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# The Impact of Invasive Earthworms on Soil Respiration and Soil Carbon Within Temperate Hardwood Forests

Bradley Wayne Jennings[1] and Shaun A. Watmough[2]*

[1]*Environmental and Life Sciences Graduate Program, Trent University, Peterborough, Ontario K9J 7B8, Canada;* [2]*Trent University, 1600 West Bank Dr, Peterborough, Ontario K9J 7B8, Canada*

## Abstract

Improving current understanding of the factors that control soil carbon (C) dynamics in forest ecosystems remains an important topic of research as it plays an integral role in the fertility of forest soils and the global C cycle. Invasive earthworms have the potential to alter soil C dynamics, though mechanisms and effects remain poorly understood. To investigate potential effects of invasive earthworms on forest C, the forest floor, mineral soil, fine root biomass, litterfall and microbial litter decay rates, and total soil respiration (TSR) over a full year were measured at an invaded and uninvaded deciduous forest site in southern Ontario. The uninvaded site was approximately 300 m from the invaded site and a distinct invasion front between sites was present. Along the invasion front, the biomass of the forest floor was negatively correlated with earthworm abundance and biomass. There was no significant difference between litterfall, microbial litter decay, and TSR between the invaded and uninvaded sites, but fine root biomass was approximately 30% lower at the invaded site. There was no significant difference in total soil C pools (0–30 cm) between the invaded and uninvaded sites. Despite profound impacts on forest floor soil C pools, earthworm invasion does not significantly increase TSR, most likely because increased heterotrophic respiration associated with earthworms is largely offset by a decrease in autotrophic respiration caused by lower fine root biomass.

**Key words:** earthworms; forests; soil respiration; carbon; forest floor.

## Introduction

Earthworms function as keystone detritivores and ecosystem engineers (Eisenhauer and others 2007) and play an integral role in the processes of soil formation and function (Fahey and others 2013). As ecosystem engineers dwelling within the soil, they are capable of influencing soil carbon (C) dynamics (Bohlen and others 2004b; Hale 2008; Fahey and others 2013). The temperate forests of south-central Ontario have developed in the absence of earthworms following the retreat of the Wisconsin glacier, 10–12,000 years ago. Dispersal and introduction by Europeans began in the 1700s (Frelich and others 2006; Tiunov and others 2006). The invasion of earthworms into temperate forest ecosystems may result in significant changes to soil structure, nutrient dynamics, biogeochemical cycling, and plant community composition (Hale and others 2005; Eisenhauer and others 2007; Holdsworth and others 2007). Forests are considered C

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**Corresponding author; e-mail:* swatmough@trentu.ca

sinks (Goodale and others 2002), with half of the terrestrial C pool contained within forests and approximately two-thirds of that pool residing in the soil or associated peat deposits (Dixon and others 1994). Potential impacts on soil C cycling of forested ecosystems could have important regional and global consequences.

Carbon dioxide ($CO_2$) is produced in the soil by respiration from microbes, micro- and macrofauna, live roots, and rhizomes, and to a lesser extent by chemical oxidation of carbon-containing materials (Bridgham and Richardson 1992). Previous studies have demonstrated that earthworm invasion can lead to an increase in soil $CO_2$ emissions (Lubbers and others 2013). As earthworms consume the forest floor litter, fermentation, and humic (LFH) layer, they mix organic material into mineral soil and redistribute organic matter within their casts and burrows (Fisk and others 2004; Aira and others 2008). Earthworms stimulate heterotrophic activity, strongly affecting decomposition processes through interactions with microbes, macro- and microfauna, which influences soil $CO_2$ emissions (Fisk and others 2004; Aira and others 2008). Fahey and others (2013) suggested that earthworm invasions have the potential to reduce soil C storage in the upper 20 cm of the soil by 37%, echoing those of Bohlen and others (2004a) who found a 28% reduction in the upper 12 cm of a temperate hardwood forest. However, several studies have suggested that mineral soil C pools can be enhanced following earthworm invasion due to a redistribution of organic matter to the mineral horizons of the soil profile (Hale and others 2005; Wironen and Moore 2006; Lyttle and others 2011).

Lubbers and others (2013) recently conducted a meta-analysis in which 237 observations from 57 published studies were evaluated to synthesize the effects of earthworms on soil $CO_2$ respiration and soil C stocks. Their assessment suggests earthworms overwhelmingly increase soil $CO_2$ emissions while having a non-significant effect on the soil C stocks. The study by Lubbers and others (2013) considers lab and short-term controlled mesocosm studies, but it is noteworthy that long-term studies ($>200$ days) and those conducted within a field setting resulted in a non-significant change to soil $CO_2$ fluxes. Many of the studies utilized by Lubbers and others (2013) examine total soil respiration (TSR), which represents the sum of autotrophic and heterotrophic respiration. It has been suggested that earthworm invasion may increase microbial heterotrophic respiration, but TSR exhibits no changes because root respiration may be reduced by invasion (Fisk and others 2004; Bohlen and others 2004b). Changes in root respiration may be explained by a decrease of root biomass in earthworm-invaded forests (Fisk and others 2004; Bohlen and others 2004b). Previous studies suggest deep dwelling anecic species may consume dead root material (Lee 1985; Curry and Schmidt 2007). However, there is a lack of evidence suggesting earthworms feed extensively on living roots and may incidentally ingest fine roots while feeding on rhizosphere soil (Curry and Schmidt 2007).

The process of earthworm invasion is largely driven by various methods of anthropogenic dispersal. After successful colonization, earthworm assemblages may experience a range expansion through the formation of an invasion front, where the extent of the population has expanded outwards and forms a distinct front between invaded and non-invaded sections of a forest (Hale and others 2005; Nuzzo and others 2009). The different assemblages of earthworms consist of species from three distinct ecological groupings, functionally classified and differentiated by feeding and burrowing behaviour and their distribution in the soil profile. Epigeic species are strict litter dwellers, endogeic species are soil dwellers, and anecic species are deep burrowing surface feeders (Hale and others 2005). Within Ontario, the range of exotic earthworms is widespread throughout much of the southern reaches of the province where species thrive within agroecosystems and urban settings (Evers and others 2012). Although present and established, populations north of the southern reaches of the Canadian Shield, display greater restriction and a decreased richness (Reynolds 1977; Evers and others 2012).

The objective of the present study was to investigate the impacts of invasive earthworms on TSR at a sugar-maple (*Acer saccharum* Marshall)-dominated forest in southern Ontario. We examined TSR for a period of 1 year at both an invaded (Worm) and non-invaded (No-Worm) site located within 300 m of each other on opposing ends of an invasion front. Litterfall, microbial litter decay rates, fine root biomass, and soil C pools (0–30 cm) were also measured at both sites.

## Materials and Methods

### Study Sites

The study site is located 10 km to the east of Catchacoma, Ontario, within the Kawartha Highlands Signature Site (Zone 17T, 716485E, 4958849N) (Figure 1). Annual regional precipitation is 920 mm, whereas the average summer and

winter temperatures are 17.8 and −8.4°C (Table 1). Underlying parental material of the region consists of clastic metasedimentary to early felsic plutonic rocks and is exposed in areas of thin soils. The forest has a history of selective logging, but currently is protected from future development by having park status. It is unknown when earthworms invaded the site. The most probable routes of invasion include nearby Beaver Lake, an unnamed intermittent stream, and adjacent Beaver Lake road. The invaded (Worm) site lies within 25–50 m of the three potential invasion pathways. The non-invaded (No-Worm) site is situated approximately 300 m NNE, and rises 10 m from the lakeshore (309 m above sea level) with no apparent physical obstructions to the spread of earthworms (Figure 1). Five additional survey subplots were established along a transect leading from the Worm to No-Worm site at distances of 10, 20, 40, 80, and 160 m. The leading edge of the invasion plot occurred roughly between the 40 and 80 m plots. At the Worm and No-Worm sites, detailed measurements of TSR, fine root biomass, soil and forest floor organic matter content, litterfall, and microbial litter decay were made. At the five survey subplots, additional assessments of earthworm biomass and forest floor mass were undertaken. The forest is dominated by a canopy of mature sugar maple with an understory consisting primarily of sugar maple and American beech (*Fagus grandifolia* Ehrh.). Soils at the two sites were acidic (pH 4.0–4.2) with similar physical properties (Table 1) and classified as Dystric Brunisols (Soil Classification Working Group 1998).

## Earthworm Sampling

Earthworms were sampled in late October of 2014, following Valckx and others (2011). At each of the seven sites, ten replicate plots of 0.18 $m^{-2}$ were sampled using a liquid mustard solution to bring

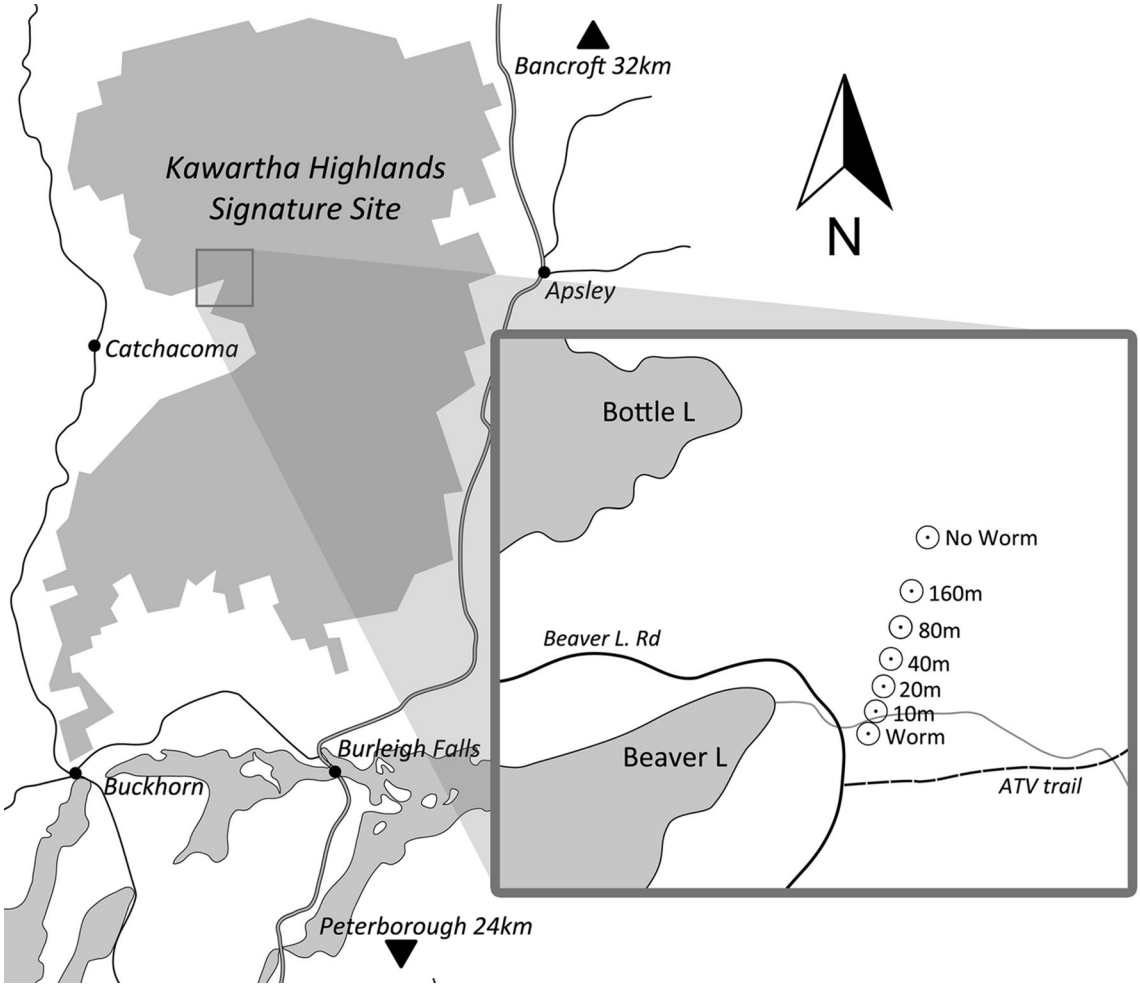


**Figure 1.** Map of study sites within the Kawartha highlands signature site.

**Table 1.** Site Characteristics for the Worm and No-Worm Study Sites

| | Worm | No Worm |
|---|---|---|
| Summer air temp average (°C) | 17.8 | |
| Winter air temp average (°C) | −8.4 | |
| Precipitation (mm) | 920 | |
| Easting | 716485E | 716496E |
| Northing | 4958849N | 4959022N |
| LFH | | |
| pH | 4.7 (0.1) | 4.6 (0.1) |
| Thickness (cm) | 0.9 (0.5) | 5.3 (0.4) |
| C:N | 39:1 (1.3) | 23:1 (1.4) |
| A-Horizon | | |
| pH | 4.2 (0.02) | 4.0 (0.05) |
| Thickness (cm) | 9.6 (0.9) | 6.8 (0.8) |
| BD (g $cm^{-3}$) | 0.93 (0.05) | 0.83 (0.03) |
| Sand (%) | 57 (4.5) | 56 (4.8) |
| Silt (%) | 41 (3.7) | 42 (3.2) |
| Clay (%) | 2 (0.1) | 2 (0.1) |
| C:N | 17:1 (0.7) | 14:1 (0.8) |
| B Horizon | | |
| pH | 4.2 (0.02) | 4.0 (0.02) |
| Thickness (cm) | +20.4[1] | +23.2[1] |
| BD (g $cm^{-3}$) | 1.1 (0.03) | 1.0 (0.04) |
| Sand (%) | 57 (4.4) | 58 (5.1) |
| Silt (%) | 41 (3.1) | 40 (3.3) |
| Clay (%) | 2 (0.1) | 2 (0.1) |
| C:N | 15:1 (0.3) | 16:1 (0.4) |
| Litterfall | | |
| Litterfall (Mg $ha^{-1}$) | 3.5 (0.5) | 3.5 (0.5) |
| Litterfall Ca (mg $g^{-1}$) | 21.8 (1.9) | 22.3 (1.5) |
| Vegetation | | |
| Cover (%) | 24 (9.7) | 14.5 (5.4) |
| Species relative cover (%) | | |
| Dominant | 66 (*Acer saccharum*) | 35 (*Acer saccharum*) |
| Secondary | 10 (*Dryopteris intermedia*) | 16 (*Carex sp.*) |
| Tertiary | 8 (*Maianthemum canadense*) | 14 (*Maianthemum canadense*) |
| Remaining | 16 (4 species)[2] | 35 (5 species)[3] |

*Values are means (±SE).*
[1] *Maximum depth of soil profile was 30 cm; full extent of B horizon was not examined.*
[2] *Aralia nudicaulis, Aralia racemosa ssp. racemosa, Carex pedunculata, Trillium grandiflorum.*
[3] *Aralia nudicaulis, Fraxinus americana, Polygonatum pubescens, Trillium erectum, Trillium grandiflorum.*

the earthworms to the surface (Lawrence and Bowers 2002; Hale and others 2005; Valckx and others 2011). The hot mustard solution technique was selected over other extraction methods (for example, formalin), as it is non-toxic to either the earthworms or surrounding vegetation and exhibits equal if not greater success (Lawrence and Bowers 2002; Valckx and others 2011).

At each earthworm sampling plot, the leaf litter was removed and 5.0 l of liquid solution containing a mustard concentration of 7.0 g $l^{-1}$ was applied. Earthworms that surfaced were collected and placed in a cooler for live preservation. Fifteen minutes after liquid mustard application, the plot was excavated to a depth of 25 cm and soil was hand sorted on a drop sheet to collect any remaining worms that did not surface (Lawrence and Bowers 2002). In the laboratory, the worms were stored in a fridge for several days to empty their guts. The worms were then euthanized in 20% alcohol and preserved in 4% formaldehyde for identification to species following the taxonomic key of Reynolds (1977). Worms were then measured for ash-free dry weight (AFD); the earthworm's biomass after the gut is emptied of soil and water (Hale and others 2004).

## Forest Floor

The forest floor was sampled at each survey plot along the transect from the Worm to No-Worm site ($n$ = 7). At each survey plot, five randomly selected samples were taken. A 25 cm × 25 cm square was placed on the forest floor and each layer was cut away, measured, bagged then oven dried at 60°C for 3 days before being weighed.

## Soil

At the Worm and No-Worm sites, 10 randomly selected soil pits were excavated to a depth of 30 cm. The forest floor was removed and mineral soil was sampled at 1 cm depths for the first 15 and at 5 cm intervals between 15 and 30 cm depth using a trowel. Soil was then oven dried at 60°C for 3 days before being sieved and pulverized. At each pit, the organic LFH layer was sampled and prepared identically in the aforementioned procedure. Subsamples of both mineral soil and LFH were placed in crucibles and incinerated in a muffle furnace at 450°C for 8 h to determine organic matter content through loss on ignition (LOI).

## Soil Respiration

Soil respiration was measured over 12 months using a system consisting of an infrared gas analyzer (IRGA), PP Systems EGM-4, and closed dynamic chamber (Rochette and others 1997; Ohashi and others 2005) placed on permanently installed soil collars. The closed chamber method is the most utilized approach for estimation of soil $CO_2$ fluxes (Kutzbach and others 2007). When used in conjunction with an IRGA, this method has been shown to effectively estimate $CO_2$ fluxes, while exhibiting minimal influence on the chambers interior environmental conditions attributed to relatively short measurement periods of $<5$ min (for example, Rochette and others 1997; Ohashi and others 2005).

The base of the chamber was fitted with a rubberized O-ring to create an airtight seal when placed within the inner dimensions of the soil collars. Soil collars constructed of PVC piping were cut to a length of 10.26 cm and buried to a depth of 5.1 cm in the soil using a mallet to evenly insert the collars without disturbing adjacent soil. Soil collars were placed in areas with minimal vegetation, but any living plants were carefully removed so as to eliminate the potential effects of photosynthesis from respiration analysis. The forest floor was not removed. The collars were left undisturbed for a month before $CO_2$ efflux measurements commenced, minimizing disturbance effects associated with collar installation (Nago and others 2012). Fifteen soil collars were installed randomly at both sites.

Measurements of soil respiration were initiated in the November of 2013, and continued on a weekly basis until the snow accumulated (mid-December), wherein measurements occurred on a monthly period until the spring thaw (April). During the winter measurement period, snow was carefully removed to the level of the collar height and stored on a tarp before being placed back over the collars after soil respiration measurements were taken.

During the measurement process, the $CO_2$ concentration was logged by the IRGA every 2.6 s for 2 min, for a total of 46 readings per soil collar. The efflux was determined from changes in $CO_2$ concentration during this 2-min period. To calculate the rate of soil respiration, $CO_2$ data were converted from the gas concentrations in ppm to μmol $l^{-1}$ according to formulas presented by Tang and others (2005) and $CO_2$ flux (μmol $m^{-2}$ $s^{-1}$) was calculated using formulas presented by (Szlavecz and others 2011). Efflux results were then averaged from all site collars $n$ = 15. Total annual soil C efflux was estimated as the sum of mean weekly C efflux readings (for example, Fisk and others 2004).

Soil $CO_2$ efflux is sensitive to temperature (Keith and Wong 2006) therefore, data loggers to measure soil temperature were installed at both sites. In doing so, a HOBO Micro Station Data Logger (H21-002) was connected to a soil temperature probe buried at a depth of 5 cm. Logging intervals were set to a period of every half hour and continued for the duration of the study. Soil moisture data were not available as the moisture probes installed at the sites failed to work.

## Litterfall and Microbial Litter Decay

Litterfall traps ($n$ = 3) constructed of 30 cm × 30 cm siding with sunken mesh catchments, were placed at the sites and collection occurred weekly during the fall of 2013 at the Worm site and at both sites in 2014 ($n$ = 5). Weekly leaf litter was collected, bagged, and then left to air dry for a minimum of 2 weeks. Branches and twigs were removed before the remaining leaf content was weighed. Remaining sugar-maple leaf litter collected in the fall of 2013 was used to examine site fungal and microbial decay rate in the absence of earthworms. In this procedure, 2 g of air-dried subsampled sugar-maple leaves were placed in 210-μm

mesh bags at each site ($n = 5$) and were left out until early September of the following summer. After a year of deployment, the leaf litter was air dried and weighed to estimate litter decomposition in the absence of detritivores, especially earthworms (microbial litter decay). The residence time (y) of C in the forest floor was estimated by dividing the C content of the forest floor (g $m^{-2}$) by the annual aboveground litter C input (g $m^{-2}$ $y^{-1}$).

### Fine Root Biomass

For the purpose of this study, fine roots describe any root under 5 mm in diameter (for example, Xu and Qi 2001). From the Worm and No-Worm sites, 10 root cores were taken at random with a cylindrical soil core (diameter 25 mm) to a depth of 30 cm. The core was measured, cut to three 10 cm lengths, and bagged. Cores were broken and oven dried at 60°C for 3 days. Dried cores were dry and wet sieved followed by the hand sorting of roots from any remaining mineral soil. Roots were then weighed and subsamples were ground for chemical analysis.

### Chemical Analysis

Dried litterfall and LFH matter were ground using a mortar and pestle followed by a Fisher Mortar Grinder. Soil was first ground in the mortar grinder and then passed through a 2-mm sieve. Samples were analyzed for carbon (CNS) using an Elementar vario MAX cube. Five subsamples from each 5 cm profile in additional to roots and LFH were analyzed ($n = 90$) and a relationship was established and evaluated between LOI and soil C. LOI was not conducted on fine roots so %C was derived from CNS (39–42%).

### Statistical Analysis

Pearson correlation analysis was employed to examine the potential relationship between both earthworm biomass and density and LFH biomass along the transect leading from the Worm to No-Worm site ($N = 7$). A multiple regression analysis was used to predict variance in soil respiration based on soil temperature and precipitation events during the growing season (excludes events during winter snow cover). Following a Shapiro–Wilk test for normality, a one-way ANOVA was used to compare soil C content by depth, C residence time, soil C:N ratio, fine root biomass, litterfall, microbial litter decay, and earthworm density and biomass between the Worm and No-Worm site. A repeated measures ANOVA was used to compare seasonal (fall, winter, spring, summer) soil respiration between the Worm and No-Worm sites. Fisher's LSD comparison test was then used to determine whether means differed significantly. All statistical analyses were performed using SPSS 22.0 for Windows.

## Results

### Earthworms

All sites were sampled for earthworms, but worms were only found at the Worm site and along the subplot transect at the 10, 20, and 40 m plots. Four species were identified in the study area, *Dendrobaena octaedra, Aporrectodea turgida, Aporrectodea rosea*, and *Lumbricus terrestris* (Table 2). Earthworm communities were relatively similar among the four sites, with the exception that *A. turgida* and the epigeic species, *D. octaedra* were only found at the Worm site. The Worm site had the highest density of earthworms (99.2 $m^{-2}$) and the highest abundance of *Aporrectodea juveniles* (43.6 $m^{-2}$). The 10, 20, and 40 m sites were all characterized by the presence of *A. rosea*, *L. terrestris*, and juveniles and exhibited little variability in worm biomass and density with the exception of the 10 m site, where the biomass was the greatest of all sites (10.5 $g_{AFD}$ $m^{-2}$) due to large *L. terrestris* and *L. juveniles*. The 40 m plot was located along the leading edge of the invasion front and had the lowest density and biomass of all sites at 53.0 $m^{-2}$ and 1.3 $g_{AFD}$ $m^{-2}$, respectively.

### Forest Floor

Within the four worm invaded plots (Worm, 10, 20, and 40 m), LFH biomass ranged between 70 and 212 g $m^{-2}$, whereas in non-invaded plots (80 m,160 m, No Worm) biomass ranged between 625 and 878 g $m^{-2}$, respectively, and the biomass of the LFH was significantly negatively related to earthworm density (Pearson's $r = -0.84$: $p < 0.002$) and biomass (Pearson's $r = -0.67$: $p < 0.03$) (Figure 2). The highest LFH biomass (878 g $m^{-2}$) was found furthest from the invasion front at the No-Worm site. The lowest LFH biomass of 70 g $m^{-2}$ was observed at the 10 m transect plot where the greatest earthworm biomass occurred (10.5 $g_{AFD}$ $m^{-2}$) (Figure 2). At the invaded plots, the fermentation (F) and humus (H) layers were nearly indistinguishable from the mineral soil, and if present, were immeasurably thin. LFH C content differed significantly between the Worm and No-Worm site ($p < 0.001$), with the No-Worm site having an estimated pool of 3.72 Mg C $ha^{-1}$

**Table 2.** Population Density of Earthworm Species ($n$ = 10) and Mean Biomass (±SE) Sorted by Taxonomic Grouping at the Sites at Which Worms were Present

| Species | Worm | | 10 m plot | | 20 m plot | | 40 m plot | |
|---|---|---|---|---|---|---|---|---|
| | Count (N $m^{-2}$) | Biomass (g $m^{-2}$) | Count (N $m^{-2}$) | Biomass (g $m^{-2}$) | Count (N $m^{-2}$) | Biomass (g $m^{-2}$) | Count (N $m^{-2}$) | Biomass (g $m^{-2}$) |
| *Epigeic* | | | | | | | | |
| *Dendrobaena octaedra* | 1.7 (1.2) | 0.1 (0.1) | – | – | – | – | – | – |
| *Endogeic* | | | | | | | | |
| *Aporrectodea turgida* | 27.8 (6.4) | 1.4 (0.3) | – | – | – | – | – | – |
| *Aporrectodea rosea* | 4.5 (2.2) | 0.1 (0.1) | 7.4 (2.5) | 0.1 (0.1) | 13.6 (4.7) | 0.2 (0.1) | 21.4 (5.4) | 0.3 (0.1) |
| *Aporrectodea juvenile* | 43.6 (7.0) | 1.2 (0.2) | 7.9 (2.4) | 0.1 (0.02) | 30.1 (4.6) | 0.3 (0.1) | 17.2 (4.3) | 0.2 (0.1) |
| *Anecic* | | | | | | | | |
| *Lumbricus terrestris* | 4.0 (1.9) | 1.9 (0.9) | 12.5 (2.9) | 6.2 (1.4) | 4.5 (2.0) | 0.2 (0.1) | 4.1 (2.2) | 0.3 (0.2) |
| *Lumbricus juvenile* | 17.6 (4.6) | 1.9 (0.5) | 27.2 (4.2) | 4.2 (1.1) | 15.3 (4.9) | 0.7 (0.2) | 10.3 (3.1) | 0.5 (0.3) |
| Sum | 99.2 (3.9) | 6.6 (0.3) | 55.0 (3.0) | 10.5 (0.6) | 63.5 (4.1) | 1.4 (0.1) | 53.0 (3.9) | 1.3 (0.2) |

and the Worm site containing an estimated 0.85 Mg C $ha^{-1}$ (Table 5). The C:N ratio of the forest floor was significantly higher ($p < 0.001$) at the Worm site (39.1 ± 1.4) compared with the No-Worm site (23.1 ± 1.3) (Table 1).

## Soil Respiration

There was no significant difference in TSR between the two sites ($\alpha = 0.05$). At both sites, $CO_2$ efflux was low during the winter (months), averaging 0.44–0.49 µmol $m^{-2}$ $s^{-1}$ before increasing during spring and reaching a maximum during the summer months (Figure 3). Although TSR exhibited pronounced seasonal variation, average yearly $CO_2$ efflux was 2.22 µmol $m^{-2}$ $s^{-1}$ at the No-Worm site and 2.38 µmol $m^{-2}$ $s^{-1}$ at the Worm site. Soil respiration was positively related to soil temperature at both sites (Worm $r^2 = 0.62$, No Worm $r^2 = 0.91$), with estimated $Q_{10}$ values of 2.57 and 2.78 for the Worm and No-Worm sites, respectively (Figure 3). No significant differences in soil temperature were observed between both sites ($\alpha = 0.05$). There was a greater variability in TSR at the Worm site during the snow-free months. The variability in TSR was better explained by temperature and precipitation (recorded in Peterborough 51 km SSE) compared with temperature alone, whereas precipitation did not improve the model in the No-Worm site (Table 3). Estimated TSR for the year was 6.39 and 6.80 Mg C $ha^{-1}$ $y^{-1}$ for the No-Worm and Worm sites, respectively (Table 5).

## Fine Root Biomass

Fine root biomass decreased with soil depth at the No-Worm Site and was significantly ($p < 0.01$) lower for the total 0–30 cm profile at the Worm site

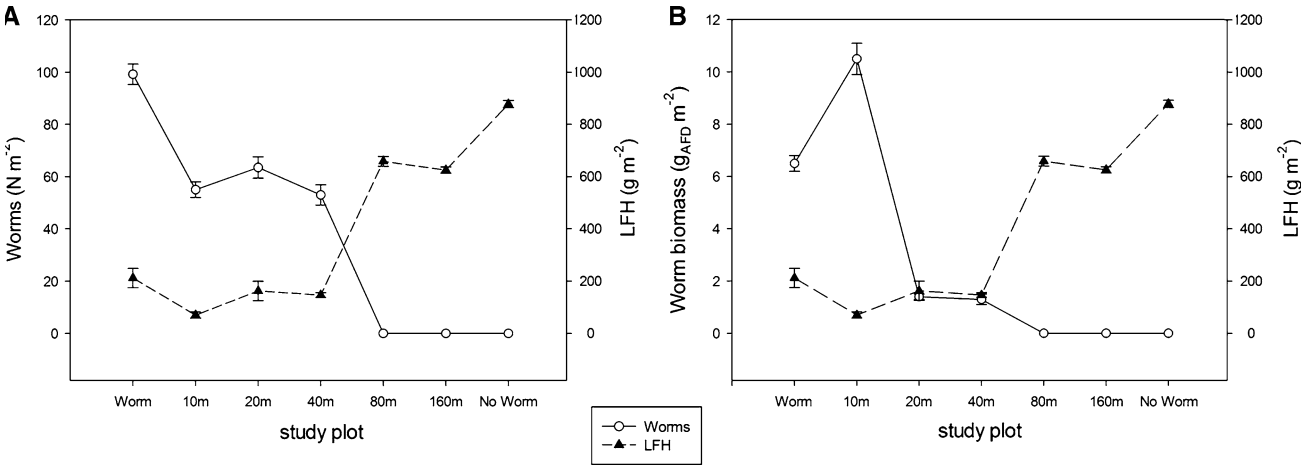


**Figure 2.** Earthworm density and LFH biomass, Pearson's $r = -0.84$ (**A**) and earthworm biomass and LFH biomass, Pearson's $r = -0.67$ (**B**) along an earthworm invasion front. ($n$ = 10) (±SE).

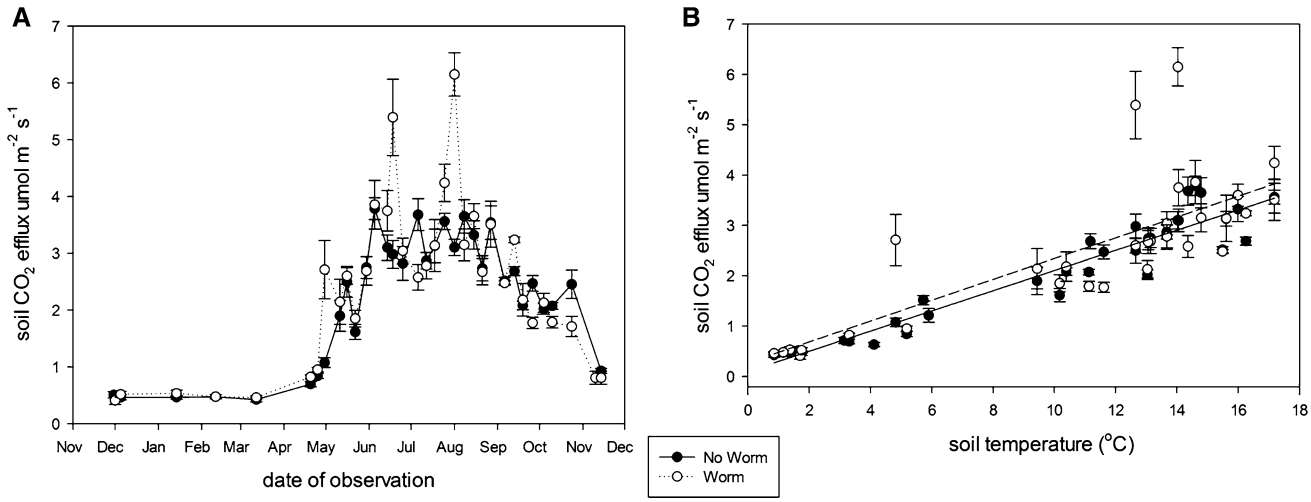


**Figure 3.** Mean $CO_2$ efflux (**A**) and linear relationship between soil temperature at 5 cm and soil $CO_2$ efflux (No Worm $r^2 = 0.913$, ($N = 34$), $f = 0.2004$ * temp + 0.0994, Worm $r^2 = 0.616$ ($N = 29$), $f = 0.2071$ * temp + 0.2721) (**B**) at Worm and No-Worm sites between November 2013 and November 2014. Observations are means of all soil collars ($n = 15$) (±SE).

compared with the No-Worm site (Figure 4). The No-Worm site contained an estimated fine root C pool of 3.63 Mg C $ha^{-1}$ while the Worm site yielded an estimated 2.38 Mg C $ha^{-1}$ (Table 5). The greatest difference occurred in the upper 10 cm of the soil where total fine root biomass was approximately 77% greater at the No-Worm site (308.5 vs 173.5 g $m^{-2}$).

## Litterfall and Microbial Litter Decay

The rate of microbial litter decay (Table 4) and leaf litter inputs (Table 5) were nearly identical at both sites. Inputs of C in litterfall at both sites were 1.8 Mg $ha^{-1}$ $y^{-1}$ ± 0.1 and earthworm excluded litter decomposition rates were 39.3 and 40.6% $y^{-1}$ for the Worm and No-Worm sites, respectively. Residence time of forest floor C was significantly lower ($p < 0.001$) at the Worm site with a mean residency of 6 compared to 25 months at the No-Worm site.

## Mineral Soil Carbon Pools

There was no significant difference in the total soil C pool (0–30 cm) between sites ($\alpha = 0.05$). The total C pool including the forest floor was 116.9 and 109.5 Mg C $ha^{-1}$ at the No-Worm and Worm Site, respectively, with the greatest difference occurring within the forest floor (Figure 4; Table 5). Below the organic horizon, estimated 0–30 cm mineral soil C carbon pools were similar at 113.1 and 108.7 Mg C $ha^{-1}$ for the No-Worm and Worm Site, respectively. The total 20–30 cm soil profile of the No-Worm site contained a significantly greater amount of C ($p = 0.03$) (Figure 4).

**Table 3.** Summary of the Multiple Regression Analysis for Soil Respiration as a Dependent Variable

| Model | Worm | | | | No Worm | | | |
|---|---|---|---|---|---|---|---|---|
| | ($R = 0.75$, $p < 0.001$) | $r^2 = 0.56$, | $F$ (2,24) = 15.207, | | ($R = 0.94$, $p < 0.001$) | $r^2 = 0.88$, | $F$ (2,29) = 107.901, | |
| Variable | $\beta$ | SE | $t$ | $p$ value | $\beta$ | SE | $t$ | $p$ value |
| Soil temp | 0.194 | 0.042 | 4.678 | <0.001 | 0.205 | 0.014 | 14.385 | <0.001 |
| Precipitation | 0.039 | 0.016 | 2.504 | 0.019 | 0.004 | 0.006 | 0.570 | 0.573 |

*Precipitation data from Peterborough weather station (51 km SSE) courtesy Environment Canada. See climate.weather.gc.ca/climateData/dailydata_e.html?StationID=48952 for data.*

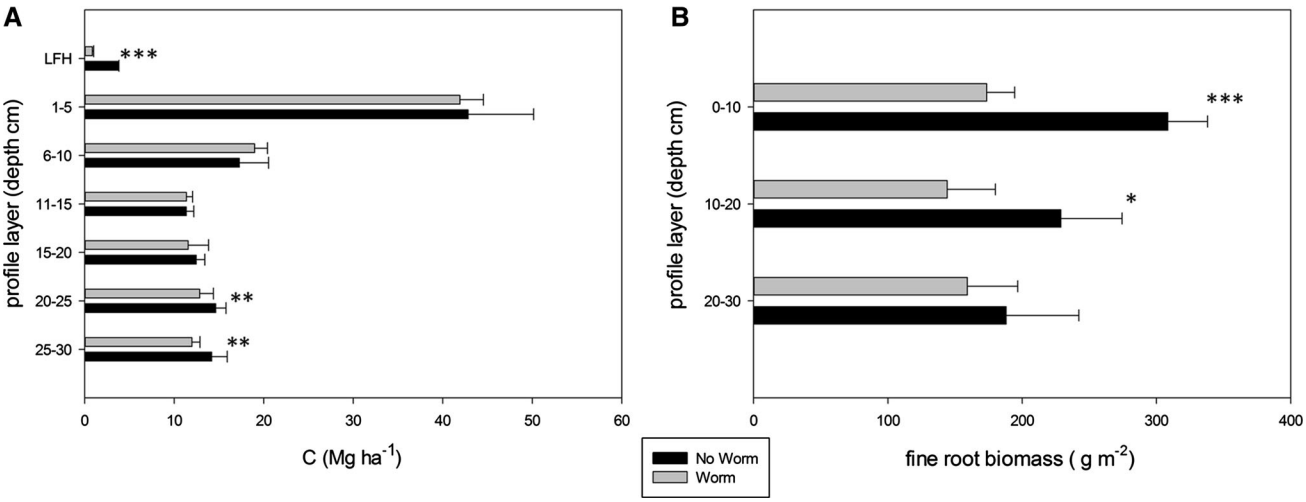


**Figure 4.** Fine root biomass (>5 mm) at different soil profile depths (No Worm 0–30 cm = 725.3 g $m^{-2}$, Worm 0–30 cm = 476.8 g $m^{-2}$, Total 0–30 cm ($p < 0.01$) (**A**) and estimated total soil C content at different profile depths (**B**) in No-Worm and Worm sites ($n = 10$) (±SE). Significance (***$p < 0.001$, **$p < 0.01$, *$p < 0.03$).

## Discussion

Earthworm abundance and biomass were negatively correlated with forest floor biomass, though the transition was notably abrupt along the leading edge of the earthworm invasion front. The leading edge marks the visible front of the potentially expanding earthworm invasion (Hale and others 2005). In the present study, earthworm biomass and abundance tended to decrease along the invasion front to the leading edge. Further, the greatest species diversity was observed at the plot furthest from the leading edge. These findings are similar to that of Hale and others (2005), who observed increasing biomass and diversity with distance from the leading edge. Hale and others (2005) also noted that increasing species diversity is often associated with increasing earthworm biomass and time since invasion. Earthworm abundance (99.2–53 $m^{-2}$) and biomass (6.6–1.3 $g_{AFD}$ $m^{-2}$) at the invaded plots compared well with those presented by others (Bohlen and others 2004b; Hale and others 2006; Stoscheck and others 2012). For comparison, Sackett and others (2013) reported a mean earthworm abundance of 99.5 $m^{-2}$ in the Haliburton Forest, roughly 60 km north of sites examined within the present study.

Earthworm invasion leads to profound and well-documented impacts on the forest floor (Lawrence and others 2003; Wironen and Moore 2006; Watmough and Meadows 2014). Following invasion, the forest floor undergoes the rapid elimination and subsequent mixing of the organic horizon within the mineral soil through earthworm feeding and burrowing activities (Li and others 2002; Hale and others 2005). Whereas uninvaded forest soils of the region often exhibit a thick LFH layer (Watmough and Meadows 2014), invaded forest soils tend to have a minimal litter layer transitioning to bare mineral soil (Bohlen and others 2004b). In this study, the invaded sites exhibited the similar depletion of the organic horizon as reported by others (Burtelow and others 1998; Lawrence and others 2003; Frelich and others 2006; Wironen and Moore 2006). No difference was found in the rate of microbial litter decay in fine (210 μm mesh) litter bags, suggesting that consumption by detritivores, primarily earthworms, was responsible for the observed decline in forest floor biomass at invaded sites. Litterfall was

**Table 4.** Litter Decomposition Rates from Detritivore Exclusion Bags ($n = 5$), and C Residence Time ($n = 10$), (±SE)

| | Worm | No Worm | $p$ value |
|---|---|---|---|
| Earthworm excluded decomposition rate (% $y^{-1}$) | 39.3 (3.1) | 40.6 (2.9) | ns |
| C residence time (months) | 6 (0.2) | 25 (0.2) | <0.001 |

**Table 5.** Estimated C Pools and Flux with Associated Significance

| | Worm | No Worm | *p* value |
|---|---|---|---|
| LFH (Mg C $ha^{-1}$) | 0.8 | 3.7 | <0.001 |
| Mineral soil 0–30 cm (Mg C $ha^{-1}$) | 108.7 | 113.1 | ns |
| Sum LFH and soil (Mg C $ha^{-1}$) | 109.5 | 116.9 | ns |
| Fine roots 0–30 cm (Mg C $ha^{-1}$) | 2.4 | 3.6 | <0.01 |
| TSR (Mg C $ha^{-1}$ $y^{-1}$) | 6.8 | 6.4 | ns |
| Litterfall (Mg C $ha^{-1}$ $y^{-1}$) | 1.8 | 1.7 | ns |

similar at both sites, suggesting that C inputs to the forest floor were accumulating at an equivalent magnitude and rate. Despite comparable C inputs, the residence time of forest floor C was significantly reduced at the Worm site. Melvin and Goodale (2013) observed a rapid decrease in forest floor C residence time as earthworm abundance increased and Watmough and Meadows (2014) suggested that C residence time of the forest floor is much longer without the presence of earthworms.

Sites without earthworms allow for increased surface decomposition time, leading to much lower C:N ratios, which facilitate an increase in mineralization rates and nitrification (Watmough and Meadows 2014). The C:N ratio of the forest floor was significantly higher in the Worm Site, which has been reported by others (Wironen and Moore 2006; Watmough and Meadows 2014). In the present study, we observed a C:N ratio of 39 at the Worm Site and 23 at the No-Worm site. These observations resemble those by Wironen and Moore (2006), who reported a C:N ratio of 23 in uninvaded sites and a linear increase of the C:N ratio with earthworm abundance approaching a value of approximately 40 when earthworm absolute biomass was 100 g $m^{-2}$.

There was no difference in TSR between the Worm and No-Worm sites and the C efflux rates measured in this study fall within previously published values for temperate hardwood forests of Northeastern North America (Toland and Zak 1994; Fisk and others 2004; Giasson and others 2013). In the present study, C efflux rates were 680 and 639 g C $m^{-2}$ $y^{-1}$ for the Worm and No-Worm site, respectively. Our rates compare well with values for temperate hardwood forests in the region which reportedly range from 880 and 715 g C $m^{-2}$ $y^{-1}$ in New York State (Fisk and others 2004), to 478 g C $m^{-2}$ $y^{-1}$ in Northern Michigan (Toland and Zak 1994). Soil moisture and especially temperature, often show a strong relationship with TSR (Epron and others 1999; Fisk and others 2004; Giasson and others 2013). Soil moisture data were not available at our sites but there was a strong relationship between TSR and soil temperature at both sites. Estimated $Q_{10}$ values fell within the reported range observed by others (Raich and Schlesinger 1992; Fisk and others 2004) and did not differ significantly between the two sites, suggesting efflux rates and biological responses exhibit similar rates of change. During the winter snow cover (December–March), TSR was low and exhibited little variability, reflecting the temperature response of C efflux with the colder soil temperatures, lowered microbial activity, and heterotrophic respiration. Although studies have observed microbial respiration during winter (Uchida and others 2005), their contributions to annual total efflux are small (Wang and others 2010), yet important in developing an annual estimation of TSR. We observed a winter TSR contribution of 9.1%, comparable to others (Epron and others 1999; Wang and others 2010). TSR increased with the spring warming period and reached its peak during the summer months. The rise in TSR occurred during the period of warming soils, reflected by $Q_{10}$ values and enhanced earthworm activity, where young emerge from cocoons (Dymond and others 1997) and biological processes and consumption increases.

In the present study, TSR was much more variable in the Worm plots compared with the No-Worm plots. Although not as significant as soil temperature, precipitation and soil moisture can affect soil $CO_2$ efflux rates (Li and others 2002; Yan and others 2014). As efflux readings for the present study were never conducted during a precipitation event, time after rainfall may be an important variable. At the Worm site, both temperature and precipitation were significant predictors of TSR, whereas temperature was the only significant predictor of TSR at the No-Worm site. Li and others (2002), observed post-rainfall $CO_2$ efflux was subject to increased rates and rain pulse effects, where efflux rates are enhanced following precipitation events. Further, Yan and others (2014) observed rain pulse events as having a significant contribution to yearly respiration estimates. Earthworm activity has been observed as increasing following precipitation events, notably through heightened casting activity (Lee 1985; Binet and Le Bayon

1999) and studies have observed increased $CO_2$ efflux in the presence of earthworm casts (for example, Wolters and Ekschmitt 1995; Maestre and Cortina 2003). Therefore, differences in TSR variance between sites may be explained by precipitation induced earthworm activity.

We observed a significant decrease of fine root biomass (34%) in the Worm site, which is comparable to findings in other studies (Fisk and others 2004; Bohlen and others 2004b; Cameron and others 2014), where decreases between 11 and 33% were reported. The decrease in fine root biomass may be due to direct consumption by earthworms. Deep dwelling anecic species may consume dead root material (Lee 1985; Curry and Schmidt 2007) and some species may graze on plant roots while burrowing (Cortez and Bouche 1992). *Lumbricus terrestris* was encountered at all worm-invaded plots. The species has been documented to consume roots (Cortez and Bouche 1992) and has been noted as present in studies where root biomass was decreased under the presence of earthworms (Fisk and others 2004; Bohlen and others 2004b). The lack of difference in TSR between the Worm and No-Worm site may be explained by a decrease in autotrophic (root) respiration at the Worm site, coupled with a comparable increase in C efflux stimulated by earthworm activity resulting in no net difference in TSR among the sites. A similar mechanism was proposed by Fisk and others (2004), who reported reductions in root biomass but non-significant differences in TSR between invaded and non-invaded sites. Along the invasion front, there were no significant differences in herbaceous species indices. The invaded plots were dominated by *Acer saccharum* seedlings which appear contrary to observations by others (Hale and others 2006; Holdsworth and others 2007).

Although large and significant differences in the C content of the forest floor are apparent between the Worm and No-Worm sites, the majority of C is stored in the mineral soil. The amount of C contained within the forest floor only represents a small fraction of the soil C pool. The forest floor of the No-Worm site contained 3.2% of the total 0–30 cm C pool, whereas the Worm site contained 0.7%. We found no significant differences in total mineral soil C content, but noted a possible re-distribution effect where the upper 2–15 cm of the mineral soil within the Worm site contained a greater amount of C. This in conjunction with the near elimination of the organic horizon suggests the occurrence of a mixing and re-distribution of C brought on by earthworm activity (Groffman and others 2004; Fisk and others 2004). Non-significant differences in mineral soil C pools have been observed by others (Lubbers and others 2013), while a re-distribution and enrichment of C storage within the upper mineral soil has been documented (Bohlen and others 2004a; Fisk and others 2004).

This study was conducted over 1 year and although there was no significant difference in TSR between the Worm and No-Worm site, TSR was slightly higher (6% $y^{-1}$) at the Worm site, which over time, may ultimately (over decades) have an impact on soil C stocks but long-term studies are needed. Gradual loss of mineral soil C in earthworm-invaded forests was observed by Alban and Berry (1994). Although it remains unknown how long the Kawartha's have been invaded by earthworms, recreational pressure was established during the twentieth century. Given the presence of worms at distance from the likely invasion points of the road and lake, it can only be hypothesized that earthworm populations have been established for some time. The slightly lower soil C pool observed in the Worm site may be a result of earthworm invasion eventually resulting in long term decreases of mineral soil C such as those documented by Alban and Berry (1994). For comparison, we measured soil respiration and soil C pools at an earthworm-invaded sugar-maple-forested site located 82 km south east of the Kawartha Sites within the Ganaraska Forest. This site has presumably been invaded for a longer period of time than the Kawartha site as no locations without earthworms were found and the region has a longer history of human disturbance and agriculture (Jennings 2015). There was no significant difference in LFH (0.6 Mg C $ha^{-1}$), litterfall (1.6 Mg C $ha^{-1}$), fine root biomass (2.5 Mg C $ha^{-1}$) and TSR (6.3 Mg C $ha^{-1}$ $y^{-1}$), between the Ganaraska and invaded site at Kawartha. Notably, however, the mineral soil C pool in Ganaraska was significantly (27%) smaller than the Kawartha Worm site (80.1 Mg C $ha^{-1}$) perhaps suggesting that in the long-term, invasion by earthworms may lead to reduced soil C pools owing to slightly greater soil C losses through increased respiration (Jennings 2015). Although other factors (for example, different land-use histories) could also potentially explain differences in soil C, the data suggest that, over time, invasion by earthworms may deplete soil C pools.

In summary, earthworms significantly reduced fine root biomass and depleted the forest floor of organic matter but had no significant impact on TSR. Although we observed no significant difference in TSR, there exists the potential that

earthworms are altering the originating sources of C respiration. TSR may undergo source shifting in earthworm-invaded forest soils, where a decrease in autotrophic respiration at the worm-invaded site coupled with a comparable increase in C efflux stimulated by earthworm activity results in no net difference in TSR. Over time, small cumulative differences in TSR may contribute to a gradual C loss from earthworm-invaded temperate hardwood forests.

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