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Effect of Application of Liquid Swine Manure on Soil Organic Carbon and Enzyme Activities in Two Contrasting Saskatchewan Soils

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Abstract

Repeated application of animal manure to agricultural fields as a source of plant nutrients has led to questions concerning the impact of this practice on soil organic carbon (C) and biochemical properties, specifically the activity of soil enzymes. There are also some environmental concerns of using livestock manure. The objectives of this study were to determine the effects of repeated applications of liquid swine manure (LSM) on total organic C (TOC), light fraction organic C (LFOC) and on the activity of the soil enzymes (arylsulfatase, alkaline phosphatase and urease) in two contrasting soil-climatic zones and cropping systems in Saskatchewan. Liquid hog manure was applied annually at 37,000 L ha^{-1} and 74,000 L ha^{-1}rates for three years at Melfort (Mollic Cryoboralf) and for four years at Plenty (Typic Boroll) in Saskatchewan, Canada. Soil samples were collected to a depth of 0-15 cm in the spring of 2003 and analyzed for TOC, LFOC and enzyme activities. Annual application (37,000 L ha^{-1}) and larger application made every two years (74,000 L ha^{-1}) of LSM at Melfort increased LFOC, which was attributed to stimulation of plant growth and thus residue inputs, from the nutrients contained within the manure. Applications of LSM at 37,000 L ha^{-1} and 74,000 L ha^{-1} at the Plenty site increased both TOC and LFOC concentration when compared to the control. Soil at the Plenty site is a Typic Boroll of heavy clay texture, which aids in protecting soil organic matter (SOM) from decomposition by soil microorganisms. Melfort was the only site that responded to LSM applications in terms of increased enzyme activity, which may be a result of a shorter application history. The Melfort site also had significantly higher LFOC in the manure treatments compared to the other site and LFOC has been linked to enzyme activity. The results of this study indicate that it may take a long period of time for addition of LSM to produce measurable changes in TOC and LFOC, as the effect from LSM is mainly from the stimulation of plant growth rather than from direct additions of C, and the nutrients contained in the LSM may potentially enhance microbial decomposition. In soils that receive repeated applications of LSM, nutrient loading may contribute to reduced enzyme activity after a period of time.

Keywords: arylsulfatase, light fraction organic C, liquid hog manure, total organic C, phosphatase, urease

1. Introduction

Livestock manure is a valuable source of plant nutrients such as nitrogen (N), phosphorus (P) and potassium (K) (Schoenau & Assefa, 2004). It is also a source for organic matter (OM). In solid cattle manure (SCM), more than 25% of the manure is in solid form and contributes directly to the soil organic matter (SOM) when applied and incorporated into the soil (Mooleki et al., 2004). Only about 2% or less of liquid swine manure (LSM) in Western Canada is solid material (Saskatchewan Agriculture and Food, 2003) and the agronomic benefits of LSM application in enhancing crop yields in the Canadian prairies has been well documented (Mooleki et al., 2002; Mooleki et al., 2003). Most of the nutrients contained in LSM are in a plant available inorganic form (Schoenau & Assefa, 2004). The OM content of the LSM is low, thus the mechanism by which LSM can potentially increase soil organic carbon (SOC) is through supplying nutrients, which increases above and below ground plant biomass, and subsequently adds OM to the soil.

Application of manures with high OM content such as SCM add organic carbon (C) directly to the soil due to the C content of feces and bedding materials such as straw in the manure. Plants utilize nutrients such as N and P contained in the LSM to increase biomass. It has long been known that the application of animal manures

increases the OM content of the soil and provides benefits such as improved soil structure, increased water infiltration and water holding capacity, increased nutrient holding capacity, better nutrient cycling, and reduced wind and water erosion (Assefa et al., 2004). Soils with low organic C contents have been identified as having large potential for C sequestration (Dumanski et al., 1998; Izaurralde et al., 1998). The LFOC is considered to be an important indicator of SOM turnover as the light fraction serves as an easily decomposable substrate for soil microorganisms (Carter et al., 1998). Increase in LFOC mass in the upper soil layer (0-10 cm) is a good indicator of soil C response to N fertilization (Malhi et. al., 2003).

Adeli et al. (2008) has reported on the effect on soil organic C (SOC) from surface applied LSM on clay soils in Mississippi. However, there is little literature on the effects of long-term addition of manure, especially liquid manures like LSM, on TOC amounts and forms in Canadian prairie soils. It is important to ascertain and understand the effects of management practices such as the application of LSM on SOC. Soil enzymes serve as the mediators and catalysts for soil functions that include decomposition of plant residue inputs, transformation of native SOM, release of inorganic nutrients, nitrification and denitrification (Dick, 1997). Soil health can be viewed as the ability of the soil to perform certain functions that are necessary to drive the biological components of an ecosystem, thus soil enzyme assays provide the potential to assess the biological status of a soil (Dick, 1997). Enzymes accumulate in soils from a variety of plant and animal sources, including but not limited to, remnants of degraded cells, as endoenzymes released from degrading cells, and as exoenzymes released from living cells (Kiss et al., 1975). Arylsulfatase is the enzyme responsible for the release of plant available sulfate by catalyzing the hydrolysis of ester sulfate (C-O-S) bonds. Mineralization of sulfur (S) in manure is an important process as a significant portion of S is contained in organic form that must be converted to sulfate to be plant available. Furthermore, manures, especially LSM, often have low availability of S relative to other nutrients and may not supply enough S to meet plant needs (Schoenau et al., 2003).

Lalande et al. (2000) reported that at a LSM application rate of 90,000 L ha^{-1} added to a previously unmanured soil, the activities of arylsulfatase, alkaline phosphatase and urease were all significantly increased ($p \leq 0.05$) in the 0-15 cm layer of a silt loam under continuous corn silage cropping system. The authors reported a trend towards more activity of arylsulfatase, alkaline phosphatase and urease at the 60,000 L ha^{-1} rate of LSM application compared to the lower rate (30,000 L ha^{-1}) and the control. At the lower rate of 30,000 L ha^{-1}, there was no significant difference in activity rates for arylsulfatase, alkaline phosphatase and urease between the manured plots and the control.

On average, LSM produced on the Canadian prairies contains 3.1 kg N m^{-3} as total N and 1.9 kg N m^{-3} as ammonium-N (NH_4–N) (Saskatchewan Ariculture and Food, 2003). However, LSM has a low C content when compared to SCM (Ndayegamiye & Cote, 1998). Addition of LSM may even reduce the TOC content in soil due to enhanced microbial oxidation of native SOM. Microorganisms will use the N in the manure in protein metabolism, but the lack of C for an energy source means the microorganisms will degrade organic residues to release C for the energy source to support bacterial growth (Lalande et al., 2000). Initially, enzyme activity may increase along with microbial activity following addition of the nutrient and OM source. However, if the net result is to decrease SOC content eventually, then enzyme activity may also decrease eventually. Enzymes in the soil environment are important catalysts for nutrient transformation and SOM turnover. Beiderbeck et al. (2005) assessed arylsulphatase and phosphatase activities in an Orthic Dark Brown Chernozem in southern Saskatchewan that had received green manure amendment and Kotkovka et al. (2008) has recently reported on arylsulfatase activities in the plant rhizosphere using rhizoboxes in a controlled environment. Balota et al. (2011) reported that application of LSM applications at 30, 60 and 120 m^3 ha^{-1} yr^{-1} influenced arylsulfatase enzyme activities in the 0-15 cm depth, as the organic carbon from plant residues form a storage location of sulfate esters that are substrates for this enzyme. The authors also reported that high rates of LSM application can create anaerobic areas in a soil which can lead to decreases in soil enzyme activity.

However, there is limited information on how manure application affects soil enzyme activities in Canadian prairie soils. Studies have reported that field management practices have an effect on soil enzyme activity, however a wide range of soil enzymes have not been studied (Bandick & Dick, 1999). The objectives of this study were (1) to assess the differences in total TOC and LFOC as related to the field application of different rates of LSM in two field experiments in Saskatchewan, and (2) to determine whether repeated applications of LSM result in differences in the activity of the soil enzymes arylsulfatase, alkaline phosphatase and urease.

2. Method

2.1 Experimental Sites

Field experiments were conducted at Melfort located at 52° 48.97' N 104° 30.75' W and Plenty located at 51° 47.86' N 108° 31.5' W in Saskatchewan, Canada. The two sites have contrasting soil type and climatic conditions (Melfort: sub-humid; Plenty: semi-arid). The soil at the Melfort site belongs to the Kamsack Association and is a Dark Gray Luvisol (US equivalent: Mollic Cryoboralf) formed in silty lacustrine materials and has a loam surface texture (Saskatchewan Soil Survey, 1987b). Sulfur deficiency also has been observed on this site (Schoenau et al., 2003). Crops grown on this site were spring wheat (*Triticum aestivum*) in 2000, canola (*Brassica napus*) in 2001, and oats (*Avena sativa*) in 2002. The soil at the Plenty site belongs to the Regina Association, and is a Dark Brown Chernozem (US equivalent: TypicBoroll) formed in clay lacustrine materials with a clay surface texture and of lower organic matter content than the Melfort site (Saskatchewan Soil Survey, 1987a). The surface soil is neutral to alkaline and formed under semi-arid environmental conditions.

Crops grown on this site were spring wheat (*Triticum aestivum*) in 1999, canary seed (*Phalaris canariensis*) in 2000, spring wheat (*Triticum aestivum*) in 2001, and spring wheat (*Triticum aestivum*) but crop failure (due to drought and grasshopper infestation) in 2002. Crops grown at both Saskatchewan sites for each year followed the land owner or producer best management crop rotation practices. The use of crop rotations is a common land best management practice in Saskatchewan, that is used to eliminate or reduce problems associated with problem weeds or plant diseases in crops. Growing conditions were also reported to be quite dry in 2000 and 2001, with lack of moisture being the main limiting factor for crop growth and development (Hultgreen et al., 2002). Total growing season precipitation for 2000, 2001 and 2002 reported by the nearest Environment Canada station located approximately 35 km east of the Plenty site, at Rosetown, SK was 263, 122 and 242 mm, respectively (Environment Canada, 2009).Total growing season precipitation for 2000, 2001 and 2002 reported by the nearest Environment Canada station located at Melfort, SK was 329, 133 and 313 mm, respectively (Environment Canada, 2009).

The Melfort site was established in the fall of 1999 and Plenty site in the fall of 1998, when LSM was applied to the plots. The Melfort plots were 3.9 m × 30 m in diameter. The Plenty site plots were 4.2 m × 30 m in diameter. At Plenty, the LSM was applied using an injector applicator with sweep furrow openers on the shanks in fall (October) of 1998, fall (October) of 1999 and the fall (October) of 2000. At Melfort, the LSM was applied using the sweep injector applicator in fall (October) of 1999 and in the fall (October) of 2000. Beginning in fall (October) of 2001 at both sites, the LSM was applied using an injector truck equipped with modified Bourgault™ low disturbance manure injector disc coulters spaced 30 cm apart. The LSM was applied at an average depth of 8-10 cm with both the sweep and disc machines. The Plenty site crops were seeded from spring of 1999 to spring of 2002 using an airseeder equipped with sweeps and harrow packed after seeding operations were conducted. The Melfort site crops were seeded from spring of 2000 to spring of 2002 using an air drill equipped with on-row packing.

2.2 Treatments and Experimental Design

Each experiment was set up as a randomized complete block design. Treatments were replicated four times at Melfort, and three times at Plenty. The LSM experiment at Melfort and Plenty consisted of four treatments listed in Table 1. The treatments were a control (no LSM), 37,000 L ha^{-1}, 74,000 L ha^{-1} and urea fertilizer applied at a rate of 80 kg N ha^{-1}. At both sites, repeated applications of various rates of LSM, or urea fertilizer at one rate were applied. The control treatment did not receive any LSM or urea fertilizer. In the manure treatments the LSM was injected into the soil with a LSM injector unit mounted on a truck. Nutrient composition of the most recent LSM addition is provided in Table 2. At each site, the lowest rate of LSM being applied (1×) each year was equal to about 100 kg total N ha^{-1}, in an attempt to apply an agronomic rate in line with the amount of N that would be recommended as fertilizer manure to meet a crop requirement. Higher rates of LSM (2×) were considered to be double (2×) the recommended agronomic rates of N fertilizer (100 kg N ha^{-1}) application for an application made every year, but were applied every second year instead of every year. In LSM systems, bedding is not usually added to the manure, but water from varied sources can be added to the manure raising the moisture contents to over 90% (Saskatchewan Agriculture and Food, 2003). At both sites, fall applications were usually made in mid-October, just before onset of freeze-up. In the urea fertilizer treatment based on regional fertilizer recommendations, commercial urea (46-0-0) was applied at 80 kg N ha^{-1} for the Melfort and Plenty sites (Table 1) for comparison to the results obtained in the LSM treatments.

Table 1. Treatments in the liquid hog manure experiment established in the fall of 1998 at Plenty, Saskatchewan and in 1999 at Melfort, Saskatchewan

Treatment	Sequence	N rate	Application method
0 L ha^{-1}	Control[a]	0 kg N ha^{-1}	No injector pass
37,000 L ha^{-1}	1×[b]	100 kg N ha^{-1}	Injection @ 30 cm spacing and 10 cm depth
74,000 L ha^{-1}	2×[c]	200 kg N ha^{-1}	Injection @ 30 cm spacing and 10 cm depth
Urea fertilizer	Annual[d]	80 kg N ha^{-1}	Banded urea 46-0-0 fertilizer

[a]Control treatment, no manure or fertilizer applied.

[b]The sequence, 1× for example, refers to the rate of hog manure application for the crop year ongoing yearly.

[c]The 2× hog manure treatment is applied every second year.

[d]Urea fertilizer banded at a rate of 80 kg N ha^{-1}.

2.3 Measurements and Procedures

2.3.1 Soil Organic C Analysis

2.3.1.1 Soil Sampling

Soil samples were collected from all replicated treatment plots at Melfort on April 25, 2003 and all replicated treatment plots at Plenty on April 30, 2003. Soil samples were taken using polyvinylchloride (PVC) pipes measuring 15 cm in height and 10 cm in diameter. Four PVC cores were inserted per plot, and 0-15 cm depth was sampled. Soil was removed from the cores, and the four cores from each plot bulked, mixed, air-dried, ground to pass a 2-mm sieve and stored in labeled plastic bags at 20 ºC, or room temperature for TOC, LFOC and enzyme analysis.

2.3.1.2 Determination of Soil Organic C

The TOC concentrations of ground soil samples (0-15 cm) of approximately 0.15 g, previously ground with a ball mill to pass a 100-mesh sieve, were measured by the dry combustion method using the LECO CR-12 Carbon Analyzer™ set at 840 ºC (Leco, 1987). The time needed for OC decomposition to CO_2 decreases with increasing temperature at 840 ºC, and OC can be continuously and completely decomposed to CO_2 in about 120 seconds, before any carbonates in the sample begin to decompose. Carbonates will decompose after 150 seconds at 840 ºC, therefore, sample size was adjusted to ensure that any carbonates were not oxidized but OC was completely oxidized (Wang & Anderson, 1998). For each sampling plot, the measured TOC concentration, measured soil bulk density and thickness (depth of sampling), were used to quantify TOC on a relative area basis (Mensah et al., 2003). The mass of TOC for each depth segment was calculated using the following equations:

$$Mass_{TOC} = Conc_{TOC} \times \rho_b \times T \times 10{,}000\ m^2\ ha^{-1} \times 0.001\ Mg\ kg^{-1} \quad \text{(Equation 1)}$$

Where: $Mass_{TOC}$= mass of organic C per unit area (Mg ha^{-1})

$Conc_{TOC}$=organic C concentration (kg Mg^{-1})

ρ_b = dry bulk density (Mg m^{-3})

T = depth segment or thickness of soil increment layer (m)

2.3.1.3 Determination of Light Fraction Organic C

The light fraction component of the OC in the soil samples was determined using the method developed by Gregorich and Ellert (1993). Twenty-five grams of air dried, sieved (2 mm mesh) soil samples were weighed into 110 mL centrifuge tubes and 50 mL of NaI (BDH, USP grade) of density 1.7 g cm^{-3} was added to each tube, covered, shaken for 60 min, and centrifuged at 1000 g for 20 min. The NaI containing the light fraction was decanted from the tubes and filtered (Millipore™ 0.45 μM filter). The light fraction retained by the filter was rinsed with 0.01 M $CaCl_2$ to remove all the NaI as iodine may interfere with C analysis and $CaCl_2$ prevents the clogging of the filter (Mensah et al., 2003). The light fraction was rinsed with deionized water to remove all the $CaCl_2$ from the light fraction. The light fraction was collected, dried for 72 hours at 45 ºC and weighed. The concentration of OC in the light fraction was determined using the LECO CR-12 Carbon Analyzer™ as described in the previous section. The mass of LFOC was calculated using the following equations:

$$Conc_{LFOC} = [(DryWt._{LFOM} \times \%C_{LFOM})/wt._{SOIL}] \times 1000 \quad \text{(Equation 2)}$$

Where $Conc_{LFOC}$ = concentration of carbon in the light fraction (kg Mg^{-1})

$DryWt._{LFOM}$ = dry weight of light fraction organic matter (g)

$\%C_{LFOM}$ = concentration of carbon in light fraction organic matter (%)

$wt._{SOIL}$= dry weight of soil (g)

$$Mass_{LFOC} = Conc_{LFOC} \times \rho_b \times T \times 10{,}000\ m^2\ ha^{-1} \times 0.001\ Mg\ kg^{-1} \quad \text{(Equation 3)}$$

Where: $Mass_{LFOC}$ = mass of light fraction organic carbon per unit area (Mg ha^{-1})

$Conc_{LFOC}$=carbon concentration of light fraction organic matter (kg Mg^{-1})

ρ_b = air dry bulk density (Mg m^{-3})

T = depth segment or thickness of soil increment layer (m)

0.001 Mg kg^{-1} = conversion factor

This calculation makes an adjustment for equivalent mass.

2.3.2 Soil Enzyme Analysis

Prior to enzyme analysis, samples were moistened to approximately 60-70% field capacity and kept at this moisture content at 20 ºC for 21 days to allow time for the biological and biochemical characteristics to stabilize before use in the enzyme experiments (Gupta, 1989; Gupta et al., 1993).

The activity of the arylsulfatase enzyme was estimated using buffered p-nitrophenyl sulfate as substrate and toluene following the procedure established by Tabatabai and Bremner (1970). This method is based on the colorimetric determination of the p-nitrophenol released by arylsulfatase activity (Tabatabai, 1982). Colorimetric determination of the p-nitrophenol released by arylsulfatase activity was conducted using a Beckman model DU-64 spectrophotometer set at a wavelength of 420 nm.

The activity of alkaline phosphatase was estimated using buffered sodium *p*-nitrophenol phosphate as substrate and toluene (Sigma-Aldrich, reagent grade) following the procedure established by Tabatabai and Bremner (1970) which measures the amount of *p*-nitrophenol released g^{-1} soil h^{-1}.

The activity of urease was estimated using the method described by Kandeler and Gerber (1988) which consists of the incubation of soil with a buffered urea solution and subsequent extraction of ammonium (NH_4) with 1 *M* KCl and 0.01 *M* HCl and colorimetric determination of NH_4. Urease activity is expressed as µg NH_4 hydrolyzed g^{-1} h^{-1} at 37 ºC.

2.4 Data Analysis

Data emanating from both sites were subjected to analysis of variance (ANOVA) determine any significant treatment effects using the general linear model (GLM) procedure of SAS (1985). Where ANOVA indicated significant treatment effects, means were compared using the least significant difference (LSD) at $p \leq 0.10$. A probability level of 0.10 was selected to assess significant treatment effects, owing to the inherently high variability in soil properties encountered in manured soils (Assefa et al., 2004; Stumborg & Schoenau, 2008).

3. Results

3.1 Soil Organic C

At Melfort, soil TOC mass in the control, 1× and 2× LSM treatments was higher ($p \leq 0.10$) than the TOC mass for the urea fertilizer treatment (61.7 Mg ha^{-1}) (Table 2). However, the mass of TOC measured in the 1× (67.1 Mg ha^{-1}) and 2× (67.3 Mg ha^{-1}) LSM treatments was not significantly different from the control. Urea fertilizer applied at 80 kg N ha^{-1} resulted in the lowest canola seed yield of all treatments (King et al., 2004). At this site, plots receiving only urea fertilizer have consistently exhibited signs of S deficiency and S deficiency greatly limited seed and straw production in the urea plots in the canola crop in 2001 (Mooleki et al., 2003). In 2002 the seed yield of the oat crop was increased by applications of LSM and urea fertilizer, but there was no significant ($p \leq 0.10$) response to LSM rate, most likely as a result of dry conditions (Mooleki et al., 2003). Reduced amounts of plant biomass being produced would limit the amount of biomass incorporated into the soil to be decomposed by microorganisms.

Table 2. Total soil organic carbon in the 0-15 cm depth at the melfort and plenty sites under various hog manure and fertilizer treatments

	Melfort		Plenty	
	Mean	Std.dev.	Mean	Std.dev.
Treatment		Mg ha^{-1}		
Control[†]	68.68a	7.37	25.80b	1.21
1×[‡]	67.10a	6.23	28.73a	1.35
2×[§]	67.30a	6.88	28.76a	1.55
Urea[¶]	61.67b	6.93	29.33a	2.54
LSD $_{(0.10)}$[††]	4.22		2.76	

[†]Control- no manure or fertilizer applied.

[‡]Hog manure applied at 37,000 L ha^{-1} annually.

[§]Hog manure applied at 74, 000 L ha^{-1} every second year.

[¶]Urea applied at 80 kg N ha^{-1} annually.

[††]Least significant difference. Mean values in a column followed by the same letter are not significantly different at $p \leq 0.10$.

The addition of the N source as urea may have also stimulated microbial activity and decomposition of native SOM, further contributing to lower TOC in the urea treatment compared to others.

At the Plenty site, soil TOC was significantly ($p \leq 0.10$) different in the 1× and 2× LSM treatments versus the control plots (Table 2). The 1× and 2× LSM treatments produced a mean TOC mass of 28.7 and 28.8 Mg ha^{-1}, respectively, versus 25.8 Mg TOC ha^{-1} in the control plot. The urea fertilizer treatment also had significantly higher ($p \leq 0.10$) TOC (29.3 Mg ha^{-1}) compared to the control plots. The TOC mass in the urea fertilized plots was not significantly different from the two rates of LSM. This site has received low amounts of precipitation since the LSM experiment began in 1999 and Grevers (2002) reported that crop production at the site was frequently limited by lack of moisture. Low inputs of moisture restrict crop biomass production, which in turn limits the amount of plant biomass incorporated into the soil, thus limiting the inputs to TOC. However, dry soil conditions also reduce SOM decomposition rates. Furthermore, the Plenty site has low amounts of SOM and there is a large capacity to build the pool of TOC and LFOC. Soil at this site has a clay texture. Higher amounts of clay in a soil can better protect the native SOM from decomposition, allowing for greater C accretion (Anderson, 1995).

At the Melfort site, LFOC in soil for the 2× LSM treatment was significantly different than the control ($p \leq 0.10$) (Table 3). The 2× LSM treatment added every second year produced 3.13 Mg ha^{-1} of LFOC, while the control had 1.84 Mg ha^{-1} of LFOC. The carryover of nutrients from the previous year's application of manure aided in producing more plant biomass in the second year, such that, no significant differences in LFOC mass between the 1X and 2X rates of LSM treatments were observed. A significant increase in LFOC compared to the control with LSM addition, despite no increase in TOC mass, reflects the influence of the LSM and urea fertilizer on stimulating plant growth and increasing recent C additions.

Table 3. Light fraction organic carbon in the 0-15 cm depth at the Melfort and Plenty sites under various hog manure and fertilizer treatments

	Melfort		Plenty	
	Mean	Std.dev.	Mean	Std.dev.
Treatment			Mg ha^{-1}	
Control[†]	1.84b	0.29	0.92b	0.17
1×[‡]	2.68ab	0.69	1.64a	0.18
2×[§]	3.13a	0.91	1.73a	0.31
Urea[¶]	2.64ab	1.18	1.49a	0.33
LSD $_{(0.10)}$[††]	1.03		0.48	

[†]Control- no manure or fertilizer applied.

[‡]Hog manure applied at 37,000 L ha^{-1} annually.

[§]Hog manure applied at 74, 000 L ha^{-1} every second year.

[¶]Urea applied at 80 kg N ha^{-1} annually.

[††]Least significant difference. Mean values in a column followed by the same letter are not significantly different at $p \leq 0.10$.

At Plenty, the LFOC in the two LSM treatments and urea fertilized treatments were higher than the control treatment, but not significantly ($p \leq 0.10$) different from each other (Table 4). The LFOC mass was 1.64 and 1.73 Mg ha^{-1} for the 1× and 2× (every second year) LSM treatments, respectively, while the control LFOC was 0.92 Mg ha^{-1}. The LFOC mass in the urea fertilized treatment (1.49 Mg ha^{-1}) was different compared to the control treatment, but was not significantly different from the two rates of LSM. The LFOC in the two rates of LSM followed the same pattern as the TOC levels at this site. The enhancement of LFOC from LSM and urea fertilizer indicate that in the future, an increase in TOC due to the LFOC being slowly transformed into more stable humus forms with time is expected (Stevenson, 1994).

3.2 Soil Enzyme Activities

Application of LSM at the Melfort site had no effect on soil arylsulfatase enzyme activity ($p \leq 0.10$) (Table 5). Soil arylsulfatase enzyme activity was similar in the control plot (20.2 µg p-nitrophenol released g^{-1} h^{-1}) to the 2× LSM treated plot (20.5 µg p-nitrophenol released g^{-1} h^{-1}) and urea 2× fertilized plot (20.3 µg p-nitrophenol released g^{-1} h^{-1}) (Table 5). At the Plenty site there was also no difference in soil arylsulfatase enzyme activity among the treatments ($p \leq 0.10$) (Table 4). Soil arylsulfatase enzyme activity slightly increased from 15.7 µg p-nitrophenol released g^{-1} h^{-1} in the control plot to 16.7 and 16.0 µg p-nitrophenol released g^{-1} h^{-1} soil in the 1× LSM treated plots and urea fertilized plots, respectively.

Table 4. Arylsulfatase enzyme activity in the 0-15 cm depth at the Melfort and Plenty sites under various hog manure and fertilizer treatments

	Melfort		Plenty	
	Mean	Std.dev.	Mean	Std.dev.
Treatment	*p*-nitrophenol released ($\mu g\ g^{-1}\ h^{-1}$)			
Control[†]	20.22a	2.50	15.73a	0.14
1×[‡]	18.70a	3.42	16.74a	2.16
2×[§]	20.51a	4.45	15.42a	3.08
Urea[¶]	20.34a	4.53	16.01a	0.64
$LSD_{(0.10)}$[††]	5.50		2.50	

[†]Control- no manure or fertilizer applied.

[‡]Hog manure applied at 37,000 L ha^{-1} annually.

[§]Hog manure applied at 74, 000 L ha^{-1} every second year.

[¶]Urea applied at 80 kg N ha^{-1} annually.

[††]Least significant difference. Mean values in a column followed by the same letter are not significantly different at $p \leq 0.10$.

Application of LSM at the Melfort site produced a small, but significant ($p \leq 0.10$) increase in soil alkaline phosphatase enzyme activity between the control plot and the LSM and urea fertilizer treated plots. Soil alkaline phosphatase enzyme activity increased from 53.6 µg p-nitrophenol released g^{-1} h^{-1} in the control plot to 63.7 and 60.3 µg p-nitrophenol released g^{-1} h^{-1} soil in the 1× and 2× LSM treated plots, respectively (Table 5). The significant positive effect of LSM addition at the Melfort site may be related to the shorter duration of manure addition at Melfort versus Plenty. The Melfort site showed no build up in soil extractable P (data not shown). Therefore a feedback inhibition from accumulated phosphate is not likely at the Melfort site.

Table 5. Alkaline phosphatase enzyme activity in the 0-15 cm depth at the Melfort and Plenty sites under various hog manure and fertilizer treatments

	Melfort		Plenty	
	Mean	Std.dev.	Mean	Std.dev.
Treatment	*p*-nitrophenol released ($\mu g\ g^{-1}\ h^{-1}$)			
Control[†]	53.61b	5.70	77.55a	2.33
1×[‡]	63.76a	4.26	77.76a	1.51
2×[§]	60.35a	0.75	74.60a	0.33
Urea[¶]	62.48a	6.76	77.18a	4.84
$LSD_{(0.10)}$[††]	6.60		3.30	

[†]Control- no manure or fertilizer applied.

[‡]Hog manure applied at 37,000 L ha^{-1} annually.

[§]Hog manure applied at 74, 000 L ha^{-1} every second year.

[¶]Urea applied at 80 kg N ha^{-1} annually.

[††]Least significant difference. Mean values in a column followed by the same letter are not significantly different at $p \leq 0.10$.

Application of LSM at the Plenty site had no effect on soil alkaline phosphatase enzyme activity ($p \leq 0.10$) (Table 5). Soil alkaline phosphatase enzyme activity was similar for all treatments at this site. Campbell et al. (1989) reported that on a southwestern Saskatchewan loam soil, the alkaline phosphatase activity increased from

378 µg p-nitrophenol released g^{-1} h^{-1} in the 0-7.5 cm depth of a fallow wheat crop rotation treatment to 568 µg p-nitrophenol released g^{-1} h^{-1} in a zero till treatment soil sample. The authors reported that the increase in alkaline phosphatase activity could have been due to higher organic P levels in the test plot soils. Dick and Tabatabai (1984) reported that arylsulfatase and alkaline phosphatase activities in the 0-7.5 cm depth were higher in no tilled fields versus conventionally tilled fields. A greater content of labile organic P extractable by bicarbonate was observed in LSM treatments at Plenty site compared to unmanured controls (Stumborg & Schoenau, 2008). However, a buildup of soluble inorganic P was also evident in plots treated with higher rates of LSM and this may have inhibited enzymatic activity.

There was no difference in soil urease activity between the control plot, the 2× LSM and the urea fertilizer treated plots ($p \leq 0.10$) at the Melfort site (Table 6). Soil urease activity decreased from 253.0 µg NH_4^+ released g^{-1} h^{-1} in the control plot to 189.4 µg NH_4^+ released g^{-1} h^{-1} in the 1× LSM treated plots (Table 7). The lack of differences among treatments, except for the 1× treatment at Melfort when compared to the control plot could be due to the 2× LSM treatment being last applied in the fall of 2001, while the enzyme measurements were made in the spring of 2003.

At the Plenty site there was no difference in soil urease activity between the control plot and the LSM and urea fertilizer treated plot ($p \leq 0.10$). However, there was a trend for urease activity to increase with addition of LSM and urea fertilizer (Table 6). Soil urease activity did show a trend in increasing activity, from 202.0 µg NH_4^+ released g^{-1} h^{-1} in the control plot to 216.7 and 223.7 µg NH_4^+ released g^{-1} h^{-1} in the 1× and 2× hog manure treated plots, respectively.

Table 6. Urease enzyme activity in the 0-15 cm depth at the Melfort and Plenty sites under various hog manure and fertilizer treatments

	Melfort		Plenty	
	Mean	Std.dev.	Mean	Std.dev.
Treatment		NH_4 released (µg g^{-1} h^{-1})		
Control[†]	253.03a	82.82	202.03a	70.99
1×[‡]	189.47b	67.09	216.73a	17.72
2×[§]	225.07ab	29.06	223.77a	32.28
Urea[¶]	220.67ab	57.58	278.80a	31.31
$LSD_{(0.10)}$[††]	51.7		77.40	

[†]Control- no manure or fertilizer applied.

[‡]Hog manure applied at 37,000 L ha^{-1} annually.

[§]Hog manure applied at 74, 000 L ha^{-1} every second year.

[¶]Urea applied at 80 kg N ha^{-1} annually.

[††]Least significant difference. Mean values in a column followed by the same letter are not significantly different at $p \leq 0.10$.

4. Discussion

Despite no significant increase in TOC at Melfort, there was an increase in LFOC with LSM application, likely due to the manure stimulating plant biomass growth and C additions. High amounts of clay in the soil, as at the Plenty site, can protect the OM from microbial decomposition (Perucci et al., 1984). This site is also low in SOM compared to Melfort and has a greater capacity to build the TOC pool (Mensah et al., 2003).

Based on the results of this study, there are several factors that need to be considered when attempting to predict the effect of LSM application on TOC and LFOC mass in the soil. Time is an important factor. The LSM applications were treatments made over 4 years at Melfort site and over 5 years at the Plenty site which is a relatively short time period. Increases in C inputs may take decades to manifest themselves in detectable TOC increases, due to decomposition of most of the added C to carbon dioxide (CO_2), with only a small portion converted to more stable humus forms. It must be noted that LSM adds to C indirectly, via the uptake of nutrients contained within the manure by plants, which can then increase above and below ground biomass.

Several studies have demonstrated that microbial activity is enhanced with animal manure application (Charles, 1999; de Frietas et al., 2003). It is possible that the nutrients contained within the LSM were utilized by microbial populations and contributed to decomposition of native TOC pools.

Plaza et al. (2004) reported that phosphatase activity was inhibited on a Spanish sandy loam soil amended with hog slurry manure. The authors reported a significant negative correlation between phosphatase activity and available phosphorus content, which confirmed the hypothesis proposed by Nannipieri et al. (1979) that inorganic P caused feedback inhibition of phosphatase. With the first additions of an organic amendment, soil organisms are stimulated to secrete high levels of enzymes, but over time, there can be a lower enzyme response with additional organic inputs (Burns, 1978). Martens et al. (1992) hypothesized that there may be feedback mechanisms that will slow or end enzyme production when adequate energy sources are available, and that addition of organic residues over time fails to increase enzyme activity. This provides an explanation for lack of response of enzyme activity to manure amendment, especially in the Plenty soil where build-up of labile P forms may be associated with inhibition of enzyme activity.

Many studies have reported some increases in soil activity of enzymes resulting from manure application to soils that had not received manure previously (Dormaar & Chang, 1995; Lalande et al., 2000). Stojanovic (1959) has reported that urease enzyme activities vary according to the season depending on temperature and moisture conditions that are present at or immediately prior to soil sampling. Conversely, Burns (1978) has reported that on soil samples collected on five separate occasions over a period of three years from the same location, urease activity remained the same, independent of season. McGarity and Myers (1967) reported that on soil samples collected from winter to late spring period, there were no major differences in urease activities. However, only a few studies have looked at enzyme activity in soils with several years' history of manure application (Eivazi et al., 2003; Lalande et al., 2000). While repeated application may, and in some studies has been reported to, contribute to higher activity due to continued addition of substrate and promotion of microbial activity, an important counteracting effect may be the buildup of end product such as phosphate or ammonium. This can slow activity through feedback inhibition. Therefore the effects of manure additions on enzyme activity can be complex and difficult to predict, and are likely to be time and rate dependent. Soil samples were obtained approximately six months after manure application, in which time substrate material could have been consumed, thereby leading to a decrease in enzyme activity at sampling time.

One time soil sampling was chosen in this study to assess what effect that four and five years of LSM application had on soil carbon and enzyme activity at the Melfort and Plenty sites. Soil samples were only analyzed for the soil enzymes arylsulfatase, alkaline phosphatase and urease activity. Assays for other soil enzymes such as nitrogenase and dehydrogenase could show greater differences than those chosen for this study. Future enzyme assay work should include timing sampling periods as close as possible after manure application and examine what effect rates and types of manure application such as direct subsurface injection of solid cattle manure have on enzyme activity in Saskatchewan soils. The effects of manure application to soils of the northern Great Plains of even drier and wetter moisture regime than those used in this study also deserves attention.

5. Conclusions

Overall, repeated application of LSM at agronomic N rates for four to five years is associated with significant increases in TOC and LFOC mass in the 0-15 cm depth in some instances, and that soil enzyme activities are also occasionally enhanced, depressed or often unaffected. Application of LSM at 37,000 L ha^{-1} per year and 74,000 L ha^{-1} every second year at Melfort resulted in a significant increase in LFOC and alkaline phosphatase activity relative to the control, but not in TOC or other enzymes. Annual application of LSM at Plenty resulted in significant increases in both TOC and LFOC compared to the control. This is attributed to the high clay and low OM content of the soil at the site that would protect OM from being rapidly decomposed and provides a large capacity for soil C storage. Manure application had no impact on enzyme activity at this site. There are other questions that should be addressed regarding the effect that repeated application of LSM has on biochemical properties. It would be beneficial to investigate whether applications of LSM for many years would consistently cause feedback inhibition and reduce the stimulus for enzyme release. Comparison of arylsulfatase, alkaline phosphatase and urease enzymatic activities from soil samples collected from solid manure soils applied at different rates and application methods at different periods of time throughout the year is another question that needs to be addressed. Other soil C fractions that may shed light on C dynamics include microbial biomass and C contained in different aggregate size fractions.

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