Understanding the effects of crop rotation on soil organic carbon stabilization
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Abstract/ Summary:

This project focussed on assessing the impact of long-term continuous monoculture of cereals (wheat, corn) and canola on the quantity of functionally important soil carbon pools. Using long-term field experiments for wheat (Swift Current, SK) and canola (Swift Current and Scott, SK and Lacombe, AB), we measured functionally important mineral-associated organic matter (MAOM) and particulate organic matter (POM) pools in monocropped and diverse crop rotations, along with microbial abundance and community structure and extracellular enzyme activities. As expected, carbon stored in the POM and MAOM fractions differed among sites in the replicated canola systems and the both the mass fraction and amount of carbon stored as MAOM was greater than POM. The mass fraction of POM and MAOM only differed due to rotation in the canola system at Swift Current; where there were differences in the carbon stored as POM and MAOM it resulted from a change in carbon concentration within the fraction. Microbial abundance in the canola systems was highest at Lacombe where no treatment differences were apparent, followed by Scott and Swift Current. We collected samples at peak canola flowering which likely subdued the long-term differences in crop rotation on the microbial community due to the presence of an actively growing common host in all treatments as differences in community structure were only apparent at Scott. Similarly, differences in microbial abundance in the wheat system at Swift Current were only detected at the post-harvest sampling time demonstrating the strong short-term influence of the wheat crop on the microbial community. Because all soils were fertilized according to soil testing recommendations, some of the long-term effects of the differences in crop residue inputs may be mediated by balanced fertility. We recommend future studies focus on sampling outside the growing season (pre-seeding or post-harvest) to better capture the long-term differences in crop rotations on soil organic matter quantity and quality, and accompanying responses of the microbial community.

Introduction:

Soil organic matter (SOM) is a cornerstone of soil health and sustained soil productivity. Increasing soil C storage is important not only for soil fertility but also for mitigating rising atmospheric CO_2 concentration and off-setting greenhouse gas emissions from crop production systems. However, not all SOM is created equally. Some types of SOM can be destabilized through microbial decay more easily than others, leading to differences in soil organic carbon (SOC) turnover, fuel for other biological processes like nitrogen transformations, and susceptibility to future loss. In order to capitalize on the benefits that farming systems have for storing C in soil, predicting how management decisions affect the stability of SOC is critical. We know that many western Canadian soils have gained large quantities of C as a result of reduced tillage and continuous cropping. We need to better understand how different crop species and their use in rotation affect the cycling, protection, and stabilization of that C.

Plants are the main source of new C in soil and microorganisms use this plant C for energy. As they break it down, they transform plant C into SOM. Thus, crop rotation is not only an important aspect of integrated pest management but also impacts soil fertility through its effects on the quantity, quality and variety of incoming plant C. The “balanced diet” provided by more diverse crop rotations subsequently impacts how microorganisms transform plant C into SOM. Unless it is protected either physically or chemically, SOM

is constantly decomposing and providing fuel to the soil foodweb. Interactions between the quantity and quality of crop residues with microbial activity therefore strongly influence the form and stability of newly formed SOM.

Soil C sequestration potentials can be better understood through a framework based on the separation between SOC pools, specifically, young particulate organic matter (POM) and mineral associated organic matter (MAOM)(Cotrufo et al., 2019). The formation of POM and MAOM occurs through a continuum of decomposition in which POM, representing large structural polymers, is progressively broken down until it is mineralized to CO_2 (Lehmann & Kleber, 2015). Particulate organic matter is mainly stabilized by physical protection within soil aggregates, while MAOM is stabilized by bonding to clay mineral surfaces (Haddix et al., 2020a). Particulate organic matter consists of coarse, light organic material that is vulnerable to loss following disturbance (e.g., tillage) and is considered the fast cycling pool of SOC. MAOM on the other hand consists of more stable compounds that are formed by microbial metabolism of plant C when microbial metabolic biproducts and dead microbial cell compounds are sorbed through chemical bonding on clay surfaces (Cotrufo et al., 2019). We will also measure water extractable organic matter (WEOM), which is the soluble fraction of SOM. Although small by comparison to POM and MAOM, WEOM is the most readily bioavailable and actively utilized pool of SOM.

Quantifying the long-term impacts of crop rotation diversity on SOC cycling and stabilization as well as microbial abundance, community structure and function is needed to support recommendations for diversified crop rotations. Currently, motivation for diversification is mainly derived from pest and disease management but there are many positive impacts for soil health as well (Renwick et al., 2021; Bowles et al., 2020; Tiemann et al., 2015). Providing evidence-based guidance for improvements to SOC stabilization and resulting benefits for microbial abundance and activity will support crop rotations that bolster long-term soil health and resilience.

Understanding how crop rotation diversity contributes to the formation of stable SOM is critical to striking the right balance between soil C sequestration and microbial decomposition and nutrient cycling. This understanding is needed to manage the risk of losing stored soil C due to a change in management practices. We propose to use archived soils from three different long term cropping trials to assess the impact of diverse crop rotations on the quantity and type of C stored in these functional pools

Methodology:

Sample collection

This study leveraged previously collected samples that were collected originally to provide functional information about nutrient cycling and plant root-microbe interactions of key rotation crops grown in long term rotations at different locations in Saskatchewan and Alberta.

<u>Canola Frequency Study:</u>

Established in 2008, this 12-year field experiment was established at 5 sites in Saskatchewan and Alberta. It examines frequency of both glufosinate-resistant Liberty Link (LL) and glyphosate-resistant RoundUp

Ready (RR) canola in continuous, 2- and 3-year rotations (continuous canola, canola-wheat and canola-pea-barley) (see Harker et al., 2015 for further details). For the current study we focussed on the more widely grown glufosinate-resistant InVigor® L241C. Plots were 3.7 × 152 m, arranged in a randomized complete block design (n=4). The plots were managed using no-tillage and crops were seeded using knife-opener equipped air seders with row spacing at 23- to 30-cm. Fertilizer was added based on soil test to achieve 100% of the recommended rate of fertilizer addition for each crop species at each site.

Of the original five sites, we targeted Swift Current, Scott and Lacombe to encompass sites in each of the three major soil zones (Brown, Dark Brown and Black) thereby capturing soil regional differences in climate and soil type (additional sites at Melfort, SK and Lethbridge, AB were not sampled in this study). The canola phase of all 3 rotations was sampled in both 2018 and 2019 which represented the 11th and 12th years of the long-term experiment.

At peak canola flowering, we collected 3-4 plants from each plot using a hand trowel. Plants and accompanying soil (1-1.5kg) were transported on ice and processed the following day. Canola plants were removed from the sampling bag, shaken rigorously 5 times to remove any loosely adhering soil and the soil remaining in the bag was used the purpose of this study. Soils were sieved to <2mm to homogenize, sub-sampled and frozen at -20°C for further analysis as detailed below.

Wheat Rotation Diversity Study:

In 2016, soils were sampled from the long-term wheat-based crop rotation study at the Agriculture and Agri-Food Canada (AAFC) Swift Current Research and Development Centre. For a full description of the agronomic history of the wheat experiment, including some changes in the crop varieties grown see (Smith et al., 2017; St. Luce et al., 2020; Zentner et al., 2003). Briefly, the experiment was established in 1987 and for the purposes of the current research, we focussed on continuous wheat (CW) and the wheat (W1)- canola (C)-wheat (W2)- pea (P) rotation. The soil was loamy textured with 17.4% clay and 42.3% sand. The plot size was 15 × 45 m arranged in a randomized complete block design (n=3). Soils were fertilized with nitrogen based on soil test recommendations and all plots received 9.6 kg ha^{-1} P as monoammoniums phosphate.

Soil organic matter fractionation

To isolate particulate and mineral associated organic matter fractions, the Par+Den1 method adapted by Haddix et al., (2020b) and Poeplau et al., (2018) was employed. In short, 35ml of deionized water was added to 10g of freeze-dried soil and placed on a rotary shaker for 15min followed by 30min centrifuged at 1069 x *g*. Water extractable organic matter (WEOM) was siphoned off using a vacuum suction and aspirator lined with 0.2um filter paper. The WEOM was collected in the collection base and frozen at -20^{oC} until the samples were analyzed for WEOM carbon content using a Shimadzu TOC-L/TNM-L liquid carbon analyzer. Following WEOM extraction 2g of glass beads and 35ml of 0.5% hexametaphosphate were added to the remaining soil sample and placed on a rotary shaker for 18 hours at a speed of 60 rpm. Dispersed samples were rinsed using deionized water in a spray bottle over a 53µm sieve with receiving tray. Aggregates were gently crushed, and POM (> 53 µm) was separated from the MAOM (< 53 µm) by scraping the MAOM particles and water through the sieve with a rubber policeman followed by wet sieving using a 53um sieve to separate the POM from MAOM. Each fraction was transferred to a weighed, lined baking tray, dried for 24hrs after which they were weighed again and transferred to storage

vials. Fractionated samples (POM and MAOM) were oven dried at 60^{oC} for 10 hours, finely ground using a MM200 Retsch ball-mill grinder at a frequency of 30 for 2 minutes. Ground soils were sub sampled from 0.15g -0.4g depending on soil organic matter percent to prevent carbon saturation during combustion-gas chromatography analysis. Higher organic matter soils (5-8% OM) from Lacombe, AB were sub sampled from 0.15-0.2g while lower organic matter soils (1.5-3% OM) of Scott and Swift Current, SK were weighed from 0.3-0.4g into ceramic boats with nickel liners. Prior to organic carbon content analysis samples were acid treated for carbonate removal with additions of H_2SO_4. Total carbon and organic carbon content (mg C g^{-1} soil) were analyzed using automated flash combustion-gas chromatography via a NC-2100 LECO C632.

Phospholipid fatty acid profiling

Phospholipid Fatty Acid (PLFA) extraction was conducted to determine the total abundance (nmol g^{-1} soil), the amount or concentration of each functional microbial group present in a soil sample, and relative microbial abundance (mol%), the composition or proportion of each functional microbial group as a percentage of the whole microbial community. Phospholipid fatty acid analysis provides insight to the viable abundance and composition of bacteria and fungal communities present at the time of sampling. Microbial fatty acids are highly sensitive to changes in environment, such as agricultural management practices, making PLFA analysis a simple yet effective tool to understand the impact crop rotation practices have on microbial communities.

Briefly, 4g of freeze-dried, ground soil was extracted according to after Helgason et al., (2010) adapted from Bligh & Dyer, (1959). Neutral lipids and glycolipids were eluted off by filtering 5mL chloroform and 5mL acetone through 500mg silicon fractionation columns (Bond Elut, Agilent Technologies, Santa Clara, CA), followed by phospholipid elution with the addition of 5mL of methanol filtered through the silicon column. Phospholipids were methylated, with the addition of the internal standard methyl nonadecanoate (19:0), and dried under N_2 gas resulting dried, stabilized fatty acid methyl esters (FMAEs). The extracted fatty acid methyl ester (FAME) peaks were identified based on the retention time relative to known FAMEs (the MIDI) standard and were quantified using the internal standard (19:0) via a Bruker 486 GC-FID. Phospholipid concentration was calculated from the GC quantified FAME peaks using the following calculation:

$$\text{nmol } g^{-1} \text{ soil} = \frac{PA_{FAME} \bullet [IS] \bullet IS_{vol}) \bullet 1000 \text{nmol } \mu \text{ mol}^{-1}}{Soil_{dw} \bullet PA_{IS} \bullet MW_{FAME}}$$

Where PA_{FAME} = peak area of the FAME, [IS] = concentration of the internal standard (µg 19:0 μL^{-1}), IS_{vol} = volume of the internal standard (µL), $Soil_{dw}$ = weight of soil extracted (g dry soil), PA_{IS} = peak area of the internal standard (19:0) and MW_{FAME} = molecular weight of the target FAME (µg μmol^{-1}). Microbial abundance was calculated by converting nmol g^{-1} soil to mol %, by dividing the nmol g^{-1} soil of an individual FAME by the sum of the nmol g^{-1} soil of all FAMEs (except the IS 19:0) and multiplying by 100. This conversion was performed for the FAMEs of each individual microbial biomarker group.

Extracellular enzyme activity

Fluorometric extracellular enzyme analysis of β-glucosidase, N-acetyl-glucosaminidase, β-D-cellobiohydrolase, β-Xylosidase and phosphatase activities was conducted to estimate the potential

carbon and nutrient cycling activities according to Bell et al., (2013). In short 1g of stored field moist soil was prepared in a buffered slurry. Buffer pH was prepared to match the pH of the soil solution. The samples were treated with 4-methylumbelliferone (MUB) followed by the addition of enzyme substrates and incubated at 23^{o}C for 3 hours. Following incubation, samples were centrifuged at 2000rpm for 5 min and the substrate extract was pipetted into 96-well microplates which were read at an excitation of 360nm and emission of 465nm in a Varioskan LUX Multimode Microplate Reader (ThermoFisher Scientific Canada) for fluorometric enzyme activity.

Arylsulfatase did not produce consistent activity under fluorometric extraction and so colorimetric enzyme analysis was utilized to assess sulfatase activity in the canola frequency study soil samples. The benchtop method according to Acosta-Martínez & Ali Tabatabai 2015) was used where three technical replicates each containing 1g of field moist soil were combined in a slurry with 1ml of toluene, 4ml of 0.5M acetate buffer at a pH of 5.8, treated with 0.025M arylsulfatase p-nitrophenol, and incubated for 1 h at 37°C. One additional vial of 1g field moist soil was included for each sample as a homogenate control in which no enzyme substrate was added. Following incubation, the reaction was terminated using additions of 0.5M NaOH and $CaCl_2$ solutions, soil slurries were filtered through number 2 Whatman filter paper and pipetted into clear 2mL cuvettes. The filtered substrate extract was then analyzed for colour intensity using a Bench Top N6000 UV-Vis Spectrophotometer (Agilent).

Phenol Oxidase was analyzed by modifying the bench top colorimetric method according to Prosser et al., (2015) and adapted for high-throughput analysis in a microplate format. Simply, 1g of stored field moist soil was prepared in soil pH matched acetate buffer slurry and incubated at 23^{o}C for 2 hours. Following incubation, samples were centrifuged at 2000rpm for 5min and the supernatent was pipetted into clear 96-well plates and read at 475nm for colourimetric absorbance using a Varioskan LUX Multimode Microplate Reader (ThermoFisher Scientific Canada).

Statistical analysis

The abundance of PLFA, carbon content of POM and MAOM fractions, and extracellular enzyme activity were analyzed using linear mixed effects model (function 'lme' in R) and ANOVA univariate statistics. Microbial PLFA biomarker data, soil organic matter fraction, and enzyme activity were considered continuous variables, canola frequency and sampling year were categorical variables, and block was considered a random factor. The models were checked for suitability compared to a two-way ANOVA through Akaike Information Criterion (AIC) value comparison where the model with the lowest AIC was determined to have greater model fit as well by visualization using ggplot2 box and whisker graphics in RStudio. To determine the effect of crop rotation treatments on PLFA biomarker data, soil organic matter fraction, and enzyme activities, the effect of sampling year, and the interaction between canola frequency and sampling year the mixed models underwent Chi-squared type III ANOVA tests where p-value <0.05 were considered significant unless otherwise stated. Pairwise comparisons of estimated marginal means (function 'emmeans' in R) with post-hoc testing with Tukey p-value adjustment as Tukey was appropriate for a single set of pairwise comparisons between canola frequency treatments. Function 'multcomp' was used to assign significance letters to each pairwise comparison of means. Function 'multcomp' auto adjusted the p-values using Sidak adjustment as it was more appropriate for multiple combinations of means versus Tukey.

Relative microbial abundance (mol%) of different PLFA functional group biomarkers was analyzed using Aligned-Rank Transformation (ART) models where the data is automatically transformed by square

root and ranked according to proportion or weight within the data set. Means contrast and ANOVA post hoc testing (significance $p<0.05$) was conducted on each microbial group ART model to determine mean differences in response to canola frequency, sampling year and the interaction between both parameters. Further analysis was conducted to determine the effect of canola frequency within each sampling year using an ART model. Means contrast, Kruskal-Wallis, and Dunn test with Benjamin-Hochberg p-value adjustment for means comparison post hoc testing was completed to determine which canola rotation had the greatest impact on microbial community composition.

Relative microbial abundance (mol%) was visualized for compositional shifts using non-metric multidimensional scaling (NMDS). A distance matrix was developed using the Bray-Curtis dissimilarity metric in the R Vegan package. Pairwise PERMANOVA multivariate analysis was conducted on square root transformed relative microbial abundance data (mol%). The abundance of functional groups were overlaid on the ordination plots for those functional groups with R^2 values>0.4 with $p<0.05$.

The abundance and relative abundance of functional microbial groups and extracellular enzyme activity underwent correlation analysis with soil organic matter fractions (POM and MAOM) through Shapiro-Wilks testing and Spearman's correlation analysis to determine which microbial indicators negatively or positively correspond with SOM C storage in response to crop rotation. Statistically significant results ($p<0.05$) determined through linear mixed model, ANOVA, aligned rank transformation modelling and univariate post hoc testing underwent correlation analysis testing.

Research accomplishments:

Objectives (as stated in the proposal)	Progress
1. Determine the stability of soil organic carbon in soils from different long term wheat- and canola-based rotations	Partially complete– see explanation
2. Quantify carbon storage in different soil functional pools	Completed
3. Relationship of microbial abundance, community structure and activity with soil organic matter storage and utilization	Completed

Results and Discussion:

1. Canola Cropping Systems

Soil organic matter fractions.

In the canola cropping systems at Swift Current, Scott and Lacombe differences in POM and MAOM fractions resulting from long-term crop rotation or canola monocropping varied between the locations (**Table 1.1**). The clay loam soils at Lacombe had more POM and MAOM carbon than the loamy textured soils at Scott and Swift Current. Further, the mass fraction of carbon stored as more stable MAOM was greater at Lacombe compared to the other two sites. Significantly more carbon was stored as MAOM than POM at all sites.

There were no differences in MAOM due to crop rotation at Lacombe or Scott (**Table 1.1**). At Swift Current, there were differences between rotations but inconsistent patterns between 2018 and 2019 bring into question these results because MAOM should be a reasonably stable pool year-to-year. The raw data showed that the MAOM fractions in the Can in 2018 ranged from 1.18 to 1.89 % carbon by mass. The concentration of carbon in the MAOM fraction was consistent but there may have been issues with either sample fractionation or homogenization of the MAOM fraction affecting the validity of this result.

There were no differences in the mass fraction of POM at any of the sites (**Table 1.1**) but the carbon concentration of the POM responded more dynamically to differences in canola frequency compared to MAOM resulting in some differences between rotations. However, these year-to-year differences between rotation treatments did not follow consistent trends within or across sites. At Lacombe and Scott there were no differences in the mass fraction of POM between rotations, but differences in the concentration of carbon in the POM lead to the highest POM carbon content in the CW soil in 2018 and the CCan soil in 2019, and the lowest in CPB in 2019. Similarly, there were no differences in the mass fraction as POM at Swift Current but the POM carbon content was highest in the CCan 2018 soils compared to all other soils sampled at that location.

Microbial abundance and community structure.

At Lacombe, was no effect of crop rotation treatment on the total microbial biomass or in the abundance of any of the functional biomarker groups in either 2018 or 2019 (**Table 1.2**). The fungal:bacterial biomass ratio was higher in continuous canola vs canola in 2- and 3-year rotations (**Table 1.3**). At Scott, continuous canola had the highest total and bacterial biomass as well as higher Gram positive and Gram negative biomass while the abundance of these groups varied by comparison in the 2- and 3-year rotations (**Table 1.4**). Actinobacterial abundance differed between treatments, but with contrasting trends between years. A significant effect of crop rotation on the Stress 1 biomarker was noted at Scott, with highest stress in continuous canola (**Table 1.5**). A significant effect of rotation was also noted on the ratio of Gram positive:Gram negative bacteria but there was no consistent pattern that could be attributed to rotation (**Table 1.5**). There were no significant effects of crop rotation on the abundance of any of the PLFA biomarkers at Swift Current (**Table 1.6**). Total microbial biomass as well as general bacterial and Gram positive bacterial biomass was higher in 2019 compared to 2018. In contrast to what was observed at Scott, the Stress 1 biomarker was higher in 2019 compared to 2018 (**Table 1.7**) but total and bacterial biomass did not decrease in response. However, the Gram negative:Gram positive ratio was higher in 2018 when Stress 1 was significantly lower. Microbial community structure at each of the three sites is shown in **Figure 1.1** where clear effects of sampling year is apparent at all three sites. Crop rotation had a significant affect on community structure at only at Scott (**Table 1.8**) where continuous canola communities were different than 2- and 3-year rotations, an effect that was more pronounced in 2018 than 2019.

Extracellular Enzyme Activity

Phosphatase and arylsulfatase activity were both significantly impacted by canola frequency in 2018. Continuous canola monocropping had significantly greater phosphatase activity compared to canola-pea-barley in 2018. In contrast arylsulfatase however was lowest for continuous canola monocropping compared to canola-pea-barley (**Table 1.9**). In 2019 Beta-D-glucosidase was significantly

less for canola-wheat compared to canola-pea-barley and no difference between either rotation to continuous canola. N-acetyl-glucosaminidase was impacted by canola frequency in both sampling years. Canola-wheat in 2018 was significantly greater than canola-pea-barley, canola-pea-barley was greater than canola-wheat and no differences were observed between two and three canola rotation treatments and continuous canola monocropping. Significant differences were found most consistently in 2019 between canola frequency crop rotation treatments when analyzed within sampling year for phosphatase, beta-glucosidase, and arylsulfatase. Results for N-acetyl-glucosaminidase and phenol oxidase can be found in Table X. Phosphatase activity (nmol g^{-1} dry soil hr^{-1}) was highly variable for continuous canola monocropping in 2018 and showed a trend of decreasing activity with greater rotation diversity. Significant differences in phosphatase activity occurred between between2018 and 2019 where 2018 had significantly greater activity. There was no impact on phosphatase activity from canola frequency within either sampling year (**Table 1.10**). Beta-glucosidase is utilized by microbes to decompose cellulose into glucose and is an indicator of soil organic carbon cycling. Figure 5-5 shows statistically greater Beta-glucosidase activity for continuous canola monocropping in 2019 compared to canola-wheat and canola-pea-barley rotations. While highly variable, there was no difference in N-acetyl-glucosaminidase activity in 2018 or 2019. There was no impact on nutrient cycling and enzyme activity in N-acetyl-glucosaminidase, phosphatase, Beta-D-glucosidase, arylsulfatase or phenol oxidase in soils after 12 years of continuous canola monocropping compared to canola grown in two- and three-year rotations (**Table 1.11**).

Correlations of MAOM and POM with microbial abundance and enzyme activity

Microbial abundance was positively correlated with the amount of MAOM and POM in 2018 (**Table 1.12**) but not 2019 (**Table A1**). Likewise, in 2018, microbial abundance was positively correlated with the proportion of organic carbon as MAOM (and negatively correlated with proportion as POM)(**Table 1.12**). These differences indicate that the overall microbial abundance was most strongly controlled by the long-term differences in soil organic carbon availability whereas in 2019 abundance was more strongly controlled by conditions at the time of sampling (e.g. soil moisture or nutrient status). Positive correlation of phosphatase, N-acetyl-glucosaminidase and Beta-D-glucosidase with MAOM in 2019 (**Table A1**) but not 2018 (**Table 1.12**) likewise indicates differences in the availability of nutrients at the time of sampling between the two years.

Summary

The lack of consistent differences in POM and MAOM carbon pools observed under different canola cropping frequencies indicates that the interplay between quantity and quality of crop root and residue inputs did not lead to differences in stabilization in these systems. Canola, wheat, barley and field peas have different above and belowground biomass both in quantity and quality. Fan et al. (Fan et al., 2016) propose that the proportionally higher belowground carbon deposition by canola can contribute to increasing soil organic carbon stocks. Since root carbon is thought to be preferentially stabilized (Cotrufo et al., 2013; Sokol et al., 2019; Sokol & Bradford, 2019) the greater quantity of root-derived carbon from canola may balance the higher quality but lower quantity field pea residue and relatively high quantity but low quality cereal crop residues. Because soils were sampled at in the canola phase of all rotations, at the peak flowering stage, the long-term impact of differences in crop diversity and residue input quality may have been

temporarily masked by the homogeneity of the canola crop. During the active growing season, the microbial community is stimulated by incoming plant photosynthate from the crop canopy in the form of rhizodeposits, which appears to have created uniformity in the community structure among the different canola rotations.

Table 1.1. Total carbon content of soil organic matter fractions sampled during peak canola flowering after 12 years of crop rotations with varied canola frequency sampled in 2018 and 2019 at Swift Current and Scott, SK and Lacombe, AB and Swift Current, SK.

		Lacombe, AB		Scott, SK		Swift Current, SK		Lacombe, AB		Scott, SK		Swift Current, SK	
		POM	MAOM	POM	MAOM	POM	MAOM	POM	MAOM	POM	MAOM	POM	MAOM
Rotation		----------mg C g^{-1} soil----------						---------- % mass of fraction ----------					
2018	CCan.[a]	6.03 A[b]	58.2 A	7.60 AB[b]	24.7 A	7.37 A[b]	15.9 B	26.8 A	73.8 A	37.8 A	62.6	30.6 A	66.8 C
		(0.89)	(2.33)	(0.16)	(0.75)	(2.33)	(1.49)	(5.49)	(6.79)	(4.15)	(3.97)A	(5.97)	(5.58)
	CW	6.92 A	58.9 A	8.22 A	24.9 A	3.35 B	18.0 A	25.8 A	73.9 A	37.3 A	63.7	21.4 AB	76.6 A
		(0.41)	(1.18)	(0.72)	(1.49)	(0.60)	(0.26)	(2.33)	(2.32)	(3.28)	(3.35)A	(3.60)	(1.67)
	CPB	7.45 A	60.9 A	6.69 AB	24.4 A	5.10 AB	17.8 AB	19.4 A	80.3 A	37.4 A	63.4	24.7 A	70.8 BC
		(0.84)	(1.31)	(0.77)	(1.72)	(0.62)	(0.79)	(2.25)	(2.34)	(4.06)	(4.19)A	(1.97)	(2.07)
2019	CCan.	9.10 A	58.1 A	8.42 A	23.9 A	3.67 B	18.5 A	26.5 A	74.3 A	37.2 A	63.6 A	19.2 B	72.7 BC
		(0.34)	(2.27)	(0.71)	(1.25)	(0.90)	(0.85)	(5.05)	(4.95)	(4.03)	(4.13)	(2.04)	(2.91)
	CW	8.58 A	58.5 A	6.55 AB	25.0 A	3.88 B	17.6 AB	21.7 A	78.7 A	33.4 A	66.5 A	26.3 A	73.3 AB
		(0.98)	(1.59)	(0.34)	(1.57)	(0.56)	(0.19)	(2.55)	(2.46)	(3.78)	(3.69)	(1.16)	(0.69)
	CPB	9.68 A	63.8 A	5.55 B	24.1 A	4.19 B	17.0 AB	16.8 A	83.0 A	32.2 A	65.9 A	26.5 A	73.0 AB
		(0.74)	(2.01)	(0.07)	(1.13)	(0.46)	(0.61)	(2.05)	(2.08)	(3.28)	(3.65)	(1.54)	(0.96)
ANOVA	*df*	---------- *F value (p-value)* ----------											
Rot.	2	1.89	1.56	4.04	0.12	6.48	6.14	3.26	2.78	0.08	0.26	3.90	6.60
		(0.39)	(0.46)	(0.13)	(0.94)	(**0.04**)	(**0.05**)	(0.20)	(.25)	(0.96)	(0.88)	(0.14)	(**0.04**)
Year	1	8.62	0.01	1.13	0.31	5.46	7.43	0.003	0.03	0.06	0.15	6.31	2.36
		(**0.003**)	(0.94)	(0.29)	(0.58)	(**0.02**)	(**0.006**)	(0.95)	(0.87)	(0.81)	(0.70)	(**0.01**)	(0.12)
Rot. x	2	0.91	1.31	5.84	0.19	3.68	7.61	0.39	0.43	2.50	0.62	7.29	2.82
Year		(0.63)	(0.52)	(**0.05**)	(0.91)	(0.16)	(**0.02**)	(0.82)	(0.81)	(0.29)	(0.73)	(**0.02**)	(0.24)

[a] Frequency of canola in rotation where CCan = Continuous Canola, CW= Canola-Wheat, and CPB = Canola-Pea-Barley
[b] Means within the same column followed by the same letter are not significantly different at $p<0.05$.

Table 1.2. Microbial phospholipid fatty acid analysis (PLFA) functional microbial group abundance means with standard error and analysis of variance significance results in soils from Lacombe, AB after 12 years of varied canola frequency.

		General Bacteria	Gram Positive	Gram Negative	Fungi	Actinobacteria	Total PLFA
	Rotation	----------nmol g^{-1} soil ----------					
2018	CCan[a]	94.4(1.93)A[b]	30.8(0.61)A	49.6(1.06)A	1.60(0.04)A	14.7(0.31)A	142(2.85)A
	CW	94.5(1.21)A	32.0(0.18)A	48.0(0.88)A	1.67(0.03)A	15.0(0.22)A	144(1.63)A
	CPB	101(1.02) A	33.5(0.57)A	53.4(1.24)A	1.89(0.05)A	15.3(0.22)A	154(2.64)A
2019	CCan	107(2.40)A	29.7(0.69)A	62.7(1.38)A	2.90(0.10)A	15.9(0.40)A	164(3.50)A
	CW	97.6(1.52)A	27.2(0.29)A	56.7(1.17)A	3.19(0.16)A	14.1(0.21)A	148(2.89)A
	CPB	98.5(2.04)A	27.9(0.60)A	57.0(1.20)A	2.49(0.08)A	14.4(0.26)A	151(3.23)A
ANOVA	*df*	----------F value (p value)----------					
Rot.	2	0.57(0.75)	0.82(0.67)	0.71(0.70)	0.36(0.84)	0.17(0.92)	0.62(0.73)
Year	1	1.63(0.20)	0.13(0.72)	3.97(**0.04**)	6.60(**0.01**)	0.56(0.45)	2.05(0.15)
Rot:Year	2	1.22(0.54)	1.34(0.51)	1.05(0.59)	1.77(0.41)	1.18(0.55)	1.33(0.51)

[a] Frequency of canola in rotation where CCan = Continuous Canola, CW= Canola-Wheat, and CPB = Canola-Pea-Barley

[b] Values in parentheses are standard error of the mean. The same letter within a column denotes no statistically significant difference ($p<0.05$) between treatments within a year.

Table 1.3. Ratio of functional microbial group abundance means of microbial phospholipid fatty acid analysis (PLFA) in soils from Lacombe, AB after 12 years of varied canola frequency.

		Stress 1[a]	Stress 2	Fungi:Bacteria	G.Neg.: G.Pos.
	Rotation	----------nmol g^{-1} soil: nmol g^{-1} soil----------			
2018	CCan[b]	3.53(0.03)[c]A	0.42(0.01)A	0.06(0.001)A	1.61(0.01)A
	CW	3.18(0.05)A	0.44(0.004)A	0.08(0.001)B	1.49(0.02)A
	CPB	3.44(0.05)A	0.38(0.01)A	0.08(0.001)B	1.59(0.02)A
2019	CCan	3.57(0.03)A	0.32(0.01)A	0.08(0.001)A	2.12(0.01)A
	CW	3.58(0.07)A	0.30(0.01)A	0.09(0.002)B	2.07(0.02)A
	CPB	3.67(0.03)A	0.29(0.003)A	0.09(0.001)B	2.05(0.002)A
ANOVA	*df*	---------- F value (*p* value) ----------			
Rot.	2	1.91[d](0.38)	2.55(0.28)	6.56(**0.04**)	2.14(0.34)
Year	1	0.03(0.87)	8.29(**0.004**)	3.44(0.06)	33.6(<**0.001**)
Rot:Year	2	0.91(0.63)	0.75(0.69)	0.48(0.79)	1.06(0.59)

[a] Stress 1 = cy17:0 to 16:1ω7c, Stress 2 = cy19:0 to 18:1ω7c, Fungi:Bacteria = ratio of fungal functional biomarker to the sum of bacterial functional biomarkers, G.Neg.:G.Pos. = abundance ratio of Gram negative bacteria to Gram positive bacteria

[b] Frequency of canola in rotation where CCan = Continuous Canola, CW= Canola-Wheat, and CPB = Canola-Pea-Barley

[c] Values in parentheses are standard error of the mean. Upper case letters within a column denote statistically significant differences ($p<0.05$) between treatments within a sampling year.

[d] F values are presented with *p* values enclosed in parentheses are presented for all years.

Table1.4. Abundance of phospholipid fatty acid biomarkers in soils sampled during peak canola flowering after 12 years of crop rotations with varied canola frequency collected in 2018 and 2019 at Scott, SK.

		General Bacteria	Gram Positive	Gram Negative	Fungi	Actinobacteria	Total PLFA
	Rotation	----------nmol g^{-1} soil ----------					
2018	CCan[c]	71.4 (1.88)[a]A	25.9(0.42)A	40.5(1.03)A	2.49(0.09 A	4.79(0.54)B	114(2.89)A
	CW	73.9 (0.40)A	25.8(0.11)A	37.9(0.38)AB	2.64(0.09) A	9.9(0.06)A	113(4.93)A
	CPB	56.9 (0.92)B	17.2(0.56)B	30.0(0.36)B	2.12(0.03) A	9.02(0.10)A	89(1.49)B
2019	CC	75.7(1.82)A	27.2(0.58)A	38.3(1.08)A	2.33(0.07)A	9.79(0.21)B	121(2.81)A
	CW	59.8(0.55)B	23.3(0.17)B	27.8(0.35)B	1.58(0.04)A	8.42(0.05)B	95(0.86)B
	CPB	60.8(0.66)B	23.7(0.13)AB	27.8(0.55)B	1.77(0.03)A	8.84(0.09)B	97(1.02)B
ANOVA	*df*	----------F value (p value)----------					
Rot.	2	7.80(**0.02**)	22.09(<**0.001**)	7.91(**0.02**)	5.27(0.07)	15.49(<**0.001**)	7.89(**0.02**)
Year	1	0.44(0.51)	0.41(0.52)	0.31(0.58)	1.65(0.28)	12.97(<**0.001**)	0.53(0.47)
Rot:Year	2	5.12(0.08)	9.01(**0.01**)	2.79(0.25)	0.11(0.94)	12.20(**0.002**)	4.38(0.11)

[a] Frequency of canola in rotation where CCan = Continuous Canola, CW= Canola-Wheat, and CPB = Canola-Pea-Barley.

[b] Values in parentheses are standard error of the mean. The same letter within a column denotes no statistically significant difference ($p<0.05$) between treatments within a year.

Table 1.5. Ratio of functional microbial group abundance means of microbial phospholipid fatty acid analysis (PLFA) in soils from Scott, SK after 12 years of varied canola frequency.

		Stress 1[b]	Stress 2	Fungi:Bacteria	G.Neg.: G.Pos.
	Rotation	----------nmol g^{-1} soil: nmol g^{-1} soil----------			
2018	CCan[a]	3.16(0.09)A[c]	0.33(0.02)A	0.09(0.001)A	1.55(0.02)AB[d]
	CW	2.62(0.04)AB	0.36(0.01)A	0.08(0.001)A	1.45(0.01)AB
	CPB	2.29(0.03)B	0.47(0.01)A	0.08(0.001)A	1.81(0.04)A
2019	CCan	3.12(0.07)A	0.30(0.01)A	0.08(0.001)A	1.40(0.02)AB
	CW	2.14(0.06)B	0.51(0.03)A	0.07(0.001)A	1.19(0.01)B
	CPB	2.25(0.07)AB	0.53(0.02)A	0.07(0.002)A	1.17(0.02)B
ANOVA	*df*				
Rot.	2	5.91[b](**0.05**)	2.55(0.28)	2.43(0.30)	7.33(**0.02**)
Year	1	0.01(0.91)	0.16(0.69)	1.99(0.16)	1.34(0.25)
Rot:Year	2	1.00(0.61)	1.93(0.38)	0.02(0.99)	7.34(**0.02**)

[a] Frequency of canola in rotation where CCan = Continuous Canola, CW= Canola-Wheat, and CPB = Canola-Pea-Barley

[b] Stress 1 = cy17:0 to 16:1ω7c, Stress 2 = cy19:0 to 18:1ω7c

[c] Values in parentheses are standard error of the mean. The same letter within a column denotes no statistically significant difference ($p<0.05$) between treatments within a year.

[d] Significance letter for the ratio abundance of Gram negative to Gram positive bacteria was calculated across both sampling years together.

Table 1.6. Microbial phospholipid fatty acid analysis (PLFA) functional microbial group abundance means with standard error and analysis of variance significance results in soils from Swift Current, SK after 12 years of varied canola frequency in Swift Current, SK.

		General Bacteria	Gram Positive	Gram Negative	Fungi	Actinobacteria	Total PLFA
	Rotation	----------nmol g^{-1} soil ----------					
2018	CCan[a]	48.7(0.82)A[b]	14.7(0.19)A	25.6(0.55)A	2.15(0.10)B	7.43(0.10)A	77.6(1.32)A
	CW	40.3(0.80)A	12.2(0.25)A	20.4(0.46)A	1.35(0.07)A	6.79(0.10)A	63.3(1.36)A
	CPB	42.5(0.75) A	13.2(0.23)A	21.7(0.43)A	1.62(0.05)AB	6.85(0.09)A	68.7(1.28)A
2019	CCan	58.4(0.97)B	22.5(0.37)A	27.2(0.51)A	1.90(0.05)A	7.70(0.09)A	95.4(1.57)A
	CW	41.3(0.88)A	16.0(0.30)B	18.5(0.46)B	1.22(0.04)B	6.12(0.10)B	67.4(1.47)B
	CPB	47.7(0.74)A	18.5(0.30)B	21.4(0.38)B	1.53(0.05)AB	7.08(0.08)B	77.7(1.31)AB
ANOVA	*df*	----------F value (*p*-value)----------					
Rot.	2	3.49(0.18)	2.68(0.26)	4.20(1.22)	4.85(0.09)	1.84(0.40)	3.39(0.18)
Year	1	4.23(**0.04**)	23.86(<**0.001**)	0.35(0.55)	0.43(0.51)	0.25(0.62)	5.13(**0.02**)
Rot:Year	2	1.68(0.43)	3.08(0.21)	0.85(0.65)	0.10(0.95)	2.03(0.36)	1.56(0.46)

[a] Frequency of canola in rotation where CCan = Continuous Canola, CW= Canola-Wheat, and CPB = Canola-Pea-Barley

[b] Values in parentheses are standard error of the mean. Upper case letters within a column denote statistically significant differences ($p<0.05$) between treatments within a year.

Table 1.7. Ratio of functional microbial group abundance means of microbial phospholipid fatty acid analysis (PLFA) in soils from Swift Current, SK after 12 years of varied canola frequency.

		Stress 1[a]	Stress 2	Fungi:Bacteria	G.Neg.:G.Pos.
	Rotation	----------nmol g^{-1} soil: nmol g^{-1} soil----------			
2018	CCan[b]	1.81(0.07)[c]A	0.78(0.04)A	0.08(0.002)A	1.73(0.02)A
	CW	1.54(0.04)A	0.82(0.03)A	0.06(0.002)A	1.67(0.01)A
	CPB	1.64(0.04)A	0.81(0.03)A	0.07(0.001)A	1.65(0.02)A
2019	CCan	2.35(0.04)A	0.60(0.02)B	0.07(0.001)A	1.21(0.01)A
	CW	1.89(0.04)B	0.85(0.02)A	0.06(0.001)A	1.15(0.01)A
	CPB	1.98(0.02)B	0.79(0.009)A	0.07(0.001)A	1.16(0.01)A
ANOVA	*df*	----------F value (p value)----------			
Rot.	2	1.52[d](0.47)	0.11(0.95)	2.16(0.34)	1.38(0.50)
Year	1	5.92(**0.02**)	1.64(0.20)	1.00(0.32)	55.19(<**0.001**)
Rot:Year	2	0.51(0.77)	1.23(0.53)	0.36(0.84)	0.13(0.94)

[a] Stress 1 = cy17:0 to 16:1ω7c, Stress 2 = cy19:0 to 18:1ω7c, Fungi:Bacteria = ratio of fungal functional biomarker to the sum of bacterial functional biomarkers, G.Neg.:G.Pos. = abundance ratio of Gram negative bacteria to Gram positive bacteria

[b] Frequency of canola in rotation where CCan = Continuous Canola, CW= Canola-Wheat, and CPB = Canola-Pea-Barley

[c] Values in parentheses are standard error of the mean. Upper case letters within a column denote statistically significant differences ($p<0.05$) between treatments within a sampling year.

[d] F values are presented with *p* values enclosed in parentheses are presented for all years.

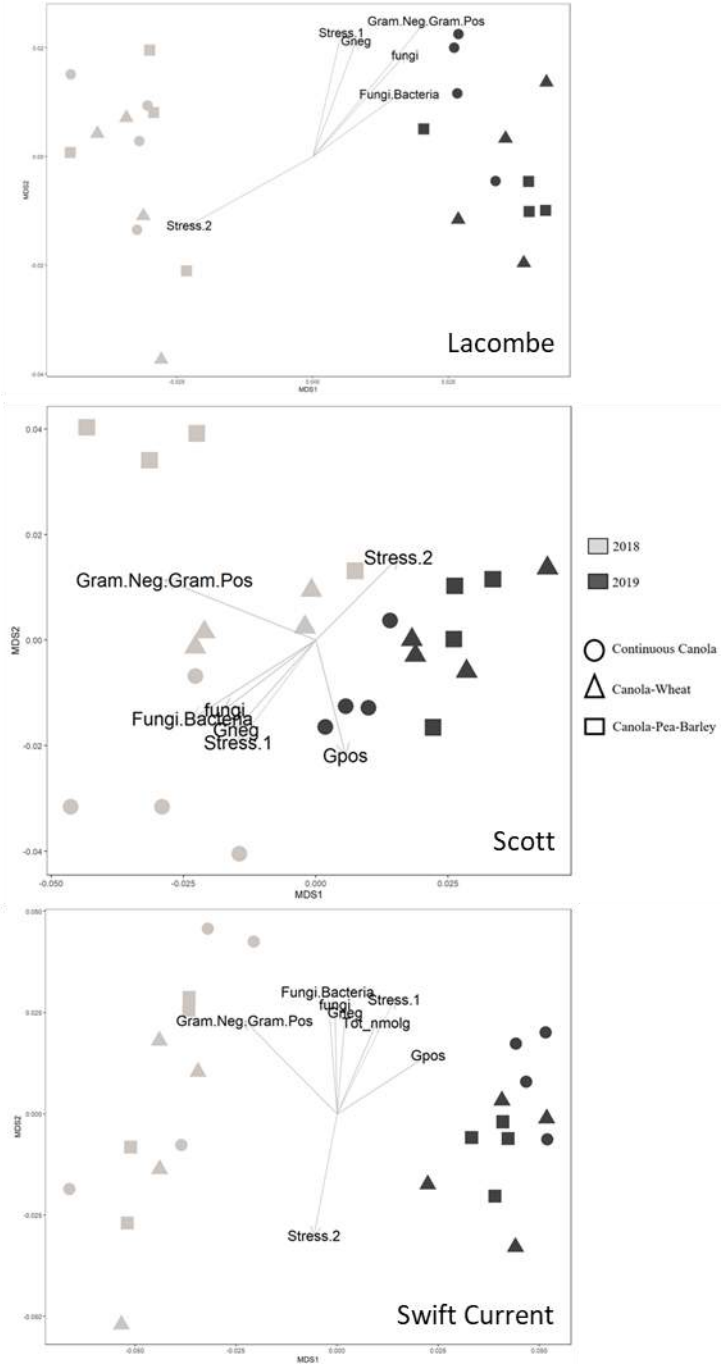

Figure 1.1. Microbial community structure from soils sampled at peak canola flowering after 12 years of crop rotations with varied canola frequency at Scott, SK, Lacombe, AB and Swift Current, SK. Functional group biomarkers correlated with overall community structure are depicted as vectors (R^2 values $\geq$ 0.4 and p values < 0.05)

Table 1.8. PERMANOVA analysis microbial community profiles (mol%) correlated with abundance in soils after 12 years of crop rotations with varied canola frequency sampled in 2018 and 2019 as determined by phospholipid fatty acid (PLFA) profiling at Scott, SK.

Site		Df	R^2	Pr(>f)
Lacombe	Rotation	2	0.07859	0.4489
	Residuals	21	0.92141	
	Total	23	1.00000	
Scott	Rotation	2	0.22382	0.0082
	Residuals	21	0.77618	
	Total	23	1.00000	
Swift Current	Rotation	2	0.0565	0.6494
	Residuals	21	0.9435	
	Total	23	1.00000	

Table 1.9. Mean values of extracellular enzyme activity of N-acetyl-glucosaminidase (NAG), Beta-glucosidase (BG), phosphatase (PHOS), arylsulfatase (ARYL), and phenol oxidase (PHN.OX) in soils collected at peak canola flowering after 12 years of crop rotations with varied canola frequency sampled in 2018 and 2019 at Lacombe, AB.

		NAG	BG	PHOS	ARYL	PHN.OX
	Rotation	----------nmol g^{-1} soil hr^{-1} ----------				
2018	CCan[a]	223(11.1)[b]AB	847(85.8)A	648(51.3)A	5205(218)A	2136(132)A
	CW	242(11.1)A	814(20.1)A	604(70.2)AB	5437(385)A	2021(287)A
	CPB	203(13.1)B	881(78.2)A	431(40.4)B	5593(130)A	2650(141)A
2019	CCan.	176(6.5)AB	1055(6.5)AB	449(26.9)A	5397(178)A	2575(60.4)A
	CW	169(7.3)B	989(18.3)B	435(17.9)A	5823(244)A	3273(474)A
	CPB	201(3.2)A	1274(32.3)A	449(6.3)A	5919(81.1)A	2864(395)A
ANOVA	*df*	----------F value (*p* value)----------				
Rotation	2	6.27[c](**0.04**)	0.38(0.83)	10.3(**0.006**)	0.34(0.84)	0.86(0.65)
Year	1	9.05(**0.003**)	3.56(**0.05**)	7.75(**0.005**)	0.08(0.77)	0.37(0.54)
Rot. x Yr	2	10.9(**0.004**)	2.26(0.32)	5.39(0.07)	0.04(0.98)	1.14(0.56)

[a] Frequency of canola in rotation where CCan = Continuous Canola, CW= Canola-Wheat, and CPB = Canola-Pea-Barley

[b] Values in parentheses are standard error of the mean. Upper case letters within a column denote statistically significant differences ($p<0.05$) between treatments within a sampling year.

[c] F values are presented with *p* values enclosed in parentheses are presented for all years.

Table 1.10. Mean values of extracellular enzyme activity of N-acetyl-glucosaminidase (NAG), Beta-glucosidase (BG), phosphatase (PHOS), arylsulfatase (ARYL), and phenol oxidase (PHN.OX) in soils collected at peak canola flowering after 12 years of crop rotations with varied canola frequency sampled in 2018 and 2019 at Scott, SK.

			NAG	BG	PHOS	ARYL	PHN.OX
		Rotation	----------nmol g^{-1} soil hr^{-1} ----------				
2018	1	CCan[a]	633(18.87)[b]A	847(85.8)A	2270(52.38)A	1040(18.1)A	805(84.0)A
	2	CW	611(12.16)A	814(20.1)A	2144(82.41)AB	1247(71.3)A	553(38.87)A
	3	CPB	616(8.31)A	881(78.2)A	1970(68.16)AB	945(50.7)A	597(19.88)A
2019	1	CCan	722(6.52)A	1055(6.5)B	1878(12.19)B	1766(155)A	533(19.64)A
	2	CW	667(7.34)A	989(18.3)A	1844(35.82)B	1186(198)A	717(41.10)A
	3	CPB	668(12.51)A	1274(32.3)A	1848(50.70)B	1110(200)A	654(10.28)A
ANOVA		*df*	----------F value (*p* value)----------				
Rotation		2	1.72[c](0.42)	0.38(0.83)	9.16(**0.01**)	1.47(0.48)	1.2(0.54)
Year		1	10.72(**0.001**)	3.56(**0.05**)	15.44(<**0.0001**)	8.10(**0.004**)	1.27(0.26)
Rot. x Year		2	0.18(0.91)	2.26(0.32)	3.78(0.15)	5.04(0.08)	1.8(0.41)

[a] Frequency of canola in rotation where CCan = Continuous Canola, CW= Canola-Wheat, and CPB = Canola-Pea-Barley

[b] Values in parentheses are standard error of the mean. Upper case letters within a column denote statistically significant differences (p<0.05) between treatments within a sampling year.

[c]F values are presented with *p* values enclosed in parentheses are presented for all years.

Table 1.11. Extracellular enzyme analysis of soils after 12 years of crop rotations with varied canola frequency sampled in 2018 and 2019 at Swift Current, SK.

		NAG	PHOS	BG	ARYL	PHN. OX
	Rotation	----------nmol g^{-1} dry soil hr^{-1}----------				
2018	CC	665(42.4)[a]A	1560(64.4)A	1023(64.7)A	1001(50.8)A	518(49.1)A
	CW	685(31.8)A	1569(30.2)A	1008(61.3)A	944(76.8)A	392(72.1)A
	CPB	682(18.8)A	1512(15.0)A	1241(79.3)A	922(42.1)A	582(61.4)A
2019	CC	572(16.1)A	1349(62.5)A	867(24.0)A	605(117.8)A	650(38.2)A
	CW	454(15.3)A	1222(60.4)A	674(32.7)A	457(72.0)A	682(33.4)A
	CPB	547(20.9)A	1452(71.2)A	811(49.8)A	500(42.7)A	597(20.8)A
ANOVA	*df*	----------F value (*p*-value)----------				
Rot.	2	0.11[b](0.95)	0.23(0.89)	3.70(0.16)	0.51(0.78)	3.92(0.14)
Year	1	1.99(0.16)	2.76(0.10)	1.32(0.25)	11.84(<**0.001**)	1.81(0.18)
Rot:Year	2	2.29(0.32)	2.55(0.28)	2.11(0.35)	0.32(0.85)	3.98(0.14)

[a] Values in parentheses are standard error of the mean. Upper case letters within a column denote statistically significant differences (p<0.05) between treatments within a sampling year.

[b] F values are presented with *p* values enclosed in parentheses are presented for all years.

Table 1.12. Relationship between POM and MAOM carbon content and mass fraction with microbial abundance (nmol PLFA g^{-1} soil) and extracellular enzyme activities at Lacombe, AB in 2018.

	MAOM OC (mg C g^{-1} soil)		POM OC (mg C g^{-1} soil)		MAOM (% mass)		POM (% mass)	
Microbial abundance	Rho[a]	p-value[b]	Rho	p-value	Rho	p-value	Rho	p-value
Total	0.66	**0.024**	0.67	**0.02**	0.73	**<0.001**	-0.83	**<0.001**
General Bacteria	0.62	**0.037**	0.63	**0.032**	0.68	**<0.001**	-0.8	**<0.001**
Gram Positive	0.56	0.063	0.76	**<0.001**	0.68	**0.015**	-0.76	**<0.001**
Gram Negative	0.63	**0.032**	0.53	0.079	0.66	**0.019**	-0.8	**<0.001**
Fungi	0.46	0.13	0.87	0.001	0.63	**0.027**	-0.69	**<0.001**
Actinobacteria	0.59	**0.049**	0.52	0.084	0.63	**0.027**	-0.76	**<0.001**
Stress 1	0.39	0.21	0.34	0.29	0.66	**0.019**	-0.44	0.15
Stress 2	-0.62	**0.037**	-0.87	**<0.001**	-0.74	**0.006**	0.71	**0.012**
Fungi:Bacteria	0.67	**0.02**	0.41	0.18	0.7	**0.012**	-0.73	**<0.001**
Gneg:Gpos	0.3	0.34	-0.084	0.8	0.19	0.56	-0.29	0.37
Enzyme Activity								
Phos.	-0.39	0.21	-0.16	0.62	-0.47	0.13	0.59	**0.049**
NAG	-0.34	0.28	-0.27	0.4	-0.51	0.087	0.66	**0.024**
BG	0.37	0.24	0.89	**<0.001**	0.56	0.058	-0.59	**0.049**
Aryl.	0.62	**0.037**	0.35	0.27	0.63	**0.028**	-0.69	**0.016**
Phn. Ox.	0.14	0.67	0.72	**0.011**	0.33	0.3	-0.49	0.11

[a] Spearman's rank correlation reported as Rho values where positive 1= positive correlation, 0=null correlation, and -1= negative correlation between factors.
[b] Statistically significant correlation between factors indicated by *p* values ≤ 0.05.

2. Wheat Cropping System

Soil organic matter fractions.

The mass fraction and carbon as MAOM was higher than POM in all soils evaluated but there were no differences between either the CW or any of the phases of the W-C-W-P rotation (**Table 2.1**). The amount of carbon as POM was highest in the CW soils and lowest in both W1 and W2 phases of the rotation. There were no differences in the mass fraction between CW or any of the phases of the rotation; differences in POM carbon were due to a higher concentration of carbon in the CW POM.

Microbial abundance and community structure.

Microbial abundance was not influenced by long-term continuous wheat monocropping vs. W-C-W-P at either the early vegetative or anthesis growth stages, demonstrating a strong influence of incoming plant photosynthate on microbial biomass (**Table 2.2**). It was surprising that differences in microbial biomass were not observed between the different phases of the four-year rotation since the quantity and composition of belowground C would have differed between crop types. Post-harvest, total, bacterial and fungal abundance was lowest in W2 (wheat after canola) and highest in C. Actinobacterial abundance was also lowest in W2, followed by W1, P and C and highest in CW (**Table 2.2**). Community structure

shifted with sampling time but was not affected by rotation treatment or by phase within the 4-year rotation (**Figure 2.1**; **Table 2.3**).

Extracellular Enzyme Activity

There were no differences in carbon cycling enzymes ß -D- cellobiohydrolase, ß-xylosidase or phenol oxidase between CW and any of the phase of the diverse crop rotation.

Correlations of MAOM and POM with microbial abundance and enzyme activity

Microbial abundance was highly correlated with POM carbon content (r^2 = 0.70-0.79; p values = 0.005 to <0.001)(**Table 2.5**). Cellobiohydrolase enzyme activity was also positively correlated with POM carbon content (r2 = 0.55; p =0.04), demonstrating a link between active cellulose breakdown and the POM pool in these soils. Since there were few rotation treatment differences in microbial abundance and no difference in cellobiohydrolase enzyme activity the mechanisms governing their links with POM appear to be controlled by common soil characteristics, rather than by differences due to treatment.

Summary

The accumulation of POM in the CW treatment may result from slower decomposition due to lack of variety in crop residue inputs. However, continuous wheat monocropping resulted in higher microbial biomass post-harvest than the four-year rotation which contradicts this observation. It is likely that greater overall residue inputs in the CW treatment may have led to accumulation of POM but this did not translate to differences in more stable MAOM. This is supported by the strong positive correlation between microbial PLFA abundance and POM in all soils.

Table 2.1. Mean values with standard error in parentheses of total carbon content in soils after 15 years of crop rotations with varied wheat frequency (n = 15) in Swift Current, SK.

		POM	MAOM	POM	MAOM
	Rotation	----------mg C g^{-1} soil----------		-------- % mass by fraction---------	
Post Harvest	CW[a]	6.71(0.61)A[b]	19.5(0.62)A	29.3(0.86)A	70.7(0.79)A
	W1	4.64(0.35)B	18.5(0.43)A	29.4(1.42)A	70.6(1.06)A
	C	5.03(0.07)AB	18.1(0.57)A	29.1(0.73)A	70.9(0.72)A
	W2	4.63(0.70)B	18.7(0.55)A	26.3(1.85)A	73.4(1.72)A
	P	5.14(0.72)AB	19.00.64)A	26.9(2.37)A	72.5(2.56)A
ANOVA	*df*	----------F-value (*p*-value)----------			
Rot.	4	13.6(**0.01**)		3.60(0.46)	
Fraction	1	974(<**0.0001**)		355(<**0.0001**)	
Rot. x Fraction	4	1.32(0.86)		6.11(0.19)	

[a] Rotation treatments where CW = Continuous wheat monocropping was compared to a W1-C-W2-P rotation where W1 = Wheat 1st year in rotation, C = Canola, W2 = Wheat 2nd year in rotation, and P = Pea.

[b] Values in parentheses are standard error of the mean. Upper case letters within a column denote statistically significant differences ($p<0.05$) between treatments within fraction.

Table 2.2. Abundance of PLFA functional group biomarkers in soils after 15 years of continuous wheat monocropping compared to wheat grown in rotation sampled at early vegetative, anthesis, and post-harvest timing in Swift Current, SK.

		Total PLFA	General Bacteria	Gram Positive	Gram Negative	Fungi	Actinobacteria
	Rotation	----------nmol g^{-1} soil----------					
Early Veg.	CW[a]	44.0(2.47)A[b]	25.8(1.32)A	10.0(0.51)A	12.7(0.70)A	1.44(0.13)A	3.44(0.15)A
	W1	48.1(1.95)A	28.8(1.23)A	11.4(0.40)A	13.8(0.72)A	1.47(0.10)A	4.09(0.14)A
	C	40.6(1.25)A	24.1(0.57)A	9.13(0.22)A	12.3(0.30)A	1.44(0.11)A	3.11(0.06)A
	W2	52.4(1.41)A	31.3(0.79)A	12.2(0.26)A	15.6(0.53)A	1.52(0.06)A	4.11(0.04)A
	P	51.5(1.59)A	30.1(0.86)A	11.8(0.35)A	14.7(0.40)A	1.88(0.11)A	4.06(0.13)A
Anthesis	CW	46.2(0.88)A	27.2(0.46)A	11.1(0.22)A	12.8(0.17)A	1.27(0.03)A	3.74(0.07)A
	W1	47.8(1.64)A	28.2(0.96)A	11.4(0.37)A	13.5(0.47)A	1.38(0.04)A	3.79(0.12)A
	C	40.1(2.27)A	23.9(1.38)A	9.5(0.53)A	11.9(0.74)A	1.24(0.08)A	2.96(0.14)A
	W2	45.1(1.87)A	26.7(1.04)A	10.7(0.40)A	12.9(0.55)A	1.40(0.09)A	3.62(0.12)A
	P	46.6(0.91)A	27.7(0.47)A	11.2(0.18)A	13.1(0.21)A	1.15(0.06)A	3.81(0.10)A
Post Harv.	CW	54.6(1.28)A	32.0(0.82)A	13.5(0.32)A	14.8(0.42)A	1.36(0.05)A	4.31(0.10)A
	W1	47.1(1.55)AB	27.5(0.90)AB	11.4(0.38)AB	12.8(0.41)AB	0.76(0.05)B	3.91(0.13)B
	C	49.6(0.68)AB	29.6(0.38)AB	12.1(0.16)AB	14.1(0.17)AB	0.73(0.01)B	3.95(0.07)B
	W2	37.7(1.78)B	22.1(1.11)B	9.26(0.46)B	10.3(0.53)B	0.70(0.03)B	2.91(0.17)C
	P	48.4(1.11)AB	28.2(0.65)AB	11.6(0.24)AB	13.6(0.37)AB	1.11(0.05)AB	3.59(0.07)B
ANOVA	*df*	----------F value (*p*-value) ----------					
Crop	4	4.59 (0.33)	5.11(0.28)	6.21(0.18)	3.90(0.42)	2.86(0.58)	8.17(0.09)
Sampling Time	2	2.88(0.24)	3.05(0.22)	5.71(**0.05**)	1.44(0.49)	0.29(0.86)	3.79(0.15)
Crop x Sampling Time	8	10.3(0.24)	12.07(0.15)	13.3(0.10)	9.81(0.28)	7.81(0.45)	17.4(0.03)

[a] Continuously cropped wheat compared to W1-C-W2-P rotation where CW = Continuous Wheat, W1= Wheat 1, C = Canola, W2 = Wheat 2, and P = Pea

[b] Values in parentheses are standard error of the mean. Upper case letters within a column denote statistically significant differences (p<0.05) between treatments within a sampling time.

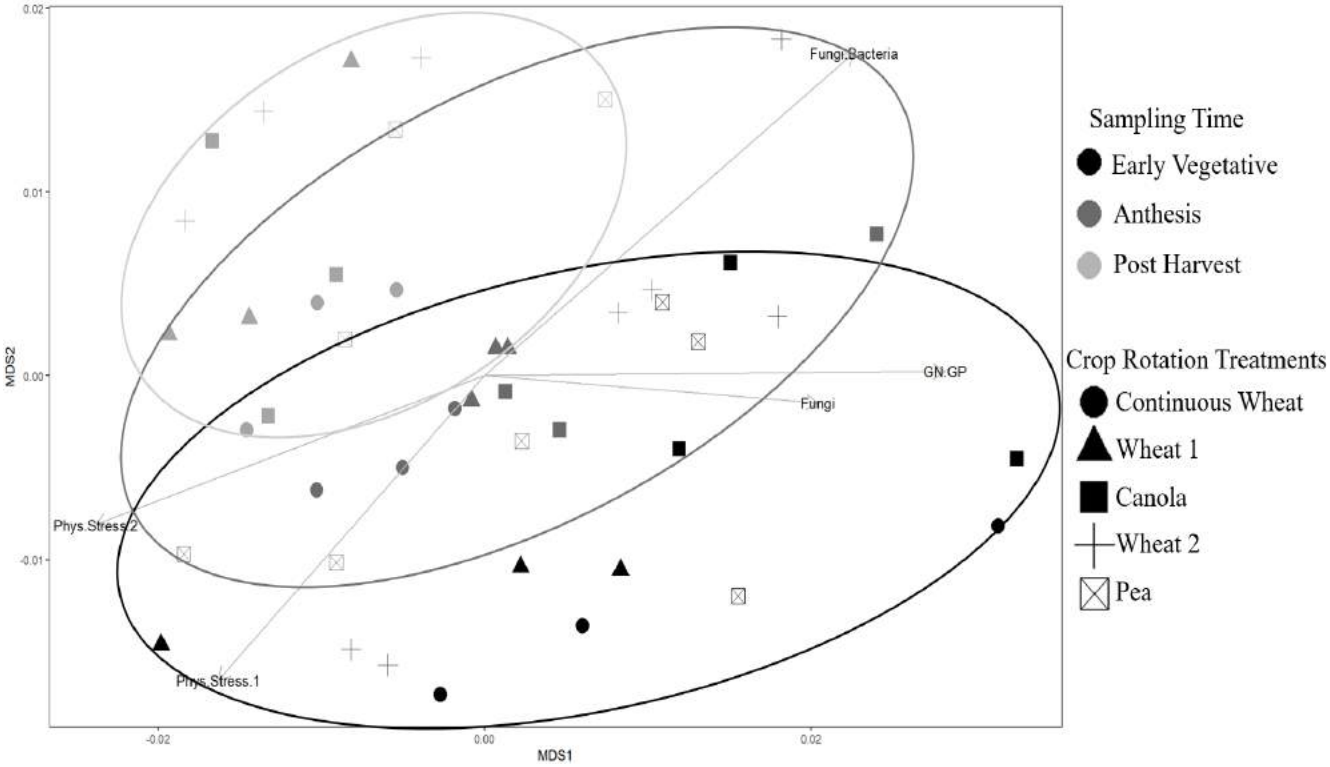

Figure 2.1. Non-metric Multi-Dimensional Scaling (NMDS) ordination of relative microbial abundance with correlated relative microbial abundance depicted as vectors (R^2 values $\geq$ 0.3 and *p* values < 0.05) in soils after 15 years of continuous wheat monocropping compared to wheat grown in rotation sampled at early vegetative, anthesis, and post-harvest timing in Swift Current, SK.

Table 2.3. PERMANOVA analysis of (NMDS) ordination of relative microbial abundance correlated with abundance of functional microbial groups in soils after 15 years of continuous wheat monocropping compared to wheat grown in rotation sampled at early vegetative, anthesis, and post-harvest timing in Swift Current, SK.

Swift Current, SK	Df	Sum of Sqs	R^2	F	Pr(>f)
Rotation	4	0.0007299	0.11441[a]	1.2919	0.2136[b]
Residuals	40	0.0056497	0.88559		
Total	44	0.0063796	1.00000		

[a] R^2 values $\geq$ 0.3 indicate significant correlation between soil relative microbial abundance and relative abundance.
[b] *Pf(>f)* values $\leq$ 0.05 were considered significant.

Table 2.4. Extracellular enzyme analysis of soils after 15 years of crop rotations with varied wheat frequency sampled at early vegetative, anthesis, and post-harvest timing in Swift Current, SK.

Swift Current, SK		CBH[a]	BX	PHN.OX
	Rotation	---------nmol activity g^{-1} dry soil hr^{-1}---------		
Early Vegetation	CW[b]	314(5.75)[c]	298(1.27)	27.9(3.63)
	W1	303(11.67)	285(7.34)	22.8(0.83)
	C	321(2.91)	296(2.57)	24.7(1.79)
	W2	357(17.10)	307(6.74)	14.9(3.20)
	P	342(8.57)	294(4.37)	22.2(0.54)
Anthesis	CW	277(8.88)	235(1.78)	26.5(2.24)
	W1	276(5.91)	252(6.01)	37.9(4.75)
	C	280(10.72)	235(5.51)	22.4(0.48)
	W2	289(8.33)	245(5.82)	23.2(1.19)
	P	269(2.62)	273(20.73)	24.9(1.67)
Post Harvest	CW	371(18.44)	322(8.57)	27.7(3.20)
	W1	324(4.72)	319(9.81)	34.1(0.61)
	C	322(8.84)	298(7.53)	29.4(3.41)
	W2	313(9.67)	292(5.71)	19.9(3.86)
	P	290(21.24)	289(3.21)	27.6(1.95)
ANOVA	*df*	----------F value (*p*-value) ----------		
Rot.	4	5.31[d](0.26)	1.27(0.87)	3.77(0.44)
Sampling Time	2	12.50(**0.002**)	20.87(<**0.0001**)	0.05(0.98)
Rot: Sampling Time	8	11.88(0.16)	10.86(0.21)	4.86(0.77)

[a] CBH = ß -D- Cellobiohydrolase, BX = ß-Xylosidase, and PHN. OX = Phenol Oxidase.

[b] Continuously cropped wheat compared to W1-C-W2-P rotation where CW = Continuous Wheat, W1= Wheat 1, C = Canola, W2 = Wheat 2, and P = Pea.

Table 2.5. Correlation of microbial abundance at post harvest sampling to POM total carbon content in soils after 15 years of continuous wheat monocropping versus wheat grown in rotation at Swift Current, SK.

	MAOM OC (mg C g^{-1} soil)		POM OC (mg C g^{-1} soil)		MAOM (% mass)		POM (% mass)	
Microbial abundance	Rho[a]	p-value[b]	Rho	p-value	Rho	p-value	Rho	p-value
Total	0.33	0.23	0.76	**<0.001**	-0.24	0.38	0.24	0.39
General Bacteria	0.36	0.19	0.77	**0.001**	-0.27	0.33	0.26	0.34
Gram Positive	0.31	0.27	0.7	**0.005**	-0.26	0.34	0.26	0.35
Gram Negative	0.44	0.10	0.79	**<0.001**	-0.2	0.47	0.19	0.49
Fungi	0.53	**0.04**	0.79	**<0.001**	-0.29	0.29	0.28	0.31
Actinobacteria	0.44	0.11	0.72	**0.003**	-0.39	0.15	0.4	0.14
Stress 1	-0.027	0.92	-0.1	0.71	-0.27	0.33	0.24	0.39
Stress 2	-0.51	**0.05**	-0.61	**0.02**	-0.025	0.93	0.068	0.81
Fungi:Bacteria	0.17	**0.05**	0.34	0.22	0.36	0.19	-0.37	0.17
Gneg:Gpos	-0.082	0.77	0.086	0.76	0.17	0.53	-0.17	0.54
Enzyme Activity								
CBH	0.41	0.13	0.55	**0.04**	0.2	0.47	-0.22	0.42
BX	0.49	0.06	0.2	0.48	-0.13	0.65	0.1	0.72
Phn. Ox.	-0.22	0.43	-0.17	0.54	-0.05	0.86	0.064	0.82

[a] Spearman's rank correlation reported as Rho values where positive 1= positive correlation, 0=null correlation, and -1= negative correlation between factors.

[b] Statistically significant correlation between factors indicated by p values ≤ 0.05.

Conclusions and Recommendations

This project demonstrated that functionally important mineral-associated (MAOM) and particulate organic (POM) matter pools were not affected by long-term continuous monocropping vs. diverse crop rotations in the wheat and canola systems studied. Previous work showed that crop yields are higher in the diverse rotations, but this does not appear to affect the long-term quantity or partitioning of MAOM and POM pools which may be determined by intrinsic soil properties, rather than composition of the organic matter inputs. Alternatively, the quantity of preferentially stabilized plant carbon (e.g. rhizodeposits) from large root systems of cereal crops and canola may compensate for differences in quality, compared to legumes.

We sampled at peak flowering in the canola systems which may not be ideal for detecting the long-term interactions of microbial abundance and MAOM and POM carbon pools. Inputs of root-derived photosynthetic carbon from the growing crop cover may overshadow the influence of MAOM and POM on microbial abundance and community structure. It is recommended that future studies studying these relationships sample in fall after harvest or in early spring before the crop is planted.

Detail any major concerns or project setbacks:

We did not determine potentially mineralizable carbon by CO_2 respiration because of the lack of differences in the MAOM and POM pools and overall microbial biomass. Instead, we focussed our efforts and re-profiled funds to repeating some of the soil organic matter fractionation assays to ensuring their accuracy because of the surprising result that there were few treatment-induced differences. Specifically, we performed both total and organic carbon analysis (i.e. untreated and acid-treated) on each of the fractions. Data reported here are organic C using the acid-treated samples.

Success stories/ practical implications for producers or industry:

This project demonstrated that functionally important mineral-associated and particulate organic matter pools were not consistently affected by long term continuous monocropping vs. diverse crop rotations in wheat and canola systems. Long-term quantity or partitioning of MAOM and POM pools in the Prairie annual cropping systems studied here may be determined more strongly by intrinsic soil properties, with quantity and quality of organic matter inputs playing secondary role.

Patents/ IP generated/ commercialized products: Not applicable.

List technology transfer activities:

- Reed, M.J. and B.L. Helgason. 2022. Impact of Short Corn Rotation on Soil Microbial Dynamics and Carbon Cycling. Soil Science Society of America, International Soils Meeting. Baltimore, MD, November 8.

- Reed, M., J. Town, R. Lemke, B. Tidemann, B.L. Helgason. Impacts of short rotation canola on soil microbial dynamics and nutrient cycling. Canadian Society of Soil Science Annual Meetings, Edmonton, AB. June 23-27, 2022.

- Reed, M., B.L. Helgason, R. Lemke and C.F. Drury. 2022. Impacts of Continuous Monocropping vs Crop Rotation on POM and MAOM Carbon Storage and Microbial Activities in Agricultural Soils. Soils and Crops online Conference. Saskatoon, SK. March 8-9.

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Appendix A.

Table A1. Relationship between POM and MAOM carbon content and mass fraction with microbial abundance (nmol PLFA g^{-1} soil) and extracellular enzyme activities at Lacombe, AB in 2019.

	MAOM OC mg C g^{-1} soil		POM OC mg C g^{-1} soil		MAOM % mass		POM % mass	
PLFA	Rho[a]	p-value[b]	Rho	p-value	Rho	p-value	Rho	p-value
Total	0.056	0.87	0.32	0.31	0.049	0.89	0.007	0.99
General Bacteria	0.043	0.90	0.36	0.26	-0.035	0.92	0.098	0.77
Gram Positive	0.07	0.83	0.35	0.27	0.056	0.87	0	1
Gram Negative	0.028	0.94	0.34	0.28	-0.042	0.90	0.11	0.73
Fungi	-0.21	0.96	0.36	0.25	0.11	0.73	-0.1	0.75
Actinobacteria	0.19	0.56	0.063	0.85	-0.098	0.77	0.12	0.72
Stress 1	-0.37	0.24	0.23	0.47	-0.15	0.65	0.2	0.53
Stress 2	0.23	0.47	-0.091	0.78	-0.46	0.13	0.39	0.21
Fungi:Bacteria	0.16	0.62	0.2	0.53	0.61	0.04	-0.63	**0.03**
Gneg:Gpos	-0.6	**0.02**	-0.014	0.97	-0.42	0.18	0.51	0.09
Enzyme Activity								
Phos.	0.64	**0.03**	0.47	0.13	0.55	0.07	-0.63	**0.03**
NAG	0.66	**0.02**	0.49	0.11	0.4	0.20	-0.48	0.12
BG	0.62	**0.04**	0.72	**0.01**	0.46	0.13	-0.46	0.13
Aryl.	0.15	0.64	0.25	0.43	0.13	0.70	-0.042	0.9
Phn. Ox.	-0.2	0.54	0.11	0.73	0.32	0.31	-0.28	0.8

[a] Spearman's rank correlation reported as Rho values where positive 1= positive correlation, 0=null correlation, and -1= negative correlation between factors.
[b] Statistically significant correlation between factors indicated by *p* values $\leq$0.05.

Table A2. Relationship between POM and MAOM carbon content and mass fraction with microbial abundance (nmol PLFA g^{-1} soil) and extracellular enzyme activities at Scott, SK in 2018.

	MAOM OC mg C g^{-1} soil		POM OC mg C g^{-1} soil		MAOM % mass		POM % mass	
PLFA	Rho[a]	p-value[b]	Rho	p-value	Rho	p-value	Rho	p-value
Total	-0.098	0.77	0.62	**0.04**	0.11	0.73	-0.081	0.80
General Bacteria	-0.15	0.64	0.51	0.09	0.14	0.67	-0.11	0.74
Gram Positive	0.007	0.99	0.66	**0.03**	0.15	0.64	-0.1	0.75
Gram Negative	-0.22	0.48	0.43	0.17	-0.014	0.97	-0.011	0.97
Fungi	0.021	0.96	0.55	0.07	0.07	0.83	-0.046	0.89
Actinobacteria	-0.049	0.89	0.35	0.27	0.17	0.59	-0.098	0.76
Stress 1	-0.36	0.26	0.2	0.54	0.13	0.70	-0.18	0.57
Stress 2	0.41	0.18	-0.084	0.80	-0.098	0.77	0.16	0.61
Fungi:Bacteria	-0.49	0.11	-0.2	0.53	-0.021	0.96	-0.14	0.66
Gneg:Gpos	-0.62	0.04	-0.58	**0.05**	-0.37	0.24	0.21	0.52
Enzyme Activity								
Phos.	-0.014	0.97	0.21	0.51	0.17	0.60	-0.039	0.91
NAG	0.042	0.90	0	1	0.25	0.43	-0.19	0.56
BG	-0.014	0.97	0.035	0.92	0	1	0.11	0.75
Aryl.	0.035	0.92	0.48	0.12	0.23	0.47	-0.07	0.83
Phn. Ox.	0.38	0.22	-0.1	0.75	0.063	0.85	0.025	0.94

[a] Spearman's rank correlation reported as Rho values where positive 1= positive correlation, 0=null correlation, and -1= negative correlation between factors.
[b] Statistically significant correlation between factors indicated by *p* values $\leq$0.05.

Table A3. Relationship between POM and MAOM carbon content and mass fraction with microbial abundance (nmol PLFA g^{-1} soil) and extracellular enzyme activities at Scott, SK in 2019.

	MAOM OC mg C g^{-1} soil		POM OC mg C g^{-1} soil		MAOM % mass		POM % mass	
PLFA	Rho[a]	p-value[b]	Rho	p-value	Rho	p-value	Rho	p-value
Total	0.091	0.78	0.64	**0.03**	0.16	0.62	0.098	0.77
General Bacteria	0.14	0.67	0.55	0.07	0.27	0.39	-0.23	0.47
Gram Positive	-0.07	0.83	0.28	0.38	0.45	0.14	-0.34	0.28
Gram Negative	0.2	0.53	0.69	**0.02**	0.21	0.51	-0.15	0.65
Fungi	0.035	0.92	0.38	0.23	0.24	0.45	-0.22	0.50
Actinobacteria	0.29	0.35	0.23	0.47	0.49	0.11	-0.49	0.11
Stress 1	0.27	0.39	0.73	**0.01**	0.11	0.74	-0.049	0.89
Stress 2	-0.28	0.38	-0.69	**0.02**	-0.22	0.50	0.17	0.60
Fungi:Bacteria	0.39	0.21	0.55	0.07	0.2	0.54	-0.2	0.54
Gneg:Gpos	0.36	0.26	0.71	**0.01**	0.11	0.74	-0.084	0.80
Enzyme Activity								
Phos.	0.2	0.53	0.22	0.48	-0.22	0.49	0.31	0.32
NAG	0.19	0.56	0.22	0.48	-0.54	0.07	0.43	0.16
BG	0.29	0.35	0.73	**0.01**	-0.088	0.79	0.16	0.62
Aryl.	0.31	0.32	0.69	**0.02**	0.018	0.96	0.028	0.94
Phn. Ox.	0.021	0.96	-0.34	0.29	0.8	**0.01**	-0.81	**0.01**

[a] Spearman's rank correlation reported as Rho values where positive 1= positive correlation, 0=null correlation, and -1= negative correlation between factors.
[b] Statistically significant correlation between factors indicated by *p* values $\leq$0.05.

Table A4. Relationship between POM and MAOM carbon content and mass fraction with microbial abundance (nmol PLFA g^{-1} soil) and extracellular enzyme activities at Swift Current SK in 2018.

	MAOM OC mg C g^{-1} soil		POM OC mg C g^{-1} soil		MAOM % mass		POM % mass	
PLFA	Rho[a]	p-value[b]	Rho	p-value	Rho	p-value	Rho	p-value
Total	-0.15	0.65	0.52	0.08	-0.07	0.83	0.29	0.35
General Bacteria	-0.25	0.43	0.59	**0.05**	-0.19	0.56	0.34	0.29
Gram Positive	-0.41	0.18	0.68	**0.02**	-0.33	0.30	0.46	0.13
Gram Negative	-0.084	0.80	0.54	0.08	-0.077	0.82	0.38	0.22
Fungi	-0.014	0.97	0.29	0.37	-0.007	0.99	0.23	0.47
Actinobacteria	-0.36	0.26	0.48	0.12	-0.007	0.99	0.21	0.51
Stress 1	0.23	0.47	0.34	0.29	0.17	0.60	0.2	0.54
Stress 2	-0.22	0.48	-0.27	0.40	-0.17	0.60	-0.11	0.73
Fungi:Bacteria	0.27	0.40	0.28	0.38	0.056	0.87	0.2	0.53
Gneg:Gpos	0.4	0.20	0.1	0.75	0.29	0.37	0.15	0.64
Enzyme Activity								
Phos.	0.2	0.53	0.3	0.34	0.17	0.59	-0.014	0.97
NAG	0.36	0.25	0.4	0.20	-0.13	0.70	0.21	0.51
BG	0.17	0.60	0.43	0.16	-0.24	0.46	0.14	0.67
Aryl.	-0.14	0.67	0.0091	0.78	0	1	0.15	0.64
Phn. Ox.	0.27	0.39	0.45	0.15	-0.24	0.44	0.19	0.56

[a] Spearman's rank correlation reported as Rho values where positive 1= positive correlation, 0=null correlation, and -1= negative correlation between factors.
[b] Statistically significant correlation between factors indicated by *p* values $\leq$0.05.

Table A5. Relationship between POM and MAOM carbon content and mass fraction with microbial abundance (nmol PLFA g^{-1} soil) and extracellular enzyme activities at Swift Current, SK in 2019.

	MAOM OC mg C g^{-1} soil		POM OC mg C g^{-1} soil		MAOM % mass		POM % mass	
PLFA	Rho[a]	p-value[b]	Rho	p-value	Rho	p-value	Rho	p-value
Total	0.57	**0.055**	0.21	0.52	-0.014	0.97	-0.41	0.19
General Bacteria	0.55	0.067	0.13	0.69	-0.014	0.97	-0.42	0.18
Gram Positive	0.5	0.099	0.17	0.59	-0.15	0.65	-0.3	0.34
Gram Negative	0.62	**0.037**	0.13	0.69	0.07	0.83	-0.52	0.08
Fungi	0.52	0.084	0.2	0.53	-0.12	0.72	-0.37	0.24
Actinobacteria	0.52	0.089	0.16	0.61	0.11	0.73	-0.18	0.57
Stress 1	0.71	**0.013**	0.28	0.38	0.25	0.43	-0.36	0.26
Stress 2	-0.76	**0.007**	-0.2	0.53	-0.26	0.42	0.49	0.11
Fungi:Bacteria	0.52	0.089	0.53	0.08	0.23	0.47	-0.049	0.89
Gneg:Gpos	0.43	0.160	0.14	0.67	0.11	0.73	-0.26	0.42
Enzyme Activity								
Phos.	0.3	0.34	0.56	**0.06**	-0.21	0.51	0.36	0.25
NAG	0.43	0.16	0.42	0.17	-0.1	0.75	-0.24	0.46
BG	0.69	**0.02**	0.37	0.23	0.14	0.67	-0.36	0.25
Aryl.	0.55	0.07	0.42	0.17	0.52	0.09	-0.14	0.67
Phn. Ox.	0.42	0.18	-0.22	0.49	0.54	0.08	-0.49	0.11

[a] Spearman's rank correlation reported as Rho values where positive 1= positive correlation, 0=null correlation, and -1= negative correlation between factors.
[b] Statistically significant correlation between factors indicated by *p* values $\leq$0.05.