased SOC is positively correlated with increased matrix porosity of arable soil at low SOC levels, but not structural pores (Dexter et al., 2008a). At higher SOC levels the matrix porosity does not change with SOC levels nor does it influence structural porosity. In their experiment, Dexter et al. (2008a), found that the equivalent pore diameter for matrix and structural pores was 1.55  $\mu\text{m}$  and 48  $\mu\text{m}$ , respectively. A

study by Pagliai and Antisari (1993) found that applying organic wastes resulted in increased micro and macroporosity. They distinguished between micro and macro porosity at 50  $\mu\text{m}$ , so while not directly comparable with the Dexter et al. (2008a) study, their results would have likely found structural porosity to be enhanced by the addition of waste. The finding by Pagliai and Antisari (1993) of organic waste additions increasing macroporosity is probably due to their application of organics from the waste in much higher quantities than would have been evaluated in Dexter et al. (2008a). A study evaluating cultivated soil samples from a number of locations on the Canadian prairies found that increased SOC resulted in an upward shift of water retention curves across a range of pressures from 0 to 10,000 kPa (De Jong et al., 1983). This suggests that there was an increased in the proportion of smaller pores, likely those associated with matrix porosity.

#### **1.4.2. Soil Porosity and Soil Disturbance**

Soil porosity is affected by physical disturbance such as tillage. The porosity changes are seen mainly in structural porosity in the surface soils (Lipiec et al., 2006). In an 18 year simulated tillage experiment, where tillage was simulated by hand tools to avoid compaction, conventional tillage was found to have more areal porosity (pores > 117  $\mu\text{m}$ ) in the plough layer than zero tillage (Lipiec et al., 2006). There were distinctions in pore size distribution as well, with zero tillage having a greater volume proportion of pores < 60  $\mu\text{m}$  and conventional tillage with a larger proportion of pores > 60  $\mu\text{m}$ . Arshad et al. (1999) found no-till to have fewer macropores (> 15  $\mu\text{m}$ ), but more micropores (< 0.75  $\mu\text{m}$ ) with no change in total porosity when compared to conventional tillage. In contrast, a literature review by Kay and VandenBygaart (2002) reported a shift in pore size distribution from 30 – 100  $\mu\text{m}$  to 100 – 150  $\mu\text{m}$  under conversion of conventional tillage to no-till. They did not report any observations on pore size distribution less than 30  $\mu\text{m}$ . These results were corroborated in a study by Chen et al. (2015) where reduced soil disturbance and decreased traffic in no-till was attributed for a larger proportion of macropores (> 100  $\mu\text{m}$ ) in surface soils (0 – 0.05 m). They also showed how the effects of tillage regimes could be isolated to certain depths within the profile and as such, specification of soil sampling depth should be made when reporting effects. The effects of disturbance on porosity are somewhat unclear, most likely due to the inconsistent classification of pore sizes and ranges of interest and it's short-term, transient nature. Another possible reason could be the effect of SOC on pore size distribution between rotations. Kay and VandenBygaart (2002) identified the interaction of porosity and SOC as a knowledge gap in tillage research.

Chen et al. (2015) compared no-till to mouldboard ploughing for two different crop rotations, maize-soybean and continuous maize, and found a significant effect of rotation between both tillage regimes.

The maize-soybean rotation had a higher proportion of pores in the range of 0.2 – 30  $\mu\text{m}$  and < 0.2  $\mu\text{m}$  for both the 0 – 0.05 m and 0.05 – 0.1 m depth intervals. In both depths the proportion of mesopores (0.2 – 30  $\mu\text{m}$ ) decreased under the maize-maize rotation. The effect of rotation on the proportion of micropores differed with depth showing an increase in the 0 – 0.05 m and decrease in 0.05 – 0.1 m range. While they did not directly discuss these results, they did provide evidence to attribute them to the SOC content of the soil. In a previous study from the same experimental plots it was noted that the soybean phase contributed fewer residues, had higher mineralization due to its lower C:N ratio and resulted in a reduced SOC content in the 0 – 0.05 m depth range compared to the maize-maize rotation (Chen et al., 2015). Irrespective of tillage regime it seems that rotations with reduced SOC contents favor a shift in the pore size distribution to pores <30  $\mu\text{m}$ .

The response of soil porosity to both SOC content and disturbance is mixed with no trends being apparent across all studies. A study using the U.S. National Soil Characterization database investigated the relationship between SOC and soil water retention (Rawls et al., 2003). Their results highlighted the complexities of the relationship showing different results depending on the soil water potential, soil texture and whether the change was in a soil with high or low SOC levels. Experimentation with a focus on the interaction between disturbance and SOC seems to be lacking as pointed out by Kay and VandenBygaart (2002).

### **1.5. Research Objectives**

The overall objective of this study was to compare the amount of mineralizable carbon between different long-term crop rotations established on two different soil types after varying degrees of sample disturbance. The comparison provides insight into the implications of long-term management choices and the legacy of soil type on SOC dynamics in these soils. The specific objectives are to determine the influence of physically uncomplexed SOC and soil pore size distribution on potentially mineralizable carbon.

Chapter two provides the methodology used to achieve these objectives and the results of the mineralization experiment. Soil characterization includes particle size, pore size distribution, total SOC and LF. Potentially mineralizable carbon is assessed through a 182-day laboratory incubation experiment. The results of the soil characterization and incubation, as well as their relationships are presented and discussed. Conclusions, limitations and future work are presented at the end of the chapter.

## 1.6. Tables

**Table 1.1 Select carbon fractions and methods of determination**

<b>Carbon Fraction</b>	<b>Definition</b>	<b>Method of Determination</b>	<b>Reference</b>
<b>Chemically recalcitrant soil organic carbon</b>	"Complex compounds that have inherently low reactivity and require high activation energy for decomposition" and as such decompose very slowly	X-ray microscopy and NEXAFS spectroscopy for "speciation of organic functional groups"	Davidson and Janssens, 2006; Kleber et al., 2011
<b>Intra-aggregate particulate organic matter (iPOM)</b>	Light fraction organic carbon that is contained within aggregates	Aggregates are isolated by sieving. The iPOM is then recovered by suspension on a dense liquid and subsequent vacuum filtration	Six et al., 2000
<b>Labile soil organic carbon</b>	Easily or readily mineralizable soil organic carbon. Half-life in soil of 0 – 2.5 years.	Indices of labile soil organic carbon are provided through several approaches including: measurement of microbial biomass, mineralized carbon in laboratory incubation or LF	Biederbeck, et al., 1994; Jenkinson and Rayner, 1977
<b>Light fraction</b>	Physically uncomplexed organic matter including plant fragments, roots and charcoal	Isolated from bulk SOC by suspension on a dense liquid, commonly with a specific gravity of 1.7 kg·L <sup>-1</sup> , followed by vacuum filtration	Gregorich and Beare, 2008; Bremer et al., 1994; Janzen et al., 1992
<b>Mineralizable carbon</b>	Soil organic carbon that is available for mineralization in laboratory incubations of a certain duration	Cumulative CO <sub>2</sub> evolution is measured from a laboratory incubation for 14 to 360 days	McDaniel et al., 2014; Campbell et al., 1991a
<b>Organomineral soil organic carbon</b>	Mineral associated soil organic carbon	Total soil organic carbon less light fraction organic carbon. The heavy fraction remaining following suspension of the light fraction on a dense liquid	Six et al., 2000
<b>Particulate organic matter</b>	"Pieces of organic debris 53 -2000 µm in size with a recognizable cellular structure [not humified]..."	"...collected on a 53 µm sieve after complete dispersion of a soil"	Baldock and Broos, 2012
<b>Soil organic carbon (SOC)</b>	All biologically derived organic materials, in all stages of decomposition on or within the soil (excludes above ground living portions of plants)	Whole soil analyzed using elemental analyzer	Baldock and Broos, 2012

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## 2. Chapter 2 – Mineralizable Carbon

### 2.1. Introduction

The conversion of grassland to arable land is associated with a reduction in soil organic carbon (SOC) under conventional tillage systems (Balesdent et al., 1988; Six et al., 1998). The initial loss is due to a number of factors favorable to SOC decomposition brought on by soil disturbance and reduced organic matter (OM) inputs (Tisdall and Oades, 1982; Huggins et al., 1998). The loss of SOC occurs primarily from the labile carbon (C) pool (Huggins et al., 1998; Janzen et al., 1998). The labile C pool is commonly estimated by the light fraction (LF) C content of a soil, which provides a measurement of soil SOC that is not in association with mineral particles (Gregorich and Beare, 2008; Janzen et al., 1992). The LF organic matter pool is isolated by floating the LF on a dense liquid, commonly with a specific gravity of  $1.7 \pm 0.15 \text{ kg} \cdot \text{L}^{-1}$ , and then aspirating the suspended material (Gregorich and Beare, 2008; Plante et al., 2006; Bremer et al., 1994; Janzen et al., 1992). In the long-term (after approximately 30 years), the SOC pool becomes dominated by non-labile SOC (Balesdent et al., 1988; Huggins et al., 1998). However, it has been suggested that what is considered the stable pool also contributes to the ongoing mineralization of SOC, accounting for some of the variation from the LF carbon content (Bremer et al., 1994).

Analysis of arable soils under long-term cultivation has shown the influence of crop rotation on SOC and LF C content (Janzen et al., 1992). Crop rotations with increasing complexity and use of cover crops in cereal phases showed increased SOC contents (McDaniel et al., 2014). Increased rotation complexity, decreased fallow frequency and the inclusion of hay phases in the rotation have also been shown to have higher LF carbon and SOC contents (Janzen et al., 1992; Bremer et al., 1994). The mineralizable carbon content of soil samples from long-term crop rotations, in ten-week laboratory incubations, has been shown to increase with increasing SOC and LF carbon contents (Bremer et al., 1994). The proportion of total SOC mineralized also increases with SOC and LF carbon contents. The proportion of SOC left remaining in the soil following removal of mineralizable carbon is indicative of the size of the stable SOC pool.

Carbon stabilization mechanisms controlled by soil physical properties show the most promise for long-term stabilization of SOC. This rationale combines concepts of SOC-mineral association and biologically relevant pore space conducive for C mineralization (Six et al., 2002; Dungait et al., 2012). Physical protection in macroaggregates is not likely to contribute to long-term stabilization mechanisms even in no-till systems due to the eventual disruption of aggregates by seed openers (Hassink, 1997; AARD, 1999). Chemically recalcitrant compounds are not likely to contribute to long-term carbon stability either due to the eventual decomposition of nearly all organic compounds (Marschner et al., 2008; Baldock and

Skjemstad, 2000). The exception would be possible recalcitrance of certain compounds due to the suppressive effect of nitrogen on decomposition, however; this amount is not anticipated to be significant relative to physically controlled mechanisms (Fog 1988). Substrate limitation is a threshold amount, which is largely determined by the soil physical properties (Don et al., 2013; Hassink 1997; Killham et al., 1993). The capacity of the soil to stabilize SOC, either in the microbial biomass located in biologically relevant pores that are inaccessible to predators or by forming association with primary soil particles, determines the threshold at which substrate limitation appears (Killham et al., 1993). The threshold would be variable depending on the soil texture, moisture content and SOC content all of which have been shown to be related.

The pore size distribution of a soil was found to be affected by tillage, which subsequently changed the distribution of biologically relevant pores, those  $\leq 30 \mu\text{m}$ , and the rate of SOC mineralization (Yoo et al., 2006). The scale of change can extend beyond that of tillage as shown by Juarez et al. (2013) where increased intensity of sample disturbance caused an increased proportion of smaller pores. Although disruption of soils, enough to alter pore geometry at scales  $\leq 13 \mu\text{m}$ , did not cause any effect on the long term rates of SOC mineralization it was suggested that different soil water contents or textures might offer different results (Juarez et al., 2013). Soil nitrogen and moisture contents have been found to significantly affect the mineralization rates of added organic matter (Abro et al., 2011). Although highly variable depending on the soil system, it was found that the SOC content of a soil shares a positive relationship with water content (Rawls et al., 2003). More specifically the gravimetric water content of soil matrix pores is positively correlated with the organic carbon content of the soil (Dexter et al., 2008a). The pore sizes associated with matrix porosity might be more significant in influencing the mineralization of SOC (Killham et al., 1993). Soils of similar texture and disturbance history, but different crop rotations and subsequent SOC contents could differ in their pore size distribution and degree of saturation influencing mineralizable SOC (De Jong et al., 1983; Killham et al., 1993; Yoo et al., 2006; Dexter et al., 2008a).

## **2.2. Research Objectives and Hypotheses**

### **2.2.1. Research Objectives**

A laboratory incubation experiment was designed to investigate quantities of potentially mineralizable carbon in long-term crop rotations. Specifically how differences in total and physically uncomplexed SOC relate to the amount of potentially mineralizable carbon. Soil sample disturbance was used to investigate the influence of a soil's physical characteristics, pore size distribution, on potentially mineralizable carbon.

Methods of soil analysis were chosen to assess the changes in LF and total SOC pools, total and physically uncomplexed SOC, during the incubation and quantify the changes induced to the soil's pore size distribution during sample disturbance.

### **2.2.2. Research Hypotheses**

We expected potentially mineralizable carbon to increase with increasing complexity of crop rotations as found by McDaniel et al. (2014). More specifically, potentially mineralizable carbon in these soils was anticipated to increase with increasing LF and SOC contents (Janzen et al., 1992; Bremer et al., 1994). The physical disturbance of these soils was not expected to increase the mineralizable carbon since the soil from these long-term crop rotations was conventionally tilled (Beare et al., 1994; Curtin et al., 2014). However, increasing intensity of physical disturbance was hypothesized to decrease the proportion of large pores while increasing the proportion of small pores. The shift in pore size distribution was expected to be unique for the different crop rotations depending on the SOC content (Pagliai and Antisari 1993; Dexter et al., 2008a). At water contents near field capacity a larger proportion of smaller pores was hypothesized to decrease mineralizable carbon due to saturation (Yoo et al., 2006).

## **2.3. Methods**

### **2.3.1. Soil Sampling Sites**

Two long term sites, Agriculture and Agri-Food Canada's (AAFC) Lethbridge and the University of Alberta Breton Plots, were chosen due to their differing soil and climate conditions (Table 2.1), yet similar rotations (Table 2.2) and management history (Natural Regions Committee, 2006; Dyck et al., 2012; Smith et al., 2012). The three rotations chosen at each location represent three different rotation philosophies ranging from intensive to extensive. Subsequently the rotations vary in the amount of carbon inputs and the soil carbon content after time (Janzen et al., 1998; Dyck et al., 2012). The plots were initiated to answer questions of how long-term rotation and fertility management effect soil fertility and crop production (Dyck et al., 2012). To maintain consistency, the plot chosen for sampling from each rotation were those under management practices that best represent typical strategies for the province. Hence, the plots receiving fertilizer for any nutrient that is or could be deficient under the current rotation was sampled.

#### **2.3.1.1. Breton**

Three rotations from two experiments were sampled from the University of Alberta Breton Plot's, located approximately 100 km southwest of Edmonton (53.089°N, 114.442°W). The plots were developed on Gray Wooded soils, which are classified as an Orthic Gray Luvisol according to the Canadian System of Soil Classification (Soil Classification Working Group, 1998). These soils were developed under mixedwood

forest vegetation. These soils are known for having a surface horizon low in organic matter and clay content with limited development of soil structure (Dyck et al., 2012). All plots at Breton are approximately 9 by 30 m and receive nitrogen, phosphorous, potassium and sulfur fertilizer in varying amounts depending on crop needs in the phase of the rotation. The amounts applied are intended to be sufficient to prevent crop deficiency. Specific fertilizer application rates are available in Dyck et al. (2012). From 1930 – 2000, biomass removed from the plots during harvest or salvage was not returned in any other form (Grant et al., 2001). However, beginning in 2000 the above ground biomass from all grain phases of the rotations was returned with the use of a combine harvester (Giweta et al., 2014). Historically weed control was achieved through tillage alone, but since 1964 has also included herbicides (Dyck et al., 2012). Tillage operations included a spring pre-seeding and fall event during cereal phases while fallow years included three summer harrow events and one fall tillage (Grant et al., 2001).

Two rotations, wheat-oat-barley-hay-hay (WOBHH) and wheat-fallow were sampled from the Classical Plots experiment, which were initiated in 1938 and 1941, respectively. The WOBHH rotation is referred to as “Hay” throughout the rest of this document. The Classical Plots were not replicated and as such only one plot was sampled for each rotation. The wheat-fallow plots are split in half, to accommodate both phases of the rotation, with the resulting dimensions 8.5 x 31.6 m. Forages from the Hay rotation were harvested twice during the first hay phase and once during the second (Grant et al., 2001). Starting in 2000 the above ground biomass from the barley was removed as silage (Dyck et al., 2012). The continuous grain rotation was sampled from the Hendrigan Plots experiment, which was initiated in 1979. Since 2000, the above ground biomass has been returned to the plots at harvest by using a combine harvester. The Hendrigan plots were replicated in triplicate with each replicate being sampled individually.

#### **2.3.1.2. Lethbridge**

Samples for all three rotations from Lethbridge were obtained from Agriculture Agri-Food Canada’s Lethbridge Research Centre located in southern Alberta, approximately 500 km south of Edmonton (49.705°N, 112.775°W). All three rotations were sampled from Rotation 120, which was initiated in 1951 (Smith et al., 2012). The plots were developed on Orthic Dark Brown Chernozemic soil according to the Canadian System of Soil Classification (Soil Classification Working Group, 1998). These soils develop under mesophytic grass and forb vegetation. The plots are 3.3 by 40 m with each treatment replicated in quadruplicate. Originally, the experiment did not receive any fertilizer, however; starting in 1985 the wheat-fallow and continuous wheat rotation plots were split with one set receiving broadcast ammonium-nitrate (80 kg N ha<sup>-1</sup>) in the spring before seeding. The rate of fertilizer applied was reduced to 45 kg N

ha<sup>-1</sup> in 2001 and has remained the same since. The fallow-wheat-wheat-hay-hay-hay (FWWHHH) rotation did not receive any fertilizer since its original development. The FWWHHH rotation is referred to as “Hay” throughout the rest of this document. Wheat crops have received phosphorous fertilizer since 1985; however, the rate has declined from an original 22.5 kg P ha<sup>-1</sup> to 11 kg P ha<sup>-1</sup> since 2001. Crop residues were retained in the field since the experiments inception with a combine harvester. The plots were tilled, harrowed and fertilizer broadcast before seeding (Smith et al., 2012). Weed control involved tillage during fallow periods and herbicides for in-crop weeds. Hay was harvested from the Hay rotations in one or two cuts each year in the hay phase depending on moisture and growth.

### **2.3.2. Soil Sampling Strategy**

Soil from three long-term crop rotations from Agriculture Agri-Food Canada’s Lethbridge research station and the University of Alberta’s Breton Plots were sampled (Table 2-2). Four soil samples were obtained from each plot by sampling along an evenly spaced linear transect that ran the length of the center of the plot. A linear transect is used to avoid edge effects in the narrow plots. A soil sample was obtained from each sample point and all four then composited. Where available plot replicates were sampled and later combined to create one sample for all analysis and experimentation. The Lethbridge plots were uniform and without any gradient or slope. The plots at Breton were on a slight slope; however, the slope was perpendicular to the direction of the transect.

### **2.3.3. Soil Sampling Procedure**

Soil samples were obtained from the Lethbridge Research Centre on April 25, 2014 while the Breton plots were sampled May 7, 2014. Sampling was performed before the spring fertilizer application and tillage event for both locations. The sampling depth was the upper 7.5 cm of the soil profile in-line with historical sampling depths as well as to characterize the soil most affected by the long-term treatment of crop rotation (Janzen et al., 1998; Izaurrealde et al., 2001; Smith et al., 2012). Approximately 1,500 cm<sup>3</sup> of soil was sampled from each sample point using a square hand shovel and directly placed into a clean 20 L plastic bucket with a lid. All crop residue and surface organic matter within the sampling area were taken with the soil sample.

### **2.3.4. Soil Sample Handling Procedure**

The soil samples were processed the same day as they were obtained from the plot. Upon returning from the field all coarse particulate organic matter (POM) greater than 2.5 cm was removed by hand. The soil was then spread into shallow drying pans, placed on drying racks and left for air-drying at room

temperature (Thomson et al., 2010). After 168 hours and sufficient air-drying, the soils were returned to their plastic buckets and stored in a cool dry location at the University of Alberta Ellerslie research facility (Thomson et al., 2010). Air-drying was chosen as the temporary storage method because air-dried soil has an appropriate moisture content for grinding and minimizes microbial activity during storage (Sheppard and Addison, 2008).

### **2.3.5. Soil treatments for the Incubation Experiment**

The soil treatments were intended to alter the pore geometry to induce observable changes in carbon mineralization (Juarez et al., 2013). Although not expected, the applied disturbance could have broken down soil aggregates and liberate encapsulated SOC (Six et al., 1999). Three different disturbance treatments, hand broken aggregates, 2 mm roller mill and dish-ring and puck mill, representing a spectrum of intensities were applied to each soil type.

#### **2.3.5.1. Hand Broken Aggregates**

The hand broken aggregates treatment was the lowest intensity disturbance treatment. Since many of the macroaggregates were broken during sample handling there was no need for the aggregates to be intentionally broken. Much of the disturbance occurred during removal of coarse POM, where the field moist soil was turned by hand to allow for removal of the coarse POM. Further disturbance occurred during weighing of the soil, both when field moist and air-dried.

#### **2.3.5.2. Roller Mill**

A roller mill with 2 mm openings was used to disturb medium and coarse aggregates. The roller mill was a custom-manufactured unit located at the University of Alberta Ellerslie Research Station. The mill consisted of steel screen drums with 2 mm openings and 16.5 cm diameter by 26 cm in length. Inside of the drum was a heavy rod 4.5 cm in diameter by 21 cm in length as well as a second rod 4.0 cm in diameter by 21 cm in length. One end of the drum was removable for loading of the air-dried soil sample. During operation, the drum was laid on its side, cradled between two rubber coated rotating shafts, which caused the drum to tumble. As the soil structure was reduced to 2 mm or less the soil particles fell from the drum and were collected in a steel tray. Coarse non-soil fragments > 2mm were discarded while OM > 2mm was recovered from the drum and recombined with the soil sample. The mill was ran until the entire soil sample had been processed, usually around five minutes.

### **2.3.5.3. Dish, Ring and Puck Mill**

A Siebtechnik dish, ring and puck (DRP) mill was used to severely disturb all classes of soil aggregates. Approximately 200 g of air-dried soil was evenly distributed between the dish and ring during loading for each run. The mill was ran for 2 minutes for each run which resulted in a finely ground and homogenous sample. Individual runs from soils representing the same treatment were recombined after milling. The mill was cleaned between different soil treatments using a brush and paper cloth.

### **2.3.6. Incubation Experiment**

#### **2.3.6.1. Experiment Setup**

Repacked soils were used for the incubation experiment due to the known conditions of the soil being incubated (Guo et al., 2013). Saturated soil samples were subjected to 60 kPa in a pressure plate extractor according to a procedure modified from Reynolds and Topp (2008). Acrylonitrile butadiene styrene (ABS) pipe 5.26 cm in inner diameter was cut to create collars 5 cm in height. The bottom of each tube was covered with cheesecloth and secured in place using an elastic band. The collars were placed on a pre-soaked ceramic plate rated to 100 kPa. Approximately 5 mm of silica sand was placed in the bottom of each tube. The silica was wetted to saturation. Approximately 25 g of air-dried soil was then added, leveled using a scupula. No efforts were made to achieve a uniform bulk density between treatments due to the intention of creating different pore characteristics through the disturbance treatments. The soil samples were then wetted to saturation. The ceramic plates holding the soil samples were loaded into the pressure extractor and pressurized at 60 kPa for ten minutes (Gregorich et al., 1991). The base of each collar was then covered with an ABS slip on test cap.

Each soil sample was incubated in its own sealed isolated incubation chamber (Juarez et al., 2013; Yoo et al., 2006). The atmosphere in the jar was equilibrated with the ambient atmosphere for five minutes following each sampling event (Plante et al., 2002). To maintain a constant moisture content during incubation a 20 ml reservoir of deionized water was established in the chamber (Juarez et al., 2013; Plante et al., 2002). The reservoir was maintained by mass, initially every week and then every two weeks after day 91. The lids of the chamber were equipped with a septum to allow for extraction of headspace gas samples.

Each incubation chamber was placed in a dark cupboard for the duration of the incubation (Plante et al., 2002). The incubation chambers were maintained at room temperature (24°C) with the temperature being recorded at each sampling event (Stenger et al., 2002; Plante et al., 2002). Room temperature is

used because warmer temperatures promote a greater mineralization of the total soil carbon, with initial rates being significantly less in cooler incubated soils (Conant et al., 2008). The incubation duration was 182 days. The sampling frequency decreased over time as the rates of mineralization decreased. A total of 38 sampling events were completed over the duration of the experiment.

The experiment was replicated four times with the replications being incubated simultaneously. In addition, there were four incubation chambers, which, served as control samples, as they did not contain any soil. The control samples were also used to correct for background CO<sub>2</sub> concentrations.

### 2.3.6.2. Gas Chromatography

Headspace samples of 25 ml were taken from the incubation chambers using a 30 ml plastic syringe and stored in pre-evacuated 12 ml Labco Exetainer® Soda Glass Vials (Guo et al., 2013). The vials were evacuated to 200 mTorr using a vacuum pump prior to being loaded with the sample. Once all of the chambers had been sampled and transferred into the vials they were analyzed for CO<sub>2</sub> concentration using a Hewitt Packard 5890 Series II gas chromatography unit equipped with an Agilent Technologies GC Sampler 80 (Guo et al., 2013). The GC was controlled using Agilent Technologies GC ChemStation Rev. A.10.02[1757] software. Prior to sample analysis the oven temperature was set to 120°C for 15 minutes. The method for CO<sub>2</sub> analysis was then loaded and the column allowed to stabilize for a minimum of two hours. The method integrated the area under the peak for CO<sub>2</sub> and provided it in the output.

The CO<sub>2</sub> standards Air, 0.5%, 1%, 1.5%, 2% and 5% were analyzed at the beginning and end of each sample run. The resulting areas were used to derive a calibration curve between area and CO<sub>2</sub> concentration, which was then used to regress the CO<sub>2</sub> concentration of each sample. A calibration curve was developed for each sample run and was highly reproducible with a mean r<sup>2</sup> of 0.997 and standard deviation of 3.6x10<sup>-3</sup> across all events. The mass of carbon evolved as CO<sub>2</sub> for each incubation chamber between sample events was determined from the moles of CO<sub>2</sub> evolved (*n*) which was determined using the ideal gas law (Halpern 2004):

$$n = \frac{PV}{RT}$$

The constants of the equation include the atmospheric pressure of the incubation chamber (*P*) assumed to be 101325 Pa for Edmonton, Alberta, the universal gas constant (*R*) assumed to be 8.314 J·K<sup>-1</sup> and the temperature of the gas at room temperature (*T*) which was 297.15 K. The dependent variable is the

volume of gas evolved ( $V$ ) in  $m^3$  as determined by gas chromatography. The mass of carbon was then found by multiplying the number of moles of  $CO_2$  evolved by the atomic weight of carbon (12.0107 g/mol).

### **2.3.6.3. Experiment Dismantling**

Immediately after the final sample, the soil cores were dismantled. The soil core was weighed and the thickness measured to obtain an approximate bulk density. A sub sample of each core, approximately 5 g, was taken and oven dried at  $105^\circ C$  for 24 hours to obtain the gravimetric moisture content. The remaining soil was air dried for one week and placed into sealed plastic bags for future analysis.

## **2.3.7. Soil Analysis**

### **2.3.7.1. Analysis Overview**

Characterization of the pre-incubation soil included particle size distribution, pore size distribution, total SOC and LF carbon. Characterization of the post-incubation soil included total SOC and LF carbon to detect changes during the incubation. A summary of the characterization analysis performed on the pre- and post-incubation samples can be found in Table 2.3.

### **2.3.7.2. Particle Size**

Particle size distribution was determined using the hydrometer method following a modified version of the method outlined by Kroetsch and Wang (2008). Particle size was only measured on pre-incubation samples, as it would not have been affected during the incubation. All six soil types were analyzed with two replicates. A total of 90 g air-dried soil was added to a beaker with 300 ml distilled-deionized (DDI) water and 100 ml of sodium hexametaphosphate ( $50\text{ g L}^{-1}$ ) and allowed to soak overnight. Two subsamples of 10 g were taken from each soil type and placed in a drying oven at  $105^\circ C$  for 24 hours to determine the moisture content.

The following day the contents of each beaker were quantitatively transferred to a dispersing cup and mixed with an electric mixer for 10 minutes. Following mixing the contents were transferred to a 1 L graduated cylinder and DDI water was added to bring the total volume to 1 L. While the contents of the cylinders were equilibrated to room temperature blank readings were taken for the hydrometers. Each cylinder had its own dedicated hydrometer. Each cylinder was then agitated for approximately one minute using a brass plunger. Hydrometer readings were taken at 40 s, 60 s, 120 s, 180 s, 240 s, 300 s, 1800 s, 1 hr, 2 hr, 3 hr, 4 hr, 5 hr, 6 hr, 7 hr, 8 hr and 24 hours. The hydrometer was left in each cylinder for the first 2 hours. The percent sand, silt and clay were then calculated after corrections were made for moisture

content. The soils were not pretreated to remove organic carbon. Texture classes were assigned based on the Canadian System of Soil Classification (Soil Classification Working Group, 1998).

### 2.3.7.3. Pore Size Distribution

The pore size distribution of each of the different soil treatments was determined using water desorption using the pressure extraction method modified from Reynolds and Topp (2008). Pore size distribution by water desorption provides an indication of the overall level of change in the soil sample caused by disturbance in a quantifiable way. All eighteen soil treatments were analyzed and replicated twice at five different matric potentials (-2, -10, -30, -60 and -100 kPa). The ceramic pressure plates were soaked overnight in de-aired room temperature tap water. ABS collars 2.5 cm in diameter by 1 cm in height were placed on the ceramic plate. The cores were filled with the soil and gently leveled. De-aired tap water was then added to the ceramic plate to a depth of approximately 0.5 cm and the cores were then wetted by capillary rise for 24 hours. The pressure extraction chambers were then pressurized and equilibrated for at least 72 hours with the exception of the 2 kPa pressure, which equilibrated for two hours. At the end of the equilibration period the soil was removed from the collar and weighed. Each sample was then placed in a drying oven for 24 hours at 105°C. The oven-dried soil was then weighed to determine the moisture content of the soil.

The effective pore diameter or largest diameter saturated pore ( $d$ ) at each water potential was calculated using the Kelvin equation (Reynolds and Topp, 2008):

$$d = \frac{4\gamma \cos \alpha}{pgh}$$

The constants of the equation include the surface tension of water ( $\gamma$ ) assumed to be 72.75 mJ m<sup>-2</sup> at room temperature, the pore water meniscus contact angle ( $\alpha$ ) which is assumed to be 0, the density of water ( $\rho$ ) assumed to be 1 mg M<sup>-3</sup> and gravitational constant ( $g$ ) which is 9.8 m s<sup>-1</sup>. The dependent variable is matric potential ( $h$ ) which is expressed in meters of water where one meter of water is equal to -10 kPa.

Water filled pore space (WFPS) was calculated as the proportion of total soil porosity ( $f$ ) that was saturated, as indicated by the gravimetric water content ( $\theta v_i$ ), at a given matric potential ( $i$ ) using the following equation:

$$WFPS_i = \frac{\theta v_i}{f}$$

Using the relationship between pore diameter and matric potential the calculated WFPS can be related to a specific diameter of pores.

#### **2.3.7.4. Total Carbon and Nitrogen**

A sub sample of approximately 1 g was obtained from each of the pre- and post-incubation samples. The subsamples were then oven dried at 105°C for 24 hours. Each sample was then homogenized using a mortar and pestle. A 0.02 – 0.04 g subsample was obtained from each homogenized sample and placed into 8 x 5 mm tin capsules (Elemental Microanalysis). The mass of soil was recorded and each sample analyzed for carbon and nitrogen content by dry combustion using a Costech ECS 4010 Elemental Analyzer equipped with a thermocouple detector (Costech Analytical Technologies Inc. Valencia, USA) Carbon results are reported as SOC although no pre-treatments or corrections were made, as the surface soils are neutral or acidic and are not expected to contain any inorganic carbon.

#### **2.3.7.5. Light Fraction SOC**

Light fraction SOC was separated from the bulk SOC pool using a modified version of the method outlined by Gregorich and Beare (2008). Pre-incubation samples were prepared in triplicate for each of the eighteen different treatments. The same soil core preparation method was used as when preparing the soil cores for incubation except silica sand was not used. Soil samples from the incubation experiment were used for post-incubation characterization. All samples were air dried prior to light fraction separation.

Air dried soil was weighed into 125 ml Nalgene® 312105-0004 wide mouth plastic bottles with the mass of soil recorded. 40 ml of 1.7 kg L<sup>-1</sup> NaI was added to each bottle and placed on a shaker for one hour. Following shaking the contents of each bottle was rinsed to the bottom using 1.7 kg L<sup>-1</sup> NaI from a rinse bottle. The bottles containing the soil and NaI solution were left undisturbed for 24 hours on the laboratory bench. A Nalgene® 300-4000 reusable 250 mL vacuum filter holder with a Whatman™ 7141-104 0.45 µm cellulose membrane filter and vacuum system was used to aspirate the light fraction SOC from each sample bottle. Once the light fraction was aspirated it was rinsed using 0.01 M CaCl<sub>2</sub> and then distilled-deionized water, both from a rinse bottle. The filter and filtrate were removed following rinsing and placed into drying tins. The drying tins were placed in a drying oven at 60°C for 24 hours. The filtrate was then scraped from the filter and the mass recorded before finally being analyzed for carbon and nitrogen content by dry combustion as explained in the previous section.

### **2.3.8. Statistical Analyses**

Results from the incubation experiment and soil characterization were compiled using Microsoft Excel. The statistical software package “R” version 3.1.2 was used for all statistical analyses. Box-Cox data transformations were applied as necessary to meet the assumptions of residual normality and homoscedasticity for linear models.

The cumulative mineralized carbon and soil characterization results were evaluated using a linear model. The model was fitted to the data and evaluated using ANOVA with Type III sum of squares at the 5% level of significance. Individual factors were compared using least-squares means with pairwise comparisons and Tukey p-value adjustment. Testing for mineralization rate stability was done by fitting a linear model to the final four sampling events (day 139 – 182) for each experimental unit. The linear models were evaluated at the 5% significance level where a p-value <0.05 indicated that the change in mineralization rate was statistically different than zero over the time period. Correlation analysis of soil parameters and incubation experiment results was completed using the Pearson method as the parameters all met the assumptions including normality. Significance of the correlations was evaluated at the 5% level of significance.

## **2.4. Results**

### **2.4.1. Texture**

Soil texture was similar across all of the soil types sampled (Table 2.4). The soil texture was classified as loam for all of the soil samples with the exception of the Lethbridge wheat-fallow, which was classified as sandy clay loam. Generally, the Lethbridge soils had a higher proportion of sand, less silt and more clay than the Breton soils (Figure 2.1). The percent sand in the Breton soils ranged from 46.5 to 47.0 % for the hay and wheat-fallow rotations, respectively. In the Lethbridge soils the sand content ranged from 48.5 to 53.1 % in the continuous grain and wheat-fallow rotations, respectively. The clay content was variable in the Breton soils, ranging from 13.38 to 17.2 % in the hay and wheat-fallow rotations, respectively. The Lethbridge soils had a more consistent clay content ranging from 20.3 to 23.0 % for the wheat-fallow and continuous grain rotations, respectively. In both locations the percentage of silt decreased from Hay to continuous grain to wheat-fallow.

### **2.4.2. Pore Size Distribution**

The gravimetric water content (GWC) at saturation of the pre-incubation soil varied considerably across the treatments, ranging from 0.43 to 0.63 g g<sup>-1</sup> (Table 2.5). The GWC at saturation (-2 kPa) was significantly

affected by the type of soil disturbance (Table 2.6). The same effect was observed for both locations (Figure 2.2). Although it was not significantly different, the Hay rotation in the Breton soils had the highest GWC at saturation, but often the lowest in the Lethbridge soils (Figure 2.3). The DRP mill treatment resulted in a higher GWC at saturation compared to the 2 mm roller mill and hand broken treatments (Table 2.7). The GWC for the -10 kPa matric potential, corresponding to pores less than 30  $\mu\text{m}$ , was significantly affected by all factors and interactions with the exception of rotation (Table 2.6). The DRP mill treatment resulted a higher GWC while the effect of location was bimodal with the Lethbridge soils having the lowest and highest GWC at -10 kPa (Table 2.8). Breton soils had a lower proportion of pores greater than 29.75  $\mu\text{m}$  compared to Lethbridge (Figure 2.4). The increase in pores larger than 29.75  $\mu\text{m}$  is apparent for all rotation and disturbance treatments in the Lethbridge soils (Figure 2.4).

Water filled pore space (WFPS), the proportion of total porosity filled with water at a given matric potential, ranged from 0.26 to 1.1 ( $\Theta_v f^{-1}$ ) at -10 kPa across all treatments. Increasing soil sample disturbance was the most significant factor in increasing WFPS at -10 kPa (Figure 2.5). To a lesser extent, rotation was a factor in WFPS at -10 kPa as well as the interaction between location and rotation, location and disturbance and rotation and disturbance. Comparing only the disturbance treatments, the WFPS at -10 kPa decreased from DRP mill to 2 mm roller mill to hand broken (Figure 2.5). Comparing only the rotations, the WFPS at -10 kPa decreased from wheat-fallow to continuous grain to Hay. The Hay and continuous grain rotations had similar WFPS at -10 kPa, but both were lower than wheat-fallow. The WFPS at -10 kPa in the Breton wheat-fallow DRP mill treatment was greater than one, which is attributed to error induced by difficulty measuring the volume of wetter soil cores, which were unable to maintain their shape (Figure 2.5 and 2.22). The WFPS at -10 kPa was found to be significantly correlated to the pre-incubation LF content of the soil ( $r = -0.7640$ ), but not to the total SOC content.

### **2.4.3. Total Carbon and Nitrogen**

Total carbon ranged from 13.30 to 29.32 g C  $\text{kg}^{-1}$  while total nitrogen ranged from 1.14 to 2.47 g N  $\text{kg}^{-1}$  in pre-incubation samples (Table 2.9). Location, rotation and disturbance were all significant factors in the total SOC content of the pre-incubation soil (Table 2.10). Rotation was the most significant factor with greatest carbon content in Hay rotations and the least in wheat-fallow (Table 2.11). There was also a significant interaction between location and rotation and to a lesser extent location and disturbance. Rotation and disturbance were significant factors in the pre-incubation total nitrogen content (Table 2.10). There was also a significant interaction between location and rotation. Hay rotations had the greatest pre-incubation total nitrogen content while wheat-fallow rotations had the least. The C:N ratio

was significantly affected by location and by the location and rotation interaction (Table 2.10). The rotation and disturbance interaction was also a factor, but to a lesser extent. Lethbridge had a higher C:N ratio in the pre-incubated soil than Breton.

Total SOC ranged from 12.24 to 25.88 g C kg<sup>-1</sup> while total nitrogen ranged from 1.10 to 2.44 g N kg<sup>-1</sup> in post-incubation samples (Table 2.9). Location and rotation were significant factors in the total SOC content of the post-incubation soil (Table 2.12). There was a significant interaction between location and rotation, which along with rotation were the most significant factors. Other significant interactions included location and disturbance, rotation and disturbance and the location, rotation and disturbance interaction. Rotation and disturbance were significant factors in the total nitrogen content of the post incubation soil (Table 2.12). There was also a significant interaction between location, rotation and disturbance. Rotation and the interaction between location and rotation were the most significant factors. Rotation and disturbance were both significant factors in the post incubation C:N ratio with disturbance being the most significant (Table 2.12). There were also significant interactions between location and rotation as well as rotation and disturbance. The C:N ratio decreased from the DRP mill disturbance treatment to the 2 mm roller mill to the hand broken treatment. The samples from Lethbridge maintained a higher C:N ratio than Breton when comparing across all treatments.

Total SOC decreased in most treatments between the pre- and post-incubation soil (Figure 2.6). Total nitrogen also decreased in most treatments over the course of the incubation (Figure 2.7). The decrease in carbon was greater than the decrease in nitrogen and subsequently the C:N ratio decreased in most treatments between the pre- and post-incubation soil (Figure 2.8).

#### **2.4.4. Light Fraction SOC**

Light fraction recovery ranged from 1.21 to 24.77 g LF kg<sup>-1</sup> in the pre-incubation soil (Table 2.13). The carbon content of the recovered LF ranged from 22.62 to 33.36 % while the nitrogen content ranged from 0.72 to 1.37% in the pre-incubation soil. The LF C:N ratio ranged from 17.69 to 44.21 in the pre-incubation soil. Location, rotation and disturbance as well as their interactions were all significant factors in determining the recoverable LF content of the pre-incubation soil (Table 2.10). The order of significance of the factors was disturbance, location and rotation, which resulted in eight significantly different recovered LF contents in the pre-incubation soil (Table 2.14). The DRP mill disturbance treatment caused a dramatic decrease in the pre-incubation LF recovered and the percent LF carbon in the whole soil compared to the 2 mm roller mill and hand broken treatments (Figure 2.9; 2.11). However, the carbon content (%) of the LF increased in the DRP mill disturbance across all rotations compared to the other

disturbance treatments (Figure 2.10). Light fraction recovery decreased from Hay to continuous grain to wheat-fallow when averaging across location and disturbance (Table 2.15).

Light fraction recovery ranged from 0.12 to 15.48 g LF kg<sup>-1</sup> in the post-incubation soil (Table 2.13). The carbon content of the recovered LF ranged from 20.67 to 31.03 % while the nitrogen content ranged from 1.09 to 1.75% in the post-incubation soil. The LF C:N ratio ranged from 15.08 to 24.24 in the post-incubation soil. Location, rotation and disturbance as well as their interactions, with the exception of the location and disturbance interaction, were all significant factors in determining the recoverable LF content of the post-incubation soil (Table 2.12).

Recovered LF decreased during incubation with the exception of the Breton continuous grain hand broken treatment (Figure 2.9). The loss of LF as a percentage of the pre-incubation LF content ranged from -30.23 to 89.84 % (Table 2.13). The carbon content of the recovered LF decreased during the incubation (Figure 2.10). The carbon contributed to the soil by LF decreased, with the exception of the Breton continuous grain hand broken treatment (Figure 2.11). The nitrogen content of the recovered LF increased during the incubation (Figure 2.12). The nitrogen contributed to the soil by LF decreased with the exception of the Breton continuous grain hand broken treatment (Figure 2.13). The C:N ratio of the recovered LF decreased in all treatments during the incubation (Figure 2.14). The decrease in C:N ratio as a percentage of the pre-incubation C:N ratio ranged from 4.18 to 45.19 % (Table 2.13).

#### **2.4.5. Total SOC and Gravimetric Water Content**

Total SOC content did not appear to influence the gravimetric water content of the soil at low or high matric potentials in the absence of aggregation. The only significant correlation was found between the total SOC content and gravimetric water content at a matric potential of -100 kPa in the 2 mm roller mill disturbance treatment (Table 2.16). The hand broken and DRP mill did not have a significant correlation between the two parameters at this matric potential. At a matric potential of -2 kPa a significant correlation between total SOC and GWC could not be found in any of the disturbance treatments (Table 2.16).

#### **2.4.6. Incubation**

Cumulative mineralized carbon respired as CO<sub>2</sub> ranged from 2.42 to 10.55 g CO<sub>2</sub>-C kg<sup>-1</sup> after 182 days incubation. The rate of CO<sub>2</sub> respiration declined over the duration of the experiment for all treatments (Figure 2.15). Treatment differences became apparent after approximately 35 days. Respiration rates declined and decreased in volatility after day 139 (Figure 2.16). After day 139, the respiration rates for 14

of the 18 treatments reached a stable rate for the remaining duration of the experiment (Table 2.17). The average rate of respiration for the different treatments at the end of the incubation experiment ranged from 0.04 to 0.09 g CO<sub>2</sub>-C (kg soil hr<sup>-1</sup>)<sup>-1</sup>.

As a whole the cumulative mineralized carbon respired as CO<sub>2</sub> was not significantly different between the two locations (Table 2.12). The rotation treatments were significantly different, with respiration increasing from wheat-fallow to continuous grain to hay. The ANOVA results suggest rotation to be the most significant factor in cumulative mineralized carbon of all the treatments. The DRP mill disturbance treatment was significantly different from the 2 mm roller mill and hand broken treatments. The DRP mill disturbance had the lowest cumulative mineralized carbon of the three disturbance treatments. While there was a significant interaction between location and rotation, the general trend of cumulative mineralized carbon increased from wheat-fallow to continuous grain to hay for both locations (Table 2.18). An interaction between rotation and disturbance was also identified with cumulative mineralized carbon increasing when moving from wheat-fallow to continuous grain to Hay rotations as well as DRP mill to 2 mm roller mill to hand broken disturbance treatments (Table 2.19). There was also a significant interaction between location, rotation and disturbance that was displayed in the hand broken treatment between locations for the Hay and wheat-fallow rotations (Figure 2.17).

Examining only the DRP mill treatment, rotation was found to be the only significant factor in cumulative carbon mineralization (Table 2.20). There were two statistically significant groupings within rotation based on least-squares means (Table 2.21). Hay rotations had the greatest cumulative mineralization, wheat-fallow had the least and the continuous grain rotations were intermediate.

The average gravimetric water content of the soil samples for each treatment at the end of the incubation ranged from 0.26 to 0.49 g g<sup>-1</sup> (Table 2.22). The ANOVA results showed location and disturbance to be significant factors in the GWC of the incubation soil (Table 2.23). Breton soils on average had a lower GWC than Lethbridge (Table 2.24). The 2 mm roller mill and DRP mill had similar mean GWC, but the hand broken disturbance was lower (Table 2.25).

During the incubation, 47 of the 72 (65%) soil samples were disturbed when the cheesecloth supporting the soil decomposed, allowing it to fall to the bottom of the ABS sleeve. A short supplemental incubation showed the presence of cheesecloth increased the cumulative mineralized carbon compared to cores without cheesecloth (Figure 2.18). In soil cores where the cheesecloth had structurally decomposed, there was on average a greater amount of cumulative C mineralization (Figure 2.19). However, including the

cheesecloth decomposition into the linear model for cumulative C mineralization did not make a significant difference (p-value = 0.8123).

#### **2.4.7. Incubation Correlation**

The light fraction content of the pre-incubation soil shared a positive correlation ( $r = 0.6622$ ) with cumulative mineralized carbon (Figure 2.20) Light fraction carbon content of the pre-incubation soil shared a positive correlation ( $r = 0.6556$ ) with cumulative mineralization (Figure 2.21). Cumulative mineralized carbon was negatively correlated ( $r = -0.7378$ ) with increasing water filled pore space at -10 kPa (Figure 2.22). The gravimetric water content at - 10 kPa shared a similar correlation ( $r = -0.5743$ ); however, it was not as strong (Figure 2.23). All four of correlations were found to be statistically significant (Table 2.26).

#### **2.5. Discussion**

Crop rotation influenced the potentially mineralizable carbon of the soils investigated during this 182-day laboratory incubation. Cumulative mineralized carbon at the end of the incubation was highest in Hay rotations, intermediate in continuous grain, and lowest in wheat-fallow. Cumulative mineralized carbon increased with rotation complexity in soils from both locations. Cumulative mineralized carbon was also well correlated with LF content as found in other studies using the same rotations from the Lethbridge Rotation 120 and Breton Plots (Carcamo, 1997; Bremer et al., 1994).

Laboratory incubations have been used extensively to study the influence of crop rotation on potentially mineralizable carbon. Due to differences in the incubation methodology such as length of incubation, direct comparisons cannot be made between experiments; however, relative comparisons of rotations with similar characteristics can be made within experiments. A 90-day laboratory incubation measured potentially mineralizable carbon for the Breton wheat-fallow and Hay rotations and found the Hay rotation to have more potentially mineralizable carbon (Carcamo, 1997). We found the pre-incubation total carbon and LF content to be much greater for both soil types than reported in Carcamo (1997). However, the relative proportions between the two rotations were similar between the two studies, with greater total carbon and LF content in the Hay rotation compared to the wheat-fallow. The large difference in total carbon and LF content is likely due to the retention of plant biomass during harvest since the adoption of the combine harvester in 2000 (Grant et al., 2001). The Breton continuous grain rotation was not included in the Carcamo (1997) study.

Different results were found when the potentially mineralizable carbon from the same Lethbridge soils was measured twenty-two years before our study in samples collected in the fall of 1992 (Bremer et al., 1994). The amount of potentially mineralizable carbon depended on the phase of the Hay rotation that was sampled. Potentially mineralizable carbon during wheat or the first hay phase was less than in the continuous grain rotation. However, sampling phases later in the Hay rotation showed potentially mineralizable carbon to be greater than in the continuous wheat rotation. Potentially mineralizable carbon in soil samples collected in the spring of 1984 from the same rotations at Lethbridge was less than continuous grain in all phases of the Hay rotation evaluated (Janzen, 1987). Other studies evaluating similar rotations on the Canadian prairies at Melfort and Indian Head, Saskatchewan have found potentially mineralizable carbon to be greatest in continuous wheat rotations, intermediate in Hay and least in wheat-fallow regardless of the phase sampled (Campbell et al., 1991a; Campbell et al., 1991b).

The hay phase sampled in our study was the first year of wheat, which would have been expected to have less potentially mineralizable carbon than continuous wheat. Our findings may have differed because as noted in Bremer et al. (1994) there is a high degree of variability in LF C and potentially mineralizable carbon with subsampling. However, we found the LF contents of the two rotations to be similar and the C:N of the Hay rotation to be lower than continuous grain. Alternatively, it could be due to changes in the SOC and LF contents of the rotations over time. The cumulative mineralized carbon at the end of the incubation in the Bremer et al. (1994) was greater for all rotations and phases than reported in Janzen (1987). Furthermore, the difference in cumulative mineralized carbon between the continuous wheat and fallow phase of the Hay rotation was smaller in Bremer et al. (1994) than in Janzen (1987). The difference in LF content was also narrower in Bremer et al. (1994) than in Janzen (1987). This trend is surprising considering that that samples collected for the Janzen (1987) study predated the use of nitrogen fertilizer in the wheat phases of the continuous wheat and wheat fallow rotations. In the rotations evaluated in this study the wheat-fallow and continuous wheat rotations received broadcast ammonia nitrate at 80 kg N ha<sup>-1</sup> from 1985 to 1995 when it was reduced to 56 kg N ha<sup>-1</sup> and then further reduced to 45 kg N ha<sup>-1</sup> in 2001 (Smith et al., 2012). Results from another rotation study at Lethbridge showed that rotations receiving nitrogen fertilizer had higher SOC content than those without (Janzen, et al., 1998). Nitrogen fertilizers were never applied to any phases of the Hay rotation and as such it would have been expected for the differences between the fertilized rotations and the unfertilized Hay to widen, but this was not the case.

Climatic factors may have been responsible for the changes in the soils LF contents. It was noted in Janzen (1987) that the hay phases had reduced growth due to moisture stress. The higher water use by the hay phases also negatively affected the subsequent cereal phases by depleting soil moisture levels. An increase in precipitation in the years preceding the 2014 sampling event could have relieved the moisture stress conditions resulting in increased biomass growth and subsequently an increase in the amount of organic matter returned to the soil.

As hypothesized, physical disturbance of the soil samples did not increase the cumulative mineralized carbon. Rather, increasing sample disturbance generally resulted in a decrease in cumulative mineralized carbon. Similar results, though not statistically significant, were presented by Juarez et al. (2013) where < 5 mm sieving of air-dried soil from a conventionally cropped soil in France resulted in a 16 % decrease in SOC mineralization during a 127-day incubation compared to undisturbed and dispersed soil samples. However, sieving or crushing aggregates from field moist soil from conventionally tilled soils has been reported to cause a very small increase in mineralized carbon, compared to undisturbed soils (Curtin et al., 2014; Beare et al., 1994). The effect of soil moisture during sample disturbance, as noted in Curtin et al. (2014), is that “dry aggregates are more susceptible to fragmentation than are moist aggregates.” In this experiment both the 2 mm roller mill and DRP mill disturbance treatments would have caused far more extensive fragmentation than in Juarez et al. (2013).

In this experiment, the decrease in mineralized carbon was proportionate to the severity of the disturbance treatment with the greatest decrease seen in the DRP mill treatment. The incubation respiration rates for all of the rotation and disturbance treatments decrease over time and many of the treatments had converged by the end of the incubation experiment suggesting the depletion of readily available carbon substrates. The respiration rates of soils in DRP mill treatment were initially lower and decreased in a shorter time period than the other disturbance treatments. This suggests that the amount of available carbon substrate was less, but not absent in the DRP mill treatment. The same applies to the 2 mm roller mill treatment compared to the hand broken treatment, but the difference is not as apparent or consistent due to relative similarity of the two treatments.

The effect of disturbance on cumulative carbon mineralization corresponded to a decrease in the LF content with increasing sample disturbance. Generally, the LF content was greatest in the hand broken treatment, less in the 2 mm roller mill and dramatically less in the DRP mill treatment. The same influence of disturbance was not observed in the total SOC content of the soils suggesting that the LF C was not lost,

but transferred to a different SOC pool. This lends that a physical stabilization mechanism is responsible for protection of the carbon that was lost from the LF.

Rapid mineralization of LF has been attributed to its lack of association with mineral particles (Gregorich and Beare, 2008). Without physical protection from soil mineral particles, the LF is susceptible to microbial attack and decomposition (Six et al., 1999). The size of mineral particles that complex with organic matter is usually at a scale far smaller than LF, which prevents association and subsequent protection (Christensen, 2001). However, the effect of increasing soil sample disturbance may have been to decrease the particle size of the LF enabling a portion of it to become complexed with primary particles (Sorensen et al., 1996). Furthermore, soils disturbed by simulated tillage events have been shown to form new aggregates and to include incorporated tracers into those aggregates (Plante et al., 2002). It has also been shown that ground plant residues (1 mm) are colonized and assimilated into the microbial biomass faster than coarse residues even after only one day of incubation (Sorensen et al., 1996). The assimilated carbon has a slower turnover rate because the assimilating biomass is protected from predators by the soil matrix. Since the soil samples for the incubation, light fraction and total SOC were all re-wetted and pressure extracted before being incubated or air-dried it could have allowed for formation of mineral-organic complexes, aggregation and assimilation by microbial biomass, which could all have contributed to the inability to recover the former LF and limited its availability for mineralization during the incubation.

In addition to the DRP mill treatment causing a loss of LF from the soil it also changed the composition of the remaining LF in the pre-incubation soil. The LF carbon content was greatest in the DRP mill treatment with little difference observed between the 2 mm and hand broken treatments. The effect was also observed in the C:N ratio, but there was an interaction with crop rotation where the greatest change was observed in the wheat-fallow rotations. There was no appreciable difference between the disturbance treatments in the total soil C:N ratio. Mineralization studies evaluating the effect of organic matter particle size and C:N ratio have suggested that more easily decomposable organic matter (low C:N) is preferentially stabilized (Nicolardot et al., 2001; Ambus and Jensen, 1997). The mechanism of stabilization is the assimilation of the organic matter by the microbial biomass, which is protected from predators by the soil matrix (Sorensen et al., 1996). Organic matter that was reduced in particle size by the disturbance may have had some of the particles with lower C:N ratio stabilized while the higher C:N ratio material remained physically uncomplexed and prone to microbial decomposition. Although the remaining LF would have a higher C:N ratio and therefore be less readily mineralizable. Since the wheat-fallow rotation already had the highest LF C:N ratio prior to disturbance, likely due to the frequency of fallow, the effect of the

disturbance was amplified compared to other rotations which may have contributed to the suppression of cumulative mineralized carbon observed in those treatments. Subsequently, the wheat-fallow DRP mill treatments had the lowest cumulative mineralized carbon values in the entire experiment.

The proportion of pore sizes was affected by disturbance as theorized. However, little difference was observed between the hand broken and 2 mm roller mill treatments. The DRP mill disturbance resulted in a slight decrease in the GWC of pores greater than 29.75  $\mu\text{m}$ , but a large increase in the GWC of pores less than 29.75  $\mu\text{m}$ . Rotation did not influence pores greater than 29.75  $\mu\text{m}$ , but the proportion of pores less than 29.75  $\mu\text{m}$  did increase slightly from wheat-fallow, continuous grain to Hay. However, there was not a significant influence of total SOC content on GWC at high and low matric potentials with the exception of the 2 mm roller mill disturbance treatment. The lack of significant correlation between water content and total SOC may have been due these soils being of fine texture, which has been found less sensitive to changes in SOC than coarse textured soils (Rawls et al., 2003).

The differences in porosity characteristics between disturbance treatments were more pronounced in the WFPS at  $-10$  kPa which showed the percentage of total porosity that was saturated at the given matric potential. Increasing disturbance resulted in increased WFPS, which at this matric potential can be interpreted as increasing disturbance resulted in an increase in small pores, those less than 30  $\mu\text{m}$ . To a lesser degree, rotation influenced the WFPS at  $-10$  kPa, possibly due to differences in the remaining LF content. Although WFPS at  $-10$  kPa was negatively correlated with cumulative mineralized carbon the relationship is confounded by the simultaneous covariance between disturbance and LF which is also correlated with cumulative mineralized carbon. It would be expected that cumulative mineralized carbon would increase with increasing WFPS at  $-10$  kPa since this would correspond to an increase in biologically relevant pore space in the soil (Yoo et al., 2006). Furthermore, since increasing disturbance caused both a loss of LF and increasing WFPS at  $-10$  kPa the correlation found between WFPS at  $-10$  kPa and cumulative mineralized carbon is likely due to the loss of LF. While there may exist a true relationship between WFPS and  $-10$  kPa as influenced by soil sample disturbance, the effect is masked in this experiment by the changes in LF content.

Contrary to our hypothesis, saturation of smaller pores likely did not result in reduced cumulative carbon mineralization due to anaerobic conditions. None of the incubation soils were saturated at the end of the incubation with GWCs similar to their GWC at  $-10$  kPa. Carbon mineralization rates have been shown to increase with increasing GWC up to 50% in cultivated soils (Yoo et al., 2006). Carbon mineralization as a percentage of maximum has been shown to increase with WFPS up to near maximum at 0.6 WFPS and

then decrease to approximately 75% at 0.9 WFPS (Franzluebbers 1998). Using <sup>14</sup>C-labelled glucose Killham et al. (1993) was able to show that turnover of added substrate carbon depended on the size of pores and matric potential to which it was added. Turnover of carbon from the added substrate was greater in 6 – 30 µm pores compared to those < 6 µm, particularly when the larger pore class was saturated.

The results of this experiment confirm that potentially mineralizable carbon increases with LF content. Since LF content is partially attributed to the amount of crop residue entering the soil there is a direct link between crop rotation and potentially mineralizable carbon. It also shows the effect of fallow phases, which due to absence of crop residue during fallow years decreases soil LF content. Eliminating fallow and including hay phases in crop rotation are likely to increase the LF and SOC contents of the soil while also increasing the rates of CO<sub>2</sub> emission from the soil. A study comparing the assimilation of surface placed or incorporated barley residue at Lethbridge, Alberta showed greater retention of the added carbon in the microbial biomass over 24 months when incorporated (Helgason et al., 2014). It was noted that the results showed the importance of physical contact between the added residue and the soil. Much the same, the results of this study show how physically disturbing the soil and subsequently the LF particles can relocate carbon from the potentially mineralizable pool. This presents a possible opportunity for altering SOC dynamics through crop residue management. Where smaller LF particles may be more readily assimilated into the microbial biomass and physically protected in the soil matrix, with the effect of increasing the stability of the carbon in the soil (Sorensen, et al., 1996). This may be a possible way to obtain the benefits of increased soil SOC while reducing the rates of CO<sub>2</sub> emission from the soil.

## **2.6. Conclusions**

The experiment sought to compare differences in long-term crop rotations from two locations within Alberta and their influence on mineralizable carbon under laboratory conditions. Differences in LF and total SOC were compared as well as the pore size distribution of the soil. The LF pool as well as the physical characteristics of the soil were manipulated through varying levels of sample disturbance. Cumulative mineralized carbon proved to be dependent on the interaction of the location sampled, crop rotation and disturbance treatment applied.

This research showed the influence crop rotation has on potentially mineralizable carbon in the laboratory setting. Where crop rotations resulting in soils with a greater LF content, representative of the most recent crop residue contributions, resulted in greater cumulative mineralized carbon. Often the soils with high LF and potentially mineralizable carbon contents also had high total SOC contents. Thus, it would be ill-

advised to consider making changes to rotations in the field based on these results to achieve CO<sub>2</sub> emissions reduction objectives.

The changes in the LF pool due to soil sample disturbance was unexpected and has generated additional hypotheses regarding the fate of the carbon lost from the LF pool and why it was seemingly unavailable for mineralization. The subsequent decrease in cumulative mineralized carbon is attributed to the stabilization of the carbon from the lost LF either by complexing with primary soil particles, microaggregation or assimilation in the microbial biomass. The stabilization of the lost LF by the microbial biomass protected by the soil matrix raises questions related to the particle size and C:N ratio of the physically unassociated organic matter in the soil. Where smaller particles with low C:N ratios can come in close association with the soil matrix and made available for rapid assimilation into the microbial biomass. Subsequently the higher C:N ratio of the remaining LF likely contributed to the depressed cumulative mineralized carbon observed with increasing physical disturbance as it was not assimilated as rapidly. Understanding these relationships could lead to improved field level management of SOC through altering the particle size of crop residues returned to the soil.

Soil physical properties were not found to be correlated with cumulative mineralized carbon in a meaningful way. The ability to characterize the influence of soil physical properties on cumulative mineralized carbon was possibly masked by the influence of both rotation and disturbance on LF and SOC characteristics. While this experiment was not able to answer how potentially mineralizable carbon might be influenced by the soils physical properties it did suggest that what influence might exist is less influential than the rotation or changes induced by soil sample disturbance to the LF pool. Despite its relative importance the relationship between potentially mineralizable carbon and soil physical properties could potentially be investigated using alternative methodology that does not induce changes to SOC pools. Understanding the relationship between soil physical properties and carbon mineralization could be useful for understanding how mechanisms of stabilization may perform differently under varied soil texture and moisture contents.

## **2.7. Limitations**

There are several limitations to the interpretation of the results of this experiment. Spring sampling was conducted under wet soil conditions, which resulted in soil clodding and possibly introduced variability, particularly with the hand broken disturbance treatment. Standardization of the hand broken disturbance treatment, possibly through sieving, would have helped to achieve homogeneity of sample preparation. The cumulative mineralized carbon results of the incubation experiment were affected by the

configuration of the soil sample holder. The cheesecloth used to hold the soil in the soil sample holder during pressure extraction contributed to the cumulative carbon measured. The uncontrolled GWC of the soil during the incubation also likely contributed to the variance of the experiment. There is some uncertainty regarding the changes in the LF pool induced by physical disturbance. It is not definitely clear where the LF lost in the 2 mm and DRP mill disturbance treatment went or why the disturbance caused it to be lost from the LF pool.

There are also limitations to the extrapolation of these incubation results to the field. Laboratory incubations are conducted under controlled conditions with moisture and temperature established at favorable levels for microbial activity compared to the variable and often inhospitable conditions in the field (Janzen et al., 1992). Furthermore, particularly with this study the 2 mm roller mill and DRP mill disturbance treatments are much more severe than what would typically be observed in conventionally tilled systems.

## **2.8. Future Research**

Understanding SOC dynamics in agricultural soils is important for making informed management decisions. This research showed a relationship between the carbon fractions and mineralizable carbon. It also showed how these relationships are affected by soil sample disturbance. Further insight into these relationships could be obtained by performing a similar experiment with modifications.

Primarily the experiment could be performed with improvements made to the soil sample holder used during incubation. The cheesecloth should be removed from the soil sample holder as soon as the pressure extraction is complete. This would eliminate any effect that the cheesecloth had on increasing the cumulative mineralized carbon mean or variance for the different treatments. To allow for greater control of the soil water content during the incubation the soil sample holder cap should be fitted on the base of the ABS sleeve so that there is no interstitial space between the cap and the soil.

In the results of this experiment, it was not statistically possible to determine the influence of the soil's physical properties on carbon mineralization. This was due to the influence of soil disturbance on both the LF content and the soil's physical properties. Disaggregation of the soil by shaking in water with glass beads, an approach used by Juarez et al. (2013), changes the physical properties of the soil and may prevent the loss of the LF during sample preparation for incubation. This is supported by the fact that the SOC mineralization from soils treated with this method did not differ from the control, but it does little to support the hypothesis that soil physical properties control SOC mineralization. However, as they

mentioned a similar experiment performed on soils of different texture or at a different matric potential may produce different results (Juarez et al., 2013).

Increasing disturbance was associated with a loss of LF, but no change in total SOC. While this appeared to suggest a relocation of the carbon from the lost LF to another SOC pool, the exact terminus could not be determined. Future incubation experiments could include the use of particulate organic matter with an isotopic signature different from the native SOC, such as labeled  $^{13}\text{C}$  barley (Helgason et al., 2014). This combined with different fractionation techniques such as density, particle size and possibly microbial phospholipid fatty acid extraction would help to identify the fate of LF following disturbance treatments. This would also quantify the degree to which SOC is stabilized by physical protection of microbial assimilated SOC.

If it is found, such as in Sorensen et al. (1996), that the particle size of the incorporated labeled residue determines the quantity mineralized, then this could be investigated in a field experiment. Residue from the combine harvester could be collected, mechanically ground to a range of specifications and then returned to the plots and incorporated as per normal practice. Soil respiration could then be measured throughout the growing season using non-steady state chambers and a photoacoustic multi gas monitor (Shahidi et al., 2014).

## 2.9. Tables

**Table 2.1 Breton and Lethbridge natural subregion and characteristics**

Characteristic	Breton	Lethbridge
Natural Subregion <sup>1</sup>	Central Mixed Wood	Mixed Grass
Mean annual temperature (°C)	0.2	4.2
Average annual precipitation (mm)	490	390
Parent geological material mode of deposition	Glacial till	Alluvial lacustrine

<sup>1</sup>(Natural Regions Committee, 2006)

**Table 2.2 Soil sampling location and rotations**

Breton	Lethbridge “Rotation 120”
Wheat-Fallow (W-F) <ul style="list-style-type: none"> <li>• Classical Plots</li> <li>• Sampled plot that was last in wheat</li> <li>• Series E, Plot 3</li> </ul>	Wheat-Fallow (W-F) <ul style="list-style-type: none"> <li>• Sampled plots that were last in wheat</li> <li>• Rep 1 – row 22, rep 2 – row 2, rep 3 – row 26, rep 4 – row 27</li> </ul>
Wheat-Oat-Barley-Hay-Hay (WOBHH) “Hay” <ul style="list-style-type: none"> <li>• Classical Plots</li> <li>• Sampled plot that was last in wheat</li> <li>• Series D, Plot 3</li> </ul>	Fallow-Wheat-Wheat-Hay-Hay-Hay (FWWHHH) “Hay” <ul style="list-style-type: none"> <li>• Sampled plots that were last in wheat</li> <li>• Rep 1 – row 14, rep 2 – row 1, rep 3 – row 24, rep 4 – row 2</li> </ul>
Continuous Grain (CG) <ul style="list-style-type: none"> <li>• Hendrigan</li> <li>• Sampled three replicates               <ul style="list-style-type: none"> <li>○ A-13, B-15, C-17</li> </ul> </li> </ul>	Continuous Wheat (CW) “Continuous Grain” <ul style="list-style-type: none"> <li>• Rep 1 – row 27, rep 2 – row 14, rep 3 – row 28, rep 4 – row 22</li> </ul>

**Table 2.3 Summary of soil analysis methods and schedule**

Test	Method	Protocol	Pre-incubation	Post-incubation
<b>Particle size distribution</b>	Hydrometer	Kroetsch and Wang, 2008	Yes	No
<b>Total SOC</b>	Elemental Analyzer	NRAL <sup>1</sup>	Yes	Yes
<b>Light Fraction SOC</b>	Dense Liquid (1.7 kg L <sup>-1</sup> ) and Elemental Analyzer	Gregorich and Beare, 2008; NRAL	Yes	Yes
<b>Pore Size distribution</b>	Pressure Extraction	Reynolds and Topp, 2008	Yes	No

<sup>1</sup>Analysis performed by the University of Alberta Natural Resources Analytical Laboratory

**Table 2.4 Soil texture as determined by hydrometer**

<b>Location</b>	<b>Rotation</b>	<b>SOC %</b>	<b>Sand (%)</b>	<b>Silt (%)</b>	<b>Clay (%)</b>	<b>Texture</b>
Breton	Hay	2.81	46.5	40.2	13.3	Loam
Breton	Continuous Grain	2.28	46.7	36.4	16.9	Loam
Breton	Wheat-Fallow	1.38	45.0	35.9	19.1	Loam
Lethbridge	Hay	2.51	49.0	29.1	21.9	Loam
Lethbridge	Continuous Grain	2.40	48.5	28.5	23.0	Loam
Lethbridge	Wheat-Fallow	1.76	53.1	26.6	20.3	Sandy Clay Loam

**Table 2.5 Gravimetric water content in two pore classes for all treatments**

Location	Rotation	Disturbance	GWC < 30 $\mu\text{m}$ (-10 kPa)	GWC > 30 $\mu\text{m}$ (-2 to -10 kPa)	GWC at Saturation ( $\text{g g}^{-1}$ )	% Porosity < 30 $\mu\text{m}$	% Porosity > 30 $\mu\text{m}$
Breton	Continuous Grain	2 mm Roller Mill	0.31	0.20	0.51	61.10	38.90
Breton	Continuous Grain	DRP Mill	0.38	0.21	0.59	64.68	35.32
Breton	Continuous Grain	Hand Broken	0.31	0.13	0.45	69.83	30.17
Breton	Hay	2 mm Roller Mill	0.35	0.21	0.56	63.03	36.97
Breton	Hay	DRP Mill	0.38	0.18	0.56	67.45	32.55
Breton	Hay	Hand Broken	0.30	0.13	0.43	70.17	29.83
Breton	Wheat-Fallow	2 mm Roller Mill	0.30	0.13	0.43	69.71	30.29
Breton	Wheat-Fallow	DRP Mill	0.37	0.15	0.52	70.82	29.18
Breton	Wheat-Fallow	Hand Broken	0.30	0.14	0.44	68.81	31.19
Lethbridge	Continuous Grain	2 mm Roller Mill	0.29	0.23	0.52	55.41	44.59
Lethbridge	Continuous Grain	DRP Mill	0.42	0.21	0.63	66.94	33.06
Lethbridge	Continuous Grain	Hand Broken	0.28	0.23	0.51	55.44	44.56
Lethbridge	Hay	2 mm Roller Mill	0.25	0.27	0.52	48.15	51.85
Lethbridge	Hay	DRP Mill	0.41	0.21	0.61	66.63	33.37
Lethbridge	Hay	Hand Broken	0.27	0.22	0.49	54.65	45.35
Lethbridge	Wheat-Fallow	2 mm Roller Mill	0.36	0.21	0.56	63.36	36.64
Lethbridge	Wheat-Fallow	DRP Mill	0.40	0.23	0.63	63.93	36.07
Lethbridge	Wheat-Fallow	Hand Broken	0.33	0.23	0.56	58.51	41.49

**Table 2.6 ANOVA table for pre-incubation gravimetric water content at select matric potentials for all treatments**

ANOVA Table (Type III tests)

Factor	P-Value for gravimetric water content at select matric potentials	
	- 2 kPa	-10 kPa
(Intercept)	< 2.2e-16 ***	< 2.2e-16 ***
Location	3.234e-11 ***	3.234e-11 ***
Rotation	0.2413017	0.2413017
Disturbance	7.526e-14 ***	7.526e-14 ***
Location:Rotation	9.982e-08 ***	9.982e-08 ***
Location:Disturbance	0.0007719 ***	0.0007719 ***
Rotation:Disturbance	2.061e-05 ***	2.061e-05 ***
Location:Rotation:Disturbance	0.0008850 ***	0.0008850 ***

Significance codes: 0 '\*\*\*' 0.001 '\*\*' 0.01 '\*' 0.05 '.' 0.1 '' 1

**Table 2.7 Pre-incubation gravimetric water content least-squares means table for disturbance treatments at -2 kPa. LSmean and standard error are back calculated to original scale**

Disturbance	lsmean	SE	df	Group*
Hand Broken	0.48	0.0040	18	1
2 mm Roller Mill	0.52	0.0039	18	2
DRP Mill	0.59	0.0038	18	3

\*Significantly different treatments represented by unique group combinations (p-value < 0.05)

**Table 2.8 Least-squares means table for pre-incubation gravimetric water content at -10 kPa for all treatments**

<b>Location</b>	<b>Rotation</b>	<b>Disturbance</b>	<b>lsmean</b>	<b>SE</b>	<b>df</b>	<b>Group*</b>
Lethbridge	Hay	2 mm Roller Mill	0.25	0.0037	18	1
Lethbridge	Hay	Hand Broken	0.27	0.0037	18	12
Lethbridge	Continuous Grain	Hand Broken	0.28	0.0037	18	23
Lethbridge	Continuous Grain	2 mm Roller Mill	0.29	0.0037	18	23
Breton	Hay	Hand Broken	0.30	0.0037	18	34
Breton	Wheat-Fallow	2 mm Roller Mill	0.30	0.0037	18	34
Breton	Wheat-Fallow	Hand Broken	0.30	0.0037	18	34
Breton	Continuous Grain	2 mm Roller Mill	0.31	0.0037	18	45
Breton	Continuous Grain	Hand Broken	0.31	0.0037	18	45
Lethbridge	Wheat-Fallow	Hand Broken	0.33	0.0037	18	5
Breton	Hay	2 mm Roller Mill	0.35	0.0037	18	6
Lethbridge	Wheat-Fallow	2 mm Roller Mill	0.36	0.0037	18	6
Breton	Wheat-Fallow	DRP Mill	0.37	0.0037	18	67
Breton	Hay	DRP Mill	0.38	0.0037	18	67
Breton	Continuous Grain	DRP Mill	0.38	0.0037	18	78
Lethbridge	Wheat-Fallow	DRP Mill	0.40	0.0037	18	89
Lethbridge	Hay	DRP Mill	0.41	0.0037	18	9
Lethbridge	Continuous Grain	DRP Mill	0.42	0.0037	18	9

\*Significantly different treatments represented by unique group combinations (p-value < 0.05)

**Table 2.9 Total carbon, nitrogen and C:N ratio for pre- and post-incubation soil**

Location	Rotation	Disturbance	Pre-Incubation			Post-Incubation			Δ Incubation		
			Total C (g C kg <sup>-1</sup> )	Total N (g N kg <sup>-1</sup> )	C:N	Total C (g C kg <sup>-1</sup> )	Total N (g N kg <sup>-1</sup> )	C:N	% Δ TC	% Δ TN	% Δ C:N
Breton	Continuous Grain	2 mm Roller Mill	21.84	2.05	10.64	20.37	1.89	10.76	-6.74	-7.83	1.19
Breton	Continuous Grain	DRP Mill	24.29	2.20	11.04	21.48	1.94	11.07	-11.59	-11.82	0.25
Breton	Continuous Grain	Hand Broken	22.79	2.05	11.12	24.75	2.30	10.77	8.58	12.07	-3.12
Breton	Hay	2 mm Roller Mill	27.05	2.40	11.27	25.88	2.44	10.60	-4.33	1.77	-5.99
Breton	Hay	DRP Mill	29.32	2.47	11.89	25.70	2.15	11.95	-12.36	-12.84	0.55
Breton	Hay	Hand Broken	28.12	2.41	11.65	20.60	1.99	10.35	-26.75	-17.54	-11.17
Breton	Wheat-Fallow	2 mm Roller Mill	13.30	1.14	11.67	12.24	1.10	11.13	-7.97	-3.51	-4.62
Breton	Wheat-Fallow	DRP Mill	14.77	1.25	11.79	13.24	1.16	11.39	-10.40	-7.25	-3.40
Breton	Wheat-Fallow	Hand Broken	13.77	1.24	11.13	13.33	1.25	10.64	-3.18	1.28	-4.41
Lethbridge	Continuous Grain	2 mm Roller Mill	23.77	1.88	12.67	21.37	1.78	12.01	-10.09	-5.15	-5.21
Lethbridge	Continuous Grain	DRP Mill	22.52	1.87	12.02	20.37	1.79	11.41	-9.52	-4.72	-5.05
Lethbridge	Continuous Grain	Hand Broken	23.95	1.95	12.28	21.14	1.84	11.47	-11.75	-5.51	-6.60
Lethbridge	Hay	2 mm Roller Mill	22.60	1.98	11.39	20.92	1.88	11.16	-7.44	-5.46	-2.09
Lethbridge	Hay	DRP Mill	23.94	1.95	12.25	21.59	1.79	12.10	-9.80	-8.62	-1.30
Lethbridge	Hay	Hand Broken	25.06	2.06	12.17	20.36	1.96	10.37	-18.77	-4.73	-14.74
Lethbridge	Wheat-Fallow	2 mm Roller Mill	16.81	1.42	11.84	16.97	1.51	11.24	0.95	6.34	-5.07
Lethbridge	Wheat-Fallow	DRP Mill	17.59	1.47	11.94	16.18	1.40	11.56	-8.01	-4.98	-3.19
Lethbridge	Wheat-Fallow	Hand Broken	17.59	1.57	11.23	17.28	1.52	11.39	-1.75	-3.14	1.43

**Table 2.10 ANOVA table for pre-incubation soil properties for all treatments**

ANOVA Table (Type III tests)

Factor	P-Value for pre-incubation soil properties				
	Total SOC	Total N	C:N	LF	LF C
(Intercept)	< 2.2e-16***	<2e-16***	< 2.2e-16***	< 2.2e-16***	< 2.2e-16***
Location	4.544e-06***	0.55773	6.509e-07***	2.994e-13***	3.059e-13**
Rotation	< 2.2e-16***	< 2.2e-16***	0.390232	1.416e-12***	3.340e-11***
Disturbance	0.002769**	0.03925*	0.138322	< 2.2e-16***	< 2.2e-16***
Location:Rotation	2.130e-14***	3.934e-13***	3.842e-05***	2.847e-08***	0.001217**
Location:Disturbance	0.011980*	0.09686.	0.527407	7.475e-11**	0.027618*
Rotation:Disturbance	0.240128	0.14434	0.003929**	0.003141**	1.278e-11***
Location:Rotation: Disturbance	0.536163	0.97467	0.251111	0.006475**	6.363e-14***

Significance codes: 0 '\*\*\*' 0.001 '\*\*' 0.01 '\*' 0.05 '.' 0.1 '' 1

**Table 2.11 Least-squares means for location-rotation-disturbance interaction of linear model for pre-incubation total SOC content (%). LSmean and standard error are back calculated to original scale**

Location	Rotation	Disturbance	lsmean	SE	df	Group*
Breton	Hay	DRP Mill	2.93	0.1521	36	1
Breton	Hay	Hand Broken	2.73	0.1279	36	12
Breton	Hay	2 mm Roller Mill	2.70	0.1249	36	12
Lethbridge	Hay	Hand Broken	2.50	0.1029	36	123
Breton	Continuous Grain	DRP Mill	2.43	0.0962	36	123
Lethbridge	Continuous Grain	Hand Broken	2.39	0.0929	36	123
Lethbridge	Hay	DRP Mill	2.39	0.0927	36	123
Lethbridge	Continuous Grain	2 mm Roller Mill	2.37	0.0912	36	123
Breton	Continuous Grain	Hand Broken	2.27	0.0819	36	23
Lethbridge	Hay	2 mm Roller Mill	2.26	0.0808	36	23
Lethbridge	Continuous Grain	DRP Mill	2.25	0.0802	36	23
Breton	Continuous Grain	2 mm Roller Mill	2.18	0.0744	36	3
Lethbridge	Wheat-Fallow	DRP Mill	1.76	0.0441	36	4
Lethbridge	Wheat-Fallow	Hand Broken	1.76	0.0440	36	4
Lethbridge	Wheat-Fallow	2 mm Roller Mill	1.68	0.0396	36	4
Breton	Wheat-Fallow	DRP Mill	1.48	0.0290	36	5
Breton	Wheat-Fallow	Hand Broken	1.37	0.0244	36	56
Breton	Wheat-Fallow	2 mm Roller Mill	1.33	0.0225	36	6

\*Significantly different treatments represented by unique group combinations (p-value < 0.05)

**Table 2.12 ANOVA table for post-incubation soil properties for all treatments**

ANOVA Table (Type III tests)

Factor	P-Value for post-incubation soil properties					
	Total SOC	Total N	C:N	LF	LFC	Mineralized C
(Intercept)	< 2.2e-16***	<2e-16***	< 2.2e-16***	< 2.2e-16***	< 2.2e-16***	< 2.2e-16***
Location	0.001678**	0.9245158	1.124e-06***	7.593e-14***	2.280e-13***	0.5732625
Rotation	< 2.2e-16***	< 2.2e-16***	0.29065	1.169e-12***	1.807e-12***	7.741e-13***
Disturbance	0.622012	0.0011977**	6.506e-09***	< 2.2e-16***	< 2.2e-16***	3.066e-09***
Gravimetric Water Content	-	-	-	-	-	0.0004768***
Location:Rotation	< 2.2e-16***	< 2.2e-16***	0.03569*	0.001366**	0.01524*	0.0094712**
Location:Disturbance	0.039654*	0.6012459	0.09920 .	0.101645	0.21575	0.4158619
Rotation:Disturbance	6.705e-10***	0.0007648***	7.645e-07***	1.382e-07***	4.968e-07***	0.0008192***
Location:Rotation:Disturbance	5.786e-07***	2.941e-05***	0.08541.	1.129e-09***	1.308e-10***	4.342e-07***

Significance codes: 0 '\*\*\*' 0.001 '\*\*' 0.01 '\*' 0.05 '.' 0.1 ' ' 1

**Table 2.13 Light Fraction total SOC, total nitrogen and C:N ratio**

Location	Rotation	Disturbance	Pre-Incubation				Post Incubation				Δ Incubation	
			LF (g kg <sup>-1</sup> )	LF %C	LF %N	C:N	LF (g kg <sup>-1</sup> )	LF %C	LF %N	C:N	%Δ LF	%Δ C:N
Breton	Continuous Grain	2 mm Roller Mill	11.09	24.52	1.19	20.58	4.45	23.51	1.24	18.90	-59.87	-8.14
Breton	Continuous Grain	DRP Mill	3.17	31.75	1.34	23.76	0.44	27.58	1.42	19.40	-86.28	-18.37
Breton	Continuous Grain	Hand Broken	11.88	24.06	1.16	20.82	15.48	21.26	1.25	17.05	30.23	-18.12
Breton	Hay	2 mm Roller Mill	22.41	26.44	1.37	19.23	12.05	21.70	1.32	16.45	-46.22	-14.43
Breton	Hay	DRP Mill	8.52	33.36	1.28	26.12	4.53	24.52	1.23	19.92	-46.83	-23.73
Breton	Hay	Hand Broken	24.77	24.80	1.30	19.15	5.67	20.67	1.13	18.35	-77.09	-4.18
Breton	Wheat-Fallow	2 mm Roller Mill	10.81	27.32	0.93	29.40	3.78	24.67	1.17	21.08	-65.02	-28.30
Breton	Wheat-Fallow	DRP Mill	3.65	31.28	0.74	42.32	0.48	24.77	1.09	23.21	-86.97	-45.15
Breton	Wheat-Fallow	Hand Broken	14.87	28.39	1.00	28.30	5.00	23.23	1.21	19.92	-66.35	-29.60
Lethbridge	Continuous Grain	2 mm Roller Mill	11.62	23.61	1.23	19.41	4.22	21.40	1.24	17.41	-63.65	-10.31
Lethbridge	Continuous Grain	DRP Mill	1.99	30.48	0.86	35.40	0.44	27.68	1.37	22.38	-78.04	-36.77
Lethbridge	Continuous Grain	Hand Broken	16.92	22.62	1.13	20.13	4.71	21.02	1.21	17.37	-72.15	-13.70
Lethbridge	Hay	2 mm Roller Mill	12.27	24.49	1.31	18.80	4.91	23.00	1.50	15.29	-60.02	-18.67
Lethbridge	Hay	DRP Mill	2.40	31.04	0.93	33.27	0.28	30.09	1.75	18.35	-88.23	-44.85
Lethbridge	Hay	Hand Broken	14.43	23.52	1.33	17.69	5.57	25.63	1.70	15.08	-61.39	-14.77
Lethbridge	Wheat-Fallow	2 mm Roller Mill	12.08	26.69	0.98	27.43	2.20	24.78	1.28	19.33	-81.78	-29.52
Lethbridge	Wheat-Fallow	DRP Mill	1.21	31.65	0.72	44.21	0.12	31.03	1.34	24.24	-89.84	-45.19
Lethbridge	Wheat-Fallow	Hand Broken	8.95	22.64	0.99	23.02	3.79	22.40	1.17	19.20	-57.61	-16.59

**Table 2.14 Least-squares means for pre-incubation soil light fraction content (g LF kg soil<sup>-1</sup>). LSmean and standard error are back calculated to original scale**

Location	Rotation	Disturbance	lsmean	SE	df	Group*
Breton	Hay	Hand Broken	24.76	2.5187	36	1
Breton	Hay	2 mm Roller Mill	22.20	2.2361	36	12
Lethbridge	Continuous Grain	Hand Broken	16.91	1.6613	36	123
Breton	Wheat-Fallow	Hand Broken	14.60	1.4150	36	23
Lethbridge	Hay	Hand Broken	13.99	1.3513	36	234
Lethbridge	Hay	2 mm Roller Mill	12.27	1.1708	36	345
Lethbridge	Wheat-Fallow	2 mm Roller Mill	12.05	1.1474	36	345
Breton	Continuous Grain	Hand Broken	11.56	1.0966	36	345
Lethbridge	Continuous Grain	2 mm Roller Mill	11.55	1.0959	36	345
Breton	Continuous Grain	2 mm Roller Mill	11.09	1.0478	36	345
Breton	Wheat-Fallow	2 mm Roller Mill	10.75	1.0136	36	345
Lethbridge	Wheat-Fallow	Hand Broken	8.76	0.8107	36	45
Breton	Hay	DRP Mill	8.52	0.7862	36	5
Breton	Wheat-Fallow	DRP Mill	3.64	0.3113	36	6
Breton	Continuous Grain	DRP Mill	3.15	0.2650	36	6
Lethbridge	Hay	DRP Mill	2.39	0.1968	36	67
Lethbridge	Continuous Grain	DRP Mill	1.96	0.1582	36	7
Lethbridge	Wheat-Fallow	DRP Mill	1.21	0.0936	36	8

\*Significantly different treatments represented by unique group combinations (p-value < 0.05)

**Table 2.15 Least-squares means for pre-incubation soil light fraction content (g LF kg soil<sup>-1</sup>), by rotation. LSmean and standard error are back calculated to original scale**

Rotation	lsmean	SE	df	Group*
Hay	10.8491	0.42	36	1
Continuous Grain	7.1171	0.26	36	2
Wheat-fallow	6.2447	0.23	36	3

\*Significantly different treatments represented by unique group combinations (p-value < 0.05)

**Table 2.16 Pearson’s correlation coefficient and p-value for pre-incubation total SOC content and gravimetric water content at -100 and – 2 kPa**

Disturbance	- 100 kPa		- 2 kPa	
	Correlation TC and GWC	p-value	Correlation TC and GWC	p-value
2 mm Roller Mill	0.8424	0.0353	0.6488	0.1634
DRP Mill	0.4824	0.3325	0.1338	0.8005
Hand Broken	0.7837	0.0651	-0.2085	0.6917

**Table 2.17 Significance testing that the rates of CO<sub>2</sub> respiration in the final four sampling events (Days 139-182 ) was not equal to zero. Respiration rate not stable when p-value < 0.05**

Location	Rotation	Disturbance	P-value
Background	Background	Background	0.593
Breton	Hay	2 mm Roller Mill	0.031
Breton	Hay	Hand Broken	0.017
Breton	Hay	DRP Mill	0.794
Breton	Continuous Grain	2 mm Roller Mill	0.080
Breton	Continuous Grain	Hand Broken	0.053
Breton	Continuous Grain	DRP Mill	0.886
Breton	Wheat-Fallow	2 mm Roller Mill	0.271
Breton	Wheat-Fallow	Hand Broken	0.218
Breton	Wheat-Fallow	DRP Mill	0.128
Lethbridge	Hay	2 mm Roller Mill	0.417
Lethbridge	Hay	Hand Broken	0.338
Lethbridge	Hay	DRP Mill	0.051
Lethbridge	Continuous Grain	2 mm Roller Mill	0.070
Lethbridge	Continuous Grain	Hand Broken	0.031
Lethbridge	Continuous Grain	DRP Mill	0.052
Lethbridge	Wheat-Fallow	2 mm Roller Mill	0.101
Lethbridge	Wheat-Fallow	Hand Broken	0.049
Lethbridge	Wheat-Fallow	DRP Mill	0.980

**Table 2.18 Cumulative mineralized carbon (g CO<sub>2</sub>-C kg soil<sup>-1</sup>) least-squares means for location rotation interaction of linear model. LSmean and standard error are back calculated to original scale**

Location	Rotation	lsmean	SE	df	Group*
Lethbridge	Wheat-Fallow	3.67	0.2277	53	1
Breton	Wheat-Fallow	4.56	0.2603	53	12
Breton	Continuous Grain	5.41	0.2819	53	23
Lethbridge	Continuous Grain	6.11	0.3056	53	34
Lethbridge	Hay	6.71	0.3129	53	4
Breton	Hay	6.76	0.3141	53	4

\*Significantly different treatments represented by unique group combinations (p-value < 0.05)

**Table 2.19 Cumulative mineralized carbon (g CO<sub>2</sub>-C kg soil<sup>-1</sup>) least-squares means for rotation disturbance interaction of linear model. LSmean and standard error are back calculated to original scale**

Rotation	Disturbance	lsmean	SE	df	Group*
Wheat Fallow	DRP Mill	3.26	0.2627	53	1
Wheat Fallow	2 mm	4.43	0.3048	53	12
Continuous Grain	DRP Mill	4.49	0.3118	53	12
Wheat Fallow	HB	4.70	0.3260	53	2
Hay	DRP Mill	5.31	0.3369	53	23
Continuous Grain	2 mm	5.67	0.3496	53	234
Hay	HB	6.57	0.3831	53	34
Continuous Grain	HB	7.29	0.4012	53	45
Hay	2 mm	8.54	0.4437	53	5

\*Significantly different treatments represented by unique group combinations (p-value < 0.05)

**Table 2.20 ANOVA table for cumulative mineralized carbon (g CO<sub>2</sub>-C kg soil<sup>-1</sup>) linear model, DRP mill disturbance treatment only**

ANOVA Table (Type III tests)

Response: Mineralization

	Sum Sq	Df	F value	Pr(>F)	Significance
(Intercept)	440.55	1	428.914	1.865e-15	***
Rotation	21.27	2	10.356	0.0007422	***
Residuals	21.57	21			

Significance codes: 0 '\*\*\*' 0.001 '\*\*' 0.01 '\*' 0.05 '.' 0.1 ' ' 1

**Table 2.21 Least-squares means for cumulative mineralized carbon (g CO<sub>2</sub>-C kg<sup>-1</sup>) and rotation linear model, DRP mill disturbance treatment only**

Rotation	lsmean	SE	df	Group*
Wheat-Fallow	3.09	0.3583	21	1
Continuous Grain	4.36	0.3583	21	12
Hay	5.40	0.3583	21	2

\*Significantly different treatments represented by unique group combinations (p-value < 0.05)

**Table 2.22 Gravimetric water content (GWC) of soil samples at the end of the incubation and at – 2 kPa (saturation)**

Location	Rotation	Disturbance	Incubation GWC (g g <sup>-1</sup> )	Saturated GWC (g g <sup>-1</sup> )
Breton	Continuous Grain	2 mm Roller Mill	0.34	0.51
Breton	Continuous Grain	DRP Mill	0.39	0.59
Breton	Continuous Grain	Hand Broken	0.39	0.45
Breton	Hay	2 mm Roller Mill	0.45	0.56
Breton	Hay	DRP Mill	0.41	0.56
Breton	Hay	Hand Broken	0.33	0.43
Breton	Wheat-Fallow	2 mm Roller Mill	0.38	0.43
Breton	Wheat-Fallow	DRP Mill	0.44	0.52
Breton	Wheat-Fallow	Hand Broken	0.26	0.44
Lethbridge	Continuous Grain	2 mm Roller Mill	0.45	0.52
Lethbridge	Continuous Grain	DRP Mill	0.49	0.63
Lethbridge	Continuous Grain	Hand Broken	0.39	0.51
Lethbridge	Hay	2 mm Roller Mill	0.42	0.52
Lethbridge	Hay	DRP Mill	0.38	0.61
Lethbridge	Hay	Hand Broken	0.41	0.49
Lethbridge	Wheat-Fallow	2 mm Roller Mill	0.42	0.56
Lethbridge	Wheat-Fallow	DRP Mill	0.45	0.63
Lethbridge	Wheat-Fallow	Hand Broken	0.43	0.56

**Table 2.23 ANOVA table for incubation soil GWC at the end of incubation (Day 182)**

ANOVA Table (Type III tests)

Response: GWC

	Sum Sq	Df	F value	Pr(>F)
(Intercept)	11.6235	1	1886.9834	< 2e-16 ***
Location	0.0431	1	6.9950	0.01068 *
Rotation	0.0015	2	0.1184	0.88857
Disturbance	0.0406	2	3.2962	0.04460 *
Location:Rotation	0.0155	2	1.2619	0.29132
Location:Disturbance	0.0110	2	0.8891	0.41697
Rotation:Disturbance	0.0256	4	1.0402	0.39510
Location:Rotation:Disturbance	0.0475	4	1.9277	0.11904
Residuals	0.3326	54		

Significance codes: 0 '\*\*\*' 0.001 '\*\*' 0.01 '\*' 0.05 '.' 0.1 ' ' 1

**Table 2.24 Least-squares means for incubation soil GWC at the end of incubation (Day 182) by location**

Location	lsmean	SE	df	Group*
Breton	0.37	0.0131	54	1
Lethbridge	0.43	0.0131	54	2

\*Significantly different treatments represented by unique group combinations (p-value &lt; 0.05)

**Table 2.25 Least-squares means for incubation soil GWC at the end of incubation (Day 182) by disturbance**

Disturbance	lsmean	SE	df	Group*
Hand Broken	0.37	0.0160	54	1
2 mm Roller Mill	0.41	0.0160	54	12
DRP Mill	0.43	0.0160	54	2

\*Significantly different treatments represented by unique group combinations (p-value &lt; 0.05)

**Table 2.26 Pearson's correlation coefficient and p-value for incubation cumulative mineralized carbon and select soil properties**

Parameter	Correlation with Cumulative Mineralized Carbon	Correlation p-value
LF (g kg <sup>-1</sup> ), pre-incubation	0.6622	0.0028
LF-C (g kg <sup>-1</sup> ), pre-incubation	0.6556	0.0031
Water filled pore space (WFPS) at -10 kPa	-0.7378	0.0004
Gravimetric water content < -10 kPa	-0.5743	0.0127

2.10. Figures

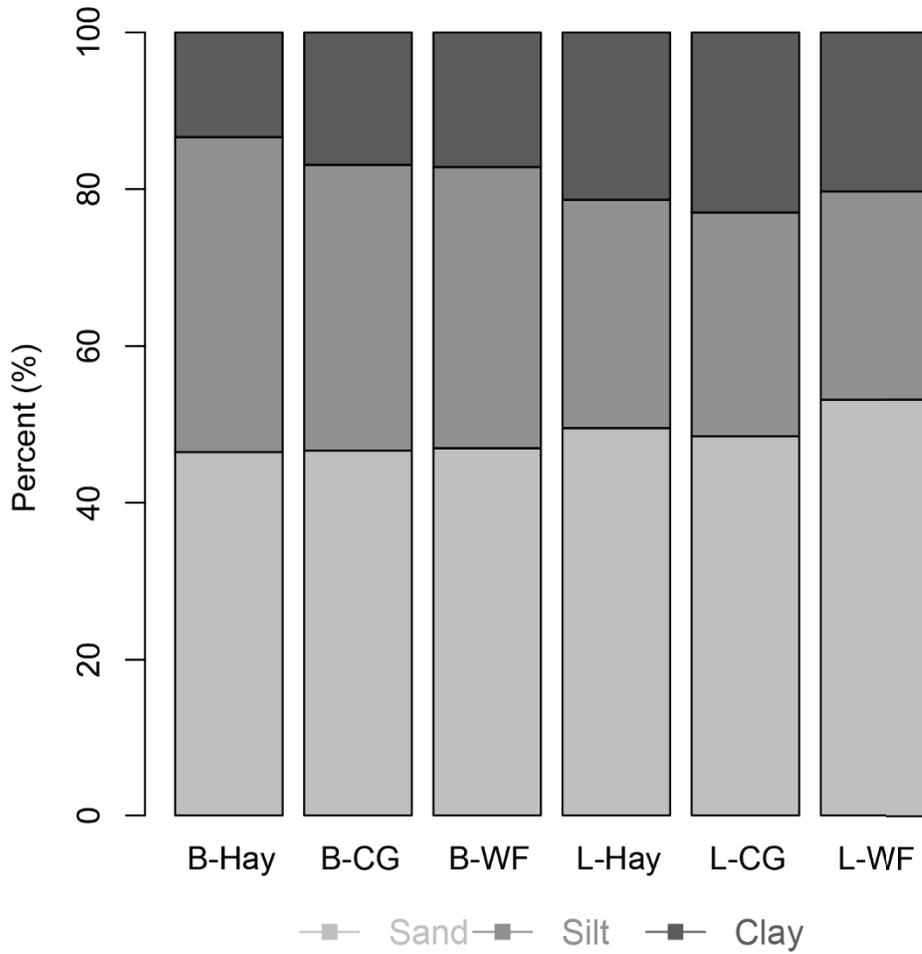


Figure 2.1 Particle size distribution of soil samples. Where B = Breton, L = Lethbridge, CG = continuous grain, WF = wheat-fallow

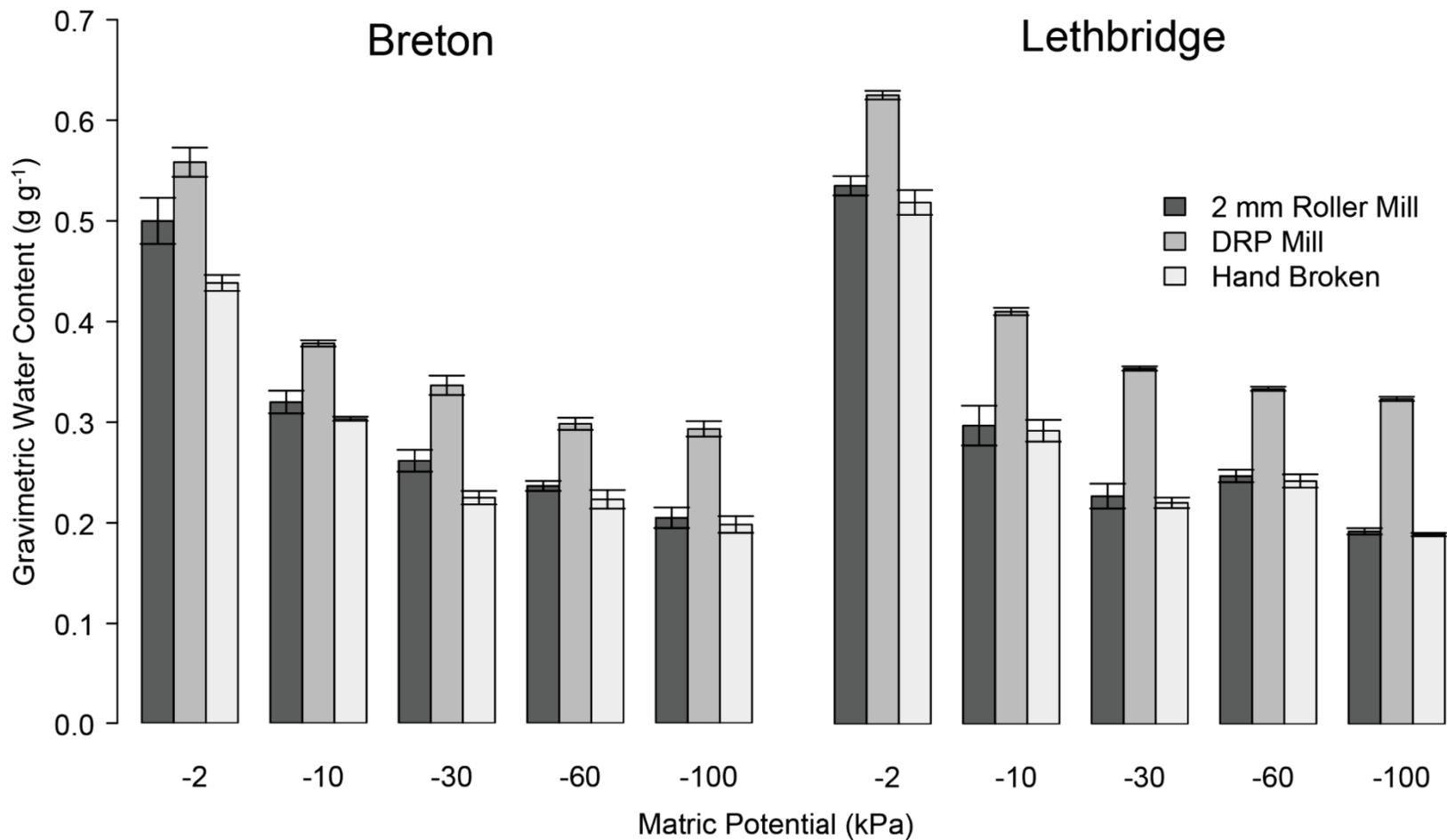


Figure 2.2 Pre-incubation soil gravimetric water content by disturbance treatment, averaged across location and rotation. Error bars represent ± 1 standard error of the mean for each treatment

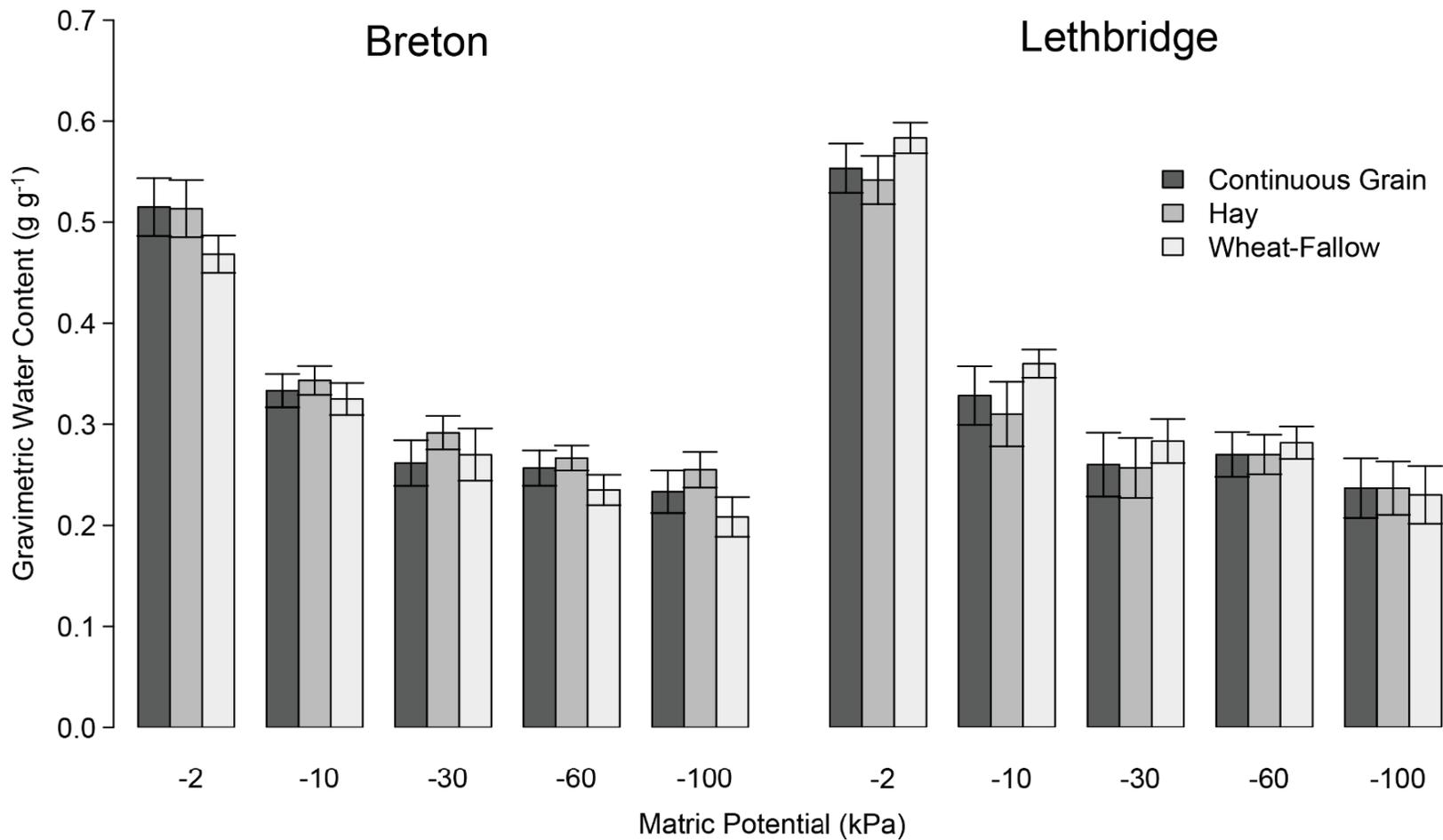


Figure 2.3 Pre-incubation gravimetric water content by rotation treatment, averaged across location and disturbance. Error bars represent  $\pm 1$  standard error of the mean for each treatment

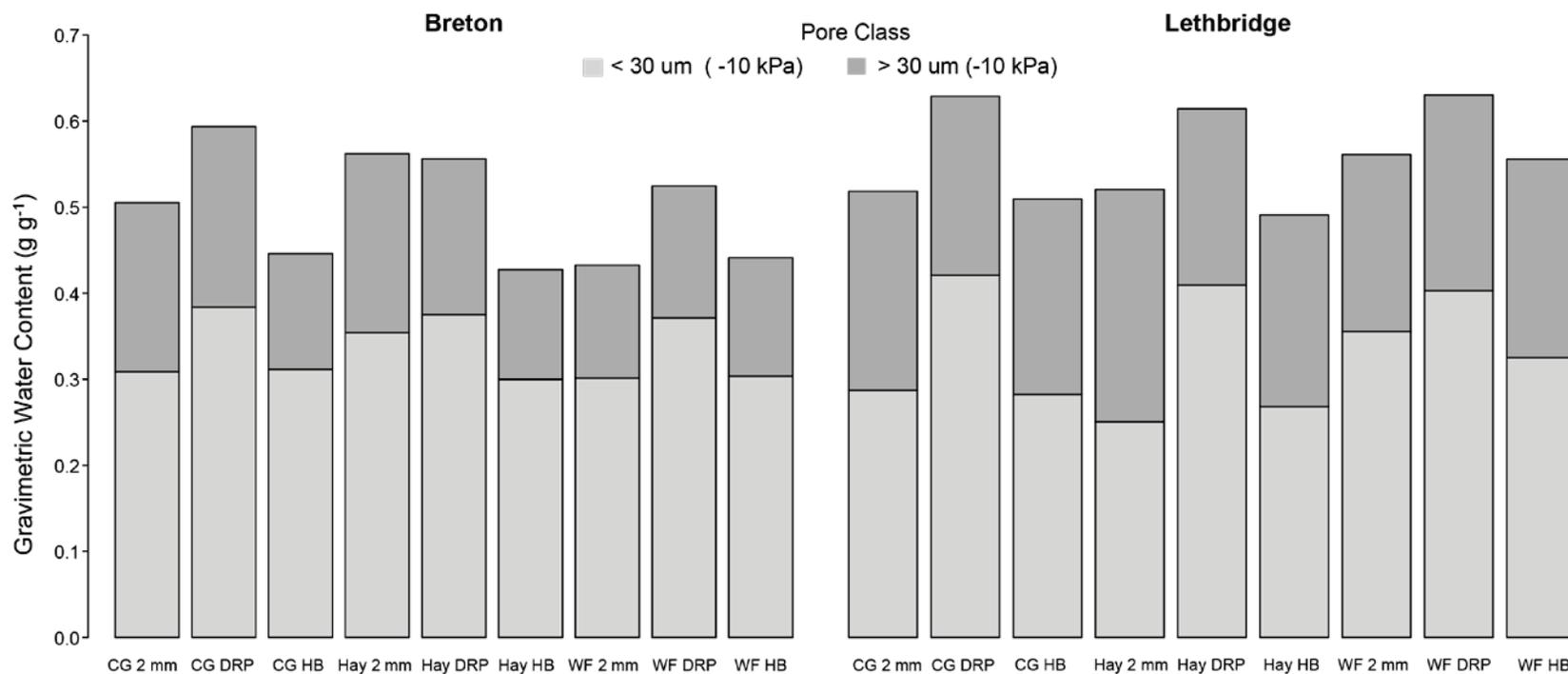


Figure 2.4 Pre-incubation soil gravimetric water content for two pore classes. Where CG = continuous grain, WF = wheat-fallow, 2 mm = 2 mm roller mill, DRP = DRP mill, HB= hand broken

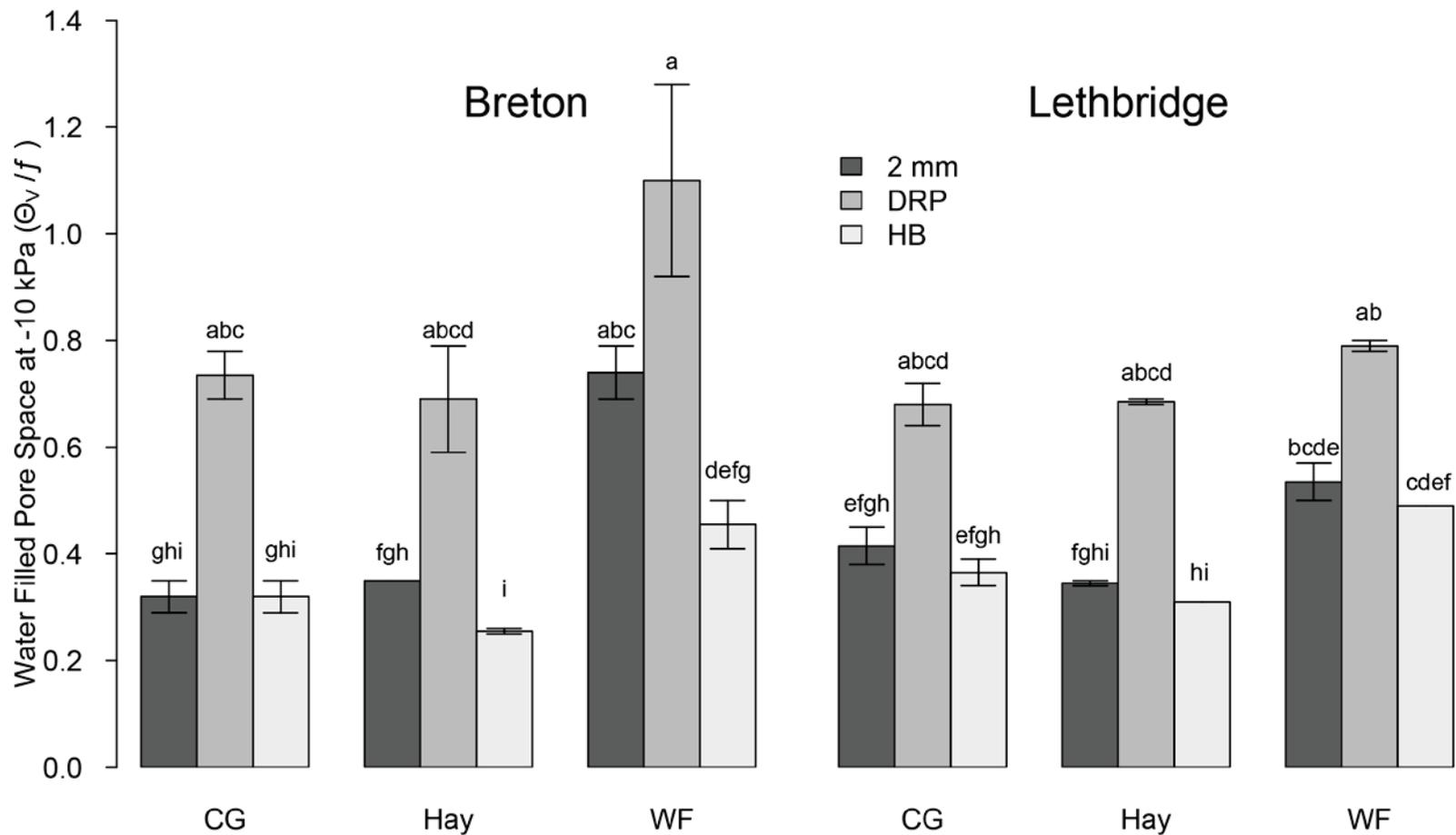
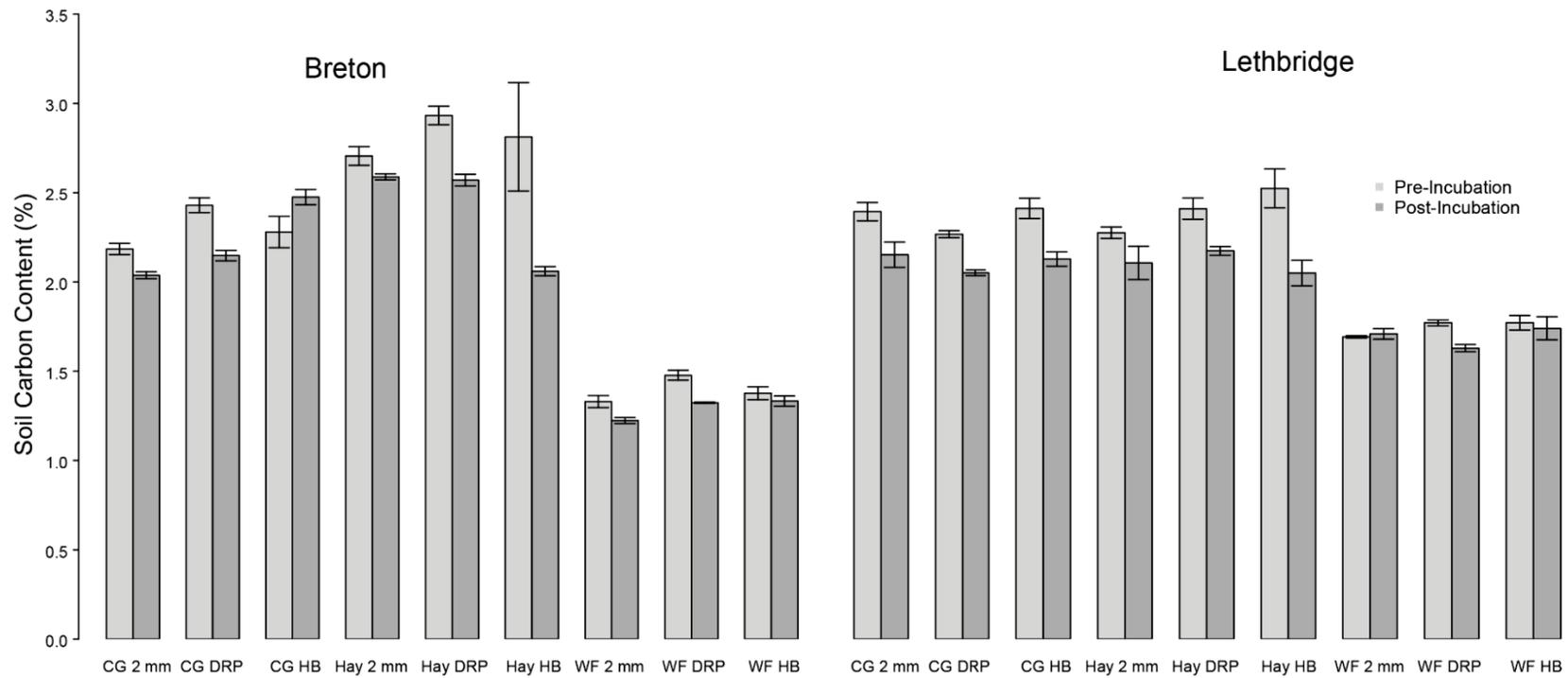
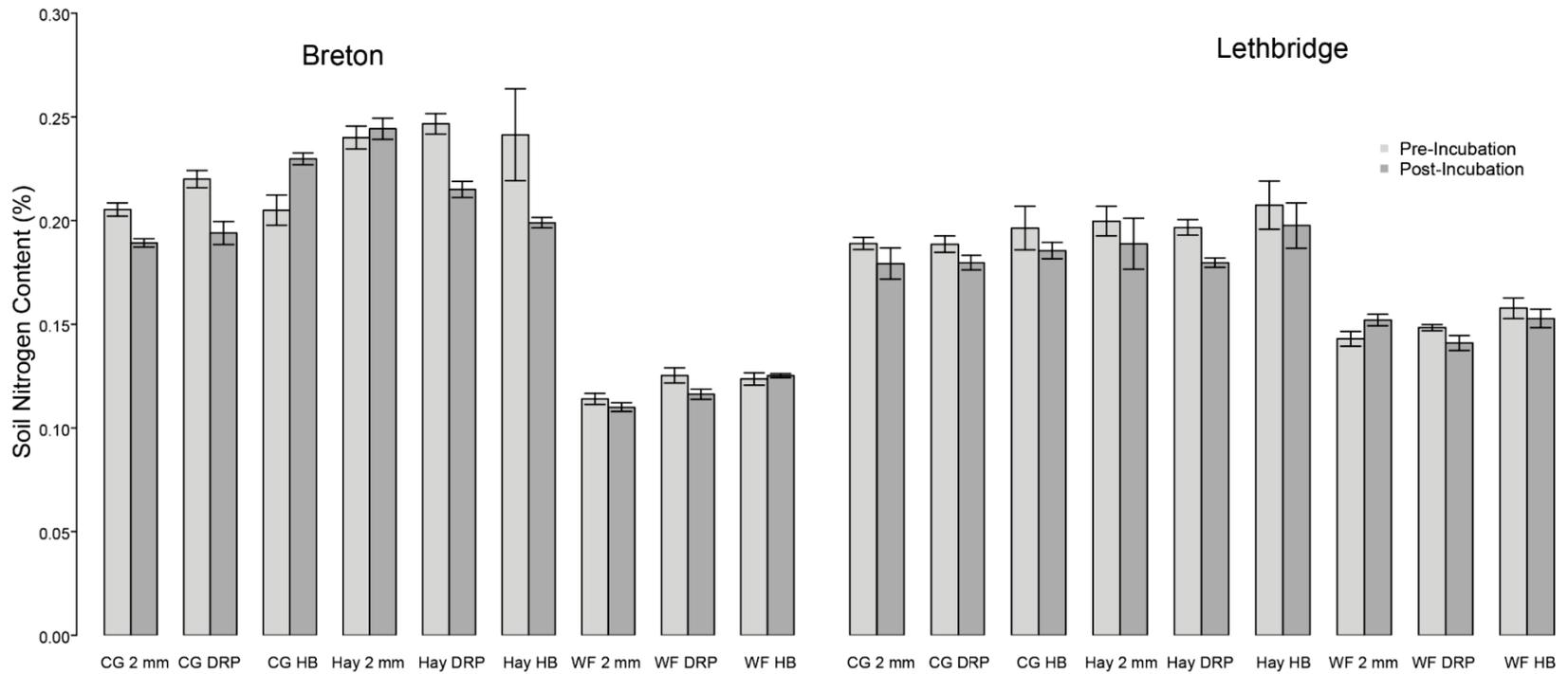


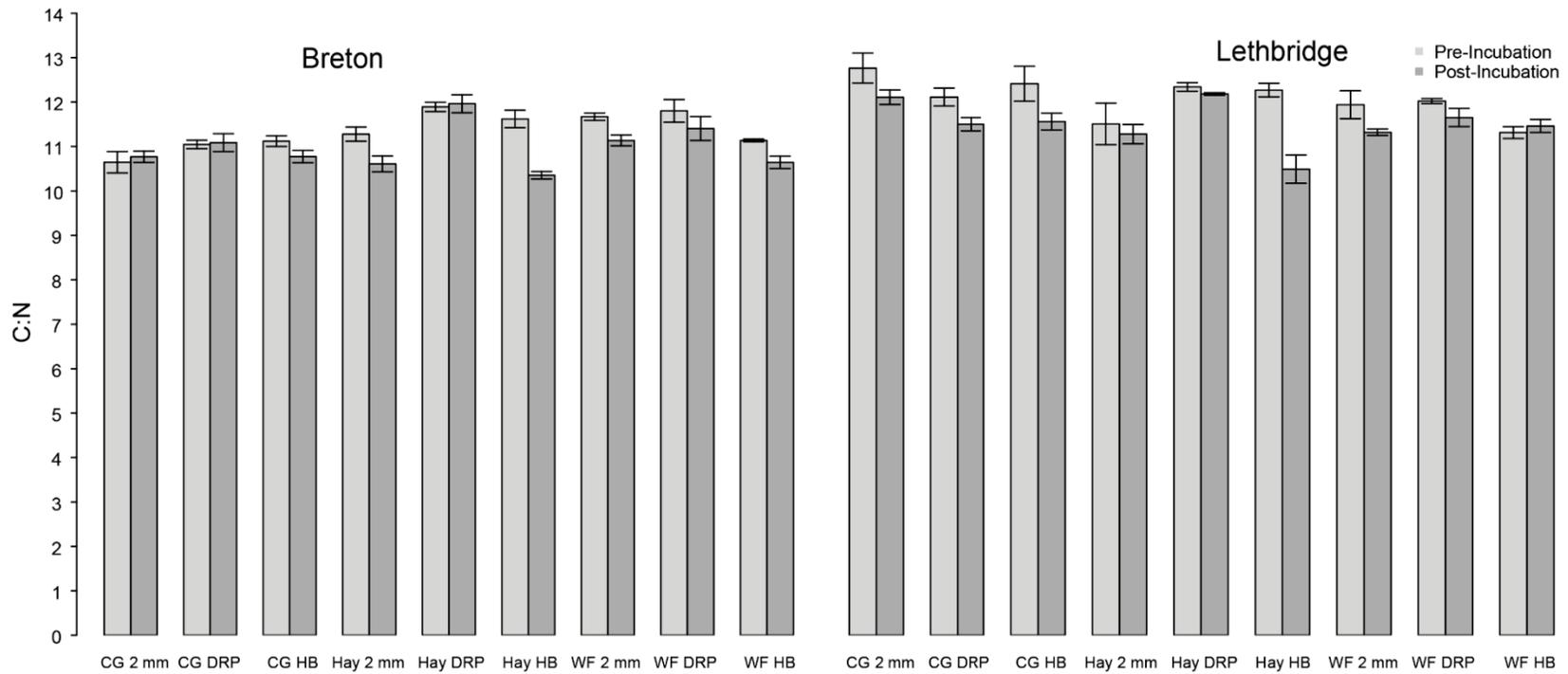
Figure 2.5 Pre-incubation soil water filled pore space at – 10 kPa for all treatments. Significantly different treatments represented by unique letter combinations ( $p$ -value < 0.05). Where CG = continuous grain, WF = wheat-fallow. Error bars represent  $\pm 1$  standard error of the mean for each treatment



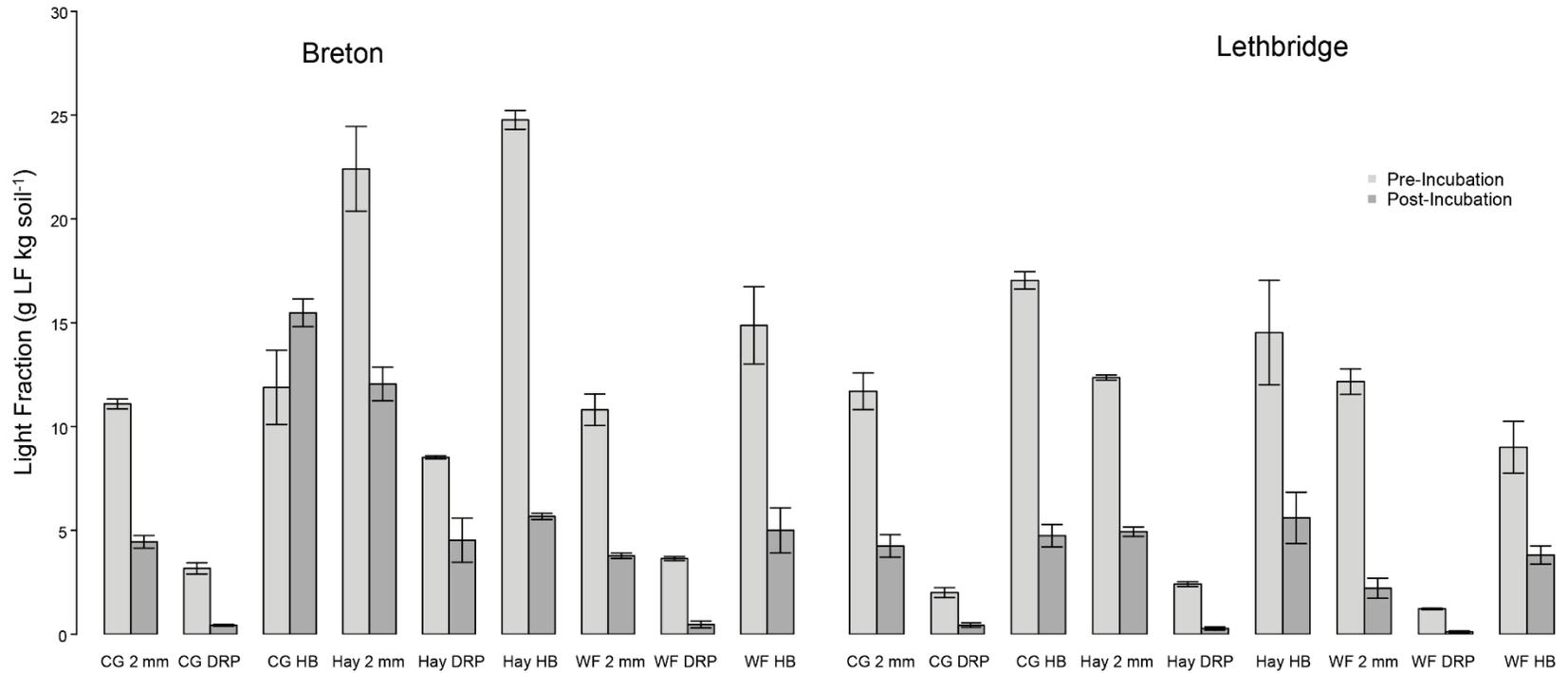
**Figure 2.6 Total SOC content pre- and post-incubation for all treatments. Where CG = continuous grain, WF = wheat-fallow, 2 mm = 2 mm roller mill, DRP = DRP mill, HB= hand broken. Error bars represent ± 1 standard error of the mean for each treatment**



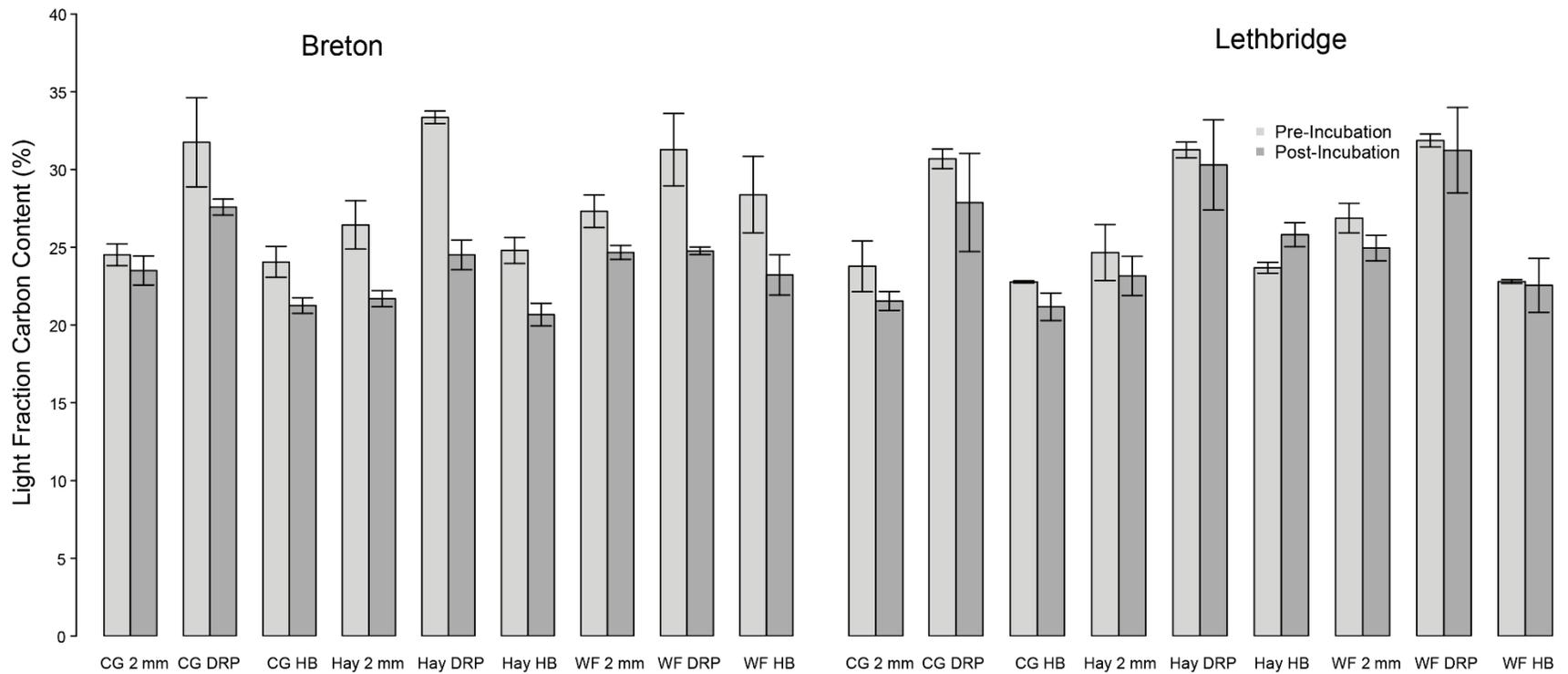
**Figure 2.7 Soil total nitrogen content pre- and post-incubation for all treatments. Where CG = continuous grain, WF = wheat-fallow, 2 mm = 2 mm roller mill, DRP = DRP mill, HB= hand broken. Error bars represent  $\pm 1$  standard error of the mean for each treatment**



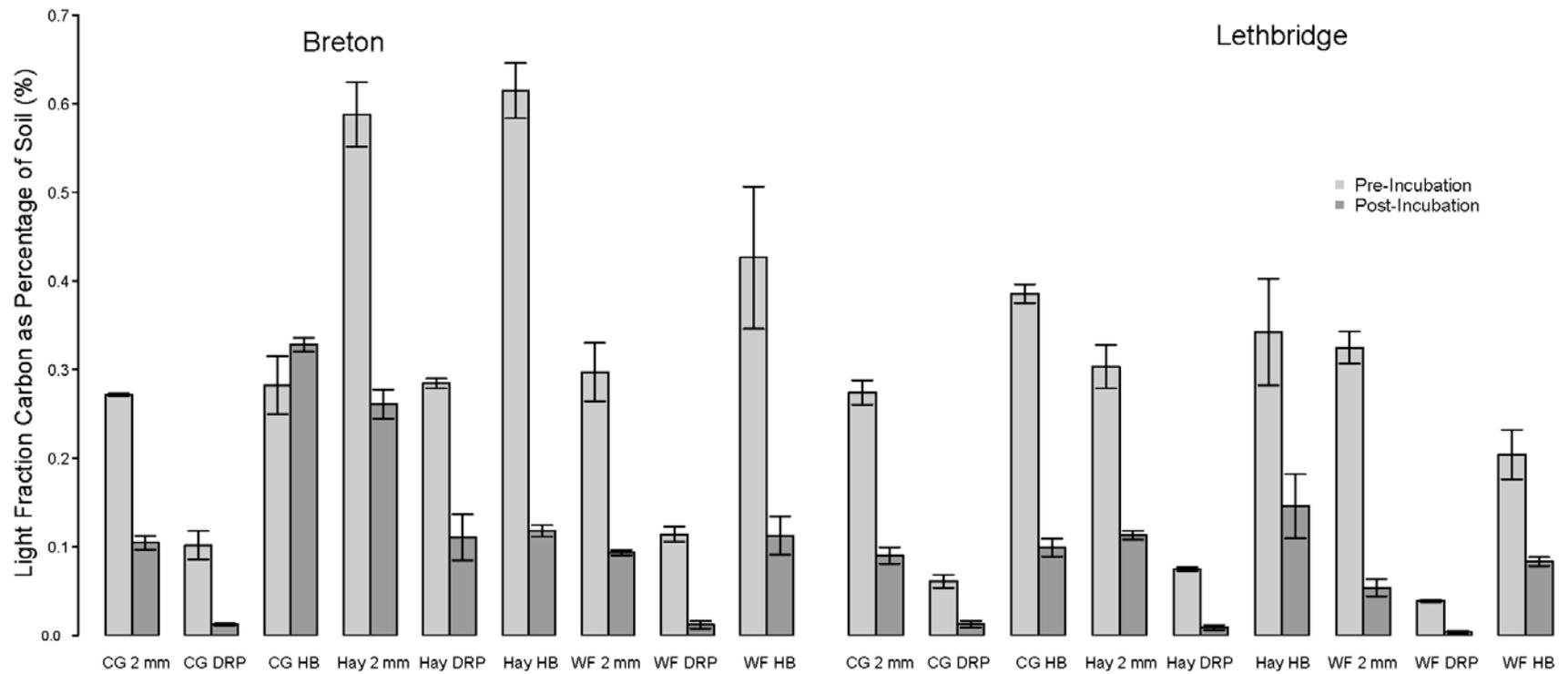
**Figure 2.8 Soil C:N ratio pre- and post-incubation for all treatments. Where CG = continuous grain, WF = wheat-fallow, 2 mm = 2 mm roller mill, DRP = DRP mill, HB= hand broken. Error bars represent  $\pm 1$  standard error of the mean for each treatment**



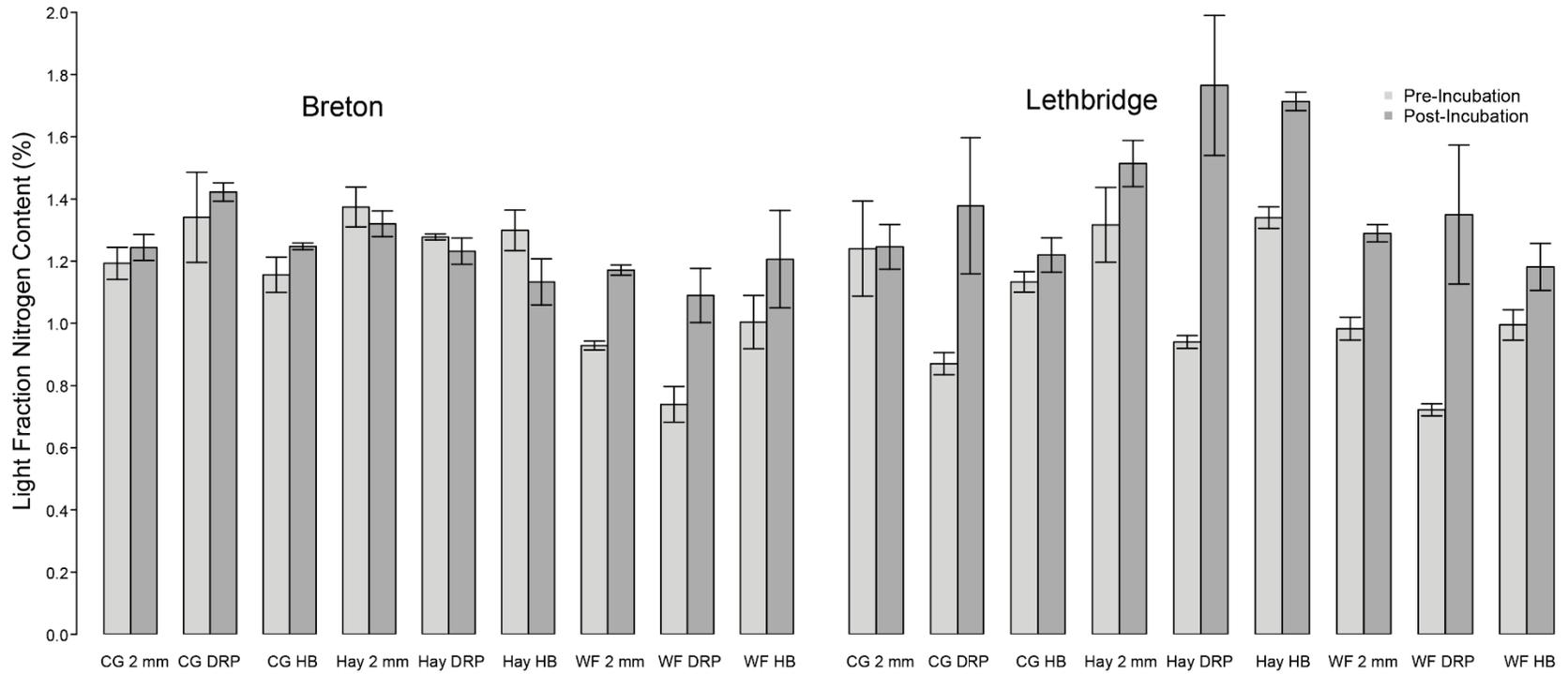
**Figure 2.9 Light fraction recovery pre- and post-incubation for all treatments. Where CG = continuous grain, WF = wheat-fallow, 2 mm = 2 mm roller mill, DRP = DRP mill, HB= hand broken. Error bars represent  $\pm 1$  standard error of the mean for each treatment**



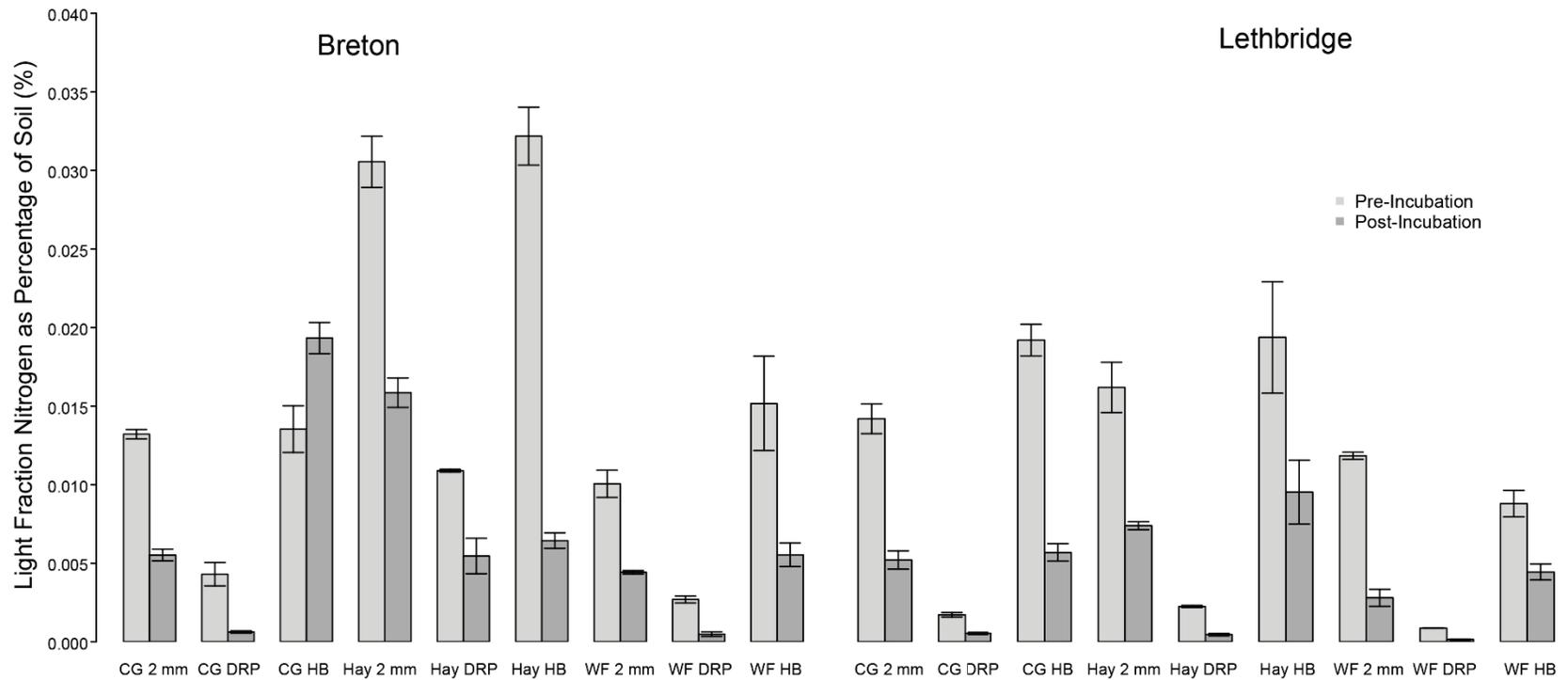
**Figure 2.10 Carbon content of recovered light fraction pre- and post-incubation for all treatments. Where CG = continuous grain, WF = wheat-fallow, 2 mm = 2 mm roller mill, DRP = DRP mill, HB= hand broken. Error bars represent  $\pm 1$  standard error of the mean for each treatment**



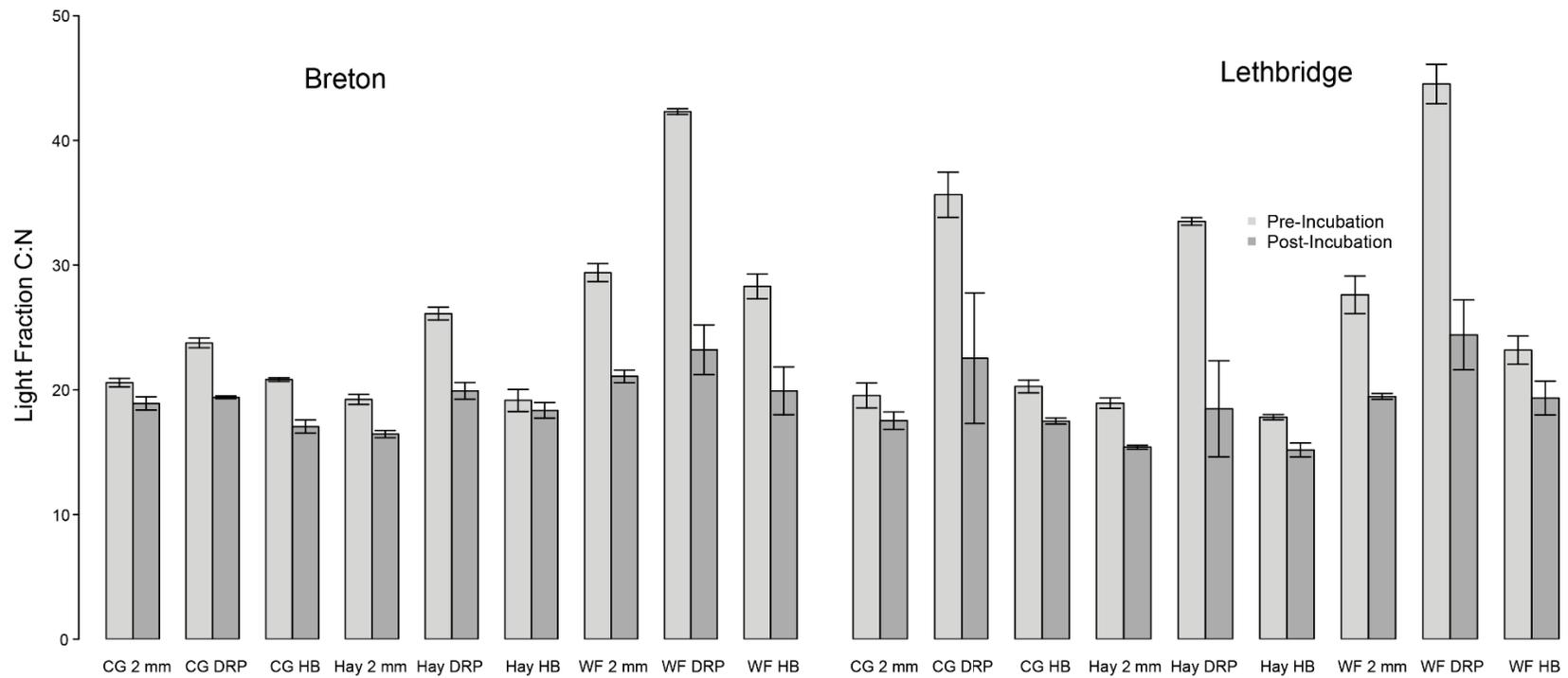
**Figure 2.11 Light fraction carbon as a percentage of soil pre- and post-incubation for all treatments. Where CG = continuous grain, WF = wheat-fallow, 2 mm = 2 mm roller mill, DRP = DRP mill, HB= hand broken. Error bars represent ± 1 standard error of the mean for each treatment**



**Figure 2.12 Nitrogen content of recovered light fraction pre- and post-incubation for all treatments. Where CG = continuous grain, WF = wheat-fallow, 2 mm = 2 mm roller mill, DRP = DRP mill, HB= hand broken. Error bars represent ± 1 standard error of the mean for each treatment**



**Figure 2.13 Soil nitrogen contributed from light fraction pre- and post-incubation for all treatments. Where CG = continuous grain, WF = wheat-fallow, 2 mm = 2 mm roller mill, DRP = DRP mill, HB= hand broken. Error bars represent ± 1 standard error of the mean for each treatment**



**Figure 2.14 C:N ratio for recovered light fraction pre- and post-incubation for all treatments. Where CG = continuous grain, WF = wheat-fallow, 2 mm = 2 mm roller mill, DRP = DRP mill, HB= hand broken. Error bars represent  $\pm 1$  standard error of the mean for each treatment**

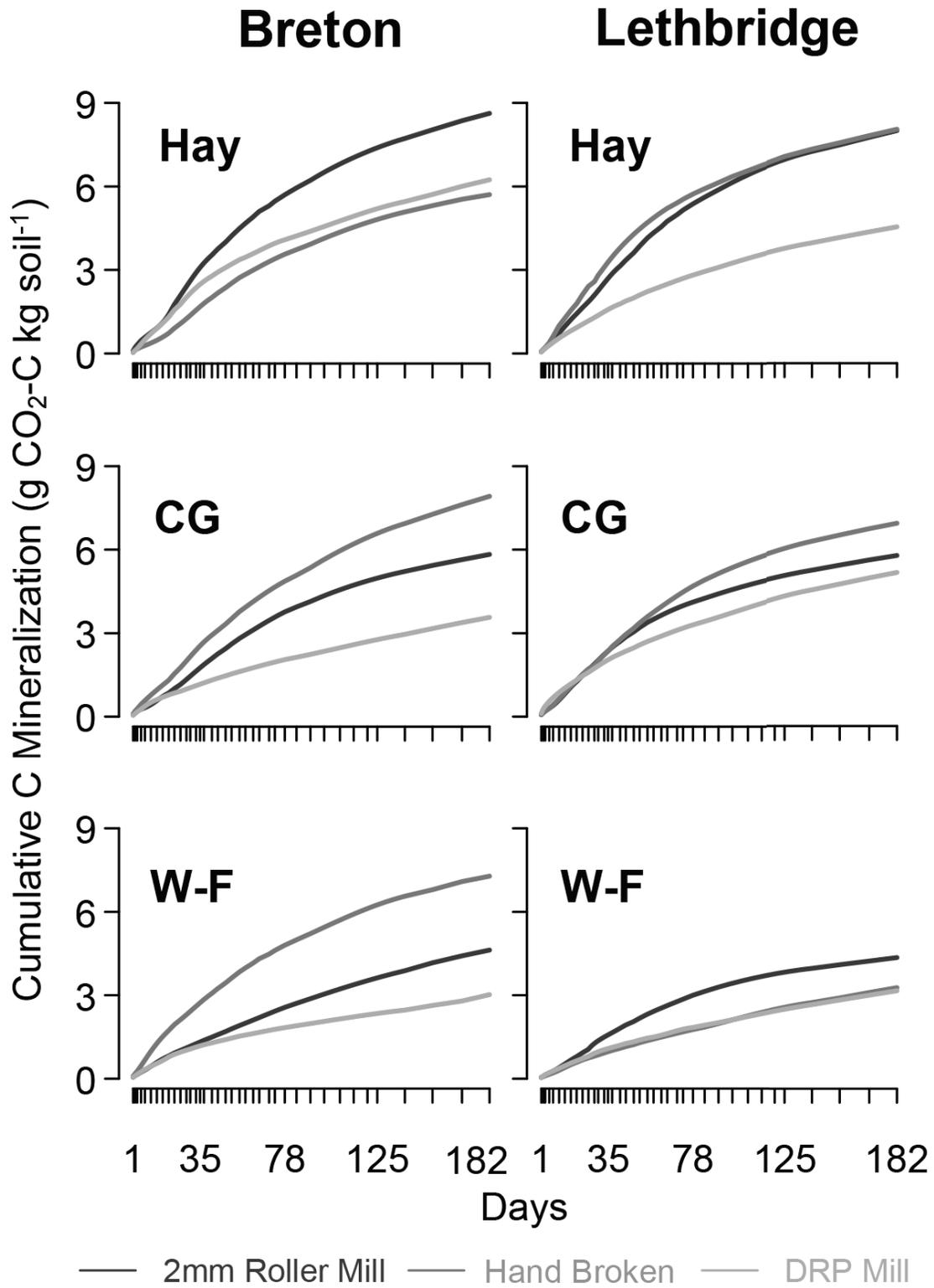


Figure 2.15 Cumulative mineralized carbon as respired CO<sub>2</sub> during incubation (182 days)

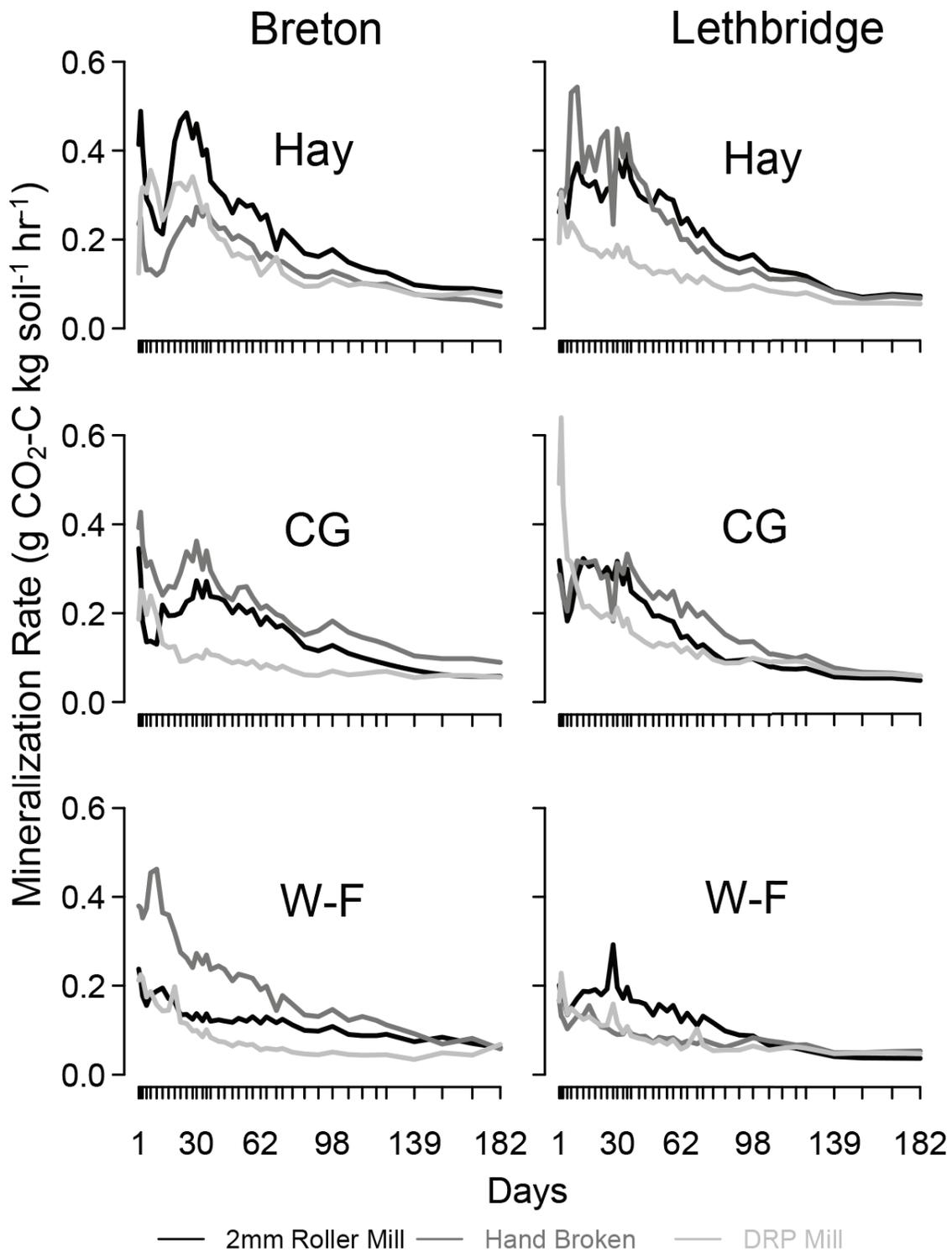


Figure 2.16 Rate of carbon mineralization as respired CO<sub>2</sub> during incubation (182 days)

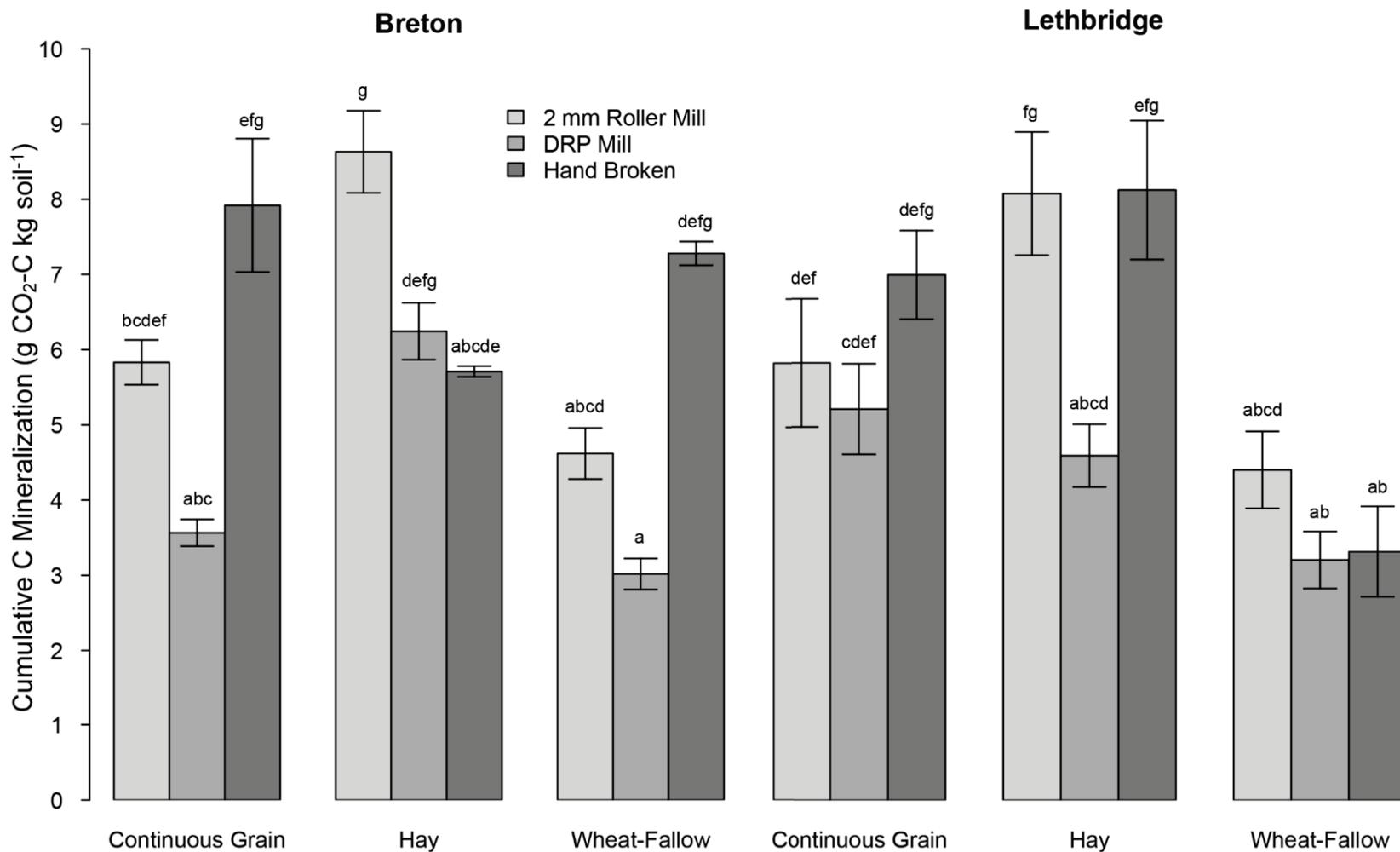


Figure 2.17 Cumulative mineralized carbon respired as CO<sub>2</sub> during incubation (182 days). Significantly different treatments represented by unique letter combinations (p-value < 0.05). Error bars represent ± 1 standard error of the mean for each treatment

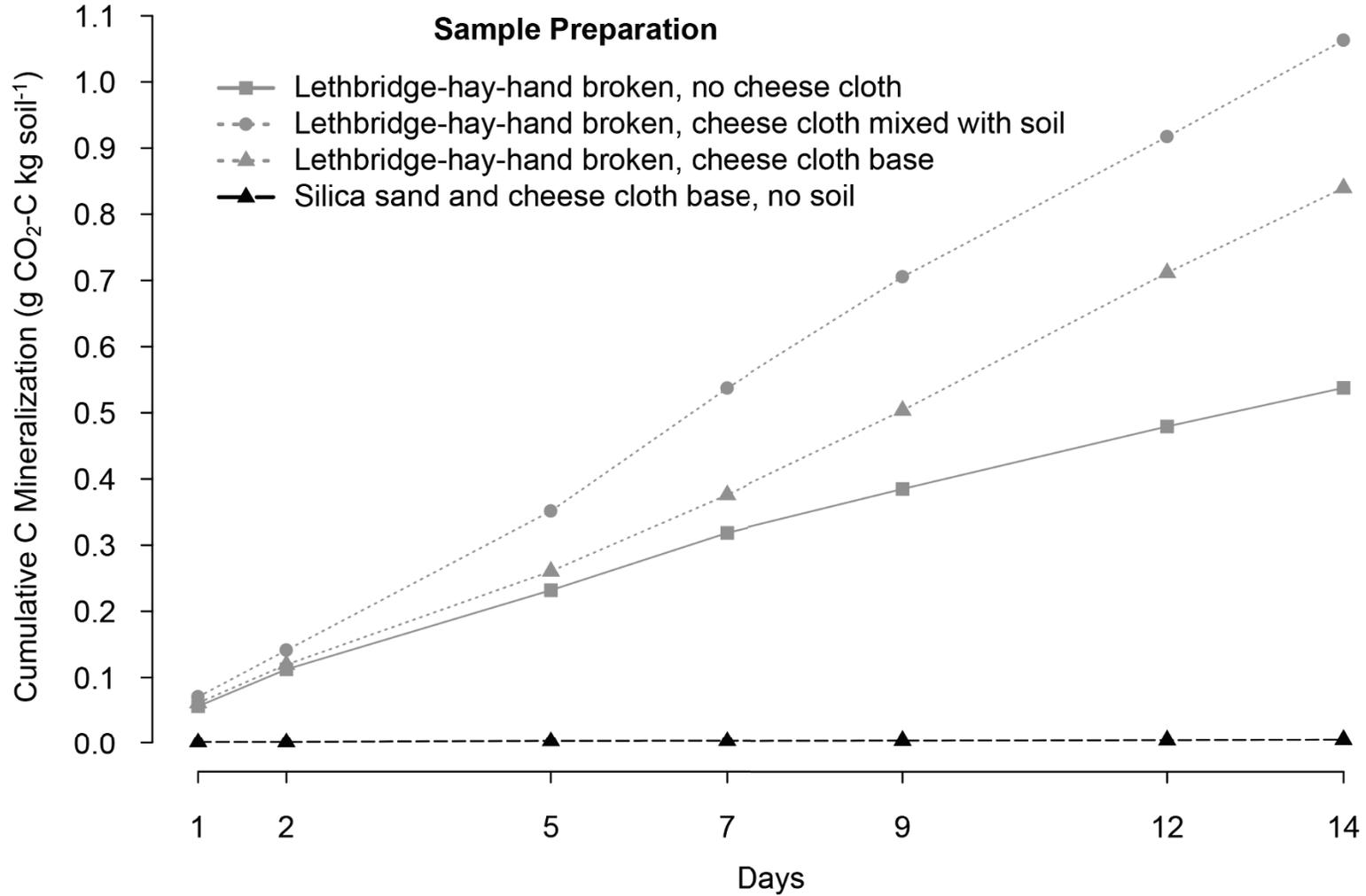


Figure 2.18 Cumulative mineralized carbon respired as CO<sub>2</sub> during supplemental incubation (14 days) with different cheesecloth configurations

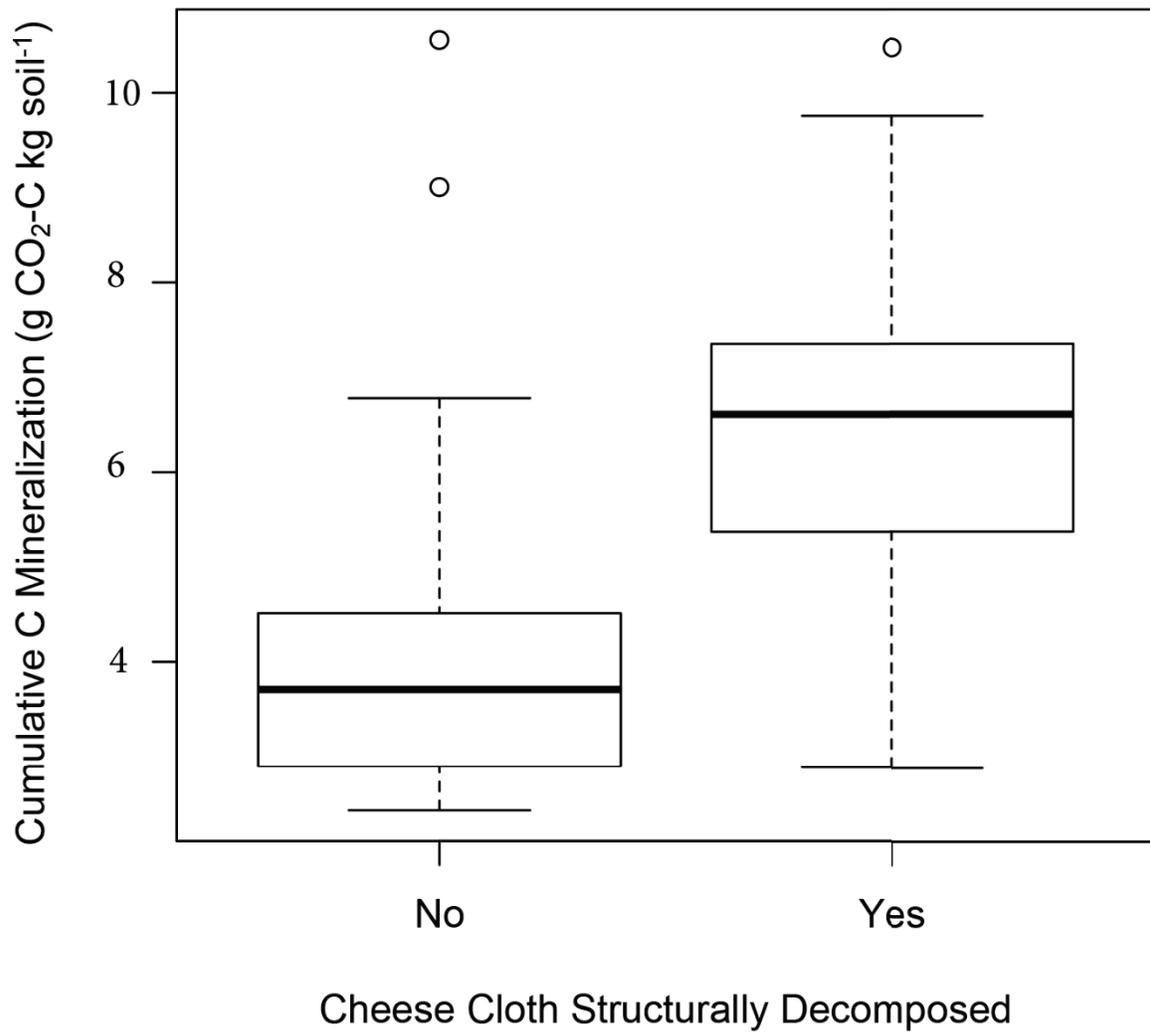


Figure 2.19 Cumulative mineralized carbon respired as CO<sub>2</sub> during incubation (182 days) for soil cores where structural decomposition of cheesecloth did or did not occur



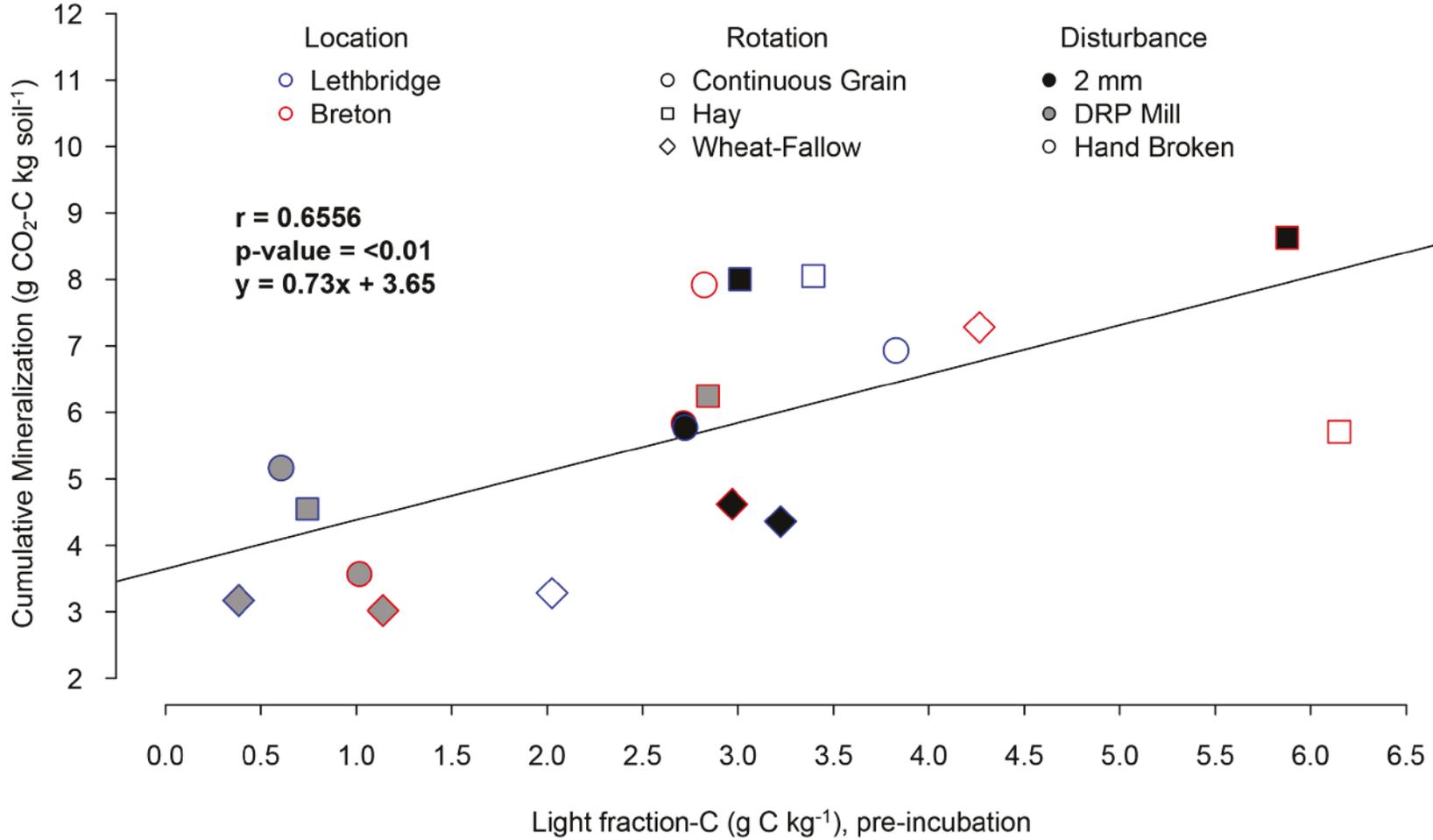
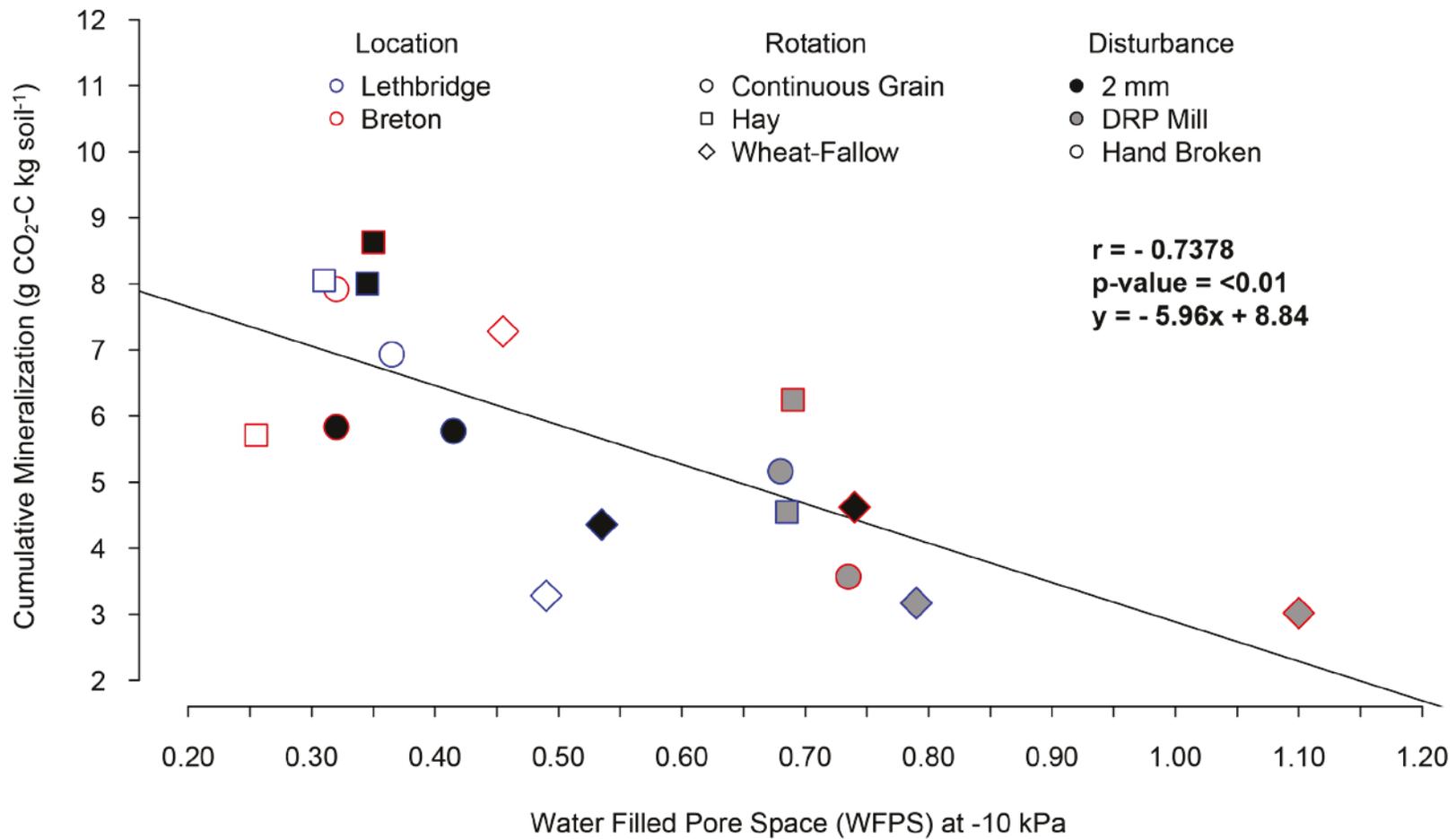


Figure 2.21 Correlation of cumulative mineralized carbon during incubation (182 days) with pre-incubation light fraction carbon content of soil



**Figure 2.22** Correlation of cumulative mineralized carbon during incubation (182 days) with the pre-incubation water filled pore space (WFPS) at -10 kPa

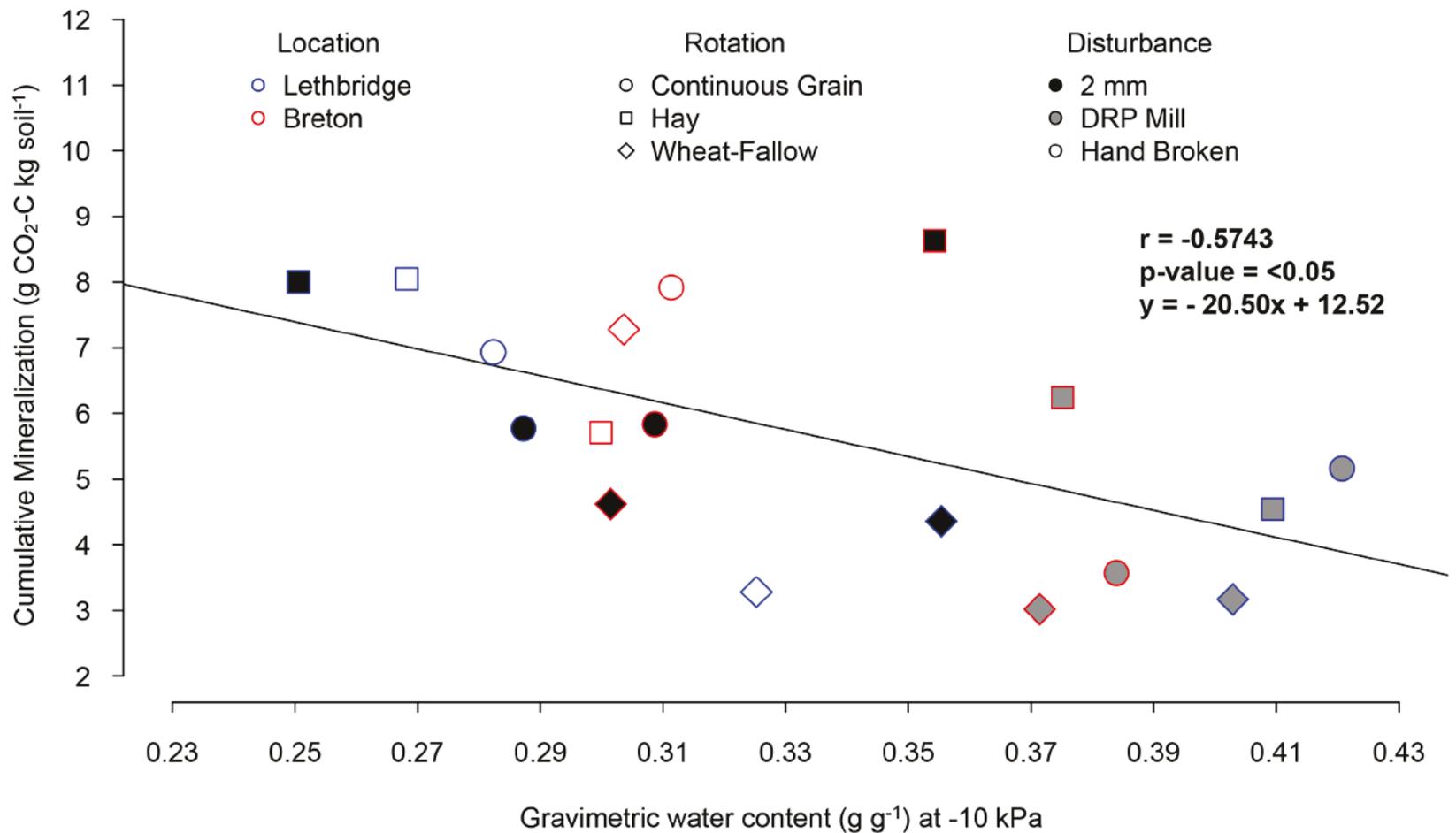


Figure 2.23 Correlation of cumulative mineralized carbon during incubation (182 days) with the pre-incubation gravimetric water content of soil at -10 kPa

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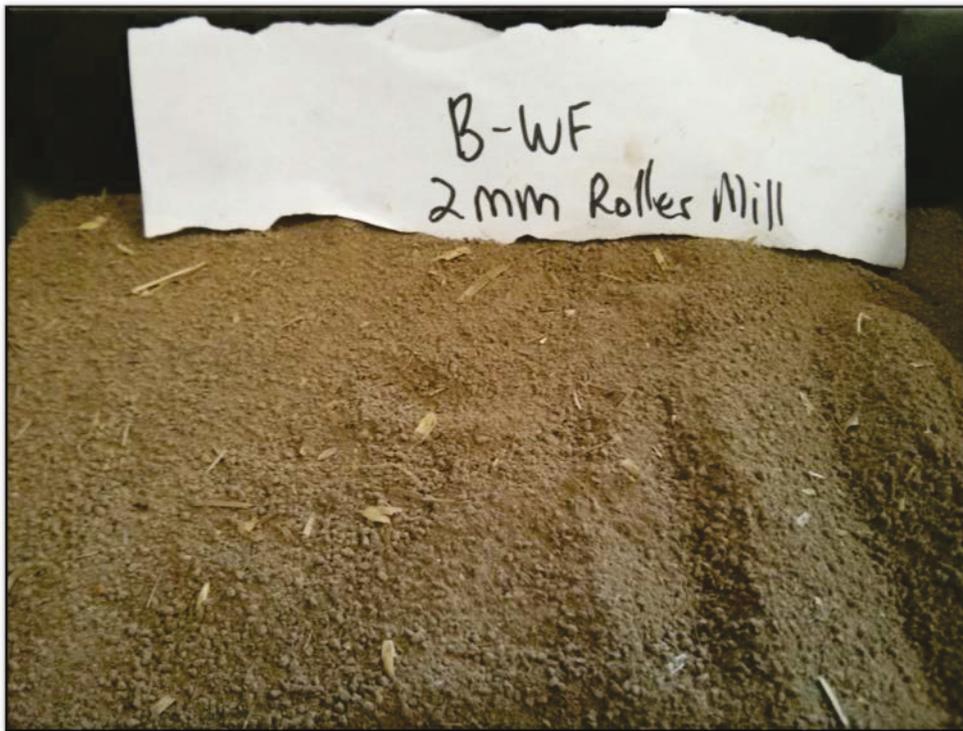
# Appendix A



**Photo 1. University of Alberta Ellerslie Research Station 2 mm roller mill**



**Photo 2. Single drum of 2 mm roller mill showing internal rollers**



**Photo 3. Soil following 2 mm roller mill treatment**



**Photo 4. Hand broken disturbance treatment (left) and coarse particulate matter removed (> 2.5 cm)**



**Photo 5. NRAL dish ring puck (DRP) mill**



**Photo 6. Soil following DRP mill treatment**



**Photo 7. Pressure extraction vessels**



**Photo 8. Saturated soil cores before pressure extraction**



**Photo 9. Prepared incubation chambers with soil samples**



**Photo 10. Headspace sampling event. Front row equilibrating with ambient atmosphere**



**Photo 11. Closet used to house the incubation chambers during the experiment**



**Photo 12. Intact soil sample after incubation**



Photo 13. Soil sample holder inside incubation chamber

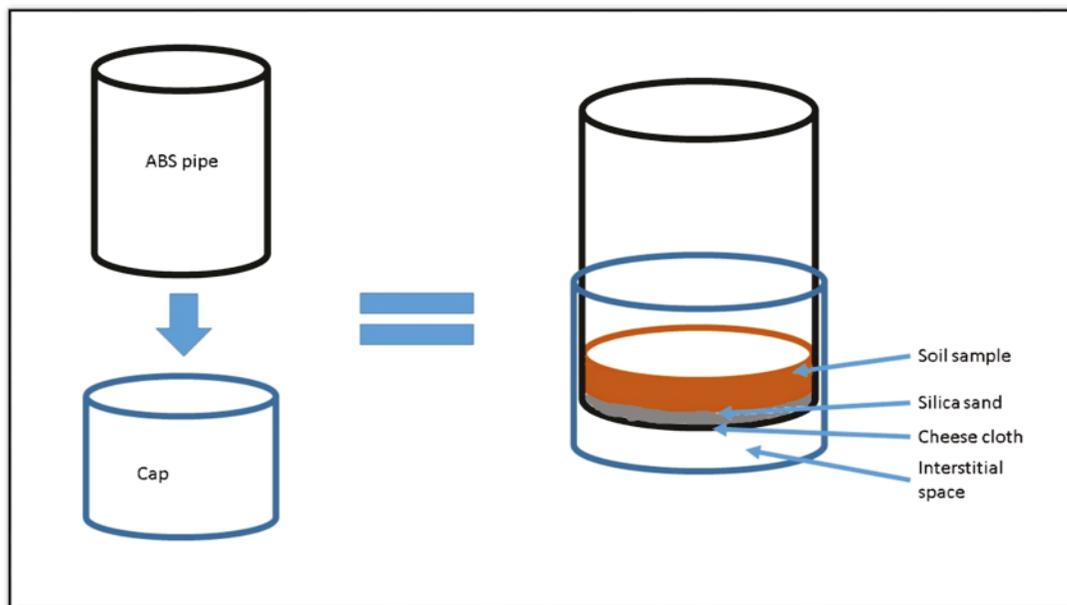


Photo 14. Incubation soil sample holder diagram



**Photo 15. Fallen soil sample due to cheesecloth decomposition**



**Photo 16. Fallen soil sample due to cheesecloth decomposition**



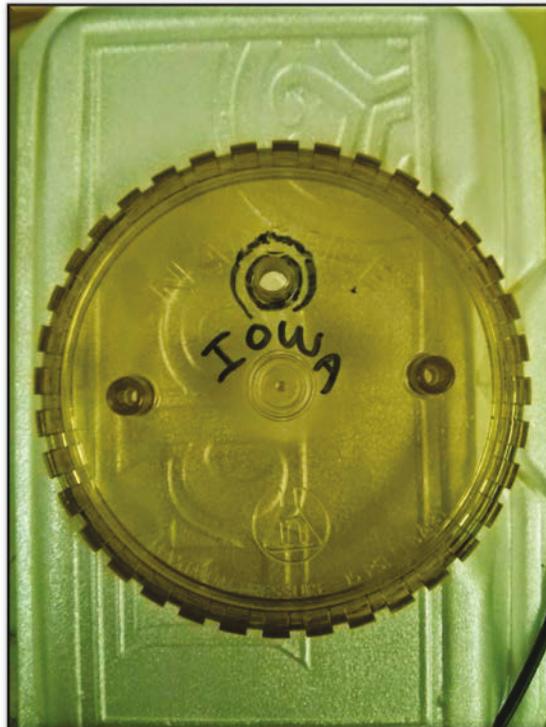
**Photo 17. Soil sample holder with intact cheesecloth at the of incubation**



**Photo 18. Cheesecloth decomposition where it was in contact with soil**



**Photo 19. Light fraction aspiration apparatus**



**Photo 20. Microlysimeter lid with enlarged port to prevent clogging of LF during aspiration**



**Photo 21. LF recovery in microlysimeter**



**Photo 22. LF recovery from Lethbridge wheat-fallow hand broken soil**



Photo 23. LF recovery from Lethbridge wheat-fallow 2 mm roller mill soil



Photo 24. LF recovery from Lethbridge wheat-fallow DRP mill soil

# **Appendix B**

**Table B.1 ANOVA table for pre-incubation gravimetric water content at -2 kPa, all treatments**

ANOVA Table (Type III tests)

Response: Gravimetric water content

	Sum Sq	Df	F value	Pr(>F)
(Intercept)	7.2602	1	40027.6037	< 2.2e-16 ***
Location	0.0365	1	201.4827	3.234e-11 ***
Rotation	0.0006	2	1.5402	0.2413017
Disturbance	0.0905	2	249.4656	7.526e-14 ***
Location:Rotation	0.0163	2	44.9644	9.982e-08 ***
Location:Disturbance	0.0040	2	10.9558	0.0007719 ***
Rotation:Disturbance	0.0103	4	14.2604	2.061e-05 ***
Location:Rotation:Disturbance	0.0055	4	7.6326	0.0008850 ***
Residuals	0.0033	18		

Significance codes: 0 '\*\*\*' 0.001 '\*\*' 0.01 '\*' 0.05 '.' 0.1 ' ' 1

**Table B.2 ANOVA table for pre-incubation gravimetric water content at -10 kPa, all treatments**

ANOVA Table (Type III tests)

Response: Gravimetric water content

	Sum Sq	Df	F value	Pr(>F)
(Intercept)	7.2602	1	40027.6037	< 2.2e-16 ***
Location	0.0365	1	201.4827	3.234e-11 ***
Rotation	0.0006	2	1.5402	0.2413017
Disturbance	0.0905	2	249.4656	7.526e-14 ***
Location:Rotation	0.0163	2	44.9644	9.982e-08 ***
Location:Disturbance	0.0040	2	10.9558	0.0007719 ***
Rotation:Disturbance	0.0103	4	14.2604	2.061e-05 ***
Location:Rotation:Disturbance	0.0055	4	7.6326	0.0008850 ***
Residuals	0.0033	18		

Significance codes: 0 '\*\*\*' 0.001 '\*\*' 0.01 '\*' 0.05 '.' 0.1 ' ' 1

**Table B.3 ANOVA results for total pre-incubation SOC content, all treatments**

ANOVA Table (Type III tests)

Response: Total SOC (%)

	Sum Sq	Df	F value	Pr(>F)
(Intercept)	7.5883	1	10726.6162	< 2.2e-16 ***
Location	0.0206	1	29.0514	4.544e-06 ***
Rotation	0.8181	2	578.2133	< 2.2e-16 ***
Disturbance	0.0099	2	6.9667	0.002769 **
Location:Rotation	0.1209	2	85.4678	2.130e-14 ***
Location:Disturbance	0.0071	2	5.0158	0.011980 *
Rotation:Disturbance	0.0041	4	1.4422	0.240128
Location:Rotation:Disturbance	0.0023	4	0.7952	0.536163
Residuals	0.0255	36		

Significance codes: 0 '\*\*\*' 0.001 '\*\*' 0.01 '\*' 0.05 '.' 0.1 ' ' 1

**Table B.4 ANOVA results for pre-incubation total nitrogen content, all treatments**

ANOVA Table (Type III tests)

Response: Total nitrogen (%)

	Sum Sq	Df	F value	Pr(>F)
(Intercept)	1698.59	1	16486.0048	< 2.2e-16 ***
Location	0.04	1	0.3501	0.55773
Rotation	85.14	2	413.1526	< 2.2e-16 ***
Disturbance	0.73	2	3.5473	0.03925 *
Location:Rotation	14.42	2	69.9935	3.934e-13 ***
Location:Disturbance	0.51	2	2.4926	0.09686 .
Rotation:Disturbance	0.75	4	1.8304	0.14434
Location:Rotation:Disturbance	0.05	4	0.1195	0.97467
Residuals	3.71	36		

Significance codes: 0 '\*\*\*' 0.001 '\*\*' 0.01 '\*' 0.05 '.' 0.1 ' ' 1

**Table B.5 ANOVA results for pre-incubation total C:N ratio, all treatments**

ANOVA Table (Type III tests)

Response: C:N

	Sum Sq	Df	F value	Pr(>F)
(Intercept)	7357.0	1	48848.3502	< 2.2e-16 ***
Location	5.5	1	36.2561	6.509e-07 ***
Rotation	0.3	2	0.9660	0.390232
Disturbance	0.6	2	2.0910	0.138322
Location:Rotation	4.1	2	13.6647	3.842e-05 ***
Location:Disturbance	0.2	2	0.6513	0.527407
Rotation:Disturbance	2.8	4	4.6555	0.003929 **
Location:Rotation:Disturbance	0.8	4	1.4077	0.251111
Residuals	5.4	36		

Significance codes: 0 '\*\*\*' 0.001 '\*\*' 0.01 '\*' 0.05 '.' 0.1 ' ' 1

**Table B.6 ANOVA table for pre-incubation soil light fraction content (g LF kg soil<sup>-1</sup>), all treatments**

ANOVA Table (Type III tests)

Response: LF content

	Sum Sq	Df	F value	Pr(>F)
(Intercept)	37.290	1	2.8766e+05	< 2.2e-16 ***
Location	0.016	1	1.2473e+02	2.994e-13 ***
Rotation	0.017	2	6.3951e+01	1.416e-12 ***
Disturbance	0.173	2	6.6677e+02	< 2.2e-16 ***
Location:Rotation	0.008	2	2.9258e+01	2.847e-08 ***
Location:Disturbance	0.012	2	4.7743e+01	7.475e-11 ***
Rotation:Disturbance	0.003	4	4.8436e+00	0.003141 **
Location:Rotation:Disturbance	0.002	4	4.2426e+00	0.006475 **
Residuals	0.005	36		

Significance codes: 0 '\*\*\*' 0.001 '\*\*' 0.01 '\*' 0.05 '.' 0.1 ' ' 1

**Table B.7 ANOVA table for pre-incubation soil light fraction carbon content (g LF C kg soil<sup>-1</sup>), all treatments**

ANOVA Table (Type III tests)

Response: LF C content

	Sum Sq	Df	F value	Pr(>F)
(Intercept)	68.596	1	952.9125	< 2.2e-16 ***
Location	6.606	1	91.7681	3.059e-13 ***
Rotation	5.611	2	38.9739	3.340e-11 ***
Disturbance	19.136	2	132.9184	< 2.2e-16 ***
Location:Rotation	1.097	2	7.6189	0.001217 **
Location:Disturbance	0.553	2	3.8388	0.027618 *
Rotation:Disturbance	7.077	4	24.5775	1.278e-11 ***
Location:Rotation:Disturbance	9.501	4	32.9945	6.363e-14 ***
Residuals	3.887	54		

Significance codes: 0 '\*\*\*' 0.001 '\*\*' 0.01 '\*' 0.05 '.' 0.1 ' ' 1

**Table B.8 ANOVA results for total post-incubation SOC, all treatments**

ANOVA Table (Type III tests)

Response: Total SOC (%)

	Sum Sq	Df	F value	Pr(>F)
(Intercept)	51.440	1	5.8783e+05	< 2.2e-16 ***
Location	0.001	1	1.0940e+01	0.001678 **
Rotation	0.129	2	7.3424e+02	< 2.2e-16 ***
Disturbance	0.000	2	4.7900e-01	0.622012
Location:Rotation	0.027	2	1.5595e+02	< 2.2e-16 ***
Location:Disturbance	0.001	2	3.4284e+00	0.039654 *
Rotation:Disturbance	0.007	4	1.9276e+01	6.705e-10 ***
Location:Rotation:Disturbance	0.004	4	1.1813e+01	5.786e-07 ***
Residuals	0.005	54		

Significance codes: 0 '\*\*\*' 0.001 '\*\*' 0.01 '\*' 0.05 '.' 0.1 ' ' 1

**Table B.9 ANOVA results for total post-incubation nitrogen, all treatments**

ANOVA Table (Type III tests)

Response: Total nitrogen (%)

	Sum Sq	Df	F value	Pr(>F)
(Intercept)	424.44	1	92082.4144	< 2.2e-16 ***
Location	0.00	1	0.0091	0.9245158
Rotation	4.15	2	450.4184	< 2.2e-16 ***
Disturbance	0.07	2	7.6396	0.0011977 **
Location:Rotation	0.91	2	99.0440	< 2.2e-16 ***
Location:Disturbance	0.00	2	0.5136	0.6012459
Rotation:Disturbance	0.10	4	5.5960	0.0007648 ***
Location:Rotation:Disturbance	0.15	4	8.2303	2.941e-05 ***
Residuals	0.25	54		

Significance codes: 0 '\*\*\*' 0.001 '\*\*' 0.01 '\*' 0.05 '.' 0.1 ' ' 1

**Table B.10 ANOVA results for total post-incubation C:N ratio, all treatments**

ANOVA Table (Type III tests)

Response: C:N

	Sum Sq	Df	F value	Pr(>F)
(Intercept)	9027.2	1	72607.0153	< 2.2e-16 ***
Location	3.7	1	30.0889	1.124e-06 ***
Rotation	0.3	2	1.2643	0.29065
Disturbance	6.8	2	27.2717	6.506e-09 ***
Location:Rotation	0.9	2	3.5473	0.03569 *
Location:Disturbance	0.6	2	2.4124	0.09920 .
Rotation:Disturbance	5.7	4	11.5423	7.645e-07 ***
Location:Rotation:Disturbance	1.1	4	2.1640	0.08541 .
Residuals	6.7	54		

Significance codes: 0 '\*\*\*' 0.001 '\*\*' 0.01 '\*' 0.05 '.' 0.1 ' ' 1

**Table B.11 ANOVA table for post-incubation soil light fraction content (g LF kg soil<sup>-1</sup>), all treatments**

ANOVA Table (Type III tests)

Response: LF content

	Sum Sq	Df	F value	Pr(>F)
(Intercept)	125.878	1	10192.3620	< 2.2e-16 ***
Location	1.228	1	99.4097	7.593e-14 ***
Rotation	1.178	2	47.6968	1.169e-12 ***
Disturbance	8.129	2	329.0968	< 2.2e-16 ***
Location:Rotation	0.185	2	7.4709	0.001366 **
Location:Disturbance	0.059	2	2.3859	0.101645
Rotation:Disturbance	0.654	4	13.2461	1.382e-07 ***
Location:Rotation:Disturbance	0.921	4	18.6335	1.129e-09 ***
Residuals	0.667	54		

Significance codes: 0 '\*\*\*' 0.001 '\*\*' 0.01 '\*' 0.05 '.' 0.1 ' ' 1

**Table B.12 ANOVA table for post-incubation soil light fraction carbon content (g LF C kg soil<sup>-1</sup>), all treatments**

ANOVA Table (Type III tests)

Response: LF C content

	Sum Sq	Df	F value	Pr(>F)
(Intercept)	57.271	1	8350.1717	< 2.2e-16 ***
Location	0.640	1	93.3464	2.280e-13 ***
Rotation	0.638	2	46.5007	1.807e-12 ***
Disturbance	3.993	2	291.1117	< 2.2e-16 ***
Location:Rotation	0.062	2	4.5252	0.01524 *
Location:Disturbance	0.022	2	1.5780	0.21575
Rotation:Disturbance	0.328	4	11.9620	4.968e-07 ***
Location:Rotation:Disturbance	0.586	4	21.3714	1.308e-10 ***
Residuals	0.370	54		

Significance codes: 0 '\*\*\*' 0.001 '\*\*' 0.01 '\*' 0.05 '.' 0.1 ' ' 1

**Table B.13 ANOVA table for incubation cumulative mineralized carbon linear model, all treatments**

ANOVA Table (Type III tests)

Response: Cumulative mineralized carbon<sup>0.45</sup> (Box-cox transformed)

	Sum	Sq Df	F value	Pr(>F)
(Intercept)	13.3524	1	477.8299	< 2.2e-16 ***
Location	0.0090	1	0.3212	0.5732625
Rotation	2.7611	2	49.4052	7.741e-13 ***
Disturbance	1.6223	2	29.0276	3.066e-09 ***
Gravimetric Water Content	0.3875	1	13.8666	0.0004768 ***
Location:Rotation	0.2847	2	5.0942	0.0094712 **
Location:Disturbance	0.0499	2	0.8921	0.4158619
Rotation: Disturbance	0.6215	4	5.5603	0.0008192 ***
Location:Rotation: Disturbance	1.3603	4	12.1704	4.342e-07 ***
Residuals	1.4810	53		

Significance codes: 0 '\*\*\*' 0.001 '\*\*' 0.01 '\*' 0.05 '.' 0.1 ' ' 1