A COMPARISON OF EARTHWORM DENSITIES IN GRASSLAND SET-ASIDES AND CULTIVATED POTATO FIELDS IN THE LOWER FRASER RIVER DELTA, BRITISH COLUMBIA

by

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ABSTRACT

The Municipality of Delta, British Columbia is an area with rich, fine textured soils, that when integrated with a long growing season, provide conditions highly conductive for food production. Fine-textured soils of this region, however, are also easily degraded in response to heavy compaction and intensive tillage practices. The Delta Farmland and Wildlife Trust (DF&WT), a local non-governmental non-profit conservation organization, provides financial support to farmers for fallowing fields for a minimum of a year through their Grassland Set-aside Stewardship Program (GSSP), in an attempt to sustain soil quality. These grasslands are expected to increase soil organic matter, aggregate stability and drainage through the development of rooted systems. Earthworms, a biological indicator of soil health, are expected to increase in undisturbed soils and in response to inputs of soil organic matter that would be associated with grassland set-asides. To better understand earthworm response to the GSSP, the difference in earthworm abundance was quantified between one year old grassland set-asides and recently harvested potato fields in the fall of 2012. Physical and chemical soil indicators including aggregate stability, bulk density, soil particle distribution, organic matter, pH and trace elements were also analyzed in order to provide insight into the factors influencing earthworm abundance in the area. Earthworm presence did not increase after one year under the GSSP when compared to potato fields (total wet biomass: p-value = 0.14; total count of individuals: p-value = 0.19). Aggregate stability was the only sampled indicator which showed a significant difference between the two treatments (p-value < 0.005). Sampled parameters which predict earthworm abundance across the two treatments with relative statistical success include a positive relationship with organic matter (p-value = 0.02, $R^2 = 0.63$), and negative relationships with percent silt (p-value < 0.01, $R^2 = 0.71$), phosphorus (p-value = 0.02, $R^2 = 0.62$) and zinc (p-value = 0.02, $R^2 = 0.63$).

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1.0 INTRODUCTION

1.1 Soil Health Indicators

Soil is a dynamic medium for plant growth and ensures the availability of a number of ecosystem services. An ecosystem service includes any product resulting from a process or function of a natural ecosystem that supports or benefits human needs (Brady & Weil, 2010). Primary soil functions include nutrient cycling, and natural water filtration (United States Department of Agriculture, 2001a). Soil health is paramount to soil function and a number of quality indicators exist to quantify, monitor and manage soil health. Soil indicators include heightened biological activity, presence of soil organic matter (SOM), and aggregate stability (Brady & Weil, 2010). Agricultural practices, including conventional row-crop cultivation, interrupt nutrient cycling, and hinder soil structure and water filtration through soil compaction. Therefore agricultural managers attempt to foster sustainable development through the management of a number of these soil health indicators (Brady & Weil, 2010).

Earthworms are commonly recognized as an important soil fauna, enhancing soil fertility and structure through a number of pathways (Coleman, Croccley & Hendrix, 2004). As so-called "ecosystem engineers", earthworms encourage water filtration and increase rooting depths through the formation of bio pores (Lagerlöf, Pålsson, & Arvidsson, 2011). Earthworms are classified into three categories based on their distribution in the soil horizon. Epigeic worms are located in the top layer and are often smallest in size, feeding on available surface litter (Tian, Olimah, Adeoye, & Kang, 2000). Endogeic worms form an array of horizontal burrows with the potential to impede water movement. Finally, anectic species form deep vertical burrows and are important in the formation of bypass pores, increasing the rate of water and solute transfer to depths (Coleman *et al*., 2004). Earthworms also aid in the mineralization of nutrients and induce stable aggregate formation through the excretion of nutrient-enriched casts, developed through the consumption and processing of SOM. Cast formation is highly dependent on soil moisture content as well as soil temperature (Coleman *et al*., 2004).

In order to quantify the role of earthworms in the soil system, information on diversity, abundance and biomass is commonly required. For arable land systems, it is expected that four to six species coexist, however individuals are not likely to be randomly distributed within the soil (Valckx, Govers, Hermy, & Muys, 2011). Earthworm population densities are heavily influenced by intensive soil cultivation; however following years of perennial crops being in the management rotation, earthworm densities will likely increase due to their short species-dependent life cycle of 42 - 72 weeks (Lagerlöf *et al*., 2011).

SOM and aggregate stability are heavily associated with earthworm activity in the soil system. SOM encompasses living biomass, dead plant residues and colloidal organic complexes (Brady & Weil, 2010). The type and percent composition of SOM provides insight into a range of soil properties including fertility, stability, and nutrient availability (United States Department of Agriculture, 2001b). Increasing SOM is key to reducing soil erosion, ultimately limiting the introduction of aerosols into the air and water bodies (United States Department of Agriculture, 2003). SOM can be enhanced through a number of management practices which promote reduced tillage and rotational grazing (United States Department of Agriculture, n.d). Aggregate stability is commonly used as an indicator of soil structure. Aggregate stability is measured by the relative size distribution and cohesive properties of soil aggregates following direct agitation or disturbance (Nimmo & Perkins, 2002). The formation of aggregates, although dependent on a number of abiotic and biotic processes, is commonly defined primarily by the presence of clay flocculates (Brady & Weil, 2010). Analysis of soil aggregates provides insight into the dynamics of soil water including infiltration rates and water retention properties. Aggregate stability is commonly used to measure the direct impact of agricultural management practices on water infiltration and retention (Nimmo & Perkins, 2002).

1.2 Regional and Local Agriculture

The agriculture and food industry of British Columbia generates a total of $18 billion in food retail annually and employs over 200,000 individuals (British Columbia Ministry of Agriculture, 2013a). In British Columbia a total of 4.7 million hectares of land are classified as agricultural land reserves (ALR) based on the quality of arable soils in the province. Of these ALR's, cultivated farmland covers approximately 618,000 hectares. British Columbia's Fraser River Delta has rich and productive soils. Within the area, predominately vegetables, fruits and nursery crops are harvested (British Columbia, Ministry of Agriculture, 2013b). Soil texture in the Lower Fraser Valley is predominately composed of silts and clays. Together these fine textured soils and a long growing season provide ideal conditions for food production (Delta Farmland & Wildlife Trust, 2011).

1.2.1 The Municipality of Delta and the Grassland Set- aside Stewardship Program

Two conservation issues plague the agriculture community of Delta, BC. Fine-textured soils of this region are easily degraded in response to heavy compaction and intensive tillage practices. Secondly, Delta represents a major migratory pathway for a number of over-wintering birds. An expanding urban population in the Municipality of Delta has reduced the area of marsh and grassland available for the conservation of these species. The practice of leaving crops fallow is a common management approach to enhance soil fertility; however, many farmers are unable to take on the financial burden of removing portions of their field from production. The Delta Farmland and Wildlife Trust (DF&WT), a not-for-profit organization, subsidizes farmers to promote fallow land use through the Grassland Set-Aside Stewardship Program (GSSP). These established grasslands also represent sanctuaries for a number of migratory raptors. Through the program, individual fields are planted with clover or forage grass. These grasses help re-introduce SOM to the soil system and increase soil drainage and aeration through the formation of natural rooting channels (Delta Farmland and Wildlife Trust, 2011).

1.3 Statement of Purpose

The Faculty of Land and Food Systems at the University of British Columbia has had an ongoing relationship with the DF&WT, working hand-in-hand to provide insight into the outcomes of the DF&WT Stewardship programs including the GSSP, Winter Cover Crop and Hedgerow programs. Reports by farmers participating in the GSSP have suggested that some fields are experiencing a reduction of nutrient availability following the deactivation of fallowed set-asides, potentially repelling farmers from participation in the DF&WT program (Yates, 2011). Current graduate research affiliated with the GSSP is in the process of developing a soil quality assessment following the introduction of grassland set-asides into crop rotation (Yates, 2011). Soil quality measurements associated with this assessment include, but are not limited to, the physical and chemical parameters of: foliar nutrient analysis, particulate organic matter, total carbon, total and available nitrogen and aggregate stability.

Therefore an opportunity to provide insight into a biological soil quality indicator in affiliation with the GSSP will further add to the overall objective of understanding soil quality under this management program. Little is known about the density of earthworms in the soils of Delta, or if there is a difference in the presence of earthworms between grassland set-asides and cultivated fields in the area. Earthworm population densities are believed to be heavily influenced by intensive soil cultivation and tillage practices. Therefore groundcover and litter fall supplied by fallow vegetation has the potential to enhance soil biological activity (Tian *et al.*, 2000).

The main objective of this research is to understand whether a significant difference in earthworm presence exists between one-year-old grassland set-asides and potato fields which have undergone cultivation in the last growing season. In addition to quantifying the presence of earthworms, analysis for other soil indicators including SOM, aggregate stability, pH, soil moisture content, soil bulk density, soil texture and soil trace metal composition, will help develop a more solid understanding of the conditions in which these earthworms thrive and ultimately may assist in future management decisions. I hypothesize that an increase in earthworm presence will occur in the grassland treatment when compared to recently cultivated potato fields. If no difference is observed, investigation into the influence of other soil indicators on earthworm abundance will occur.

2.0 METHODOLOGY

2.1 Site and Soil Information

The location of focus for this study was Ladner, British Columbia, a town in the Municipality of Delta. The area receives annual total precipitation of 918 mm and experiences average temperatures of 11.1^{o}C in October and 7.6^{o}C in November (The Weather Network, 2012). Two study treatments were identified (1) grassland set-asides planted in the fall of 2011 (2) harvest potato fields which had recently undergone cultivation in fall 2012. Four replicate fields per treatment were used during this study. All set-asides and potato fields were selected with the assistance of the DF&WT's D. Bradbeer (personal communication, September 17, 2012). Within each field, three 25 cm x 25 cm x 25 cm subplots were sampled in order to capture spatial variability within these large fields (1.2 ha - 15.4 ha), for a total of twenty-four subplots in this study.

Soils in Ladner predominately belong to the gleysolic order, developing under saturated conditions and holding high clay content. These characteristics induce poor soil drainage and a reduction in the rate of SOM transformation (University of Saskatchewan Department of Soil Science, n.d). Following heavy rainfall events, pooling commonly occurs on the soil surface and the water table remains near the surface for most of the year (British Columbia Ministry of Environment, 1980). Soil types for each field of study are provided in table 1. Drainage characteristics and soil classifications based on identified soil types are provided in table 2. All set-asides were planted with the DF&WT seed mix comprised of 25% orchard grass, 28% tall fescue, 15% chewig's fescue, 15% creeping fescue and 2% double cut red clover. In addition to DF&WT seed mix, SAS-F3 was also planted with a barley nursery crop. Cover crops of winter wheat were planted on HP-F1 and HP-F3 only (Christine Terpsma, personal communications, March 25, 2013).

Table 1- Soil types for the fields of study (SAS = set-aside field, HP = harvested potato field). Soil Types: **BU** – Blundell, **CT** – Cresent, **DT** – Delta, **L** – Ladner, **WS** – Westham. Topographic ID: **b** - Gently undulating (% slope 0.5 -2), **bc** - Gently undulating - undulating (% slope 0.5-5) (British Columbia Ministry of Environment, 1980).

Site	Soil Type
SAS- F1	DT-BU/b
SAS- F2	DT-BU/b, CT-L/bc
SAS- F3	CT/b, L-BU/b
SAS- F4	CT-WS/ bc, WS/b
HP- F1	DT-BU/b, CT-L/bc
HP- F2	L/b
HP- F3	CT/b, L-BU/b
HP- F4	CT-WS/ bc, WS/b

Table 2- Drainage characteristics and soil classification based on soil types experienced in fields of study (British Columbia Ministry of Environment, 1980).

Soil Type	Drainage Characteristics	Classification
BU - Blundell	Poorly to Very Poorly	Rego Gleysol: Saline and Peaty Phase
CT - Cresent	Moderately Poorly to Poorly	Orthic Gleysol
DT - Delta	Poorly	Orthic Humic Gleysol: Saline Phase
L - Ladner	Moderately Poorly to Poorly	Humic Luvic Gleysol
WS- Westham	Poorly to Very Poorly	Rego Humic Gleysol

2.2 Field Sampling

Field sampling was completed between October 27th and November 5th. Rainfall accumulation during this time period was 105.2 mm and annual accumulation at the end of the sampling period was 789 mm (Conarroe, 2012). Field sampling was completed in numerical order, with a best attempt to alternate subplot type between set-aside and harvested potato treatments, in order to minimize the influence of temporal weather variability between the two treatments types.

Locations of sample plots within each field were chosen at random. Each plot was chosen based off of a randomly generated compass bearing and a step count between 100 - 250. This was in attempt to correct for autocorrelation of earthworm ranges of distribution, described as 16 m in Canadian arable land (Valckx *et al*., 2011). At each plot, soil samples for SOM, pH, and moisture content were removed from three depths using a Dutch Auger. Sampled depths from the surface were completed as follows: *depth one*, 0-7.5 cm; *depth two*, 7.5-15 cm; and *depth three*, 15-22.5 cm. All sample depths were removed from each subplot unless a high water table prevented retrieval from a given depth. Samples for aggregate stability were removed from the surface to a depth of 3 cm. An approximate volume of 0.0156 m^3 or (25 cm x 25 cm x 25 cm) of soil was removed for hand sorting earthworms in the field (Valckx *et al*., 2011). The volume of soil was separated into quarters and a sampling effort of approximately 20 - 25 minutes was carried out on each quarter in order to maintain a uniform sampling effort throughout the entire plot and across all study sites.

2.3 Laboratory Sample Processing

Moisture content measurements were completed immediately after returning from the field to the laboratory. Percent soil water content was determined through the gravimetric method, oven drying at 105^{o}C for over 24 hours (Brown, 2011). SOM, aggregate stability and pH samples were stored at 4^{o}C until processing was completed in order to prevent drying and to slow down biological and chemical activities.

Earthworm samples were recounted and sorted in the laboratory as adults, juveniles, or parts. Individuals were classified as adults if a clitellum was observed (Lagerlöf *et al*., 2011). Total wet biomass was reported for each plot and for the three individual classifications of adults, juveniles and parts measured within each subplot. For earthworm data, values obtained within the sample field plot were extrapolated to a squared meter.

Aggregate stability was determined using the nested Yoder fast wet - sieve method, a method best representing normal field conditions at the soil surface (Dickson, Rasiah, & Groenevelt, 1991). Samples were sieved using a nested 6 mm and 2 mm system. Samples passing through the 6 mm sieve but trapped by the 2 mm sieve were assumed to be an average of 4 mm. A portion of the sample remaining on the 2 mm sieve was removed for moisture content measurements. Approximately 15 g of the average 4 mm soil was introduced to a second sieve nest system comprised of three sieves with a diameter of 2 mm, 1 mm, and 0.25 mm respectively. Sieve nests were submerged in water using a motorized agitator set at forty reps per minute and removed after 10 minutes, followed by oven drying for a minimum of 10 hours. Prior to fast wetting, samples were steamed to prevent the effects of high-pressure build up, which has the potential to lead to aggregate explosion during the fast wetting process (Dickson *et al.,* 1991). Oven dried sample weights found on each sieve were subsequently measured. Corrections were made for the accumulation of coarse material in each sieve nest by grinding the dried sample with a mortar and pestle. The grinded soil was re-sieved and material that remained on the sieve was classified as coarse within that sieve class (Yoder, 1936).

Physical and chemical analysis of the remaining soil parameters was carried out on milled soil samples. Soils were air dried for approximately a week and then crushed using a rolling pin then sieved through a 2 mm mesh sieve in order to remove coarse mineral fragments. In order to quantify soil pH, two soil samples were analyzed; one, suspended in distilled water and the other, suspended in 0.01 *M* $CaCl_2$ (Brown, 2011). Both sample types were analyzed using an Orion 42014 calibrated pH probe. Both methods were carried out on 1:2 ratio of soil to solution. Measurements completed in 0.01 *M* $CaCl_2$ are more commonly reported, as $CaCl_2$ masks the variability in measurements introduced through the presence of salts and suspended particles, and often provides a better approximation of the pH of the soil under field conditions (Murray, 2011).

SOM in g/g was quantified through weight loss on ignition of a dry soil sample. Samples experienced three heating cycles beginning with 105^{o}C for 24 hours in order to remove hydroscopic water held by soil water tension. Following re-weighing, samples were placed in a muffle furnace at 350^{o}C for approximately 12 hours. The final heating cycle included approximately 12 hours at 550^{o}C (Brown, 2011). Due to the high clay content of gleysolic soils, SOM was therefore calculated as the percentage difference between the 105^{o}C and 350^{o}C weights as depicted in equation 1, as ignition temperatures of less than 400^{o}C are suggested to minimize the influence of weight loss from carbonates and structural water within the clays. Percent carbon content of soil was then predicted from calculated SOM based on the relationship that within soils, the organic carbon content is approximately 58% (Hoskins, 2002).

$$\% \boldsymbol{SOM} = \left(\frac{(\boldsymbol{Weight\ after\ 105^{oC}}) - (\boldsymbol{Weight\ after\ 350^{oC}})}{\boldsymbol{Weight\ after\ 105^{oC}}}\right) * \mathbf{100} \qquad \textbf{(1)}$$

Determination of soil particle size distribution was completed on a composite sample made for each depth within each field, for a total of twenty-four composite samples. Soil texturing was completed using a simplified method for approximation of soil particle distribution based on size fractioning through a 0.055 mm sieve described as the Rapid Method by Kettler, Doran and Gilbert, 2001. The results from the Rapid Method were then utilized to predict soil mineral density through contour analysis of figure 1, developed by Walter Rawls of the United States Department of Agriculture (USDA). Once soil mineral density was predicted from figure 1, and percent SOM was obtained in g/g from weight loss on ignition, soil bulk density was calculated using a pedo-transfer function (equation 2) where *x* is percent SOM g/g, ρ_m = the predicted soil mineral density in g/cm^3 from figure 1 and ρ_o = 0.224 g/cm^3, the average calculated organic bulk density in soil (Rawls, 1982). Calculated standard deviations for the spectrum of soil mineral densities accompanied figure 1 in Rawls initial publication. Standard deviations provided were then incorporated in the calculation of standard error for bulk density measurements.

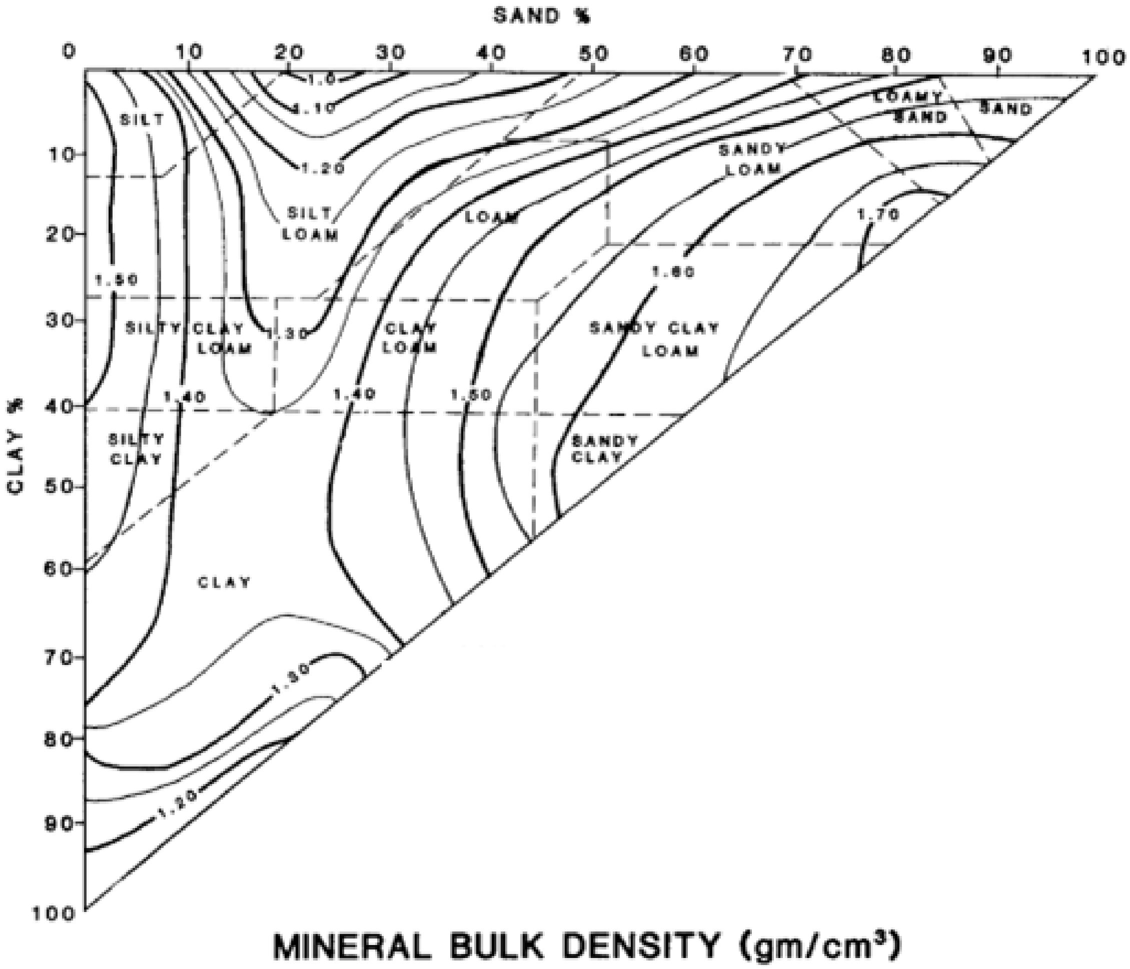

Figure 1- Soil mineral density contour map based on soil particle distribution. Modified from Rawls (1982).

$$\boldsymbol{Soil\ Bulk\ Density} = \frac{\mathbf{100}}{\left\{\left(\frac{x}{\rho_0}\right)+\left(\frac{100-x}{\rho m}\right)\right\}} \quad \textbf{(2)}$$

Measurements of percent SOM and carbon content are most relevant to agricultural management practices when in units of g/cm^2. Therefore, predicted bulk densities for each composite sample were then used in partnership with an average sample depth of 7.5 cm to convert percent SOM in g/g to a measure in g/cm^2.

Soil elemental composition was profiled for all twenty-four *depth one* samples acquired from the field. Soil trace metal composition was quantified through inductivity coupled plasma atomic emissions spectrometery (ICP-EOS) analysis following HCl and $HN0_3$ digestion, measuring the liable fraction for each element. The following elements were sampled: aluminum, calcium, magnesium and phosphorus along with the heavy metals: cadmium, copper, lead, nickel and zinc. During HCl digestion, approximately 5 g of soil were placed in 50 ml of 1 *M* HCl, shaken for one hour and left to stand for approximately 12 hours. The sample was then filtered through a Whatcom # 42 filter paper. $HNCO_3$ was then added to the filtrate till a volume of 100 ml was achieved (Snape, Scouller, Stark, Stark, Riddle, & Gore, 2004). Results produced from ICP-EOS analysis were then corrected with a dilution factor of approximately twenty (5 g soil/100 ml of solution) and reported in parts per million (ppm). Results for trace and major elements were then averaged if more than one wavelength was used during detection, with the exception of lead and magnesium in which respective wavelengths of 217.000 nm and 279.553 nm were chosen.

2.4 Statistical Analysis

Data organization for statistical analysis was dependent upon the number of sampled depths for a given soil parameter. The soil parameters of earthworm abundance, aggregate stability and ICP-EOS analysis were completed once within each subplot. In this scenario, representative field averages for each replicate field were calculated based on the three completed subplots. A treatment average was then calculated based on four replicate fields. For parameters including pH, SOM, moisture content, soil texture, and bulk density, representative averages were calculated for each field by depth.

Statistical analysis was completed using the open source program RStudio version 2.15.2 (RStudio Team, 2012). Prior to the formation of statistical inferences between the two treatments, the distribution of field sample means within each treatment were tested for normality using Shapiro-Wilk's test of formal normality (Whitlock & Schluter, 2009). If both treatments failed to reject the null hypothesis of originating from a normal distribution with a p value > 0.05, then further statistical inferences were made on the original means. If the distribution of means within one or more of the treatments rejected the null hypothesis of formal normality with a p value < 0.05, replicate field means were transformed using a natural logarithmic transformation. Equation 3 details the transformation administered if values within the dataset were greater than one and equation 4 was performed on all values if at least one value was less than one.

$$\boldsymbol{y} = \mathbf{ln}(\boldsymbol{x}) \tag{3}$$

$$\boldsymbol{y} = \mathbf{ln}(\boldsymbol{x} + \mathbf{1}) \tag{4}$$

Statistical inferences were made between the two treatments using Welch's Two Sample t-test due to unequal variances observed. If transformations failed to provide a normal distribution within each treatment, non-parametric statistical analysis was completed, utilizing the Mann-Whitney-Wilcoxon sum-rank test (Whitlock & Schluter, 2009).

The method of least squares was used to develop linear regressions between analyzed soil indicators and total earthworm wet biomass. Relationships were developed using transformed earthworm biomass in order to meet the assumptions of normality of the dependent variable during linear regressions (Whitlock & Schluter, 2009). Visual analysis of (1) Residual vs. Fitted, (2) Scale-location, (3) Normal Quantile and (4) Leverage Plots was completed to more confidently accept the results of the developed linear regressions. Transformations were completed if visual analysis suggested a poor fit.

Standard error is reported along with the mean of each sample parameter. Results of statistical analysis are organized as * p-value ≤ 0.05, ** p-value ≤ 0.01 or N.S. (not significant) within displayed figures. Results of all statistical analysis can be found in Appendix A.

3.0 RESULTS

3.1 Earthworms

Values of interest in earthworm numbers between the two treatments primarily include total wet biomass and the total number of individuals excluding parts. Parts are to be excluded from the total count of individual as they are not capable of performing qualities of interest (for example the formation of casts or release of nutrients through soil digestion).

Mean total wet biomass observed in the set aside treatment was 33.50 ± 13.62 g/m^2. For the harvested potato treatment, the average total wet biomass was 13.02 ± 11.39 g/m^2. There was no significant difference between the log transformed values (figure 2).

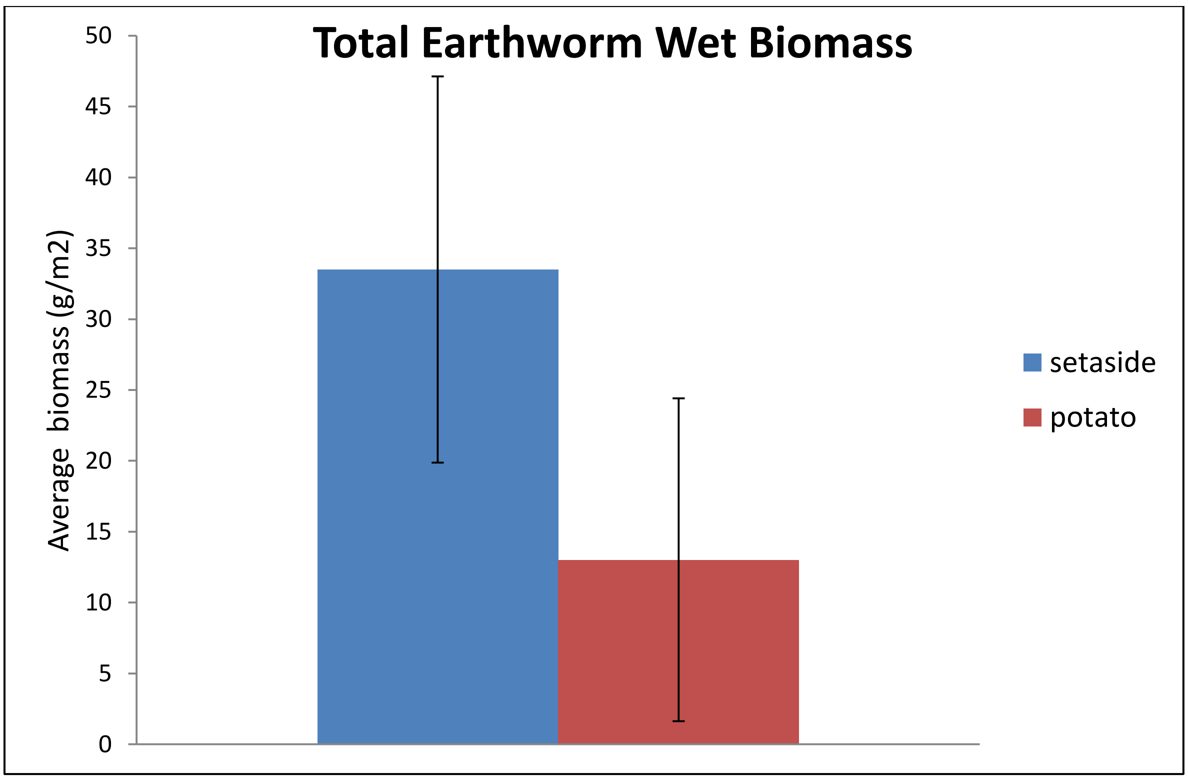

Figure 2-Presence of earthworms presented as total wet biomass in g/m^2 of untransformed data. Error bars represent standard error of untransformed data. Welch's Two Sample t-test was N.S. on transformed data (n = 4, p-value = 0.14).

Nor was there a significant difference between transformed values of the total number of individuals excluding parts (figure 3). The mean number of individuals experienced in the set-aside treatment was 535 ± 196 ind/m^2 and for the harvested treatment 161 ± 126 ind/m^2.

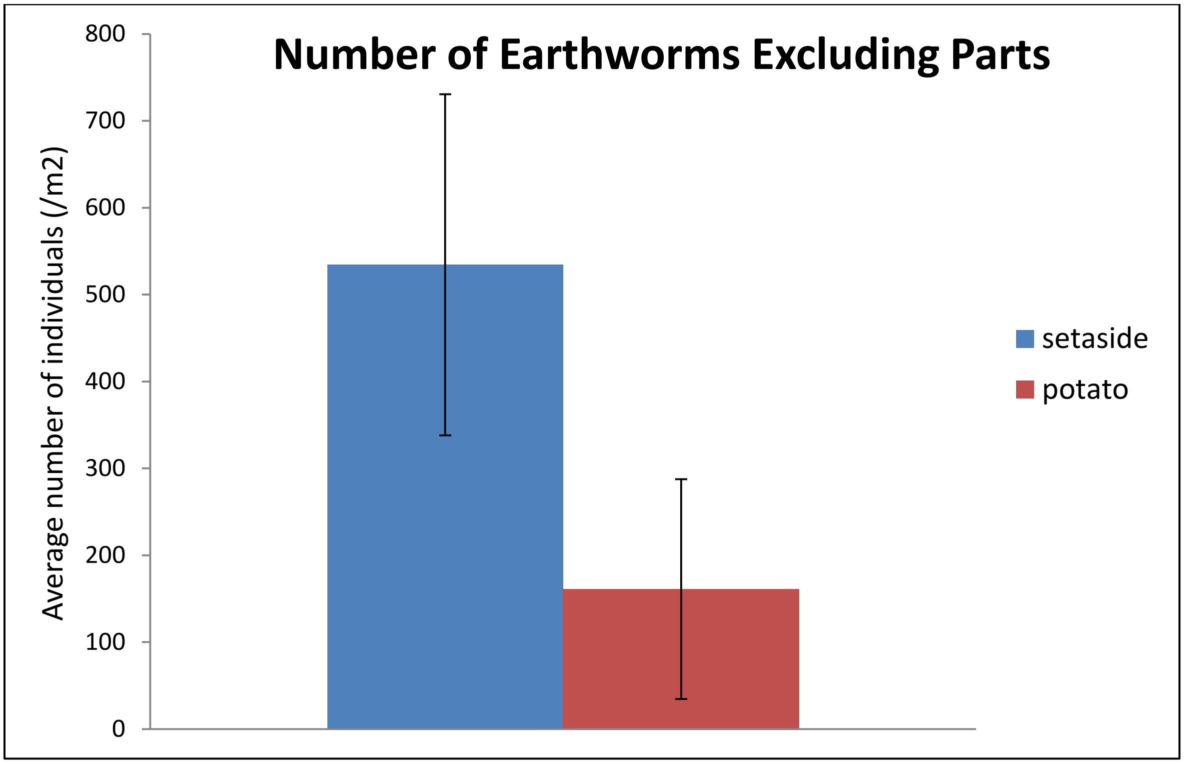

Figure 3- Total number of earthworms excluding parts as ind/m^2 of untransformed data. Error bars represent standard error of untransformed data. Welch's Two Sample t-test was N.S. on transformed data (n = 4, p-value = 0.19).

3.2 Soil Physical and Chemical Properties

3.2.1 Aggregate Stability

Sample results for soil aggregate stability (figure 4) are expressed as mean wetted diameter in millimeters following the sieving, wetting, agitating and drying regime prescribed by Yoder, 1936. The set-aside treatment experienced a higher mean wetted aggregate diameter, with an average of 1.591 ± 0.046 mm, where the harvested potato treatment experienced a mean of only 1.066 ± 0.087 mm. Results of Welch's two sample t-test suggests a significant difference exists between the two treatments with a resultant p-value < 0.01.

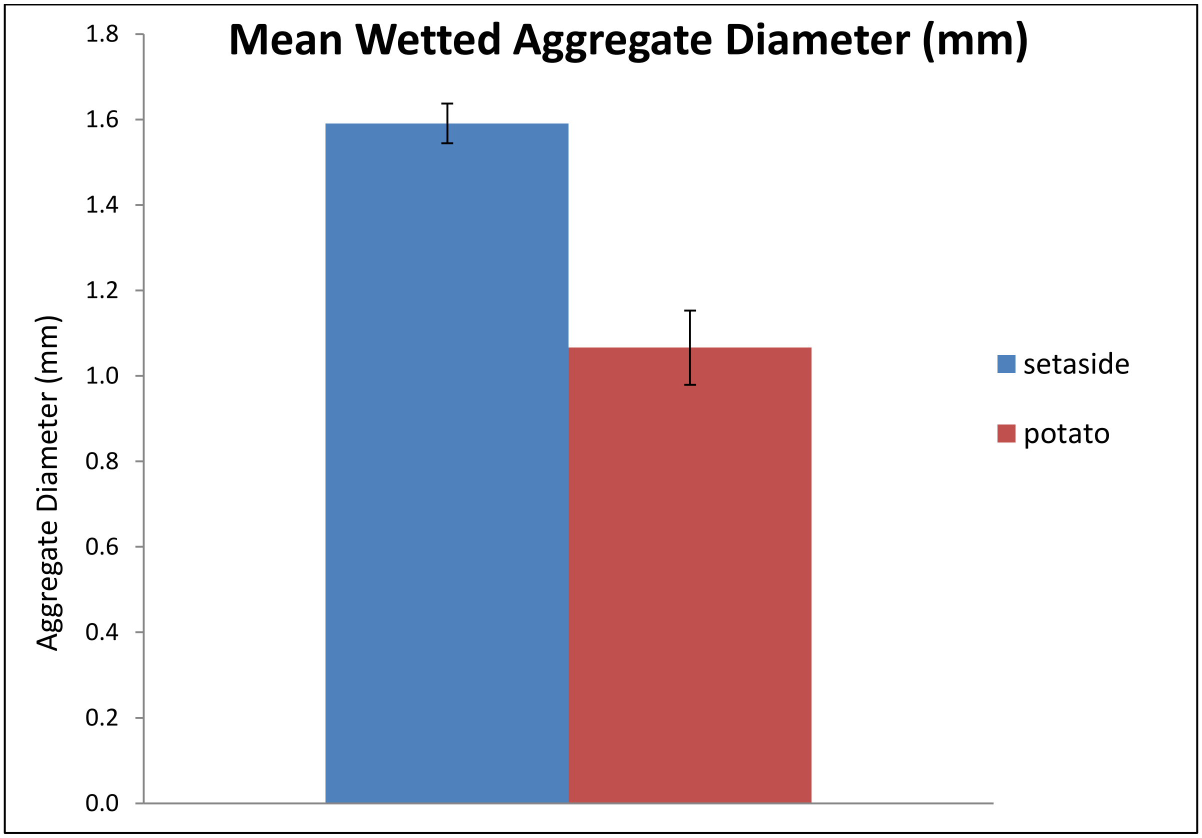

Figure 4- Mean wetted diameter of non-transformed data post Yoder wet-sieving measured in mm. Error bars represent standard error of non-transformed data. Welch's Two Sample t-test was significant on non-transformed data (n = 4, p-value < 0.01).

Linear regression analysis was performed to model mean wetted diameter from total earthworm biomass, *depth one* percent clay content and *depth one* SOM in g/g (figure 5). No significant relationship resulted from these analyses. Percent clay showed the strongest relationship with a p-value of 0.07 and an R^2 value of 0.46.

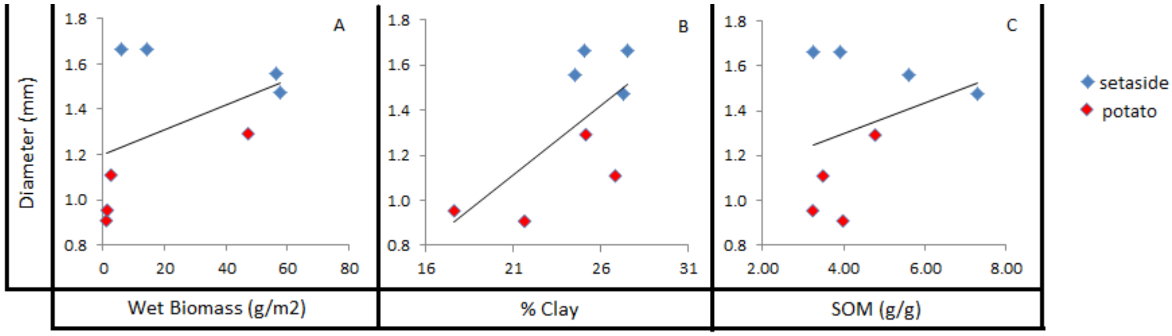

Figure 5- Predicting soil aggregate stability with non-transformed: **A** = total wet earthworm biomass (p-value: 0.26, R^2 = 0.20), **B** = percent clay content (p-value: 0.07, R^2 = 0.46), **C** = soil organic matter (p-value: 0.45, R^2 = 0.10). Results from linear regression analysis on non-transformed data were N.S. in each case.

3.2.2 Standard soil parameters through soil horizon

Results for the standard soil parameters measured at all three depths within the topsoil horizon are reported in table 3. Specific statistical results for all relationships can be found in Appendix A. Standard error reported for bulk density inferred from equation 2 along with the standard error calculated for SOM in g/m^2 were obtained through propagation of error (Appendix B) using Wolfram Mathematica 9 computing software (Wolfram Research, 2013). Calculated error for SOM in g/m^2 was high due to the magnitude of the correlation coefficient calculated during error propagation.

There was no significant difference found for calculated bulk density between the cultivated and grassland fields throughout the sampled horizon. This was also the case for measurements of SOM in g/m^2. Laboratory measured soil pH experienced no significant difference between the two treatments for all three depths measured. However, mean soil pH always measured lower when suspended in 0.01 M $CaCl_2$ than when suspended in distilled water. Analysis of soil particle distribution for percent sand, silt and clay suggests that both treatments were not statistically different for particle composition within each depth. Texture identification for all sampled depths within each field was inferred from results of soil particle distribution and the use of the USDA soil texture triangle, depicted in figure 1. Resulting soil textures are displayed in table 4. The fields in this study range from Silty Loam to Silty Clay Loam where silt is always the predominant particle size, followed by clay.

Table 3 - Treatment means and standard error for standard soil parameters measured at three depths within soil horizon for set-asides (S) and harvested potato (H) fields on non-transformed data. Standard error for bulk density and SOM were calculated through error propagation (Appendix B). (**BD** = Soil bulk density, **SOM** = Soil organic matter). Welch's Two Sample t-test on non-transformed data was N.S. for all relationships (n = 4).

	BD (g/cm3)		**SOM (g/cm2)**		**pH 0.01*M* CaCl2**		**pH DI**		**% Clay**		**%Silt**		**%Sand**	
Depth (cm)	S	H	S	H	S	H	S	H	S	H	S	H	S	H
0-7.5	1.11 ± 0.07	1.14 ± 0.07	35.04 ± 56.88	25.77 ± 7.29	5.14 ± 0.26	5.41 ± 0.15	5.80 ± 0.20	6.02 ± 0.06	26.1 ± 0.8	22.8 ± 2.0	63.4 ± 1.9	64.4 ± 1.7	10.5 ± 2.1	12.8 ± 1.4
7.5 -15	1.13 ± 0.07	1.14 ± 0.07	32.33 ± 54.84	25.47 ± 11.65	5.07 ± 0.28	5.38 ± 0.17	5.74 ± 0.25	5.90 ± 0.05	26.6 ± 1.2	22.0 ± 2.0	63.5 ± 1.9	65.1 ± 1.0	9.9 ± 2.4	12.8 ± 1.6
15 -22.5	1.15 ± 0.06	1.11 ± 0.07	27.94 ± 35.30	26.07 ± 6.05	5.08 ± 0.25	5.39 ± 0.24	5.73 ± 0.22	5.82 ± 0.16	26.9 ± 0.9	22.6 ± 1.7	63.1 ± 2.1	62.9 ± 0.4	10.0 ± 2.0	14.4 ±1.6

Table 4 - Inferred soil textures for eight fields of study. Texture classes inferred from the USDA soil texture triangle depicted in figure 1.

Depth (cm)	**SAS – F1**	**SAS –F2**	**SAS –F3**	**SAS –F4**	**HP – F1**	**HP –F2**	**HP –F3**	**HP –F4**
0 -7.5	Silty Clay Loam/ Silt Loam	Silt Loam	Silt Loam	Silty Clay Loam	Silt Loam	Silt Loam	Silt Loam	Silty Clay Loam/ Silt Loam
7.5 -15	Silty Clay Loam	Silt Loam	Silt Loam	Silty Clay Loam	Silt Loam	Silt Loam	Silt Loam	Silty Clay Loam
15 -22.5	Silty Clay Loam/ Silt Loam	Silty Clay Loam/ Silt Loam	Silt Loam	Silty Clay Loam	Silt Loam	Silt Loam	Silt Loam	Silty Clay Loam/ Silt Loam

3.2.3 Elemental analysis of depth one soils

Results of major and minor element analysis completed on *depth one* samples are presented in table 5. Treatment means are reported, accompanied by standard error. No significant differences between the two study treatments were produced for the nine elements of interest. Non-parametric statistical analysis was required on aluminum. Welch's two sample t-test was performed on non-transformed data for all other elements but nickel, requiring transformation under the assumptions of Welch's two sample t-test.

Table 5- Treatment means and standard error of non-transformed data measured in ppm following ICP-EOS analysis of *depth one* samples for set-asides (S) and harvested potato (H) fields. Mann-Whitney-Wilcoxon sum-rank test was N.S. for aluminum (n = 4). Welch's Two Sample t-test on non-transformed data was N.S. for all relationships. Welch's Two Sample t-test on transformed nickel data was N.S. (n = 4).

	Aluminum	**Cadmium**	**Calcium**
S	6150.12 ± 184.62	25.06 ± 0.25	2793.50 ± 304.33
H	5736.87 ± 420.50	25.52 ± 0.12	2956.47 ± 191.95
	Copper	**Lead**	**Magnesium**
S	21.45 ± 1.45	18.28 ± 1.57	4293.72 ± 291.48
H	21.45 ± 3.20	19.69 ± 1.79	4178.14 ± 195.46
	Nickel	**Phosphorous**	**Zinc**
S	33.98 ± 0.92	923.89 ± 66.03	28.98 ± 1.63
H	34.57 ± 0.87	1037.93 ± 84.82	31.07 ± 3.61

3.3 Regression analysis, predicting earthworm biomass

No significant results were observed for earthworm biomass between the two treatments. Therefore an investigation into the influence of other soil quality indicators on earthworm presence was completed. Results from proposed linear regressions between transformed total earthworm biomass and measurements for a variety of physical and chemical soil health indicators are presented in figure 6. All independent values used to predict earthworm biomass are *depth one* samples. The top panel illustrates relationships between earthworm biomass and physical soil parameters of calculated soil bulk density, moisture content and soil particle distributions. The central panel shows developed relationships with chemical indicators of soil pH in 0.01 *M* $CaCl_2$, SOM in g/g and elements of calcium, magnesium and phosphorous. The bottom panel shows the relationship between biomass and a number of heavy metals identified during ICP-EOS analysis. Linear regressions were developed using transformed biomass. All independent variables are based off of non-transformed data, with the exception of SOM. The relationship developed for lead suggested a very poor fit with the normal quantile plot, and transformations failed to improve the relationship.

Significant relationships were computed for a number of regressions. The only positive relationship resulted from transformed SOM in g/g (p-value = 0.02, R^2 = 0.63). Negative relationships resulted for percent silt, phosphorus and zinc. Percent silt fraction had the most significant negative linear relationship (p-value < 0.01, R^2 value = 0.71). Soil particle fractions of sand and clay were not significantly correlated with earthworms. Both the presence of phosphorus (p-value = 0.02, R^2 = 0.62) and zinc (p-value = 0.02, R^2 value = 0.63) held similar strength in predicting earthworm wet biomass. Lead showed the next strongest relationship within the remaining trace metals (p-value = 0.06, R^2 value of 0.47). All other trace metals failed to show a significant relationship to earthworm biomass.

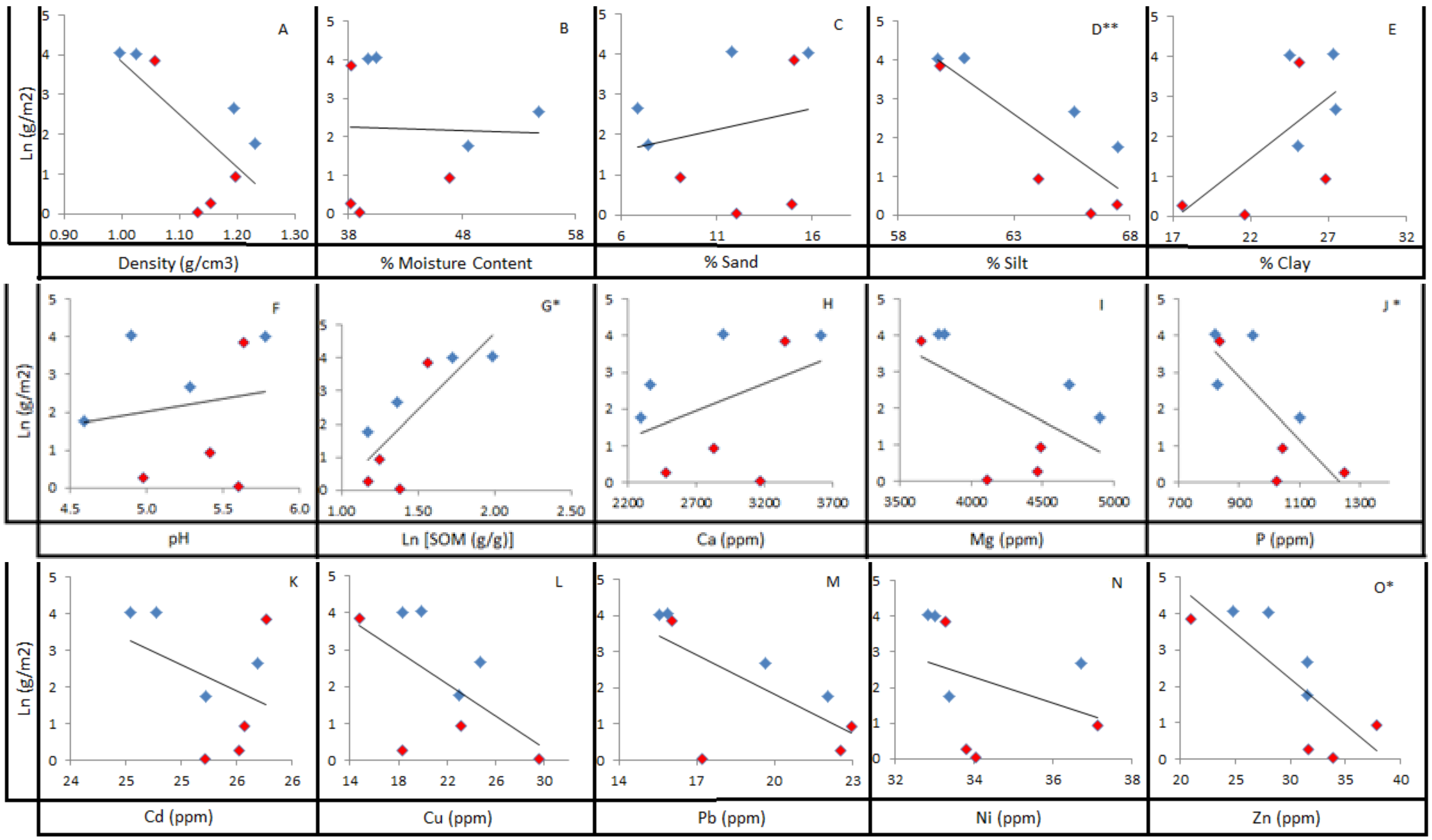

Figure 6 - Results of linear regressions for *depth one* physical and chemical parameters when predicting transformed earthworm total wet biomass. Set-aside fields are in blue, harvested potato fields in red. ***A***-Bulk Density, ***B***-Moisture Content, ***C***-% Sand, ***D***-%Silt, ***E***-% Clay, ***F***-pH 0.01 *M* $CaCl_2$, ***G***- Soil organic matter, ***H***-Calcium, ***I***-Magnesium, ***J***-Phosphorous, ***K***-Cadmium, ***L***-Copper, ***M***-Lead, ***N***-Nickel, ***O***- Zinc. All data for independent variables are non-transformed, with the exception of SOM. (* p-value ≤ 0.05, ** p-value ≤ 0.01, n = 8).

4.0 DISCUSSION

4.1 Earthworms, aggregate stability and soil organic matter

Aggregate stability was the only physical or chemical measurement that was significantly different between the two treatments. When trying to discern the importance of the processes influencing aggregate formation, a number of biotic and abiotic processes must be considered. In fine textured soils, physical-chemical interactions tend to dominate the process and are highly dependent on the presence of clay in the acceleration of aggregation through flocculation and attraction of SOM (Brady & Weil, 2010). When evaluating the ability of clay, earthworm biomass, or SOM to describe aggregate stability, clay showed the strongest relationship, however, was insignificant ($p = 0.07$, $R^2 = 0.46$).

Investigations into the understanding of earthworm interaction with soil structure and other physical parameters was completed by Fonte, Winsome, & Six, 2008 in Northern California's tomato cropping system, viewing the impact of three management regimes on earthworm abundance, soil structure and physical properties. Fonte *et al.* findings suggest that although the presence of a cover crop provides a number of available added benefits, including inputs of SOM and overhead cover for earthworms, when compared to bare fallow management, only a slight increase in earthworm presence was found. They concluded that recent tillage intensity and activity may play a significant role in contributing to a reduction in earthworms. They also concluded that factors other than earthworm presence were likely controlling aggregate associated SOM dynamics within their study plots.

Given that soil percent clay composition was not statistically different between the two treatments at all three soil depths, it is likely that there is not one direct component adding to the significant difference in the mean aggregate stability between the two treatments. It is likely a combination of biological and chemical factors within these young grassland set-asides that are associated with increased aggregate stability.

4.2 Parameters predicting earthworm biomass

4.2.1 Soil Organic Matter

Transformed SOM in g/g held a significant correlation when predicting transformed total earthworm wet biomass (p-value = 0. 02, R^2= 0.63). Addition of organic matter is expected to increase earthworm biomass as it is a direct source of food and when managed with reduced tillage can increase earthworms in abundance and biomass (Lagerlöf *et al*, 2011). Increased microbial activity and its influence in the colonization of paletable organic matter is also important in describing the relationship between earthworms and SOM (LeBayon & Milleret, 2009). It is likely that a strong a relationship has not yet developed between SOM and earthworm biomass due to the young age of the set-asides used in this study. There were no differences in SOM detected between the set-asides and potatoes after one year, however with time groundcover and litter fall supplied by fallow vegetation has the potential to enhance soil biological activity (Tian *et al.,* 2000) .

4.2.2 Silt

The strong negative correlation of transformed earthworm total wet biomass and the composition of silt (p-value < 0.01, R^2= 0.71) was not expected. Little is known regarding the influence of soil particle distribution on earthworm species (LaPied, Nahmani, Rousseau, 2009). More specifically, little is known surrounding the influence of high silt content in soil, other than its direct effect of reducing fractions of sand and clay.

El- Duweini & Ghabbour 1965, describe favourable soil conditions for earthworms as "transient" in global arable land. Prescribed favourable conditions include, undisturbed soil, adequate supply of soil water and organic matter and fine textured soil (ultimately for its influence in water retention) (El- Duweini & Ghabbour, 1965). LaPied *et al* 2009, attempted to quantify the influence of soil particle distribution on the presence of specific earthworm species and ultimately on total earthworm counts between clay loams and sandy loams of Canada's Saint Lawrence Valley. Results of analysis of earthworm density and biomass were significantly greater in clay loam soils than in sand loams, with a high correlation to clay content. This finding can be countered by the model developed by Klok, Faber, Heijmans, & Bodt, 2007, in which an increase in clay content resulted in a decrease in individual biomass, implying that earthworms in high clay content soils require more energy to be obtained per unit weight gained. Klok *et al*. suggest this is a result of the increased resistance experienced by earthworms burrowing in search for food through clayey, non-penetrable soils.

No significant positive correlation between earthworms and clay content was found in this study. Similarly, no obvious pattern occurred where an increase in silt resulted in a direct marked decline in the fraction of clay present as in some instances both clay and silt were high and the fraction of sand low within the two treatments.

Conflicting literature on the influence of soil particle distribution do not help explain the observed decrease in earthworm biomass with an increase in silt fraction in this study. As for many soil parameters describing earthworm biomass, there are likely a number of underlying indirect influences introduced through an increase in silt content. Potential influences may include responses to soil compaction and alterations in soil water potential experienced in a loam soil versus that of clay soil (Brady & Weil, 2010). Excessive compaction of high silt content soil may induce similar conditions found by LaPied *et al.*, where earthworms likely struggle to penetrate the soil. Bulk density, often a measurement of soil compaction, was not statistically different between the two treatments within all sample depths (*depth one:* p-value = 0.72; *depth two:* p-value = 0.92; *depth three:* p-value = 0.51), allowing for excessive compaction to be influential in describing this relationship across the two treatments.

4.2.3 Phosphorous

Labile phosphorus showed a significant negative relationship with transformed earthworm biomass (p-value = 0.02, R^2= 0.62). Within soil systems, phosphorous is highly influential in describing soil fertility. Natural phosphorous compounds are insoluble and when additions of phosphorus occur, fixation with soil minerals has the potential to reduce bioavailability (Brady & Weil, 2010).

Investigation into relationships between phosphorus and other indicators that showed a significant correlation with transformed earthworm biomass suggests that phosphorus likely has an indirect negative effect on earthworms. Phosphorus was negatively correlated with SOM and positively correlated with silt.

O'Halloran, Stewart & Kachonoski, 1987 investigated the influence of texture and management practices on the forms and distribution of soil phosphorous in Chernozemic loam soils in Swift Current, Saskatchewan. Investigation into the relationship of labile inorganic phosphorus increased with the presence of a crop, but it was suggested that it may be associated with a decrease in soil pH. This relationship between soil pH and labile phosphorus was not found in the soils of Delta. O'Halloran *et al*. also found that an increase in percent silt content of the soil was also significantly correlated to relative amounts of phosphorous in a number of soil fractions. It was postulated that an increase in percent fine fractions (silt + clay) would hold a higher number of adsorbable surfaces for phosphorus. However, O'Halloran *et al.* did conclude that acid extractible inorganic phosphorus was the only phosphorus fraction that was positively correlated with a decrease in the fine fraction.

4.2.4 Zinc

Labile zinc was the only heavy metal to hold a significant relationship with earthworm biomass (p-value = 0.02, R^2= 0.63). Zinc is a common component of several commercial fertilizers, and is added primarily as zinc sulphate due to its relatively high solubility and often low cost (Schulte, 2004). Zinc is a micronutrient in the soil-plant system and has a relatively wide sufficiency range, requiring high concentrations to reach toxic levels (Brady & Weil, 2010). Zinc is primarily held in soils through interactions with clay surfaces or through chelation with SOM (Schulte, 2004). As a result, zinc bioavailability is most often strongly correlated with soil pH and cation exchange capacity (Lock & Janssen, 2001).

Exotoxicity models developed by Rombke, Jansch, Junker, Pohl, Scheffczyk, & Schallnab 2006, investigated the influence of zinc nitrate additions to a number of artificial and natural soils. Half maximal effective concentrations based on earthworm reproduction were correlated with soil parameters of pH organic carbon content and cation exchange capacity, described as influential in the bioavailability of zinc by Rombke *et al.* 2006. The correlations developed between measured effective concentrations continuously failed to be significant for the soil parameters tested in Rombke *et al.*'s study. This highlights that for earthworms, a complex set of interactions ultimately describes toxic levels of zinc.

Lev, Mattheix, Snodgrass, Casey, Ownby, 2010 investigated the potential zinc storage in the gut and body tissues of earthworms when exposed to levels expected in urban ecosystem. The use of artificial soils allowed this study to isolate the direct impacts of zinc. Results suggest a non-linear relationship between zinc and earthworm populations. At low levels, zinc limits populations as a micronutrient and at high concentrations, induces negative effects of bioaccumulation. Zinc concentrations experienced in samples of soils in Ladner hold a range of 20-40ppm which correlate to mid to low concentrations in Lev *et al.* study. This suggests that the decline in earthworm biomass with an increase in moderate zinc levels is likely associated with the cumulative impact of heavy metals in the system and while there was some correlation with lead (p-value = 0.06, R^2 value = 0.47), there was no relationship with cadmium (p-value = 0.36, R^2 value = 0.14), copper (p-value = 0.12, R^2 value = 0.36) or nickel (p- value = 0.37, R^2 value = 0.13).

4.3 Earthworm presence and sampling constraints

Substantial literature on methods to optimize earthworm sampling is available (East & Knight 1998; Eisenhauer, Straube, & Stefan, 2007; Valckx *et al.,* 2011). However, specific knowledge on the efficiency of various methods under a number of different soil conditions is scarce (Eisenhauer *et al*., 2007). The method of hand sorting for earthworms, although time consuming, has for a long time been considered the most accurate technique for quantifying earthworm abundance (Valckx *et al.,* 2011; East & Knight, 1998). Downfalls of sampling earthworms through hand sorting include an underestimation of juveniles within the sampled plot and the inability to capture larger, deep burrowing anectic species who reside at unreachable depths during sampling (Valckx *et al.,* 2011). Underestimation of juveniles is often addressed through a mixed hand sorting and sieving effort (Valckx *et al.,* 2011). Anectic species sampling is often optimized through the use of mustard vermifuge extraction, where earthworm's surface in response to a natural chemical irritant found in mustard powder (Eisenhauer *et al*., 2007).

It is likely that an underestimation of total juveniles and high biomass anectic earthworms occurred in both treatments. The wet and fine textured nature of the soils in Ladner prevented sieving, likely leading to an underestimation of juveniles. Similarly, attempts to optimize sampling efforts for deep burrowing anectic species were made. Prior to field sampling, a preliminary field study was carried out in Ladner, utilizing methods of earthworm extraction through mustard vermifuge. The highly compact surface and poor drainage characteristics in this area prevented infiltration of the vermifuge solution into soil horizon, therefore making it an ineffective solution.

Rainfall accumulation for the duration of the field sampling reached approximately 105.2 mm (Conarroe, 2012). Pools had begun to form on the poorly drained, highly compacted gleysolic soils. In some instances when the water table was edging closer to the surface and seepage of ground water into the sampling pit occurred, larger worms were found swollen and suspended in water. Regression analysis on transformed earthworm biomass in response to moisture content was not significant ($p = 0.94$, $R^2 = 0.001$); however, observations in the field, along with the increased difficulty in handling saturated soil samples suggest that differences in rainfall between plots could have influenced the number of total counted worms.

Another probable reason no difference in earthworm presence was detected between the two treatments could be the low sample size. The small sample size caused any variability within the treatment to have a major effect on the statistical results. One replicate field within each sample treatment failed to follow the trend expected. SAS-F3 experienced a much lower number of worms when compared to other replicate fields. SAS-F3 differed from the other set-asides in that it was planted with both DF&WT mix and barley nursery crop, where all other set-asides were planted with only DF&WT (Christine Terpsma, personal communications, March 25, 2013). SOM from *depth one* in this set-aside was the lowest within the treatment with a value of 3.24 g/g, not even half of what was experienced in SAS-F1 (7.30 g/g). In the case of the harvest potato field, HP-F1 held a much higher number of worms in comparison to other potato fields. Sampling of this field occurred early in the study, following a day of heavy rainfall. On the day this field was sampled, a number of large bodied earthworms could be observed emerging from the soil and moving horizontally on the surface of the potato field. This phenomenon of "earthworm migration" is supported by Mather & Christensen, 1992 in a discussion on surface migration of earthworms in grasslands, where surface activity is most highly pronounced following a heavy rainfall event. The surface activity of earthworms is also primarily in response to a search for resources, a mate or in an attempt to avoid unfavourable habitat conditions (Ellis, Hodson & Wege, 2010; Mather & Christensen, 1992). It is therefore possible that a large number of worms were removed during sampling of this potato field as they attempted to migrate to the surface where more favourable conditions could be experienced. Similarly, this potato field held the highest amount of *depth one* SOM within this treatment with 4.7 g/g.

4.4 Further Research

The fact that only one sampling effort was completed reduces this studies ability to quantify the overall influence of the grassland treatment on earthworm abundance and other soil quality indicators. However, this study does provide baseline information for the influence of young set-asides on earthworm presence, and provides a platform for which a continual monitoring program can be established. Set-asides may remain in commission for upwards of six years within this area. Therefore, monitoring these set-asides along with their control potato fields could help quantify the time requirements for a significant increase in beneficial earthworm populations.

The DF&WT through the GSSP, acts as a liaison for farmers intent on converting to organically certified production by leaving their field fallow or chemically free for up to three years (Delta Farmland and Wildlife Trust, 2011). La Pied *et al.,* 2009, found that fertilization based on inputs of organic residues completed during organic agricultural management was highly beneficial for earthworms and overall soil quality. Investigation into the influence of organic cultivation versus conventional management on earthworm populations also represents an important avenue of research in quantifying biological indicators in this area.

5.0 CONCLUSIONS

A primary study of the presence of earthworms under two management practices revealed no statistical difference in earthworm presence between the set-aside and harvest potato treatments. There were however, significant differences in aggregate stability between the two treatments. While aggregate stability has been shown to be correlated with earthworm presence, through mucus inputs and cast formation, and influenced directly by clay and SOM, these factors did not explain the observed increase in this study. The variation of total earthworm abundance observed in Delta was explained largely by SOM, percent silt composition and the presence of phosphorus and zinc. Increasing values of percent silt, phosphorus and zinc predicted a decrease in earthworm biomass, where an increase in SOM predicted an increase in earthworm abundance. Percent silt composition was the strongest predictor of earthworm biomass.

More information, including multiple sampling events and a larger sample size, may assist in further describing the relationships highlighted in this study. Efforts to continue developing knowledge of soil parameters and management decisions which can help develop and maintain populations of earthworms in Ladner's grassland fields will be beneficial to the farmers that choose to participate in the GSSP provided by the DF&WT.

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APPENDIX A: COMPLETE DATA SETS AND RESULTS OF STATISTICAL ANALYSIS

Table A.1 -Results of statistical analysis between grassland set-asides (S) and harvested potato fields (H). Provided are the non-transformed averages experienced within each field along with the calculated treatment average. Standard error is reported for each treatment mean. Reported p-values resulted from Welch's Two Sample t-test on non-transformed data. However (*) indicates that statistical analysis was completed on transformed data and (#) indicates that non-parametric statistical analysis was required.

	Total EW Biomass (/m2)		Total Ind. Without Parts (ind/m2)		Mean Wetted Aggregate Diameter (mm)	
FIELD ID	S	H	S	H	S	H
F1	57.65	47.17	981	539	1.475	1.292
F2	56.13	1.04	629	16	1.558	0.908
F3	5.82	1.31	32	21	1.665	0.954
F4	14.40	2.55	496	69	1.666	1.109
AVERAGE:	**33.50**	**13.02**	**535**	**161**	**1.591**	**1.066**
STD ERROR:	**13.62**	**11.39**	**196**	**126**	**0.046**	**0.087**
P-VALUE:	***0.1345 ****		***0.1845 ****		***0.004023***	
	Bulk Density: *Depth One*		Bulk Density: *Depth Two*		Bulk Density: *Depth Three*	
SOIL ID	S	H	S	H	S	H
F1	1.00	1.06	1.02	1.06	1.05	1.07
F2	1.02	1.13	1.03	1.13	1.07	1.07
F3	1.23	1.15	1.25	1.15	1.25	1.12
F4	1.19	1.20	1.24	1.23	1.24	1.18
AVERAGES:	**1.11**	**1.14**	**1.13**	**1.14**	**1.15**	**1.11**
STD ERROR:	**0.07**	**0.07**	**0.07**	**0.07**	**0.06**	**0.07**
P-VALUE:	***0.7195***		***0.922***		***0.5124***	
	SOM (g/g): *Depth One*		SOM (g/g): *Depth Two*		SOM (g/g): *Depth Three*	
SOIL ID	S	H	S	H	S	H
F1	7.30	4.77	6.93	5.09	6.19	4.48
F2	5.60	3.98	5.44	3.85	4.58	4.21
F3	3.24	3.23	3.10	3.26	2.86	3.30
F4	3.91	3.49	3.30	3.13	3.03	3.36
AVERAGES:	**5.01**	**3.87**	**4.69**	**3.83**	**4.17**	**3.83**
STD ERROR:	**0.91**	**0.34**	**0.91**	**0.45**	**0.78**	**0.30**
P-VALUE:	***0.3065***		***0.4422***		***0.715***	

	SOM (g/m2): *Depth One*		SOM (g/cm2): *Depth Two*		SOM (g/m2): *Depth Three*	
SOIL ID	S	H	S	H	S	H
F1	54.89	33.87	51.14	35.89	44.14	31.43
F2	41.00	26.38	39.55	25.56	32.23	29.57
F3	19.73	21.01	18.66	21.36	17.13	22.02
F4	24.53	21.84	19.96	19.08	18.27	21.27
AVERAGES:	**35.04**	**25.77**	**32.33**	**25.47**	**27.94**	**26.07**
STD ERROR:	**56.88**	**7.29**	**54.84**	**11.65**	**35.30**	**6.05**
P-VALUE:	***0.343***		***0.473***		***0.7998***	
	pH 0.01 *M* CaCl2: *Depth One*		pH 0.01 *M* CaCl2: *Depth Two*		pH 0.01 *M* CaCl2: *Depth Three*	
SOIL ID	S	H	S	H	S	H
F1	4.90	5.63	4.77	5.74	4.88	5.97
F2	5.77	5.60	5.75	5.51	5.63	5.47
F3	4.59	4.98	4.47	4.96	4.50	4.81
F4	5.28	5.41	5.31	5.31	5.33	5.31
AVERAGES:	**5.14**	**5.41**	**5.07**	**5.38**	**5.08**	**5.39**
STD ERROR:	**0.26**	**0.15**	**0.28**	**0.17**	**0.25**	**0.24**
P-VALUE:	***0.4035***		***0.3977***		***0.4105***	
	pH DI H20: *Depth One*		pH DI H20: *Depth Two*		pH DI H20: *Depth Three*	
SOIL ID	S	H	S	H	S	H
F1	5.43	6.09	5.29	6.04	5.35	6.25
F2	6.31	6.11	6.40	5.90	6.24	5.75
F3	5.52	5.85	5.39	5.80	5.37	5.49
F4	5.93	6.01	5.87	5.85	5.95	5.78
AVERAGES:	**5.80**	**6.02**	**5.74**	**5.90**	**5.73**	**5.82**
STD ERROR:	**0.20**	**0.06**	**0.25**	**0.05**	**0.22**	**0.16**
P-VALUE:	***0.3684***		***0.5784***		***0.7524***	
	% CLAY: *Depth One*		% CLAY: *Depth Two*		% CLAY: *Depth Three*	
SOIL ID	S	H	S	H	S	H
F1	27.3	25.1	27.6	21.7	26.9	23.2
F2	24.5	21.6	24.1	19.8	26.6	20.0
F3	25.1	17.6	25.3	18.8	24.9	19.9
F4	27.5	26.8	29.4	27.8	29.2	27.3
AVERAGES:	**26.1**	**22.8**	**26.6**	**22.0**	**26.9**	**22.6**
STD ERROR:	**0.8**	**2.0**	**1.2**	**2.0**	**0.9**	**1.7**
P-VALUE:	***0.2042***		***0.1096***		***0.0859***	

	% SILT: *Depth One*		% SILT: *Depth Two*		% SILT: *Depth Three*	
SOIL ID	S	H	S	H	S	H
F1	60.9	59.8	61.3	63.4	61.8	62.2
F2	59.7	66.3	59.9	67.6	58.3	62.7
F3	67.5	67.5	68.3	65.9	68.4	64.2
F4	65.6	64.1	64.5	63.8	64.1	62.7
AVERAGES:	**63.4**	**64.4**	**63.5**	**65.1**	**63.1**	**62.9**
STD ERROR:	**1.9**	**1.7**	**1.9**	**1.0**	**2.1**	**0.4**
P-VALUE:	***0.7051***		***0.4663***		***0.9317***	
	% SAND: *Depth One*		% SAND: *Depth Two*		% SAND: *Depth Three*	
SOIL ID	S	H	S	H	S	H
F1	11.8	15.1	11.1	14.9	11.3	14.7
F2	15.8	12.0	16.0	12.6	15.1	17.3
F3	7.4	14.9	6.4	15.3	6.7	15.9
F4	6.9	9.1	6.0	8.4	6.8	9.9
AVERAGES:	**10.5**	**12.8**	**9.9**	**12.8**	**10.0**	**14.4**
STD ERROR:	**2.1**	**1.4**	**2.4**	**1.6**	**2.0**	**1.6**
P-VALUE:	***0.4017***		***0.3466***		***0.1359***	
	Aluminum		Cadmium		Calcium	
SOIL ID	S	H	S	H	S	H
F1	6074.96	5363.22	24.54	25.77	2894.79	3348.72
F2	5757.71	5304.05	24.78	25.21	3614.28	3168.07
F3	6119.25	5282.86	25.22	25.52	2298.34	2479.67
F4	6648.56	6997.35	25.69	25.57	2366.57	2829.41
AVERAGES:	**6150.12**	**5736.87**	**25.06**	**25.52**	**2793.50**	**2956.47**
STD ERROR:	**184.62**	**420.50**	**0.25**	**0.12**	**304.33**	**191.95**
P-VALUE:	***0.3429 #***		***0.1708***		***0.6693***	
	Copper		Lead		Magnesium	
SOIL ID	S	H	S	H	S	H
F1	19.85	14.79	15.87	16.04	3773.77	3651.32
F2	18.31	29.55	15.55	17.21	3816.46	4111.30
F3	22.94	18.31	22.06	22.55	4902.27	4463.89
F4	24.68	23.13	19.65	22.97	4682.38	4486.06
AVERAGES:	**21.45**	**21.45**	**18.28**	**19.69**	**4293.72**	**4178.14**
STD ERROR:	**1.45**	**3.20**	**1.57**	**1.79**	**291.48**	**195.46**
P-VALUE:	***1***		***0.5752***		***0.7547***	

	Nickel		Phosphorous		Zinc	
SOIL ID	S	H	S	H	S	H
F1	32.83	33.28	821.54	834.5	24.77	20.94
F2	33	34.05	944.28	1023.96	28.04	33.87
F3	33.36	33.81	1102.84	1249.42	31.53	31.63
F4	36.73	37.14	826.88	1043.84	31.56	37.83
AVERAGES:	**33.98**	**34.57**	**923.89**	**1037.93**	**28.98**	**31.07**
STD ERROR:	**0.92**	**0.87**	**66.03**	**84.82**	**1.63**	**3.61**
P-VALUE:	***0.6832 ****		***0.3319***		***0.6241***	

Table A.2 -Results of particle size distribution analysis at three depths within the eight fields of study. Soil classifications based off of the USDA soil texture triangle (figure 1) are given for all depths within each field. (SL = Silt Loam, SCL = Silty Clay Loam).

		SAS – F1	**SAS –F2**	**SAS –F3**	**SAS –F4**	**HP – F1**	**HP –F2**	**HP –F3**	**HP –F4**
Depth 1: ***0 cm – 7.5 cm***	*% Clay*	27.3	24.5	25.1	27.5	25.1	21.6	17.6	26.8
	% Silt	60.9	59.7	67.5	65.6	59.8	66.3	67.5	64.1
	% Sand	11.8	15.8	7.4	6.9	15.1	12.0	14.9	9.1
	Classification	SCL/SL	SL	SL	SCL	SL	SL	SL	SCL/SL
Depth 2: ***7.5 cm – 15 cm***	*% Clay*	27.6	24.1	25.3	29.4	21.7	19.8	18.8	27.8
	% Silt	61.3	59.9	68.3	64.5	63.4	67.6	65.9	63.8
	% Sand	11.1	16.0	6.4	6.0	14.9	12.6	15.3	8.4
	Classification	SCL	SL	SL	SCL	SL	SL	SL	SCL
Depth 3: ***15 cm – 22.5 cm***	*% Clay*	26.9	26.6	24.9	29.2	23.2	20.0	19.9	27.3
	% Silt	61.8	58.3	68.4	64.1	62.2	62.7	64.2	62.7
	% Sand	11.3	15.1	6.7	6.8	14.7	17.3	15.9	9.9
	Classification	SCL/SL	SCL/SL	SL	SCL	SL	SL	SL	SCL/SL

Table A.3 - Predicting non-transformed soil aggregate stability through linear regressions with non-transformed soil parameters of earthworm biomass, percent clay and SOM.

	Earthworm Total Wet Biomass	% Clay	SOM (g/g)
p-value	0.2641	0.06601	0.4549
Multiple R2	0.2018	0.4562	0.09609
Equation	y = 0.0054x + 1.2028	y = 0.0619x - 0.1849	y = 0.00677x + 1.0277

Table A.4 - Results of linear regressions for *depth one* physical and chemical parameters when predicting transformed earthworm total wet biomass. Statistical analysis was completed on non-transformed independent variables with the exception of SOM.

Physical	Bulk Density (g/cm3)	% Moisture Content	% Sand	% Silt	% Clay
p-value	0.06038	0.9382	0.5909	0.008742	0.1112
Multiple R2	0.4704	0.001088	0.05095	0.7088	0.3674
Equation	y = -13.298x + 17.142	y = - 0.0093x + 2.6039	y = 0.1046x + 0.9856	y = - 0.4241x + 29.307	y = 0.3049x - 5.2514

Chemical	pH 0.01 M CaCl2	LN [SOM(g/g)]	Calcium (ppm)	Magnesium (ppm)	Phosphorous (ppm)
p-value	0.6978	0.01882	0.3004	0.1342	0.0211
Multiple R2	0.02693	0.6292	0.1763	0.333	0.6157
Equation	y = 0.671x - 1.3346	y = 4.6444x- 4.5424	y = 0.0015x - 2.047	y= - 0.0021x + 11.1	y = - 0.0086x + 10.665

Heavy Metals	Cadmium (ppm)	Copper (ppm)	Lead (ppm)	Nickel (ppm)	Zinc (ppm)
p-value	0.3635	0.1178	0.05905	0.3718	0.01934
Multiple R2	0.1387	0.357	0.4739	0.1344	0.626
Equation	y = -1.4351x + 38.492	y = - 0.2195x + 6.9091	y = - 0.3626x + 9.087	y = - 0.3666x + 14.767	y = - 0.2518x + 9.7605

APPENDIX B: PROPAGATION OF ERROR

Standard error reported for bulk density along with the standard error calculated for SOM presented in g/m2 were approximated from standard deviation with an n = 4 in equation A (Whitlock & Schluter, 2009). Standard deviations (σ) were calculated using Wolfram Mathematica 9 computing software (Wolfram Research, 2013).

$$\boldsymbol{Std.\ Error} = \frac{\sigma}{\sqrt{n}} \qquad \textbf{(A)}$$

Equation (B) describes the relationship used to calculate bulk density (BD). Equation (C) provides the general equation for error propagation between independent variables (Stull, 2011). This equation was utilized to develop standard deviations for bulk density, propagating the standard deviation measured for SOM in g/g (σSOM) and provided for mineral density (σMD) in Rawls, 1982. Mathematica script used for calculating partial derivatives is displayed below equation C.

$$\boldsymbol{BD} = \frac{\mathbf{100}}{\left\{\left(\frac{x}{\rho_0}\right)+\left(\frac{100-x}{\rho_m}\right)\right\}} \qquad \textbf{(B)}$$

$$\boldsymbol{\sigma\ BD} = \sqrt{\left(\left(\frac{\delta D}{\delta SOM}\right)^2 * (\sigma SOM)^2\right) + \left(\left(\frac{\delta D}{\delta MD}\right)^2 * (\sigma MD)^2\right)} \qquad \textbf{(C)}$$

```
fun [om_, md_] := 100 / ((om / 0.224) + ((100 - om) / md))
partom = Simplify[D[fun[om, md], om]]
partmd = Simplify[D[fun[om, md], md]]
```

$$\frac{\delta D}{\delta SOM} = -\frac{22.4\,md\,(-0.224 + 1.\,md)}{(22.4 + (-0.224 + 1.\,md)\,om)^2}$$

$$\frac{\delta D}{\delta MD} = \frac{501.76 - 5.0176\,om}{(22.4 + (-0.224 + 1.\,md)\,om)^2}$$

Equation (D) was used to calculate SOM in g/cm^2 from bulk density and SOM in g/g. Equation (E) was used to calculate standard deviation for SOM in g/cm^2, taking into consideration the correlation coefficient calculated (C) between soil bulk density (BD) and SOM in g/g for each field (Stull, 2011). Mathematica script used for calculating partial derivatives is displayed below equation E.

$$SOM\ g/\mathrm{cm}^2 = \left(\frac{SOM\left(\frac{g}{g}\right)}{BD}\right) * sample\ depth \tag{D}$$

$$\sigma\ SOM\frac{g}{\mathrm{cm}^2} = \sqrt{\left(\left(\frac{\delta D}{\delta BD}\right)^2 * (\sigma BD)^2\right) + \left(\left(\frac{\delta D}{\delta SOM}\right)^2 * (\sigma SOM)^2\right) + \left(2C * \left(\left(\frac{\delta D}{\delta BD} * \frac{\delta D}{\delta SOM} * \sigma BD * \sigma SOM\right)\right)\right)}; \tag{E}$$

$$where\ C = \left(\frac{1}{(n-1) * \sigma BD * \sigma SOM}\right) * \sum_{i=1}^{n=4} [(BD_i - BD_{Average})(SOM_i - SOM_{Average})]$$

```
som [bd_, om_] := (om / bd) * 7.5
partbd = Simplify[D[som[bd, om], bd]]
partom = Simplify[D[som[bd, om], om]]
```

$\frac{\delta D}{\delta BD} =$ $-\frac{7.5\ om}{bd^2}$

$\frac{\delta D}{\delta SOM} =$ $\frac{7.5}{bd}$