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Earthworm population dynamics as a consequence of long-term and recently imposed tillage in a clay loam soil

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Earthworm abundances were tracked from 1997-2012 in established tillages (since 1983) and recently imposed tillages (since 1997) for a Brookston clay loam soil (Orthic Humic Gleysol) at Woodslee, Ontario. Tillages included: long-term fall moldboard plowing (CT83) and its 1997 conversion to no tillage (NT97-CT83); long-term no tillage (NT83) and its conversion to moldboard plowing (CT97-NT83); long-term ridge tillage (RT83) and its conversion to moldboard plowing (CT97-RT83); and long-term bluegrass sod (BG83) and its conversion to moldboard plowing (CT97-BG83). *Lumbricus terrestris* and *Aporrectodea turgida* were the most abundant of six species identified. NT83 had the greatest earthworm numbers except for 2012 when RT83 had equal abundance because of increased *Ap. turgida* juveniles. Populations in NT97-CT83 increased significantly from 1997-2012 as a result of reduced mechanical disturbance and greater surface residues. During 1997, 1999 and 2003, mean abundance in CT97-BG83 was not different from BG83, which likely occurred because buried sod continued to provide ample food. CT97-RT83 showed a decline in earthworm populations relative to RT83. The CT97-NT83 treatment had the most significant earthworm decline reflecting substantial increase in soil disturbance. Characterizing tillage system effects on earthworm dynamics (e.g., diversity, occurrence, adult and juvenile abundance) will provide essential input for landscape models.

Key words: Earthworm abundance, Population dynamics, Tillage change, Soil Ecology, Landscape models

Introduction

A goal of soil quality assessments has been to determine whether specific tillage operations impact earthworm populations (Kladivko et al. 1997; Chan 2001). The reason for documenting changes to earthworm populations relates to the important soil functions contributed by earthworms in agricultural systems, namely: 1) producing channels through burrowing that aid in air and water infiltration; 2) deposition of casts which stabilize soil aggregates; and, 3) translocation of crop residues below the soil surface, which contributes to residue breakdown, sequestration of soil carbon, and dissemination of nutrients throughout the crop root zone (Linden et al. 1994; Edwards and Bohlen 1996; Bottinelli et al. 2010). The intensity and depth of conventional tillage (i.e., moldboard plowing with disking) can lead to decreased earthworm abundance when compared to no till or other conservation tillage systems (House 1985; Peigné et al. 2009; and, Pelosi et al. 2014b). Land managers often question whether implementing a new tillage system will lead to reductions or improvements in soil quality as a result of tillage-induced changes in earthworm populations and species.

Soil disturbance and presence of surface residues are key factors affecting earthworm abundance (Simonsen et al. 2010) in addition to soil moisture and soil organic matter (Crittenden et al. 2014). Bohlen et al. (1995) identified cropping pattern, tillage, climate and geographic location as factors determining earthworm abundance and diversity. Ivask et al. (2007) defined specific compositions of the earthworm community as being an indicator of cultivation intensity: *Ap. caliginosa*, *Ap. rosea*, and *L. rubellus* species were tolerant to disturbance from intensive tillage and management practices or a limiting ecological factor; whereas, highly sensitive species, *L. terrestris*, *Ap. longa*, *A. chlorotica*, and *L. castaneus* were indicative of more

favourable agricultural or ecological conditions. Peigné et al. (2009) found increases in anecic species in no-tillage and reduced tillage systems compared with moldboard plowing systems.

The sensitivity of earthworms to tillage intensity and to soil properties such as organic matter and soil texture have been used to develop broad-based modelling relationships which can predict population responses and potential occurrences across landscapes and for specific field operations. For example, earthworm abundance and soil attribute data (Fox et al. 2004) from the Agriculture and Agri-Food Canada benchmark sites across Canada were used as part of soil biodiversity indicator development by Fox (2005) to estimate potential earthworm abundance distribution in eastern Ontario at the field level; i.e. $\text{Abundance potential} = -35.37 + 51.43 \text{ Organic carbon} - 2.011 \text{ Silt\%} + 7.011 \text{ clay\%}; r^2 = 0.61$). Römcke et al. (2005) estimated expected values of five soil properties (i.e., soil texture, pH, moisture, C/N ratio, and per cent organic matter) for 15 frequently observed earthworm species in Central Europe, and used this information to predict earthworm species that would likely occur in various soil environments. Krück et al. (2006) found distinctive groupings of earthworm species based on interactions with respect to the topsoil and subsoil texture and soil organic matter content enabling specific prediction ranges for expected earthworm abundances and species. Joschko et al. (2006) observed from a 151 km transect study on arable lands on morainal landscapes northeast of Berlin that although total earthworm abundance and biomass fluctuated randomly at a regional scale, species number and composition changed in relationship to increasing soil pH, and soil organic carbon, total nitrogen and clay content along the north-south transect. Rutgers et al. (2016) using soil, land use, vegetation and climate factors predicted earthworm diversity, richness, and abundance for Europe.

To account for population differences among locations, Kladvko et al. (1997) recommended conducting a detailed field history. To build on anticipating the history of population changes, the next steps for preparing earthworm abundance predictions is to predict the nature of population change for various tillage systems; that is, define how earthworm populations will potentially change from that of established systems when growers implement a new tillage practice. To achieve this, the existing population structure in the established tillage system must be determined, and then the spatial and temporal changes in earthworm populations under the new tillage must be characterized.

There are many examples of comparison studies of earthworm populations sampled under different tillage and crop management systems such as Marinissen (1992), Brown et al. (2003), Ernst and Emmerling (2009), Capowiez et al. (2009), Simonsen et al. (2010), Pelosi et al. (2014b), Ashworth et al. (2017), and the review by Chan (2001) that provide information about impacts on earthworm abundance and species composition. Even though the same earthworm species may be present (Pelosi et al. 2016) in different tillage systems, the proportion of these species may differ. For example, Clapperton (1999) observed that *Ap. turgida*, *Ap. tuberculata* and *Ap. rosea* were the main earthworm species in a long-term irrigation study on plowed and no-tillage treatments in Lethbridge, Alberta; but, their proportions differed among tillage treatment. In a two year study (1980-81) undertaken on long-term (since 1976) monoculture corn on silt loam at Guelph, Ontario, De St. Remy and Daynard (1982) reported the following species proportions for mature earthworms for all tillage systems combined: *L. terrestris* (20%), *Ap. turgida* (29%), *Ap. tuberculata* (9 %), *Ap. trapezoides* (9%), *Ap. rosea* (20%), and unknown (13%); and they noted that the immature earthworms had increased numbers relative to adults. Both the diversity and abundance of earthworm species will determine the contributions

provided by earthworms to soil functions (Whalen and Fox 2007). Potential earthworm contributions to soil functions such as rainfall infiltration and breakdown of organic matter can be either compromised or enhanced by tillage (Crittenden et al. 2015).

A better understanding of the response dynamics of earthworm abundance and speciation are essential for determining tillage effects on the earthworm community. Pertinent questions are: does implementing a new tillage system have an effect on earthworm abundance and speciation; how soon will significant changes be observed and over what time period do changes take place, what is the nature of these changes and in what direction do these changes occur, i.e., increases, decreases or no change?

The purpose of this study was two-fold: first, to provide data for advising land managers on temporal changes in earthworm population when a new tillage system is superimposed on an established (long-term) tillage system; and second, to provide input data for models predicting earthworm populations across the landscape as a result of tillage system changes at field scale. The specific objectives were to:

- 1) Observe and quantify the relative occurrence of earthworm species in both long-term and newly implemented tillages;
- 2) Determine if there was a significant difference in mean earthworm abundance between the new tillage system and the established long-term tillage system; and
- 3) Determine if the temporal trend was significantly different between new and established tillages with respect to earthworm abundance and population structure of adults and juveniles.

Materials and Methods

Field Site and Experimental Design

The field site is located at the Honourable Eugene F. Whelan Research Farm, Agriculture and Agri-Food Canada, Woodslee, Ontario (Lat. 42° 21'N, Long. 82° 75'W). This study is part of an on-going tillage-soil quality investigation – see Reynolds et al. (2007), Yang et al. (2008), Shi et al. (2012); Fan et al. (2014), and Zhang et al. (2014) for details.

The field site was originally established in 1983 to determine tillage and cropping effects on crop yield and water quality (Drury et al. 1993). The treatments included conventional tillage (moldboard plowing and spring disking), ridge tillage, and no-tillage. The cropping treatments included monoculture corn (under conventional, ridge or no tillage) and continuous bluegrass sod. The statistical design was randomized complete block with 2 replicates. In 1996, a corn (*Zea mays* L.) – soybean (*Glycine max* L.) rotation was initiated; and in 1997, a new tillage system was initiated on one half (12.2 m wide by 35 m long) of each treatment plot while the other half retained its original 1983 tillage and crop. The half receiving the new tillage was chosen randomly.

The paired new-old tillages included “new” no-tillage (designated NT97-CT83) initiated on one half of the “old” conventional tillage (designated CT83); “new” conventional tillage (CT97-RT83) on one half of “old” ridge tillage (RT83); new conventional tillage (CT97-NT83) on one half of old no-tillage (NT83); and new conventional tillage (CT97-BG83) on one half of old bluegrass sod (BG83). The treatments were further divided to allow both phases of the corn-soybean rotation to appear each year.

Tillage Treatment and Influence on Earthworm Populations

Conventional tillage included annual fall moldboard plowing to 17.5 to 20 cm depth [mean 18.7 cm, based on McLaughlin et al. (2008)], followed by spring disking and harrowing to prepare the seed bed. In studies reviewed by Edwards and Bohlen (1996) and Chan (2001) conventional tillage was shown to lower earthworm population numbers relative to no-tillage; and this was attributed to: 1) cutting and disturbing burrows, thereby disrupting direct pathways especially for surface feeding of *L. terrestris* which have deep vertical burrows; 2) burying the surface crop residues making them no longer available for surface feeders (although at same time providing additional food source within the plow layer for endogeic species); 3) disturbing and possibly damaging earthworm cocoons; 4) exposing the soil surface layer to drying out during Fall and Winter; and, 5) direct injury/killing from the tillage operation and predation by birds.

No-tillage is the opposite pole to conventional tillage, i.e. mechanical disturbance of the soil is minimal and occurs only at planting (and side-dress injection of N fertilizer when applicable). No-till consequently provides a highly stable soil ecosystem, allowing burrows and cocoons to remain largely undisturbed for both endogeic and anecic earthworms. Furthermore, surface residues from previous crops provide substantial food resources for surface feeding earthworms.

Ridge-tillage is a conservation tillage method (> 30 % of soil surface covered by crop residues) that is a compromise between the two extremes of moldboard plow and no-tillage (Shi et al. 2012). Ridge tillage consists of raised ridges on which the crop is planted and depressed troughs where residues collect resulting in improved moisture and temperature conditions for earthworm survival during dry, hot periods. Mechanical disturbance occurs when ridges are reformed in late fall which may reduce earthworm numbers.

The continuous bluegrass sod provides the least amount of mechanical soil disturbance having no cultivation at all, and only periodic surface traffic when mowed and sprayed for weed control.

Soil Conditions

The soil is a Brookston clay loam, of lacustrine origin, with nearly level slope (<0.5% slope). It is classified in the Canadian soil system as an Orthic Humic Gleysol (Soil Classification Working Group 1998); and, in USDA Soil Taxonomy (Soil Survey Staff 2010) as a fine, loamy, mixed, mesic, Typic Argiaquoll. Mean annual air temperature and precipitation (45 year average) at the field site were 8.9°C and 832 mm, respectively (Reynolds et al. 2015). Average soil texture in the Ap horizon (0-20 cm) is 28 wt. % sand, 35 wt. % silt, and 37 wt. % clay (Yang et al. 2008).

Soil organic carbon (SOC), as a mean across in-row and between-row positions for 4 depth ranges, was reported by Shi et al. (2012) as follows: No-tillage (0-5 cm, 29.1 g kg⁻¹; 10-20 cm, 17.5 g kg⁻¹; and 20-30 cm depth, 8.8 g kg⁻¹); conventional moldboard plowing (0-5 cm, 18.6 g kg⁻¹; 10-20 cm, 19.0 g kg⁻¹; and 20-30 cm depth, 10.4 g kg⁻¹); ridge tillage (0-5 cm, 25.5 g kg⁻¹; 10-20 cm, 16.4 g kg⁻¹; and 20-30 cm depth, 9.8 g kg⁻¹). The SOC concentrations decreased to 3 g kg⁻¹ at 40-60 cm depth under all tillage treatments. For 1983 bluegrass sod, soil organic matter recorded in 2012 (C. Drury, field data) at 0-5 cm was 40.9 g kg⁻¹; 5-10 cm, 22.1 g kg⁻¹; and 10-20 cm, 19.7 g kg⁻¹.

Earthworm Field Sampling

Earthworm sampling was undertaken in 1997, 1998, and 1999, but then temporarily halted because continued annual sampling could potentially deplete earthworm populations. Sampling

then resumed in 2001 and 2003 to provide a total of 5 sampling events over a 7 year period. In 2012, there was an opportunity to re-sample the treatments as a follow-up to evaluate if the temporal trends established in 1997 to 2003 had continued.

Earthworm sampling was conducted during the early to mid-fall period at the end of soybean harvest; hence, sampling dates varied somewhat from year to year. Earthworm collection was limited to the soybean half of the rotation because: 1) soil temperature and moisture conditions rapidly become unfavourable for earthworm recovery in late fall (soil too cool and wet) and the soybeans were harvested before grain corn; 2) earthworm collection occurred before fall tillage; and 3) potential additional variability introduced by changing crop stover (soybean vs corn) was avoided. Three samples were taken from each plot (i.e., one sample randomly placed near each end and one randomly placed at mid-plot) for a total of six samples per treatment.

Visibly trafficked rows were avoided, as traffic compaction may reduce earthworm captures. Each tillage plot received annual wheel traffic that varied across the plot as a result of planting, weed control, and the harvesting of the soybeans and corn. Additional traffic occurred on conventional tillage due to spring and fall cultivation, and on bluegrass due to mowing and weed control. Because of the nature of ridge-till, the quadrat was placed to straddle a ridge and non-ridge portion not subject to traffic. For the sod treatment, the quadrat was randomly placed in an area without visible wheel traffic.

Earthworm Extraction Methodology

During the first 3 earthworm collections (1997, 1998, 1999), the earthworm extractant was 37% formalin (50 ml) in 7L water. For the next 3 collections (2001, 2003, 2012), hot mustard powder was used (i.e., 53 grams of dry hot mustard powder pre-mixed in 150 ml water the day

prior, then added to 7L water). Formalin was discontinued after 1999 due to potential health risks (i.e. human carcinogen) and potential groundwater contamination. The effectiveness of hot mustard compared to formalin was tested and described below. Hand sorting of the soil for earthworms was not undertaken in order to preserve the near-surface soil structure.

As field earthworm collection protocols using both formalin and hot mustard are detailed in Clapperton et al. (2008) only a brief summary will be repeated here. A wooden quadrat, 60 cm x 60 cm frame (Edwards and Lofty 1977, p.121), was placed on the soil surface. With a shovel, soil was packed around the outside edges of the frame to prevent leakage of the applied extractant solution. All 7L of extractant was sprinkled over the soil surface. Emerging earthworms were immediately removed from the soil surface using long-nosed tweezers and placed into a 500 ml Mason jar containing 125 – 200 ml of 67% ethanol solution (70% by volume of 95% ethanol). Time for collecting earthworms was limited to 15-20 minutes which was well beyond the time period that earthworms emerged. Any worms emerging outside of the frame were not included. All plots were sampled in one day to minimize any potential artefact effects from weather-induced changes in soil moisture and temperature. Following field sampling, the Mason jars were stored at 4°C until earthworm identification. The earthworms were identified to species level as per earthworm taxonomic systematics outlined in Reynolds (1977). Each species was separated into adults (matures: having a pronounced clitellum, by colour and morphology) and juveniles (immatures: no clitellum observed, by colour and morphology; includes both sub-adults and juveniles) and counted for abundance.

A field experiment was undertaken in 1995 at Agriculture and Agri-Food Canada sites in Ontario and Nova Scotia to determine if hot mustard is a viable earthworm extractant for Canadian soils (Table 1, Fig. 1). Fifty-three grams hot mustard powder per 7 L water was

compared with the standard formalin method of 50 ml of 37% formalin in 7L water. The 1:1 reference line (Fig. 1) indicates graphically where the earthworm numbers obtained by formalin and by hot mustard would be identical. A simple correlation analysis of the data found close correspondence: (Pearson correlation $r = 0.83$ ($R^2 = 0.694$) with the prediction equation of $\text{Formalin} = 11.13 + 0.879(\text{Hot Mustard})$. A non-significant difference t-test resulted when the mean (μ) earthworm abundance m^{-2} for formalin ($\mu_1=103.4$) and hot mustard ($\mu_2=104.9$) were compared; $t \text{ stat} = -0.168$; $t\text{-critical} = 2.14$; $n=15$). Because of the close similarity and non-significant difference, it was deemed that the formalin and hot mustard methodologies were equivalent for use as an earthworm extractant. Gunn (1992) and Högger (1993) also concluded that hot mustard may be as effective as formalin for extracting earthworms. Since then, Eichinger et al. (2007) have noted negative effects of using formalin on other soil fauna, soil respiration, dehydrogenase activity, fatty acid contents, vegetation cover and shoot and root biomass. In support for using hot mustard for earthworm extraction, Lawrence and Bowers (2002) found hot mustard a suitable extractant across a range of soil and land-use types. Pelosi et al. (2009) found that commercial hot mustard powder was not significantly different from formalin for earthworm biomass. In addition, when commercial mustard was compared with chemical solutions of allyl isothiocyanate, the active ingredient in mustard, Pelosi et al (2014a) found no significant differences in efficiency to expel earthworms in terms of abundance, biomass or diversity. In view of the above, earthworm numbers obtained in this study using formalin (1997, 1998, 1999) and hot mustard (2001, 2003, 2012) were considered equivalent and amalgamated for analysis.

Mean Population Abundance Analyses

Per Cent Occurrence of Earthworm Species

To identify the earthworm species with the highest occurrence in each treatment and all treatments combined (i.e. objective 1), the total number of captured earthworms (m^{-2}) obtained for each tillage treatment from both field blocks were tabulated as well as all of the tillage treatments combined for the six sampling events 1997 to 2012. The relative abundance of each species for each tillage treatment was expressed as a percentage of the total number of captured earthworms for that treatment. Similarly, for all treatments, the total number for each species was expressed as a percentage of the total number of captured earthworms from all treatments combined.

Statistical Analyses of Tillage Pair Mean Earthworm Abundance

Because earthworm samples were taken from the same experimental plot in each sampling year and in order to undertake a paired comparison of the treatments (Sokal and Rohlf 1987), a Student's distribution [paired t-test (Sigma-Plot® and Excel®)] was used to test if there is a significance difference in mean earthworm abundance in each sampling year between the original (1983) treatment and the corresponding new (1997) tillage (i.e., objective 2). The new tillage vs old tillage comparisons were: (CT97-BG83) vs BG83; (NT97-CT83) vs CT83; (CT97-NT83) vs NT83; (CT97-RT83) vs RT83. Normality tests were conducted and any non-normal data were $\log_{10}$ transformed when required to achieve normality and these data are indicated in the results (Refer to Table 3).

Mean Abundance Trend Determination

Soil moisture and temperature can have a strong effect on earthworm captures in any sampling year by affecting the depth of extractant penetration, and proximity of earthworms to the soil surface. Soil conditions are often very different from year to year due to differences in annual precipitation and temperature regime. For example, a field site approximately 500 m away, Drury et al. (2014) showed for October-November for 2007 to 2009 that the volumetric soil water content at the 0-10 cm depth can vary from 15 to 30%.

For each tillage treatment, bar graphs of mean abundance and standard deviation were plotted for each sampling year. The bar graphs for each tillage treatment also reflect the inherent environmental influences in each individual year.

To determine if a temporal trend existed due to tillage treatment, an abundance ratio, “R”, was plotted against sampling year,

$$R_i = \frac{NT_i}{OT_i}; \quad i = 1, 2, 3, 4, 5, 6$$

where, NT_i is mean earthworm abundance in the new tillage for year i , and OT_i is mean earthworm abundance in the corresponding old tillage for year i . Calculating R for each year ensured that precipitation and temperature inputs received during the growing season would be the same. A similar approach was used by Edwards and Lofty (1982) to show temporal change in earthworm abundance between direct drilling and plowed systems.

Information for evaluating the direction of change in earthworm numbers in tillage studies is provided by Beylich and Graefe (2009) who concluded from 60 sites on forest, cropland and grassland in northwestern Germany that temporal trends in earthworm population and biomass were valid if they persisted over three consecutive samplings. A moving average trend line

based on two sampling times was inserted to determine visually if there was a consistent trend over the six sampling times during 1997 to 2012.

To account for successive sampling on the same experimental plot (Sokal and Rohlf 1987), a one-way repeated measures ANOVA (Sigma-Plot® v.13.0) was used to compare among each of the tillage pairs (i.e., objective 3) if the mean abundance ratio values were significantly different over the sampling time period. First, the normality test (Shapiro-Wilk; $P=0.545$) and equal variance test (Brown-Forsythe $P=0.010$) required passing to enable testing if a significant difference ($P \leq 0.001$) in the mean abundance ratio values existed among the tillage pairs. Second, if a significant difference existed, a *post hoc* test using a pair-wise multiple comparisons (Tukey-Test, $p < 0.05$) was used to identify which of the tillage pairs were significantly different with respect to the ratio values during the sampling period.

Adult and Juvenile Population Change in Dominant Species

For each tillage treatment, the mean abundance (i.e., mean of 6 observations from combining the two field replicates) for *L. terrestris* and *Ap. turgida* adults and juveniles for each sampling event was plotted as a bar graph. The standard deviation indicates the degree by which the six abundance observations differed from the sample mean for each sampling event.

To identify for each sampling year, the degree to which the mean abundance of adults and juveniles changed in the new tillage after being implemented on the established tillage, a mean abundance difference was calculated between the new tillage and original 1983 tillage pairs (i.e., *new tillage mean abundance – 1983 established tillage mean abundance*). The calculation of a mean abundance difference between the new tillage and established tillage would provide a parameter that would discount the annual environmental variability over the sampling period on

earthworm population in showing the extent of difference in population abundance that took place in each sampling year. For example, a mean abundance difference value of 0 in a particular year would indicate there was no change in mean abundance between either the new or the established tillage system. Positive mean abundance differences would identify the degree to which adult and/or juvenile numbers were increasing in the new tillage system; and similarly, negative differences would identify the degree to which adult and/or juvenile numbers were declining in the new tillage system.

A trend line based on a moving average of two observations (i.e., two sampling times) was inserted into each mean difference plot for each tillage pair. The trend line visually identifies the direction of change whether the mean abundance difference remained stable or had fluctuated in a positive or negative direction over the sampling period, thereby, defining the earthworm population response of the new tillage compared to the established long-term tillage.

Again, a one-way repeated measures ANOVA (Sigma-Plot® v.13.0) was undertaken to test if the mean abundance differences specifically for *L. terrestris* adults and juveniles and for *Ap. turgida* adults and juveniles were significantly different among the tillage pairs over the sampling time period (i.e., study objective 3). If the normality test (Shapiro-Wilk, $P = 0.053$) and equal variance test (Brown-Forsythe, $P = 0.330$) were statistically passed, the mean abundance differences among the tillage treatments was greater than would be expected by chance and a statistically significant difference ($P \leq 0.001$) existed among the tillage pairs. To test which of the tillage pairs significantly differed from each other with respect to mean abundance differences, a *post hoc* multiple comparison procedure (Holm-Sidak method, $P < 0.05$) was applied.

Results and Discussion

Proportion of Earthworm Species among Tillage Treatments

Six earthworm species (Table 2) were observed during 1997-2012 from all of the tillage treatments: Anecic species, *L. terrestris*; the endogeic species *Ap. turgida*, *Ap. rosea*, *Ap. tuberculata*, and *Ap. trapezoides*; and, the epigeic species, *L. rubellus*. In this study, *L. terrestris* and *Ap. turgida* were the dominant species (Table 2). For all treatments, *L. terrestris* comprised 40.4% of total numbers with a range of 24.1% (CT83) to 54.8% (BG83) when individual tillage treatments are considered. *Ap. turgida* comprised 56.6% of total earthworms extracted for all treatments, with a range of 42.7% (BG83) to 71.2% (CT97-BG83) and 71.1% (CT83) for individual tillage treatments. Except for bluegrass sod, BG83, *Ap. turgida* was found to be proportionately greater than *L. terrestris* in each tillage treatment. *L. rubellus*, an epigeic species that feeds on surface residues, comprised 2.0 % of the total numbers when all treatments and sampling times were considered; but, occurrence was very variable from 0 % in NT83 to a maximum occurrence of 7.8% in (CT97-NT83) as compared to 4.9% in (NT97-CT83). *L. rubellus* was found (data not shown) first in 2001 as a single occurrence only in (CT97-NT83). Then in 2003, *L. rubellus* was observed only in field replicate 2 in BG83, (CT97-NT83), and RT83; and again only in the second field replicate in 2012 in all tillage treatments except for RT83 and NT83 suggesting possible encroachment from adjacent grassed headlands surrounding the treatment plots. This appearance of *L. rubellus* in the later sampling periods particularly 2003 and 2012 and only in the second field replicate requires future investigation. *Ap. tuberculata*, *Ap. trapezoides*, and *Ap. rosea* occurred less than 1% (Table 2) overall. *Ap. rosea* was observed (data not shown) only in 1997 (adults) and in 2012 (both adults and juveniles); *Ap.*

tuberculata in 2001 and 2003 only as adults; *Ap. trapezoides* in 1997 (both juveniles and adults) and in 2003 and 2012 (only as adults).

Because *L. rubellus*, *Ap. tuberculata*, *Ap. trapezoides* and *Ap. rosea* occurred sporadically throughout the sampling period, it was not possible to determine a temporal pattern from the time of implementation of the new treatment for these species. This study will focus on *L. terrestris*, an anecic species, and *Ap. turgida*, an endogeic species as they were both abundant in all treatments at every sampling time. Both *L. terrestris* and *Ap. turgida* are common species in Canada occurring in all Canadian provinces (Tomlin and Fox 2003)

Mean Abundance Comparison between the New and Established Tillage Pair

Long-term bluegrass (BG83) had highly variable earthworm captures from year to year (Fig. 2) with means ranging from a high of 70.4 m⁻² in 1997 to a low of 9.7 m⁻² in 1999 suggesting soil moisture effects or a highly variable population distribution. The conversion of BG83 to conventional moldboard plow tillage (CT97-BG83) had moderate earthworm numbers in the first two years (mean abundance 63.4 and 66.7 m⁻², respectively), presumably, due to residual food reserves and improved soil moisture caused by the burial of sod in plowing to mean 18.7 cm depth. For CT97-BG83, determination of significant difference in mean earthworm abundance between the new and established tillage was variable (Table 3). Although there were significant differences in 2012, this was only for the two-tailed test. There were no significant differences in mean abundance found in 1997, 1999, and 2003. The 2012 results suggest that additional sampling (i.e., replicates) was needed to provide a more definitive assessment.

The impact of long-term conventional plow tillage (CT83) when compared to the corresponding new no-tillage treatment, NT97-CT83, is evident for CT83 with significantly

lower overall earthworm abundance (under 35 m^{-2}) for all sampling events (Fig. 2, Table 3). Following the conversion of conventional tillage, CT83, to no-tillage, NT97-CT83, there were population increases in all years in the no-tillage treatment compared to CT83, with abundances approaching that of the long-term NT83 in 2003 and 2012. For (NT97-CT83) vs CT83 tillage pair, there are variable results (Table 3) in the first three years with respect to significant differences in contrast to 2001, 2003 and 2012 which are increasingly highly significantly different with respect to mean abundance. This suggests that abundance increases in the new NT97 treatment as an outcome of much decreased disturbance from the former conventional tillage CT83 treatment may require greater than 5 years for stability of earthworm abundance to be achieved.

For (CT97-NT83) vs NT83 tillage pair, mean earthworm abundance between the established NT83 and newly implemented conventional tillage, CT97, is significantly different (Table 3) for all sampling times. The established 1983 no-tillage mean abundance is significantly greater than the mean abundances of CT97 reflecting the dichotomy between minimum and maximum disturbance of the soil surface layer. Long-term no-tillage (NT83) had the highest mean earthworm numbers for every tillage treatment for every year except in 2012 (Fig. 2) when mean abundance (Table 3) at 106.6 m^{-2} was similar to CT83 with 107.5 m^{-2} .

For CT97-NT83, earthworm abundance (Fig. 2) in the first year 1997 of conversion to conventional tillage from no-till (i.e., CT97-NT83 vs NT83) had a major decline in numbers compared to established NT83 (i.e., CT97-NT83, 26.9 m^{-2} and NT83, 170.0 m^{-2}). This first year, 1997, decrease in CT97-NT83 produced a similar mean abundance to the long-term CT83 (19.0 m^{-2}).

Long-term ridge tillage (RT83) earthworm population (Fig. 2) was less overall than under long-term no tillage (NT83) but markedly improved in comparison to long-term conventional moldboard plow (CT83). For new implemented conventional moldboard plow tillage, CT97-RT83, on the established RT83, mean earthworm abundance was often variable showing marked decreases in 1999 and 2003. Comparing CT97-RT83 vs RT83 tillage pair, in the second year, 1998 (Table 3), there was a non-significant result in mean abundance between the established RT83 and new tillage CT97. RT83 had significantly greater mean abundances than CT97-RT83 for each of the other sampling periods.

It is interesting to note in Table 2 that the total earthworm numbers captured for all sampling times from each of the new moldboard plow CT97 tillage treatments (i.e., 1701, 959, 1245) that were implemented respectively on established BG83, NT83, and RT83 were higher in total numbers than the established 1983 conventional tillage, CT83 with 634 earthworms. This suggests a) that earthworm populations were still being affected 16 years after tillage conversion (1996 to 2012) by the respective established tillage treatment (i.e., BG83, NT83, RT83) and b) a long-term biological legacy influence (Crotty et al. 2016) has taken place for the new tillage systems with a different soil environment from CT83 having been established.

Mean Abundance Ratio Value Comparisons between New and Established Tillage

For each sampling event, Fig. 3 shows the ratio of mean earthworm abundance of the new (1997) tillage relative to the corresponding established 1983 tillage system. A ratio close to 1 indicates similar mean abundance e.g., (CT97-BG83):BG83 in 1997, 1999 and 2003. Ratios > 1 indicate increases in mean abundance in the new tillage such as for (NT97-CT83):CT83, and sometimes in 1998, 2001 and 2012 for (CT97-BG83):BG83. Ratios < 1 indicate decreases have resulted following its implementation from the established tillage for (CT97-NT83):NT83 and

(CT97-RT83):RT83. To visualize the nature of change during the sampling period (1997-2012), trend lines were inserted through the ratio values (Fig. 3).

For (NT97-CT83):CT83, the trend line increases sharply through 1997 to 2012 following initial variability suggesting much improved habitat conditions under NT97 relative to CT83. Mean abundances for (CT97-BG83):BG83 were initially variable with an overall decline (Fig. 3) following the plowing of $\frac{1}{2}$ of the BG83 plot. In the first year after the conversion to CT97 from established BG83, population numbers were very similar to BG83 (Fig. 2). The second year, 1998, mean numbers in the new tillage CT97-BG83 increased almost 4 times (Fig. 2) as a result of food resources being supplied from the decomposition of the buried thick sod turf. The slight decreasing trend (Fig. 3) between the new (CT97-BG83) tillage and established BG83 suggests that although the population numbers in (CT97-BG83) initially benefitted from the incorporation of sod, there was still a gradual decline in numbers with time due to the conventional moldboard plow tillage. The trend line for (CT97-RT83):RT83 reflects an extremely gradual decline (Fig. 3) which may reflect the increased mechanical disturbance of moldboard plow and spring disking relative to ridge tillage. The (CT97-NT83):NT83 trend line (Fig. 3) indicates through the sampling period consistently lower population numbers in (CT97-NT83) from that of the established NT83, as expected because of the minimal disturbance in NT83 and maximum disturbance in (CT97-NT83). In 2012, there is a very slight upward tendency which reflects the increase in *L. terrestris* and *Ap. turgida* juveniles (Refer to Figs. 4a and 5a and discussed below).

Table 4 presents the results from the one-way repeated measures ANOVA mean abundance ratio comparisons among the tillage pairs for *L. terrestris* and *Ap. turgida*. Of the tillage pair comparisons, (NT97-CT83):CT83 vs (CT97-NT83):NT83 was highly significantly different. This finding can be confirmed in Fig. 3 with the very apparent maximum separation between the

trend lines suggesting distinctly different soil environments. Of note (Table 4) in comparing among the various conventional tillage treatments implemented on the established 1983 treatments, i.e., (CT97-NT83): NT83 compared with both (CT-BG83):BG83 and (CT97-RT83):RT83, and also (CT97-BG83):BG83 compared to (CT97-RT83):RT83, the mean abundance ratios over the sampling period were not statistically significantly different suggesting similar impact on earthworm numbers under conventional tillage as an outcome from maximum mechanical intensity in the surface soil layer.

***L. terrestris* and *Ap. turgida* adults and juveniles population structure**

We have seen above how the combined mean abundance of the dominant species *L. terrestris* and *Ap. turgida* varied for each tillage treatment over the sampling period (Fig. 2 and 3, Table 3). In addition, it was shown (Table 2) that the percentage abundance of *L. terrestris* and *Ap. turgida* varied considerably among the tillage treatments. Because *L. terrestris* and *Ap. turgida* occupy very different ecological niches in the soil, being anecic and endogeic respectively, a closer examination of their population structure was undertaken with respect to influence of tillage treatment on mean abundance patterns of adults and juveniles.

For all treatments, except in 1998, more *L. terrestris* juveniles than adults were found (Fig. 4). Exceptions in 1998 for increased *L. terrestris* mean abundance m^{-2} of the adults vs juveniles were observed only in the established treatments, that is, BG83 (6.9 vs 4.2 m^{-2}), for NT83 (25.9 vs 19.9 m^{-2}) and for RT83 (15.3 vs 13.4 m^{-2}), and for CT83, mean abundances for adults and juveniles were equivalent (0.44 m^{-2}). Similarly, mean abundances m^{-2} for *Ap. turgida* adults (Fig. 5) were greater than juveniles in 1998 in only the established treatments BG83 (5.1 vs 1.4 m^{-2}) and NT83 (27.3 vs 17.2 m^{-2}). *Ap. turgida* varied differently in occurrence (Fig. 5) from *L.*

terrestris in 1999 (Fig. 4) with adults predominating approximately 2 to 3 times more in comparison to juveniles for BG83 (0.9 vs 0 m⁻²); CT97-BG83 (4.2 vs 2.3 m⁻²); CT83 (1.4 vs 0.5 m⁻²); NT97-CT83 (16.2 vs 6.9 m⁻²); NT83 (13.4 vs 7.4 m⁻²), and RT83 (6.4 vs 2.8 m⁻²). *L. turgida* adults had also slightly greater mean abundance than juveniles in 2001 for NT97-CT83 (14.4 vs 8.8 m⁻²) and in 2003, for NT83 (39.4 vs 34.3 m⁻²). The established treatments converted in 1997 to moldboard plow tillage had fewer *L. terrestris* adults than juveniles: CT97-BG83 (8.3 vs 17.1 m⁻²); CT97-NT83 (4.2 vs 11.6 m⁻²); and CT97-RT83 (6.5 vs 10.6 m⁻²) indicating an increased impact on the adults from the implementation of plowing operations. For *Ap. turgida* results for adults vs juveniles were more variable depending on the established tillage: CT97-BG83 (6.5 vs 31.5 m⁻²); CT97-NT83 (25 vs 25 m⁻²), and CT97-RT83 (10.6 vs 8.7 m⁻²).

In 1997, Fig. 5a, mean abundance m⁻² for *Ap. turgida* juveniles in CT97-BG83 dominated over BG83 (31.5 vs 17.6 m⁻²) indicating a benefit for this species, particularly juveniles, from the decomposing thick sod turf buried by plowing to mean 18.7 cm depth. *Ap. turgida*, an endogeic species, would likely thrive from the availability of the slow decomposition of sod in the upper soil layer and the buried corn stover in alternate years. Boström (1995) also observed increases of endogeic earthworms (i.e., *A. caliginosa*) when organic matter was plowed under serving as a food source.

For the 2012 sampling event, mean abundance m⁻² of combined *L. terrestris* and *Ap. turgida* (Fig. 2) for CT97-NT83 showed a marked increase in numbers compared to the previous three sampling events. This observation can now be attributed to the marked increase (Figs. 4a and 5a) in *L. terrestris* and *Ap. turgida* juveniles (14.4 and 25.0 m⁻² respectively) in the surface soil layer at the time of sampling.

We have shown for *L. terrestris* and *Ap. turgida* adults and juveniles the effect of treatment on the variation of mean abundance m^{-2} over the sampling period. For the new tillage systems started on the 1983 established tillage it remains to discuss to what extent has change occurred with time. To evaluate the nature of this change for both adults and juveniles, mean differences between the new and established tillage pairs were calculated and graphed (Figs. 6 and 7) with a moving average trend line inserted to visually aid identifying the direction of change over the 6 sampling times.

The mean abundance difference between the new and established tillage systems will approach zero if population numbers were very close for both the new and established tillage. This is the case for tillage pairs (CT97-BG83)-BG83 and (CT97-RT83)-RT83 for *L. terrestris* adults (Fig. 6b) following after 1999 to 2012 and in the initial years (1998-2001) for *Ap. turgida* adults (Fig. 7b). This implies that the soil habitat developed under the new tillage system of conventional tillage maintained similar adult population numbers to the respective established tillage (BG83 and RT83), likely an on-going legacy benefit effect from the established tillage. This is not the situation for the *Ap. turgida* juveniles with marked increases in the new tillage (CT97-BG83)-BG83 initially 1997 and 1998 likely from added resources from the buried sod, then a decline 1999 followed by steady mean difference

Increasing differences in mean abundance in the negative direction (i.e. below zero line) indicates decline in populations under the new tillage. This is the case for (CT97-NT83)-NT83 for *L. terrestris* and *Ap. turgida* adults (Fig 6b and 7b respectively). In addition, a large negative difference for *Ap. turgida* juveniles was observed in 1997 and 2001 (Fig 7a); and, for *L. terrestris* juveniles (Fig. 6a) in 1997 and 2001. The data for (CT97-NT83)-NT83 being very variable; yet, for the adults the mean difference was more stable. The variable and often large

mean differences separations suggest soil inversion by moldboard plowing and spring disking of the new tillage (CT97-NT83) is having considerable negative impact especially on earthworm juveniles.

Increasingly greater difference in mean abundance in the positive direction (i.e. above the zero line), indicates increase in population numbers in the newly implemented tillage. This occurred for *L. terrestris* juveniles and adults for (NT97-CT83)-CT83 where the difference becomes increasingly greater to 2003 and then stabilizing by 2012 (Fig. 6). For *Ap. turgida* adults and juveniles (Fig. 7), the pattern for (NT97-CT83)-CT83 is similar showing positive increases. The extent of difference of population numbers for *Ap. turgida* between the established CT83 tends to remain within a range from close to 0 to approximately 30 with a decrease from 1997 to 2001, then from 2003 to 2012, a marked increase in populations again to 1997 levels.

For both *L. terrestris* and *Ap. turgida* adults and juveniles, there was a strong significant difference (Table 5) in comparison of the mean abundance difference over the sampling period for (NT97-CT83)-CT83 vs (CT97-NT83)-NT83 (i.e., where no tillage, NT97, had been implemented on established conventional moldboard plow, CT83, and conventional tillage, CT97, had been started on established no-tillage, NT83). For adults and juveniles of both earthworm species, there was also a significant mean abundance difference for comparisons between conventional tillage started on 1983 bluegrass and on 1983 no-till, i.e., (CT97-BG83)-BG83 vs (CT97-NT83)-NT83. For both *L. terrestris* and *Ap. turgida* adults and *L. terrestris* juveniles, there was no significant difference in comparing conventional moldboard plow treatments established on 1983 bluegrass and 1983 ridge tillage, i.e., (CT97-BG83)-BG83 vs (CT97-RT83)-RT83. However, there was a significant difference for *Ap. turgida* juveniles which can be attributed (Fig. 5a) to the greater mean abundances for (CT97-BG83). Because

mean abundance differences for (CT97-RT83)-RT83 and (CT97-NT83)-NT83 tended to be more variable over the sampling period for *L. terrestris* and *Ap. turgida* juveniles (Fig. 6a and 7a) than for the respective adults (Fig. 6b and 7b) may account for the non-significant difference for *L. terrestris* and *Ap. turgida* juveniles as opposed to the significant difference for adults. This suggests for (CT97-RT83)-RT83 and (CT97-NT83)-NT83 the adult population rather than the juvenile population was more stable under the new conventional tillage.

Conclusions and Recommendations

Conversion of conventional till to no till (NT97-CT83) resulted in consistent and statistically significant increases in earthworm abundance over the sampling period (1997 to 2012).

Conversion of no-till to conventional tillage (CT97-NT83), on the other hand, resulted in the greatest reduction in earthworm abundance. Changes in earthworm abundance when converting one tillage to another can have considerable impact on soil and environmental quality, such as change in soil organic carbon content and alteration of water runoff and infiltration characteristics. Converting bluegrass to conventional tillage (CT97-BG83) resulted in improved abundance of *Ap. turgida* juveniles, which likely occurred because the buried sod increased subsurface food supply.

Adults of *L. terrestris* and *Ap. turgida* tended to be more stable in mean abundance m^{-2} than juveniles. For CT97-RT83 and CT97-NT83, mean abundance differences m^{-2} were more variable over the sampling period for *L. terrestris* and *Ap. turgida* juveniles in comparison to adults. This implies that a relatively stable population of adults might help sustain the earthworm population despite mechanical disturbance.

Unique differences for the tillage treatments were found in the response by *L. terrestris* and *Ap. turgida* adults and juveniles. Such data about earthworm species are essential for developing robust landscape models at field scale with the goal of predicting the extent of tillage impacts on earthworm abundance when new tillage systems are implemented. Having temporal based data with insight into the nature of change whether losses or increases, potential population abundances can be extrapolated for tillage systems with time. Specific information about mean abundance differences between different tillage systems for both adults and juveniles will lead to better appreciation of the contributions made by earthworms to soil functioning, particularly soil pore formation and sequestration of organic matter. To achieve this, enhanced information on earthworm abundance and species composition as an outcome of the kind of tillage and management system will be crucial input data. Understanding the nature of earthworm population dynamics in specific tillage systems, and knowing the proportion of anecic, endogeic or epigeic species, will provide essential information for making credible interpretations about earthworm contributions to soil quality and soil functions. By understanding earthworm population dynamics and the subsequent effects from tillage and tillage changes on earthworms and their ecosystem services, the land manager will be able to maximally control the soil system by using reduced tillage and improved crop management strategies to increase populations, and thereby, improve earthworm contributions to organic matter cycling, soil pore formation, water infiltration and soil structure via reference to prediction models.

To improve earthworm population dynamic models to enable predictions across landscape zones, fluctuations in the adult and juvenile populations, i.e., *L. terrestris* and *Ap. turgida* and the occurrences of minor species, still require additional ecological research with respect to specific soil and environmental impacts. Long-term response information is required on soil moisture

and temperature conditions affecting survival and viability of earthworm cocoons. Risk information is especially required on per cent survival rates of both adults and juveniles during harsh winter periods, spring tillage preparations, and under extended summer droughts. Clear definition of the cumulative long-term impacts on earthworm survival are needed for addressing temporal changes with respect to 1) major climatic changes in soil temperature and moisture; and, 2) changes in quantity and quality of food resources (crop residues) as influenced by management decisions in crop production systems.

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[Note: Ratio new:old tillage comparisons: (**CT97-BG83**):**BG83**, new conventional tillage started 1997 on one-half of old bluegrass (CT97-BG83) is compared with the old 1983 bluegrass BG83; (**NT97-CT83**):**CT83**, new no-tillage started 1997 on one-half of old conventional tillage (NT97-CT83) is compared with the old 1983 conventional tillage, CT83; (**CT97-NT83**):**NT83**, new conventional tillage started 1997 on one-half of old 1983 no-tillage (CT97-NT83) is compared with the old 1983 no-tillage, NT83; (**CT97-RT83**):**RT83**, new conventional tillage started 1997 on one-half of old 1983 ridge-tillage (CT97-RT83) is compared with the old 1983 ridge-tillage, RT83. Refer to Table 4 for results from repeated measures analysis of variance.]

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[Note: **BG83**, long-term bluegrass since 1983; **CT97-BG83**, conventional tillage begun 1997 on one-half of bluegrass, BG83; **CT83**, long-term conventional tillage since 1983; **NT97-CT83**, no-tillage begun 1997 on one-half of long-term conventional tillage CT83; **NT83**, long-term no-tillage since 1983; **CT97-NT83**, conventional tillage begun 1997 on one-half of long-term no-tillage NT83; **RT83**, long-term ridge-tillage since 1983; **CT97-RT83**, conventional tillage begun 1997 on one-half of long-term ridge tillage RT83.]

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[Note: **BG83**, long-term bluegrass since 1983; **CT97-BG83**, conventional tillage begun 1997 on one-half of bluegrass, BG83; **CT83**, long-term conventional tillage since 1983; **NT97-CT83**, no-tillage begun 1997 on one-half of long-term conventional tillage CT83; **NT83**, long-term no-tillage since 1983; **CT97-NT83**, conventional tillage begun 1997 on one-half of long-term no-tillage NT83; **RT83**, long-term ridge-tillage since 1983; **CT97-RT83**, conventional tillage begun 1997 on one-half of long-term ridge tillage RT83.]

Fig. 6. Difference between the new and established tillage pair in mean abundance m^{-2} for *L. terrestris* (a) juveniles and (b) adults showing the direction of change with the moving average (i.e., 2 sampling events) trend line over 1997-2012 time period.

[Note: Mean abundance difference for tillage pairs: **(CT97-BG83)-BG83** is the difference between the new conventional tillage (CT97-BG83) and the established bluegrass BG83; **(NT97-CT83)-CT83** is the difference between the new no-tillage (NT97-CT83) and the established conventional tillage CT83; **(CT97-NT83)-NT83** is the difference between the new conventional tillage (CT97-NT83) and the established no-tillage (NT83); **(CT97-RT83)-RT83** is the difference between the new conventional tillage (CT97-RT83) and the established ridge-tillage (RT83). Refer to Table 5 for repeated measures analysis of variance results]

Fig. 7. Difference between the new and established tillage pair in mean abundance m^{-2} for *Ap. turgida* (a) juveniles and (b) adults showing the direction of change with the moving average (i.e., 2 sampling events) trend line over 1997-2012 time period.

[Note: Mean abundance difference for tillage pairs: **(CT97-BG83)-BG83** is the difference between the new conventional tillage (CT97-BG83) and the established bluegrass BG83; **(NT97-CT83)-CT83** is the difference between the new no-tillage (NT97-CT83) and the established conventional tillage CT83; **(CT97-NT83)-NT83** is the difference between the new conventional tillage (CT97-NT83) and the established no-tillage (NT83); **(CT97-RT83)-RT83** is the difference between the new conventional tillage (CT97-RT83) and the established ridge-tillage (RT83). Refer to Table 5 for repeated measures analysis of variance results.]

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[Note: Ratio new:old tillage comparisons: **(CT97-BG83):BG83**, new conventional tillage started 1997 on one-half of old bluegrass (CT97-BG83) is compared with the old 1983 bluegrass BG83; **(NT97-CT83):CT83**, new no-tillage started 1997 on one-half of old conventional tillage (NT97-CT83) is compared with the old 1983 conventional tillage, CT83; **(CT97-NT83):NT83**, new conventional tillage started 1997 on one-half of old 1983 no-tillage (CT97-NT83) is compared with the old 1983 no-tillage, NT83; **(CT97-RT83):RT83**, new conventional tillage started 1997 on one-half of old 1983 ridge-tillage (CT97-RT83) is compared with the old 1983 ridge-tillage, RT83. Refer to Table 4 for results from repeated measures analysis of variance.]

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(a) *L. terrestris* juveniles (b) *L. terrestris* adults

Fig. 6. Calculated mean abundance m^{-2} difference between the new and established tillage pair for *L. terrestris* (a) juveniles, and (b) adults showing the direction of change with the moving average (i.e., 2 sampling events) trend line over 1997-2012 time period.

[Note: Mean abundance difference for tillage pairs: (CT97-BG83)-BG83 is the difference between the new conventional tillage (CT97-BG83) and the established bluegrass BG83; (NT97-CT83)-CT83 is the difference between the new no-tillage (NT97-CT83) and the established conventional tillage CT83; (CT97-NT83)-NT83 is the difference between the new conventional tillage (CT97-NT83) and the established no-tillage (NT83); (CT97-RT83)-RT83 is the difference between the new conventional tillage (CT97-RT83) and the established ridge-tillage (RT83). Refer to Table 5 for repeated measures analysis of variance results]

(a) *Ap. turgida* juveniles

(b) *Ap. turgida* adults

Fig. 7. Difference between the new and established tillage pair in mean abundance m^{-2} for *Ap. turgida* (a) juveniles and (b) adults showing the direction of change with the moving average (i.e., 2 sampling events) trend line over 1997-2012 time period.
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Table 1. Site information for Ontario (ON1 and ON2) and Nova Scotia (NS) test sites (shown in Fig. 1) for evaluating equivalency of hot mustard and formalin for extracting earthworms.

Site	Soil Classification	Soil Management
Nova Scotia		
<u>NS</u> East Stewiacke AAFC Benchmark Site(19-NS) [K. Webb]	Gleyed Brunsisolic Gray Luvisols (fine-loamy glacial till derived from shale and sandstone)	Forage-corn rotation under conventional tillage
Ontario		
<u>ON1</u> . Essex County, Holiday Beach Demonstration Farm	Grey-Brown Luvisol (clay loam on lacustrine morainal deposits)	Corn soybean rotation No-till
<u>ON2</u> . AAFC London Research Centre [C. Fox, J. Miller]	Grey-Brown Luvisol (loam on glacial morainal materials)	Conventional tillage

Table 2. Per cent occurrence of observed earthworm species during 1997-2012 for all tillage treatments combined and for individual tillage treatments with the total number of earthworms captured for all sampling times indicated.

Earthworm Species*	All Tillages	BG83	CT83	NT83	RT83	CT97-BG83	NT97-CT83	CT97-NT83	CT97-RT83
----- Per cent Occurrence -----									
<i>Lumbricus terrestris</i> (Linnaeus, 1758)	40.4	54.8	24.1	46.7	46.2	26.6	37.5	35.9	31.7
<i>Aporrectodea turgida</i> (Eisen, 1873)	56.6	42.7	71.1	52.5	52.8	71.2	56.9	54.2	63.8
<i>Lumbricus rubellus</i> (Hoffmeister 1843)	2.0	2.2	4.0	0.0	0.1	1.0	4.9	7.8	2.5
<i>Aporrectodea rosea</i> (Gates 1976)	0.2	0.2	0.0	0.1	0.0	0.2	0.0	1.2	1.1
<i>Aporrectodea tuberculata</i> (Eisen 1874)	0.4	0.0	0.4	0.2	0.6	1.0	0.3	0.9	0.2
<i>Aporrectodea trapezoides</i> (Duges 1828)	0.3	0.0	0.4	0.5	0.2	0.0	0.3	0.0	0.7
Total Earthworms Captured 1997-2012	15002	1126	634	4085	2593	1701	2660	959	1245

* Identifications as per Reynolds (1977).

Table 3. Results for each sampling year of Student's paired t-test comparing mean earthworm abundance m^{-2} of 1983 established tillage with 1997 new tillage.

	CT97-BG83 (df=5)						t-critical 2.57	P-value 0.05	t-critical 2.015	P-value 0.05	Assessment of
	Mean BG83	SEM BG83	Mean CT97	SEM CT97	Mean Diff.	t Statistic	P (T<=t) two-tail	Sign. Diff.(2)	P (T<=t) one-tail	Sign. Diff.(1)	Significance
1997	70.4	7.1	63.4	25.3	6.9	0.296	0.779	No	0.389	No	BG83=CT97
1998	17.6	4.5	66.7	6.5	-49.1	-8.317	0.00041	Yes (<0.001)	0.000205	Yes (<0.001)	***BG83<CT97
1999	9.7	2.9	10.6	3.5	0.9	-0.185	0.8604	No	0.4302	No	BG83=CT97
2001	15.8	2.2	41.2	7.5	-25.5	-3.934	0.011	Yes (<0.05)	0.0055	Yes (<0.01)	**BG83<CT97
2003	48.2	7.6	54.7	21.3	-6.5	-0.236	0.823	No	0.4115	No	BG83=CT97
2012	21.3	3.3	40.8	6.5	-19.5	-2.208	0.078	No	0.0391	Yes (<0.05)	*BG83<CT97
	NT97-CT83 (df=5)						t-critical 2.57		t-critical 2.015		
	Mean CT83	SEM CT83	Mean NT97	SEM NT97	Mean Diff.	t Statistic	P (T<=t) two-tail	Sign. Diff.(2)	P (T<=t) one-tail	Sign. Diff.(1)	
1997	19.0	5.7	58.8	13.0	-39.8	-2.189	0.0801	No	0.0401	Yes (<0.05)	*CT83<NT97
1998	19.9	5.1	50.0	5.6	-30.0	-3.262	0.0223	Yes (<0.05)	0.01119	Yes (<0.05)	*CT83<NT97
1999	6.9	2.9	36.6	11.4	-29.6	-2.228	0.0763	No	0.0381	Yes (<0.05)	*CT83<NT97
2001	31.5	7.9	74.1	6.4	-42.6	-5.362	0.00303	Yes (<0.01)	0.00151	Yes (<0.01)	**CT83<NT97
2003	13.5	6.8	110.3	10.5	-96.8	-9.328	0.000238	Yes (<0.001)	0.000119	Yes (<0.001)	***CT83<NT97
2012	9.7	3.4	89.0	5.1	-79.2	-10.414	0.000141	Yes (<0.001)	0.0000703	Yes (<0.0001)	****CT83<NT97
	CT97-NT83 (df=5)						t-critical 2.57		t-critical 2.015		
	Mean NT83	SEM NT83	Mean CT97	SEM CT97	Mean Diff.	t Statistic	P (T<=t) two-tail	Sign. Diff.(2)	P (T<=t) one-tail	Sign. Diff.(1)	
1997	169.9	18.1	26.9	8.0	143.1	9.364	0.000234	Yes (<0.001)	0.000117	Yes (<0.001)	***NT83>CT97
#1998	90.3	13.2	28.7	9.9	61.6	4.102	0.00934	Yes (<0.01)	0.00467	Yes (<0.01)	**NT83>CT97
1999	43.1	6.3	11.6	5.6	31.5	5.78	0.00218	Yes (<0.01)	0.00109	Yes (<0.01)	**NT83>CT97
2001	139.9	17.0	18.1	2.8	121.9	6.877	0.00099	Yes (<0.001)	0.000497	Yes (<0.001)	***NT83>CT97
2003	125.6	23.7	14.4	8.8	111.2	4.459	0.00664	Yes (<0.01)	0.00332	Yes (<0.01)	**NT83>CT97
2012	106.6	12.4	44.5	14.9	62.1	3.257	0.0225	Yes (<0.05)	0.01125	Yes (<0.05)	*NT83>CT97
	CT97-RT83 (df=5)						t-critical 2.57		t-critical 2.015		
	Mean RT83	SEM RT83	Mean CT97	SEM CT97	Mean Diff.	t Statistic	P (T<=t) two-tail	Sign. Diff.(2)	P (T<=t) one-tail	Sign. Diff. (1)	
#1997	89.8	13.5	36.6	7.2	53.2	7.062	0.000881	Yes (<0.001)	0.00044	Yes (<0.001)	***RT83>CT97
1998	57.4	13.4	32.4	5.3	25.0	1.8578	0.1223	No	0.06115	No	RT83=CT97
#1999	28.2	11.1	11.6	5.1	16.7	2.902	0.0337	Yes (<0.05)	0.0169	Yes (<0.05)	*RT83>CT97
2001	68.1	9.5	42.2	7.5	25.9	3.6577	0.0146	Yes (<0.05)	0.00731	Yes (<0.01)	**RT83>CT97
2003	76.9	19.9	21.3	4.0	55.6	3.175	0.02466	Yes (<0.05)	0.0123	Yes (<0.05)	*RT83>CT97
2012	107.5	16.6	54.2	6.2	53.3	2.9112	0.03335	Yes (<0.05)	0.01667	Yes (<0.05)	*RT83>CT97

Note: SEM is standard error of the mean; Mean Diff. is Mean Difference between 1983 tillage and 1997 alternative tillage; Sign. Diff.(2) and Sign. Diff.(1) is Significant Difference evaluation based on two-tailed t-test and one-tailed t-test respectively; # indicates the observation data were $\log_{10}$ transformed to achieve statistical normality for t-test analyses.

Table 4. Statistical significance ($P < 0.05$; Tukey method) according to one-way repeated measures ANOVA *post hoc* pair-wise comparisons of ratio values of mean abundance m^{-2} among treatments during sampling period 1997 to 2012.

Pair-wise Treatment Comparisons	Difference of Means	P
(NT97-CT83):CT83 vs (CT97-NT83):NT83	4.860	<0.001
(CT97-BG83):BG83 vs (CT97-NT83):NT83	1.673	0.338
(CT97-BG83):BG83 vs (CT97-RT83):RT83	1.445	0.459
(CT97-RT83): RT83 vs (CT97-NT83):NT83	0.228	0.995

Table 5. Statistical significance ($P < 0.05$; Holm-Sidak method) according to one-way repeated measures ANOVA *post hoc* pair-wise comparison of mean abundance m^{-2} differences (new tillage minus (-) established tillage) among treatments during sampling period 1997 to 2012.

Pair-wise Treatment Comparisons	<i>L. terrestris</i>	<i>L. terrestris</i>	<i>Ap. turgida</i>	<i>Ap. turgida</i>
	Adults	Juveniles	Adults	Juveniles
	<i>P</i>	<i>P</i>	<i>P</i>	<i>P</i>
(NT97-CT83)-CT83 vs (CT97-NT83)-NT83	<0.001	<0.001	<0.001	0.003
(CT97-BG83)-BG83 vs (CT97-NT83)-NT83	0.006	0.014	0.001	0.001
(CT97-BG83)-BG83 vs (CT97-RT83)-RT83	0.674	0.097	0.135	0.016
(CT97-RT83)-RT83 vs (CT97-NT83)-NT83	0.011	0.210	0.015	0.249