

## **Soil Organic Matter Dynamics in Long-term Temperate Agroecosystems:**

### **Rotation and Nutrient Addition Effects**

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## Abstract

Soil organic matter (SOM) is a major driver of key agroecosystem functions. Our objective was to examine the dynamics of organic matter in whole soil, particulate (POM; > 53  $\mu\text{m}$  size) and mineral-associated (MAOM) fractions under varying crop rotations and nutrient managements at two long-term experimental sites (Breton and Lethbridge). Soil samples were collected from simple (2 yrs) and complex (5 or 6 yrs) crop rotations at the 5-10 cm depth. We found associations between SOM pools versus microbial community and soil aggregation. Compared to cropped soils, an adjacent forest exhibited a significantly higher soil total organic carbon (TOC) and a shift in SOM fractions with substantially higher POM. However, the forest soil had the lowest microbial biomass C (MBC) among all the assessed land use systems ( $P < 0.05$ ), suggesting that other factors than the amount of labile SOM (i.e., POM-C) were controlling the microbial community. When contrasted to simple 2-yr rotations, the complex rotations including perennials and legumes significantly raised TOC and soil total nitrogen (TN) as well as the stable SOM fraction (i.e., MAOM-C and -N) consistently for both Breton and Lethbridge sites. Our findings highlight that varying land managements have profound feedbacks on soil quality as mediated by alterations in long-term SOM dynamics.

## Keywords:

Agroecosystem, Microbial biomass, Soil organic matter, Aggregation, Soil quality

## Introduction

Retaining and developing soil quality is crucial for achieving sustainable agriculture systems. When focusing on soil quality status, the quantity and composition of soil organic matter (SOM) emerges as one of the key criteria that govern the overall performance of most land use systems (Coulter et al., 2009). Among soil properties, SOM plays a driving role on soil quality responses by supporting soil nutrient turnover and availability (Curtin et al., 2012). Most nutrients necessary for the growth of plants and soil organisms are sourced by SOM mineralization. Within common agriculture systems, implementation of beneficial land management practices and cropping strategies is a feasible approach to improve SOM contents and enhance soil quality (Coulter et al., 2009). As suggested by earlier long-term studies (Shrestha et al., 2015), when changing crop management practices such as nutrients additions and crop rotation sequences, the contents, distribution and transformation of SOM pools responded in conjunction with alterations in other physical, chemical and biological soil properties (Islam and Weil, 2000).

Examination of SOM partitioning and allocation between labile particulate organic matter (POM) and stabilized mineral associated organic matter (MAOM) under contrasting land management can be an effective approach to understand how soil, plants and microbes interact with management and environmental conditions, influence C cycling, and change soil quality (Figueiredo et al., 2010; Castro et al., 2015). While the labile POM pool cycles rapidly and controls the short-term availability of nutrients (including carbon that supports microbial activity and

decomposition processes), the stable MAOM pool serves as a long-term nutrient storage reservoir with relatively slow turnover rate in soils; hence, both POM and MAOM pools mediate the effects of management practices on the capacity or quality of soils to sustain agricultural systems (Gregorich et al., 1994; Muñoz-Romero et al., 2017). As nitrogen and carbon dynamics influence and interplay with several other key soil properties and processes (nutrient availability, biological activity, soil structure, and water availability), the dynamics of these SOM pools is a multi-faceted, paramount controlling factor for soil quality in soil-plant systems (Gregorich et al., 1994; Bhowmik et al., 2017).

Particulate organic matter (POM), which typically represents the most recent addition of the plant material to the soil, has been proven as a sensitive indicator of the change in soil C and N under different managements (Cambardella & Elliott, 1993; Bu et al., 2015). When comparing two simple biennial crop rotations, Bu et al. (2015) documented increases in N mineralization and microbial biomass with higher presence and decomposition of POM. They attributed these relationships to varying quality and bioavailability of POM fraction as a substrate and source for N supply. Previous reports describe the impacts of common cropping systems and intensity on SOM accumulation in the short term, but few studies have focused on the change of labile POM and stabilized MAOM pools under long-term experimentation where management effects and SOM partitioning can be assessed once the system has reached steady state. A recent study reported no long-term effects of various simple

biennial rotations on POM concentrations (Diochon et al. 2016); however, reports assessing complex, diverse crop rotations are still lacking in the literature. Moreover, intensive manure or fertilizer addition has been regarded as an approach to increase soil TOC and fertility (Liu et al, 2013). However, few studies have focused on how long-term application of manuring versus inorganic fertilization may differentially affect the concentration and distribution of organic C fractions and soil quality indicators. Such new knowledge will aid to understand the different functions and feedbacks of varying cropping and nutrient addition practices on SOM transformations and accrual.

Earlier studies show that crop rotation systems including diverse crop species have significant effect on stabilizing the soil macroaggregates as well as improving porosity and bulk density (Balota et al., 2004). Such improvement in soil macroaggregation has been associated with the enhancement of the soil POM fraction (Aoyama et al., 1999). Castro et al. (2015) also recently reported increased POM as a function of increased crop diversity in their rotations. Moreover, changes in SOM pools and transformations can also have a great impact on soil biology. Soil biological properties have increasingly been studied by researchers as another sensitive indicator of changes in soil quality as caused by the choice of cropping management options (Kennedy & Papendick, 1995). This preeminent focus on soil biology is in part because microbial community plays a key mediating role in SOM turnover processes (Powlson et al., 1987). Previous reports showed that a greater soil microbial activity

was observed in diverse crop rotation systems compared to traditional mono-cropping management (Dick, 1984). However, the specific relationships between SOM pools and the associated microbial community are still subject to examination and further characterization. Hence, there is a need to continue investigating how shifts in SOM pools relate to changes in microbial community as well as how the presence of particular microbial species changes under different soil conditions and contrasting land use systems. The objectives of our study were to: (i) evaluate the long-term impacts of different crop rotations and nutrient additions on SOM accrual, pools, and distribution, and (ii) assess functional relationships between SOM size-fractions and selected soil quality indicators including structure and microbial biomass.

## **Materials and Methods**

### **3.1. Study sites**

Our study was conducted at two long-term field sites. The University of Alberta Experiment Plots in Breton (53.089°N, 114.442°W) and the Agriculture and Agri-Food Canada's Lethbridge Research and Development Centre (49.705°N, 112.775°W). The loamy soils in Breton and Lethbridge plots are classified as Orthic Gray Luvisols and Orthic Dark Brown Chernozems, respectively, based on the Canadian System of Soil Classification (AGRASID, 2015). Particle size, analyzed through the hydrometer method, showed a granulometric distribution of 362 g kg<sup>-1</sup> of sand (2000–50 µm size diameter), 444 g kg<sup>-1</sup> of silt (50–2 µm), and 194 g kg<sup>-1</sup> of clay (<2 µm) for Breton and 432 g kg<sup>-1</sup> of sand, 304 g kg<sup>-1</sup> of silt and 264 g kg<sup>-1</sup> of clay for

Lethbridge (Kiani, et al. 2017). Soil clay mineralogy in both sites is dominant by smectite, kaolinite and illite, with presence of evaporates such as calcite and gypsum in Lethbridge (Pawluk et al., 1961; Kohut and Dudas, 1993; Kohut and Dudas, 1994; Plante et al., 2010). Bulk density of Breton soils under cropping ranged from 1.27-1.41 g cm<sup>-3</sup>, while for soils in Lethbridge, bulk density lies within the range of 1.32 to 1.50 g cm<sup>-3</sup>. According to the long-term climate data (20 yrs, Jan 1997 to Dec 2006) collected from permanent weather stations located in each field sites, the mean annual air temperature is 2.1 °C in Breton and 5 °C in Lethbridge, and the mean annual precipitation is 547 ± 37 mm in Breton (ranging from 374 to 726 mm), and 402 ± 43 mm in Lethbridge (ranging from 204 to 608 mm) (Alberta - Weather Conditions and Forecast, 2016).

### 3.2. Soil sample collection and analysis method

Two crop rotations, both established in 1929, were selected for our study at the Breton Plots site. One is a 2-yr wheat–fallow rotation and the other is a 5-yr rotation with a sequence of wheat–oat–barley/hay–hay1–hay2. Alfalfa and brome-grass were included in the hay phases since 1967 (Kiani et al., 2017). Three nutrient managements were applied for each rotation in Breton, including balanced fertilization (N-P-K-S fertilizer), manure, and control which received no additional nutrients. In balanced fertilization management, inorganic fertilizers were applied at the rates of 22 kg P ha<sup>-1</sup>, 46kg K ha<sup>-1</sup>, 5.5 kg S ha<sup>-1</sup>, and the N rate depended on the crop and its place in the rotation (wheat on fallow: 90 kg N ha<sup>-1</sup>; wheat after forage:

50 kg N ha<sup>-1</sup>; oats or barley after wheat: 75 kg N ha<sup>-1</sup>; barley under seeded to hay: 50 kg N ha<sup>-1</sup>; legume-grass forages: 0 kg N ha<sup>-1</sup>). The N application via manure also depended on the rotation (2-yr rotation: 90 kg N ha<sup>-1</sup> during cropped years; cereal crops in 5-yr rotation: 175 kg N ha<sup>-1</sup> every 5 years applied in two equal applications) (Kiani et al., 2017).

Field samples at the fallow phase in 2-yr rotation and the samples at oat and Hay2 phases in 5-yr rotation were collected in June 2014 and 2015 shortly following spring crop planting in order to examine the effects of the previous year's rotation phase (i.e., wheat phase in 2yr rotation, wheat phase in 5yr rotation and hay1 phase in 5yr rotation, respectively). Soil samples from an adjacent forest were also collected as a reference location that represented the native vegetation in Breton site. With three nutrient managements for each of the selected phases, there were in total ten sampling locations at the Breton site.

For the Lethbridge site, there were also two rotations involved: a 2-yr wheat–fallow rotation established in 1985 and a 6-yr fallow–wheat1–wheat2–alfalfa1–alfalfa2–alfalfa3 rotation established in 1951. Alfalfa and crested wheat grass were included in the alfalfa phases in the 6yr rotation. The fertilization management included ammonium nitrate broadcasted in the early spring prior to any tillage or seeding at a rate of 45 kg N ha<sup>-1</sup> on the wheat phase of the 2-yr rotation. Fertilizer P (0-45-0) was applied to wheat at 22.5 kg P ha<sup>-1</sup> from 1985 to 1994, 20 kg P ha<sup>-1</sup> from 1995 to 2000, and 11 kg P ha<sup>-1</sup> from 2001 to present. For the 6-yr rotation, triple super



phosphate was broadcasted at a rate of  $50 \text{ kg P ha}^{-1}$  prior to the spring tillage during the establishment of the alfalfa1 plots. The experiment is arranged in a randomized complete block design. The four replicates in this experiment were sampled for each selected phase. In further detail, all phases within the two rotations were sampled except alfalfa2 in the 6-yr rotation, so there were 28 sampling plots in total (Kiani et al., 2017). In contrast to the Breton site where an adjacent native vegetation (forest) location was available for soil sampling, an immediately adjacent location to sample soils under native vegetation (prairie) was unavailable in Lethbridge site. At both study sites, aboveground plant residues were left on the soil surface following the grain harvest operations for the annual crops and the forage hay removal for the grass-legume mixes.

For chemical and microbial analyses, disturbed soil samples were collected in each field by mixing 4 subsamples taken using a 2-cm inner diameter push probe (three samples per plot for Breton, and one for Lethbridge). These disturbed samples were placed in the Whirl-Pak® (Nasco, Fort Watkins, Wisconsin) sterile sampling bags and transported in an icebox to the laboratory. Samples for microbial characterization were kept frozen at  $-86 \text{ }^{\circ}\text{C}$  until they were freeze-dried in preparation for analysis. Within each sampling plot, all soil samples were taken at randomly selected sampling points and at the depth increment of 5-10 cm. Soil undisturbed clods ( $\sim 500 \text{ cm}^3$ ) were also excavated from each plot with a shovel at the 5-cm depth (three samples per plot for Breton, and two for Lethbridge). Clods

were wrapped in aluminum foil and plastic sampling bags to prevent significant moisture loss, and placed in a plastic container to minimize disturbance during transport from the field. The field protocol for collecting undisturbed samples consistently excluded the top 5 cm of soil to prevent potential extreme effects due to high presence of plant roots and litter near soil surface in particular in soils under forest and perennial crops as well as surface crusting and excessive dryness in particular in soils under fallow. As a sole soil sampling depth, the 5-10 cm increment was collected to reflect and compare the potential effects of contrasting land management systems on soil quality and SOM dynamics.

### **3.3. Soil particulate organic matter**

The particulate organic matter (POM) fraction in our study referred to all soil organic matter particles between 0.053 to 2 mm in size diameter (Cambardella and Elliot, 1992, Hernandez-Ramirez et al., 2011). Prior to dispersing the soil, moisture content of each air-dried soil sample was measured by drying 10 g of soil sample in the oven (50 °C) for 24 hours to conduct a moisture content correction. Soil dispersion was conducted using sodium hexametaphosphate as recommended in international standard ISO 11277; this chemical method aims at dispersing aggregates into soil particles (Ryzak and Bieganowski, 2011). Ten grams (oven dry equivalent weight) of non-ground, air dried soil sample, 20 mL of degassed deionized water, and 10 mL of 1.5% Na-hexametaphosphate were added into a 50 mL centrifuge tubes and dispersed overnight (24 hr) by horizontal reciprocal shaker at a rate of 120 rpm. After

dispersion, soils were transferred through a set of nested sieves with mesh size of 2 and 0.053 mm to obtain different soil size fractions. Organic matter remaining on the 0.053-mm sieve was the POM fraction with a size range of 0.053 to 2.0 mm. POM on the 0.053 mm sieve was transferred into a beaker and oven dried at a temperature of 50 °C to remove all the moisture until constant mass was achieved (72 hours). The POM samples in the beaker were weighted, ground to powder consistency and stored in 20 mL vials at room temperature.

### 3.4. Soil carbon and nitrogen

Particulate organic matter carbon (POM-C) and particulate organic matter nitrogen (POM-N) of each soil sample were measured by the dry combustion method using a Costech ECS 4010 Elemental Analyzer (Costech Analytical Technologies Inc., Valencia, CA, USA). This method was also used to measure the content of total soil organic carbon (TOC) and soil total nitrogen (TN) from composited, ground soil samples. In our research, we assumed that the total organic carbon (TOC) present in the whole soil can be further divided into two fractions: POM-C and MAOM-C. To calculate the concentration of MAOM-C, we simply subtracted the concentrations ( $\text{g kg}^{-1}$  soil) of total organic carbon in whole soil from the concentration of the POM-C for each soil sample as follow:

$$\text{MAOM-C} = \text{Whole soil TOC} - \text{POM-C} \quad [1]$$

Fractions of POM-C and MAOM-C relative to the total soil carbon (ratios) were also calculated to examine the proportional change, partitioning and allocation

of organic matter into these C pools as a function of land management. These calculation approaches were also applied to the soil nitrogen data to derive MAOM-N concentration and fraction, and POM-N fraction.

### 3.5. Soil fractal aggregation

One approach to quantify the development of soil aggregation is by determining the mass fractal dimension of the soil aggregates. Fractal dimension ( $D_m$ ) is a measure of how soil aggregates are hierarchically organized in soils and can be obtained using the following equation:

$$M(v) = K_m V^{D_m} \quad [2]$$

In this equation,  $M(v)$  is the mass of aggregates (g) with volume  $v$  ( $\text{cm}^3$ ),  $k_m$  is a constant representing the mass of aggregate unit volume, and  $D_m$  is the fractal dimension (Hebb et al., 2017; Kiani et al., 2017). Larger aggregates have a lower mass to volume ratio as explained by the porosity exclusion principle (Hirmas et al., 2013); denser micro-aggregates are bonded to form macro-aggregates with greater porosity. Therefore, a lower the  $D_m$  value represents an increasing hierarchical aggregation and improved soil structure. The laboratory analyses consisted of quantifying mass and volume for a range of undisturbed soil aggregates using multistriple laser triangulation scanning (3D Scanner Ultra HD, NextEngine, California). Parent clods ( $\sim 500 \text{ cm}^3$ ) were broken down into five smaller size classes (i.e. 4-8, 2-4, 1-2, 0.5-1, and 0.25-0.5 cm diameter) and two aggregates from each class were randomly selected and scanned to obtain a 3D image to determine

aggregate volume. Repeated laser scanning ( $n=20$ ) were used to assure the precision of the volume measurement. Details about measuring fractal dimension were described by Hebb et al. (2017).

### **3.6. Microbial community**

Soil microbial communities were characterized using phospholipid fatty acid (PLFA) analysis (Kiani et al., 2017). Polar lipids were extracted from freeze-dried samples using a modified Bligh and Dyer protocol (Hannam et al., 2006). The resulting fatty-acid methyl esters were separated using an Agilent 6890 Series capillary gas chromatograph (Agilent Technologies, Wilmington, DE, USA). Individual peaks were identified and quantified ( $\text{nmol g}^{-1}$ ) using the MIDI peak identification software (MIDI, Inc., Newark, DE, USA). The PLFAs containing 14 to 20 carbon atoms were representative of the soil microbial community (Quideau et al., 2016). They were summed and used as an index of microbial biomass carbon. In addition, specific PLFA biomarkers were assigned to different microbial groups, including gram-positive and gram-negative bacteria, fungi, and protists.

### **3.7. Statistical analysis**

For the Lethbridge data, we use Bartlett's and Shapiro-Wilk's tests for assessing the normality and equal variance of the data. A two-way analysis of variance (ANOVA) with mixed models followed by Tukey's tests were used to determine significant differences across rotations and phases for TOC, POM-C concentration and fraction, MAOM-C concentration and fraction, TN, POM-N concentration and

fraction, MAOM-C concentration and fraction, whole soil C:N, POM C:N, MAOM C:N, MBC and Dm. When a significant effect was detected based on the ANOVA models ( $p < 0.05$ ), Tukey HSD tests were subsequently run to compare means and identify any treatment grouping structure.

For the Breton site (established in 1929), since there is no experimental design or randomized replication to allow for conventional parametric statistical tests, we used nonparametric Dunn's test as well as Kruskal–Wallis test to determine significant differences across rotations, phases and the different nutrient managements at the Breton plots. All our statistical analyses were accomplished using SAS 9.1 (SAS Institute, 2015) and SigmaStat 13 (Systat Software, 2014) with a critical level alpha of 0.05.

Pearson and Spearman correlations were used for both Lethbridge and Breton sites separately to assess the association among all available properties and SOM fractions.

## **Results**

### **4.1. Organic Carbon and Total Nitrogen in Whole Soil**

In our study, the TOC and TN concentrations were affected by rotation type and nutrient management in the two long-term experimental sites. At the Breton site, TOC from adjacent forest plots was significantly greater than in the crop rotations ( $P < 0.001$ ) (Table 1). Both TOC and TN were significantly higher in the 5-yr rotation

than the 2-yr rotation ( $P_s < 0.03$ ). Manure addition also had significantly greater TOC and TN than both the inorganic balanced fertilization and the control soils. With respect to soil C:N ratio, although there was no significant difference between the two rotation types and nutrient management practices, cropland systems as a whole had significantly narrower C:N ratio compared to the undisturbed forest soil ( $P < 0.001$ ; Table 1). Additionally, at the Lethbridge site, TN was significantly higher in the 6-yr complex crop rotation; TOC was also numerically greater in this 6-yr rotation although not statistically significant (Table 2). Within all the phases of the 6-yr rotation, alfalfa phase had significantly greater TN than both wheat phase and fallow phase. No significant difference in soil C:N ratio was found among treatments for the Lethbridge site.

#### **4.2. Carbon and Nitrogen within POM and MAOM Fractions**

Soil organic carbon and nitrogen in the different SOM size fractions were strongly affected by the crop rotation type. At the Breton site, a significant increase in mineral associated (MAOM) carbon fraction was observed in crop sites when compared to the adjacent forest site ( $P < 0.001$ ). The POM-C fraction in the forest soil was 44% of the total soil organic C; and after about 86 years of continuous cropping, this POM-C had sharply decreased to 25% of the total soil carbon (Table 1), likely reflecting an increased disturbance and reduced C inputs to the soils under agricultural uses. A similar trend was also found in POM nitrogen fraction of the whole soil. Implementation of continuous cropping in this landscape where native vegetation had

been forest had significantly depleted the POM-N fraction from 34% to 20% on average ( $P < 0.001$ ; Table 1). However, when comparing between the 2-yr rotation and 5-yr rotation in the Breton Plots, although there was a significant increase in MAOM-C and -N concentration in complex crop rotation ( $P = 0.014$ ;  $P = 0.004$ ), no significant difference was observed in POM and MAOM fractions between these two crop rotation types. Conversely, at the Lethbridge site, the complex rotation including legumes and perennials had significantly greater POM-C and -N fractions than the 2-yr rotation (Table 2). Moreover, within the 6-yr complex rotation at Lethbridge, the two evaluated alfalfa phases consistently resulted in significantly higher MAOM-N concentrations and fractions than the two wheat phases in this same rotation.

The POM C:N ratio became significantly narrower in the soils under the Breton crop rotations compared to the adjacent forest land ( $P < 0.001$ ; Table 1). Nevertheless, there was no significant difference in POM C:N ratio across the rotation types or the nutrient management practices. Regarding nutrient management options, both manure and inorganic balanced fertilization addition had significantly greater POM-C and -N fraction than the control soils ( $P < 0.001$ ) (Table 1).

#### **4.3. Fractal dimension (Dm) of soil aggregation**

Focusing on soil fractal aggregation as indicator of hierarchical soil structure development, soils were significantly fractal under both the forest and complex rotation in Breton ( $P < 0.05$ ; Table 1) and a significant difference in fractal aggregation between forest and croplands was detected ( $P < 0.05$ ; Table 1); more



specifically, the simple 2-yr rotation did not result in fractal aggregation. When comparing across each crop rotation phase, soils were not significantly fractal at fallow phases of both simple and complex rotations in Lethbridge (0.987 and 0.993, respectively; Table 2), indicating the lack of soil structural development. A similar result was also found in Breton with a nonfractal soil wheat phase in the simple wheat-fallow rotation (i.e., 0.991). On the contrary, the wheat phases in complex rotations exhibited clear fractal aggregation ( $D_m$  values significantly different than one). Focusing on nutrient addition, regimes at the Breton site, only long-term balanced fertilization showed significantly fractal aggregation ( $D_m = 0.965$ ,  $P < 0.001$ ; Table 1), suggesting progressive development of soil hierarchical aggregation. Likewise, these results also showed an improved soil hierarchical fractal aggregation under complex rotations including perennial legumes and grasses.

#### **4.4. Soil microbial biomass carbon**

At both study sites, we found that soil MBC ( $\text{nmol g}^{-1}$ ) had increased significantly with the complex rotations. At the Breton site, MBC in the 5-yr rotation was about 1.5 times greater than in both the forested and 2-yr rotation soils ( $P = 0.01$ ; Table 1). The same trend was found at the Lethbridge site where MBC in the complex crop rotation was about 19% greater than in the simple rotation ( $P = 0.05$ ; Table 2). No significant differences in MBC were found between phases within these long-term rotations. Regarding the nutrient management comparisons at the Breton site, a large increase in MBC was observed for the manured soils when compared to the control

fields (Table 1).

#### 4.4. Correlation analyses among soil properties

Several significant correlations were found among soil properties across the assessed land-use systems and nutrient management regimes. At the Breton site, both POM carbon and nitrogen fraction were significantly correlated with TOC ( $P < 0.01$ , Table 3), while in Lethbridge, no significant relationship was observed. When examining the association between soil fractal aggregation and SOM fractions, we found that TOC and POM-C concentration were both inversely correlated with the  $D_m$  value in Breton soils ( $\rho = -0.363$ ;  $\rho = -0.354$ ;  $P_s < 0.01$ , Table 3). Significantly improved soil fractal aggregation was also observed with increasing C:N ratios in both POM and MAOM fractions ( $\rho = -0.628$ ;  $\rho = -0.421$ ;  $P < 0.05$ ;  $P < 0.01$ ; respectively). These observations were further supported by a significant linear regression ( $D_m = 1.07 - 0.0073 \text{ POM-C:N}$ ;  $P < 0.001$ ) which also registered a moderate goodness-of-fit ( $R^2$ ) of 39% (data not shown). No such significant patterns were found for the Lethbridge site as these Chernozemic soils can be considered more resilient by nature when compared to the Luvisolic soils at Breton, and hence, the soils at the Breton site are expected to be relatively more responsive to management choices.

In terms of MBC, a weak but significant correlation was found with TN ( $\rho = 0.338$ ;  $P < 0.05$ ), and a slightly stronger correlation was observed with MAOM-N ( $\rho = 0.424$ ;  $P < 0.05$ ) (Table 3). A regression analysis described well the direct, linear

relationship between these soil properties (i.e.,  $MBC = 1186 + 815 MAOM-N$ ,  $P = 0.006$ ,  $R^2 = 0.19$ ; data not shown). Regarding the association between soil properties and microbial biomarkers at the Breton site, fungal biomass was strongly correlated with both the TOC and POM-C ( $\rho = 0.400$ ;  $\rho = 0.371$ ; respectively;  $P_s < 0.05$ ; Table 3). Furthermore, occurrence of fungi biomarkers tended to increase with POM-C while it decreased with MAOM-C, although these patterns were not statistically significant. As opposed to fungi, protozoa biomarker abundance was strongly correlated with TN ( $\rho = 0.467$ ;  $P < 0.001$ ; Table 3) and also associated with MAOM-N ( $\rho = 0.561$ ;  $P < 0.001$ ). Concurrently, wider C:N ratios were clearly associated with an increasing fungi biomarker presence, and an opposite response was observed for protozoa, which also corresponded with our MBC results as noted above (i.e., declining MBC and protozoa occurrence with wider C:N ratios; Table 3).

## Discussion

### 5.1 Cropping leads to depletion of SOM

Cultivating undisturbed forest soil for agricultural use in the long term can result in a significant alteration in soil properties including depletions of SOM concentrations, decreasing labile SOM contents (i.e., POM) as well as microbial presence (Beheshti et al., 2012; Kiani et al., 2017). Soil TOC content was significantly depleted in the Breton crop rotations compared to the forest ( $P < 0.001$ ) (Table 1), while the 2-yr and 5-yr rotations did not differ with each other in their overall TOC contents. Moreover, the particulate organic carbon (POM) contents in the crop rotations were also significantly lower than that of the forest ecosystem. Congruently, when focusing on the mineral associated organic matter (MAOM) as a fraction, the 2-yr simple rotation and 5-yr complex rotation were not different with each other, but they were collectively higher than the adjacent forest soils in the SOM partitioning into this stable pool (Table 1). Similar tendencies were also found in soil nitrogen contents and fractions.

Recurrently annual cropping practices such as tillage and seeding can cause degradation of soil structure and aggregation resulting also in decreasing SOM (Ashagrie et al., 2007). The POM losses from our soils under cropping can have been triggered by low plant C inputs or by accelerated rates of biological decomposition of both plant residues and labile SOM in soil in part due to repeated disturbance by cultivation as well as the transfer of portion of the existing or recently-added C from

labile into a recalcitrant SOM pool (Cambardella and Elliott, 1992). The seminal study by Cambardella and Elliott reported that POM is frequently protected inside of soil aggregates and that soil aggregates broken-up by cultivation would expose POM to microbial decomposition, reducing its concentration, and consequently, part of POM-C is lost to the atmosphere via biological oxidation and any remaining C from these processes can be transformed into more stable mineral-associated organic matter (MAOM) (Figueiredo et al., 2010). In our study, changes in SOM (both TOC and TN) were directly associated with labile POM fraction (Tables 3 and 4). Moreover, POM measurements lead to the detection of consistent differences between wheat and perennial phases within the complex rotation even under the long-term management and putative steady state condition associated with our study (POM-C for Hay >> Wheat within Breton 5-yr rotation, Table 1), while TOC and TN data had yielded no detectable differences for these comparison. These results further support POM as a sensitive indicator for identifying early, short-term changes in SOM and soil quality as function on land management options.

In annual croplands, disturbance-induced changes in driving factors such as soil moisture, temperature or pH influence the enzymatic and microbial activities inside the soil which collectively determine the SOM decomposition rate (Curtin et al., 2012; Curtin et al., 2014). We infer that management-imposed disturbance stimulates these multiplicative effects by altering the microclimate and immediate boundary layer conditions, and consequently, resulting in a feedback towards increasing SOM

decomposition.

## 5.2 Complex rotations alter SOM pools and partitioning

Soil organic matter plays a key role in nutrient cycling function in the soil. In our study, this vital soil attribute has been improved in under the diverse, complex rotations as evidenced by enhanced TOC, TN, POM and MAOM (Tables 1 and 2). The SOM accrual and allocation is mostly determined by the balance between inputs of animal manure and plant residues versus losses via decomposition (Curtin et al., 2012). By introducing perennial legumes and grasses in long-term complex rotations, an enhanced N availability may result in both a faster SOM turnover rate and an increased crop yield which subsequently may also feedback into an even greater plant residue input into the soil.

Evidence of accumulating carbon and nitrogen into certain SOM pools under complex rotations compared to simple wheat-fallow rotation was observed in our study. Specifically in Lethbridge, we also found significant change in greater soil POM carbon and nitrogen fraction in complex rotation (Table 2), likely indicating that the partitioning of SOM into labile pools has been increased by the diversity of crop species included in this rotation. Compared to simple rotations, diverse crop rotations can change soils by affecting depth of rooting, nutrient status, soil aggregation and microbial habitat (Balota et al., 2004; Castro et al., 2015). Since most crop residues contain much more carbon than nitrogen, introducing N-enriching legumes into long-term crop rotations benefits the decomposition of the plant residues in the soil

and increase the efficiency of the conversion rate from plant residue into stable SOM accrual (Bowren et al, 1995). The increased fresh organic material and nutrient inputs provided by perennial legumes, forages and grasses within complex rotations is prone to be transformed into labile SOM pools such as POM and readily used by soil microbial communities (Ohtonen et al., 1997; Castro et al., 2015). By contrast, with slower SOM decomposition rate and less N availability, a scarcity of nutrient availability such as in forest soils limits the growth of the soil microbes (Quideau et al., 2013).

Across all assessed soils, the quality of labile SOM appears to be improved under complex crop rotations, and the labile SOM pool seems to be more utilized and cycled by soil microbial communities. In this context, a greater quality of labile SOM implies enhanced bioavailability and decomposability of the POM fraction as indicated by narrower C:N ratios (Bu et al., 2015; Castro et al., 2015; Stehlíková et al., 2016), suggesting that utilization of this labile SOM by microbes is propensive to occur. Moreover, it is noteworthy that although the forest soil had much higher SOM concentration than both simple and complex rotations, the MBC values in forest land was the lowest among all these land use systems, with 1.5 times significantly lower MBC in the forest soil than under the complex rotation (Table 1), which may reflect that the soil microorganisms could have limited access to decompose SOM in forest land. The POM fraction has been often described as a labile pool of SOM (Gregorich et al., 1994; Castro et al., 2015), but with an intermediate turnover time in afforested

soils (Hernandez-Ramirez et al., 2011) as well as a relatively easily decomposable fraction acting as a primary energy source for most soil microorganisms (Zou et al., 2005). In our study, forest soils had a significantly greater POM-C content than the soils under both simple and complex rotations in Breton (Table 1). This suggests that instead of being enriched with recalcitrant C, forest soils do have abundant labile organic carbon (as POM), but perhaps the concentration of this labile organic C is not the key factor that drives the soil microbial activity in such forest ecosystem. When focusing on the C:N ratios in both forests and croplands in our study, we found the forest soils had significantly wider C:N ratios in both whole SOM and POM fraction ( $P < 0.01$ ; Table 1). This indicates that the POM lability (i.e., as propensity to undergo utilization and decay) in forest soils may function under a very different dynamics and controls than in cropland soils as suggested by a higher SOM-N availability under much narrower C:N ratios in the cropland soils (Table 1) where exogenous N inputs are common. Moreover, when examining the relationship between soil MBC and C:N ratios in our study, we found a moderate but very consistent association of decreasing MBC with wider C:N ratios in whole soil, POM and MAOM pools (Tables 3 and 4). This functional relationship between soil C pools as substrates and microbial presence has not been fully documented in the literature. Although forest ecosystems have much lower or negligible disturbance compared to conventional croplands, nutrient availability in forest soils could still be constrained due to wide C:N ratios (Quideau et al., 2013), and this higher proportionality of C in forest soils may be caused by further limited N inputs and availability which prevents the soil organic compounds



from being utilized by microorganisms.

Another perspective to explain the observed TOC accruals in forest soils compared to cropped soils may be related to differences in the temperature and soil moisture conditions under forest cover. Warm-wet conditions can lead to lesser organic carbon content in the soils compared to the comparatively colder-drier scenarios as driven by the impacts of microclimate on microbial decomposition processes. Shaded by greater, permanent canopies, soils in forest ecosystems tend to exhibit cooler surface temperatures than cropland (Wickham et al., 2012). Such lower temperature as well as water uptake by trees in forest soils may depress the production and activity of soil exoenzymes, which are essential for microbes to breakdown SOM molecules into bioavailable compounds (Miller and Dick, 1995; Curtin et al., 2012).

### **5.3 Complex rotations further improve other soil quality indicators**

Besides an improved SOM accrual and dynamics, other key soil quality indicators such as soil fractal aggregation (as indicated by decreasing  $D_m$  values) and MBC were also enhanced under the longer, diverse, complex crop rotations. By including perennial grasses and legumes (e.g., alfalfa) into complex rotation systems, significantly fractal aggregation ( $D_m < 1$ ) was readily observed. The root systems of these perennial plant species can facilitate the development and enhancement of soil structure (Danga et al., 2009). More interestingly, in the Breton soils,  $D_m$  was seen to be correlated with POM-C concentration and POM C:N ratio. Collectively, these findings intriguingly suggest that increases in POM carbon due to long-term

implementation of diverse crop rotations are strongly interlinked with improvements of hierarchical soil aggregation (Table 3). Earlier studies focusing on the reciprocal relationship between soil aggregation and SOM have shown the mechanistic contribution of POM in forming and preserving soil aggregates, and vice versa (i.e., aggregates protecting existing soil TOC). Both factors – aggregation and POM – can putatively act synergistically leading to an enlarged whole SOM pool in the long-term. The aggregate hierarchy model postulated by Tisdall and Oades (1982) proposes that soil structure arises from aggregation of fine mineral particles into microaggregates (53 to 250  $\mu\text{m}$  size diameter) which continue aggregating into macroaggregates (> 250  $\mu\text{m}$ ) with increasing dependence on more transient, organic binding agents as the aggregate sizes increases. As subsequently, hypothesized by Oades (1984), these aggregating processes are considered to be closely linked with soil POM presence and turnover; soil microaggregates will first form around POM, and then enmeshed within stable macroaggregates. Thus, a large loss of soil TOC under recurrent cultivation management could often be explained by a short macroaggregate turnover time, and hence, a reduced opportunity for formation of stable microaggregates around POM cores (Six et al., 2002). Under our long-term complex rotation management, benefiting from new inputs of plant materials and a more dynamic C and N cycling, enhanced soil aggregation was effectively detected because it putatively facilitates the microaggregate formation and gradual stabilization of labile POM into the MAOM pool (Six et al., 2000).

Focusing on soil microbial community properties, we found that microbial biomass tended to be more sensitive to the variations in soil nitrogen pools (Table 3), and the complex crop rotations had greater MBC than the simple rotations. Based on results for Breton site, both TN and MAOM-N were well associated with soil MBC ( $P < 0.05$ ) (Table 3). These findings support our initial hypothesis that soil microbial communities can benefit from the N inputs as provided by perennial legumes in the complex rotations. Increasing TN and MAOM-N can benefit the soil microbial community by providing organic N substrate that can also become eventually available via mineralization to enhance plant productivity, which in turn contributes to increasing soil TOC and further promoting the growth of microorganisms (Treseder et al., 2008). Nevertheless, it should be noted that soil MBC increased with increasing organic N pools within our specific N concentration range (up to approx.  $2.7 \text{ g N kg}^{-1}$  soil; data not shown). It could be further hypothesized that beyond a certain N accumulation threshold, both soil microbial diversity and MBC plateau off or even decline due to soil N saturation (Treseder et al., 2008). These relationships need to be examined under a broader variety of agroecological conditions.

Both protozoa and fungi can be sensitive to intense cropping disturbance management, such as simple 2-yr rotations. As previously reported in a related work by Kiani et al. (2017), biomarker indicators for protozoa and fungi were distinctly present in the undisturbed Breton forest soil, and these two biomarkers were not evident under any cropping systems. Based on our correlation analyses, fungi showed

a close association with TOC and concurrently wider C:N ratios, while the presence of protozoa was highly correlated to TN, and even more directly associated with the stabilized N pool (i.e., MAOM-N) (Table 3). Existing literature supports that mycorrhizal fungi can hold up to two-thirds of the soil total carbon stored in leaf litter and soil profile in forest ecosystems (Clemmensen et al., 2013) and this has been regarded as the dominant pathway by which living carbon enters the soil carbon pool in poplar forest systems (Godbold et al., 2006). Godbold et al. (2006) further stated that the turnover of the mycorrhizal external mycelium might be a fundamental pathway for recently-added C inputs to be transferred into stable SOM pools. Moreover, our study suggests that long-term cultivation practices, in particular annual cropping, impacted and shifted the unique and dominant microbial groups in the Breton soils. By converting boreal forest soils into repeatedly-cropped soils, fungi would have lost their ecological advantages including adaptation to wide C:N ratios generated by tree litter.

With respect to protozoa, as they primarily feed on bacteria and SOM, the sharp decrease of SOM in croplands relative to forest soils would likely depress them (Table 1). However, with a plausible reestablished and enhanced soil food web and microbial community under diverse, complex rotations, the soil conditions could in the long-term become suitable again for the survival and activity of protozoa in response, the excess N that protozoa feeding on bacteria release in the form of ammonium can become available for other soil organisms and plants within these

agroecosystems (Hoorman et al., 2011). In our study, this inference is supported by a strong correlation between presence of protozoa biomarkers and stabilized N pools (e.g., TN and MAOM-N) (Table 3).

#### **5.4 Nutrient additions lead to varying enhancements in SOM dynamics**

Under the long-term management and steady state conditions at the Breton site, contrasting nutrient addition managements equally improved overall soil quality, although this response seemed to be mediated by diverging effects on SOM accrual and pools across the different nutrient regimes. Manure additions showed accelerating effects on accruing larger magnitudes of POM and MAOM pools compared to soils receiving a balanced fertilization (Table 1) although these two nutrient managements notably converged on the same allocation of SOM into POM vs. MAOM fractions. And this consistent SOM partitioning was clearly different from the soils with no nutrient additions (control) which disproportionally allocated less SOM into the labile pool (represented by POM fraction). An earlier study showed that manure addition can increase both the root and microbial biomass debris which are both key contributing factors of soil POM (Purakayastha et al., 2008). Likewise, long-term additions of manure have been shown to favor soil carbon cycling and macroaggregate formation (Liu et al., 2013; Stehlíková et al. 2016). While manure adds carbon directly to the soil profile, balanced fertilization supports plant growth and biomass production which subsequently feedbacks into increased plant residues leading to long-term accretion of SOM (Hati et al., 2008). Due to recurrent harvest

removals (i.e., grain, forage), the amount of essential nutrients such as nitrogen, phosphorus and potassium that can be returned to the soil through plant residues and roots may be insufficient for replenishing the removals. Therefore, adding nutrients on a long-term management basis could not only compensate for outputs (removals and losses) but also lead to improvements of SOM accrual and dynamics (Purakayastha et al., 2008). Manure, plant residues and roots all constitute fresh C inputs that can be at least initially allocated into the soil POM pool potentially leading to similar POM to SOM ratios. In our study two very distinct nutrient sources (i.e., manure and fertilizer) shifted, increased and sustained very similar partitioning of newly-accrued SOM towards the dynamic, labile POM fraction. This result expands earlier findings by Kiani et al. (2017) who indicated that an effective accretion of new SOM can be caused by direct manure additions in the long term.

By repeatedly applying manure into the soil, a great number and diversity of microbes along with additional input of organic carbon, nitrogen and other nutrients are expected to subsequently increase soil fertility in the long-term compared to equivalent amounts of N added via inorganic fertilization (Chakraborty et al., 2011). This postulate is in part supported by our finding of greater soil TOC, TN, and MBC in manured soils compared to fertilized soils (Table 1).

## **Conclusion**

Retention and cycling of soil organic matter is important for developing soil quality as well as sustaining agricultural production. The study of the long-term

effects of rotation and nutrient addition on SOM accrual and dynamics is essential to better understand these responses in agroecosystems under steady state conditions. Results from two long-term experimental sites indicate that certain repeated agricultural practices such as short, simple crop rotations and annual cropping decrease SOM and particularly reduce the labile SOM quantified as POM. The TOC and TN decreased by up to 46 and 14% when soils originally under native forest vegetation were continuously cropped for nearly nine decades. However, compared to the traditional wheat-fallow rotation, the introduction of perennial legumes, forage and grasses in diverse, complex rotation systems showed evidence of improved soil quality with increasing TOC and TN, as well as POM-C and -N. Moreover, the substantial amount of nitrogen likely contributed by the perennial legumes (e.g., alfalfa) may have facilitated microbial transformation of the labile SOM pool (compare to the N-limited condition in forest soils). The plant residues and roots derived from legumes and grasses in complex rotations would not only act as an additional C source in the system but would also interplay a key role in soil C and N cycling that directly involves supporting the microbial community and the progressive development of soil aggregation.

Nutrient management options resulted in varying accretion and allocation of SOM pools. Long-term manure additions can enhance soil nutrient cycling by increasing TOC, TN, POM concentrations, and MBC, while balanced fertilization shows much more benefits towards improved soil fractal aggregation. Moreover, our

study revealed that two contrasting nutrient sources – manure or inorganic fertilizer – equally result in increased allocation of SOM into labile POM pool, while unamended fields led to the depletion of this C fraction.

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**Tables:****Table 1.** Effect of crop rotation and nutrient management on soil organic matter attributes and fractions as well as fractal dimension (Dm) and microbial biomass carbon (MBC) at the 5 to 10 cm soil depth increment at the Breton site. The MBC and Dm data were presented in Kiani et al. (2017) and are repeated here on average basis for completeness of this study. Within a column, treatment means with different letters differ,  $P < 0.05$ . Three contrasts are presented.

Description	TOC (g kg <sup>-1</sup> soil)	POM-C Concentration (g kg <sup>-1</sup> soil)	POM-C Fraction	MAOM-C Concentration (g kg <sup>-1</sup> soil)	MAOM-C Fraction	TN (g kg <sup>-1</sup> soil)	POM-N Concentration (g kg <sup>-1</sup> soil)	POM-N Fraction	MAOM-N Concentration (g kg <sup>-1</sup> soil)	MAOM-N Fraction	POM C:N	MAOM C:N	Whole Soil C:N	MBC <sup>b</sup> nmol g <sup>-1</sup> soil	Dm unitless
Forest vs. Croplands															
Forest	36.9 <sub>a</sub>	15.13 <sub>a</sub>	0.44 <sub>a</sub>	21.74	0.56 <sub>b</sub>	2.10	0.68 <sub>a</sub>	0.34 <sub>a</sub>	1.42	0.66 <sub>b</sub>	22.30 <sub>a</sub>	14.60 <sub>a</sub>	17.38 <sub>a</sub>	1734 <sub>b</sub>	0.914 <sub>a</sub> <sup>a</sup>
Croplands	20.0 <sub>b</sub>	5.18 <sub>b</sub>	0.25 <sub>b</sub>	14.78	0.75 <sub>a</sub>	1.77	0.36 <sub>b</sub>	0.20 <sub>b</sub>	1.40	0.80 <sub>a</sub>	14.66 <sub>b</sub>	10.50 <sub>b</sub>	11.27 <sub>b</sub>	2443 <sub>a</sub>	0.962 <sub>b</sub> <sup>a</sup>
P-value	<0.001	<0.001	<0.001	0.059	<0.001	0.192	0.001	<0.001	0.984	<0.001	<0.001	<0.001	<0.001	0.062	0.004
2-yr vs. 5-yr Rotation															
2-yr Rotation	14.7 <sub>b</sub>	4.25	0.26	10.50 <sub>b</sub>	0.74	1.28 <sub>b</sub>	0.30	0.21	0.98 <sub>b</sub>	0.79	14.56	10.72	11.46	1912 <sub>b</sub>	0.970
5-yr Rotation	21.9 <sub>a</sub>	5.53	0.25	16.39 <sub>a</sub>	0.75	1.95 <sub>a</sub>	0.38	0.20	1.56 <sub>a</sub>	0.80	14.70	10.42	11.20	2421 <sub>a</sub>	0.957 <sup>a</sup>
P-value	0.028	0.093	0.856	0.014	0.856	0.02	0.102	0.824	0.004	0.824	0.431	0.701	0.505	0.026	0.527
5-yr Wheat vs. 5-yr Hay															
5-yr Rotation Wheat	19.2	4.46 <sub>b</sub>	0.23 <sub>b</sub>	14.75	0.77	1.79	0.32	0.18	1.47	0.82	14.08 <sub>b</sub>	10.08	10.73	2718 <sub>a</sub>	0.976 <sub>a</sub> <sup>a</sup>
5-yr Rotation Hay	23.6	6.17 <sub>a</sub>	0.27 <sub>a</sub>	17.38	0.73	2.04	0.42	0.20	1.62	0.80	15.07 <sub>a</sub>	10.62	11.49	2627 <sub>a</sub>	0.956 <sub>a</sub> <sup>a</sup>

P-value	0.056	0.037	0.027	0.210	0.027	0.189	0.152	0.210	0.474	0.210	0.002	0.136	0.107	0.83	0.12
Nutrient managements (crop rotations)															
Manure	26.0a	7.45a	0.28b	18.55a	0.72b	2.28a	0.53a	0.23b	1.75a	0.77b	14.24	10.62	11.38	3018a	0.987a
Balance Fertilization	17.4b	4.72b	0.27b	12.72b	0.73b	1.57b	0.33b	0.21b	1.24b	0.79b	11.14	14.50	10.29	2258ab	0.965a <sup>a</sup>
Control	15.3b	2.77b	0.19a	12.50b	0.81a	1.34b	0.18b	0.14a	1.17b	0.86a	15.44	10.62	11.38	1982b	0.985a
P-value	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.620	0.083	0.466	0.02	0.26
Overall Mean	22.0	6.54	0.28	15.46	0.70	1.77	0.40	0.21	1.37	0.76	15.44	11.85	11.91	2334	0.954
Standard Error	1.8	0.82	0.02	1.17	0.02	0.11	0.04	0.01	0.08	0.01	0.58	0.36	0.45	132	0.006

Note: Means within a column not sharing a lowercased italic letter differ significantly at the  $p < 0.05$  level.

C: Carbon; N: Nitrogen; TOC: total organic carbon; POM: particulate organic matter; MAOM: mineral associated organic matter; TN: total nitrogen.

<sup>a</sup>Indicates that the Dm value is significantly different from 1 at  $P < 0.05$  or it is fractal.

<sup>b</sup>The total PLFAs were used as an index of microbial biomass carbon.

**Table 2.** Effect of crop rotation on soil organic matter attributes, fractal dimension (Dm) and microbial biomass carbon (MBC) at the 5 to 10 cm soil depth at the Lethbridge site. The MBC and Dm data were presented in Kiani et al. (2017) and are repeated here on average basis for completeness of this study. Within a column, treatment means with different letters differ,  $P < 0.05$ .

Description	TOC (g kg <sup>-1</sup> soil)	POM-C Concentration (g kg <sup>-1</sup> soil)	POM-C Fraction	MAOM-C Concentration (g kg <sup>-1</sup> soil)	MAOM-C Fraction	TN (g kg <sup>-1</sup> soil)	POM-N Concentration (g kg <sup>-1</sup> soil)	POM-N Fraction	MAOM-N Concentration (g kg <sup>-1</sup> soil)	MAOM-N Fraction	POM C:N	MAOM C:N	Whole Soil C:N	MBC <sup>b</sup> nmol g <sup>-1</sup> soil	Dm unitless
2-yr vs. 6-yr Rotation															
2-yr Rotation	16.1	2.97 <sup>b</sup>	0.18 <sup>b</sup>	13.09	0.82 <sup>a</sup>	1.34 <sup>b</sup>	0.18 <sup>b</sup>	0.14 <sup>b</sup>	1.16 <sup>b</sup>	0.86 <sup>a</sup>	16.05	11.31	11.96	576 <sup>a</sup>	0.986 <sup>a</sup>
6-yr Rotation	19.4	4.45 <sup>a</sup>	0.23 <sup>a</sup>	14.96	0.77 <sup>b</sup>	1.64 <sup>a</sup>	0.30 <sup>a</sup>	0.18 <sup>a</sup>	1.34 <sup>a</sup>	0.82 <sup>b</sup>	14.85	11.24	11.88	707 <sup>a</sup>	0.984 <sup>a</sup>
P-value	0.236	<0.001	<0.001	0.115	<0.001	<0.001	<0.001	0.003	0.039	0.003	0.912	0.185	0.926	0.05	0.83
Within the 2-yr Rotation															
2-yr fallow	16.7	3.17	0.19	13.53	0.81	1.37	0.19	0.14	1.18	0.86	16.29	11.45	12.13	617 <sup>a</sup>	0.984 <sup>a</sup>
2-yr wheat	15.4	2.77	0.18	12.64	0.82	1.31	0.18	0.13	1.13	0.87	15.81	11.16	11.79	535 <sup>a</sup>	0.987 <sup>a</sup>
P-value	0.569	0.395	0.343	0.623	0.182	0.514	0.08	0.267	0.618	0.618	0.706	0.809	0.719	0.45	0.81
Within the 6yr Rotation															
6-yr fallow	19.6	4.27	0.22	15.31	0.78	1.54 <sup>bc</sup>	0.28	0.18 <sup>ab</sup>	1.26 <sup>b</sup>	0.82 <sup>a</sup>	15.15	12.12 <sup>ab</sup>	12.67	625 <sup>a</sup>	0.993 <sup>a</sup>
6-yr wheat1	17.3	4.25	0.25	13.07	0.75	1.45 <sup>c</sup>	0.31	0.21 <sup>a</sup>	1.14 <sup>b</sup>	0.79 <sup>b</sup>	11.99	11.51 <sup>ab</sup>	13.75	655 <sup>a</sup>	0.989 <sup>a</sup>
6-yr wheat2	21.1	5.34	0.25	15.75	0.75	1.55 <sup>bc</sup>	0.34	0.22 <sup>a</sup>	1.21 <sup>b</sup>	0.78 <sup>b</sup>	15.38	12.96 <sup>a</sup>	13.49	737 <sup>a</sup>	0.985 <sup>a</sup>
6-yr alfalfa1	18.5	4.01	0.23	14.44	0.77	1.79 <sup>ab</sup>	0.27	0.15 <sup>b</sup>	1.53 <sup>a</sup>	0.85 <sup>a</sup>	14.84	9.35 <sup>b</sup>	10.22	792 <sup>a</sup>	0.975 <sup>a</sup>

6-yr alfalfa3	20.6	4.38	0.21	16.23	0.79	1.86 <sub>a</sub>	0.29	0.16 <sub>b</sub>	1.57 <sub>a</sub>	0.84 <sub>a</sub>	15.12	10.27 <sub>ab</sub>	11.02	723 <sub>a</sub>	0.979 <sub>a</sub>
P-value	0.654	0.581	0.487	0.628	0.487	0.001	0.106	<0.001	<0.001	<0.001	0.085	0.856	0.042	0.64	0.42
Overall Mean	18.5	4.03	0.22	14.42	0.78	1.55	0.27	0.17	1.29	0.83	15.19	11.26	11.90	669	0.985
Standard Error	0.7	0.22	0.01	0.57	0.01	0.04	0.01	0.01	0.04	0.01	0.39	0.34	0.33	31.0	0.003

Note: Means within a column not sharing a lowercased italic letter differ significantly at the  $p < 0.05$  level.

TOC: total organic carbon; POM: particulate organic matter; MAOM: mineral associated organic matter; TN: total nitrogen.

<sup>a</sup>Indicates that the Dm value is significantly different from 1 at  $P < 0.05$  or it is fractal.

<sup>b</sup>The total PLFAs were used as an index of microbial biomass carbon.

**Table 3.** Spearman correlation coefficients ( $\rho$ ) for relationships among soil properties in Breton site. The fungi and protozoa data were presented in Kiani et al. (2017).

	TOC	POM-C	POM-CF	MAOM-C	MAOM-CF	TN	POM-N	POM-NF	MAOM-N	MAOM-NF	WS-C:N	POM-C:N	MAOM-C:N	MBC	Fungi	Protozoa
POM-C	0.874**															
POM-CF	0.509**	0.829**														
MAOM-C	0.940**	0.655**	0.208 NS													
MAOM-CF	-0.509**	-0.829**	-1.00**	-0.208 NS												
TN	0.859**	0.608	0.255 NS	0.908**	-0.255 NS											
POM-N	0.853**	0.915	0.744**	0.682**	-0.744**	0.764**										
POM-NF	0.578**	0.855	0.954**	0.298 NS	-0.954**	0.342*	0.839**									
MAOM-N	0.726**	0.368*	-0.016 NS	0.870**	0.0156 NS	0.953**	0.532**	0.055 NS								
MAOM-NF	-0.578**	-0.855**	-0.954**	-0.298 NS	0.954**	0.342*	-0.839**	-1.00**	-0.055 NS							
WS-C:N	0.724**	0.847**	0.697**	0.529**	-0.697**	0.285 NS	0.612**	0.708**	0.086 NS	-0.708**						
POM-C:N	0.537**	0.681**	0.619**	0.355 NS	-0.619**	0.121 NS	0.350*	0.478**	-0.006 NS	-0.478**	0.854**					
MAOM-C:N	0.734**	0.756**	0.476**	0.609**	-0.476**	0.327*	0.574**	0.571**	0.160 NS	-0.571**	0.936**	0.680**				
MBC	0.021 NS	-0.102 NS	-0.217 NS	0.106 NS	0.217 NS	0.338*	0.021 NS	-0.275 NS	0.424*	0.275 NS	-0.404*	-0.253 NS	-0.402*			
Fungi	0.400*	0.371*	0.305 NS	0.360*	-0.305 NS	0.169 NS	0.219 NS	0.265 NS	0.119 NS	-0.265 NS	0.530**	0.550**	0.481**	-0.355*		
Protozoa	0.123 NS	-0.074 NS	-0.171 NS	0.244 NS	0.171 NS	0.467**	0.110 NS	-0.194 NS	0.561**	0.194 NS	-0.361*	-0.287 NS	-0.356*	0.637**	-0.249 NS	
Dm	-0.363*	-0.354*	-0.266 NS	-0.308 NS	0.266 NS	-0.124 NS	-0.141 NS	-0.177 NS	-0.093 NS	0.177 NS	-0.508**	-0.628**	-0.421*	0.267 NS	-0.489**	0.235 NS

Note: F stands for fraction; WS, whole soil.

\* and \*\* indicate significant correlation at  $P < 0.05$  and  $P < 0.01$ , respectively.

NS indicate no significant relationship between variables.

**Table 4.** Pearson correlation coefficients (r) for relationships among soil properties in Lethbridge site.

	TOC	POM-C	POM-CF	MAOM-C	MAOM-CF	TN	POM-N	POM-NF	MAOM-N	MAOM-NF	WS-C:N	POM-C:N	MAOM-C:N	MBC
POM-C	0.800**													
POM-CF	0.129 NS	0.688**												
MAOM-C	0.973**	0.639**	-0.10 NS											
MAOM-CF	-0.129 NS	-0.688 NS	-1.00**	0.100 NS										
TN	0.645**	0.523**	0.173 NS	0.625**	-0.173 NS									
POM-N	0.554**	0.860**	0.779**	0.378*	-0.779**	0.517**								
POM-NF	0.230 NS	0.653**	0.788**	0.043 NS	-0.788**	-0.014 NS	0.845**							
MAOM-N	0.549**	0.312 NS	-0.057 NS	0.583**	0.057 NS	0.961**	0.260 NS	-0.289 NS						
MAOM-NF	-0.230 NS	-0.653**	-0.788**	-0.043 NS	0.788**	0.014 NS	-0.845**	-1.00**	0.289 NS					
WS-C:N	0.732**	0.586**	0.026 NS	0.712**	-0.026 NS	-0.045 NS	0.270 NS	0.321 NS	-0.138 NS	-0.321 NS				
POM-C:N	0.602**	0.486**	0.048 NS	0.583**	-0.048 NS	0.127 NS	-0.013 NS	-0.141 NS	0.148 NS	0.141 NS	0.681**			
MAOM-C:N	0.694**	0.516**	-0.049 NS	0.691**	0.049 NS	-0.088 NS	0.248 NS	0.328 NS	-0.180 NS	-0.328 NS	0.989**	0.577**		
MBC	-0.106 NS	-0.053 NS	0.083 NS	-0.117 NS	-0.083 NS	0.347 NS	0.193 NS	0.0145 NS	0.325 NS	-0.014 NS	-0.448*	-0.447*	-0.439*	
Dm	-0.246 NS	-0.064 NS	0.230 NS	-0.295 NS	-0.230 NS	-0.287 NS	0.094 NS	0.282 NS	-0.347 NS	-0.282 NS	-0.074 NS	-0.250 NS	-0.050 NS	-0.172 NS

Note: F stands for fraction; WS, whole soil\* and \*\* indicate significant correlation at P<0.05 and P<0.01, respectively.

NS indicate no significant relationship between variables.