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# EFFECTS OF INVASIVE EARTHWORM FEEDING GUILDS AND THEIR INTERACTIONS WITH PHYSIOGRAPHIC CONDITIONS ON THE RELATIVE ABUNDANCE AND DISTRIBUTION OF WOODLAND SALAMANDERS

HUNTER J. BRUNGES<sup>1</sup>, JAMES P. DUNN<sup>1,3</sup>, DAVID R. HELDER<sup>1</sup>, AND SANGO OTIENO<sup>2</sup>

<sup>1</sup>Biology Department, Grand Valley State University, 1 Campus Drive, 3300a Kindschi Hall of Science, Allendale, Michigan 49401, USA

<sup>2</sup>Statistics Department, Grand Valley State University, 1 Campus Drive, A-1-178 Mackinac Hall, Allendale, Michigan 49401, USA

<sup>3</sup>Corresponding author email: dunnj@gvsu.edu

**Abstract.**—Most species of earthworm that inhabit the forests of the northern United States are invasive and have been shown to alter soil composition and decrease the leaf-litter layer. These environmental changes could impact salamanders that live within leaf-litter and the invertebrates that they prey on. Our objective was to determine the potential impact that invasive earthworms have on salamander abundance and distribution at 36 sites within four forested ravines of Southwest Michigan, USA. We sampled earthworms using mustard extraction and estimated salamander abundance using cover boards to obtain salamander counts from north and south facing slopes, and at low and high elevations. We also collected environmental data that might influence the impact of earthworms, including soil moisture, leaf-litter cover, and soil pH. Salamander counts were negatively associated with epigeic earthworms, but not endogeic earthworms; whereas, increasing woody debris and soil moisture had a positive association with salamander counts. Leaf-litter cover and organic content of the A horizon had no significant effects on salamanders counts. Elevation and slope aspect, as independent factors, had no significant effect on salamander or earthworm distribution. Epigeic earthworms feed within the leaf-litter layer, which could lead to higher rates of salamander desiccation and allow them less time to forage for prey, along with a decline in soil-litter invertebrates. This potential impact could be a part of an already suggested complex decline syndrome of Plethodontid salamanders, which are considered detrital keystone species in deciduous forests and ideal biological indicators of forest biological diversity and ecological integrity.

**Key Words.**—*Ambystoma laterale*; Blue-spotted Salamander; endogeic earthworms; epigeic earthworms; leaf-litter layer; *Plethodon cinereus*; Red-backed Salamander; Plethodontid salamanders

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## INTRODUCTION

European and Asian earthworm species with the aid of human activities have invaded much of the northern temperate forests of North America, which have few native earthworm populations north of the Wisconsin glacial limit (Reynolds 1977; Snider 1991; James 1995; Reynolds 1995; Bohlen et al. 2004b). These exotic earthworms have had a negative effect on the organic (leaf-litter) and mineral soil horizons of forests that they have invaded by reducing leaf-litter cover or mass by mixing it with lower mineral soil and thereby altering microbial populations from slow decomposing fungal to fast decomposing bacterial dominated communities (Wardle 2002; Bohlen et al. 2004b; Hale et al. 2005). These changes result in a decrease in stable humus and the availability of carbon, nitrogen, and phosphorus (Bohlen et al. 2004b; Fahey et al. 2013), which can lead to decreased abundance, diversity, and productivity of vegetation by allowing aggressive invasive species and native graminoids to dominate (Hale et al. 2006; Nuzzo et al. 2009; Hopfensperger et al. 2011). These changes

to the forest litter layer and underlying organic content and structure of the mineral soil can also affect animals that live in or rely on these habitats, including native invertebrates, small mammals, birds, and salamanders (Salmon et al. 2005; Migge-Kleian et al. 2006; Fox et al. 2010; Burke et al. 2011; Loss et al. 2012).

Salamanders are an important part of forest ecosystems, acting as top predators of litter and soil dwelling invertebrates (Wyman 1998; Rooney et al. 2000), and as prey for birds, mammals, and snakes (Petranka 1998). Salamanders are highly abundant, making up more of the biomass of some forest communities than birds and mammals (Burton and Likens 1975). Salamanders are considered by some to be keystone species, as they regulate the populations of litter-decomposing invertebrates, damping the breakdown of forest leaf-litter and regulating the availability of soil nutrients (Davic and Welsh 2004). Plethodontid salamanders that mostly occupy woodlands have also been acknowledged as indicators of overall species diversity and forest ecological integrity (Welsh and Droege 2001). Since invasion, earthworms may

have become an important part of salamander diet (Maerz et al. 2005) especially during cool spring and autumn evenings when there is rain, and it has been suggested that an earthworm diet may increase female fecundity due to their high protein content (Ransom 2017). Alternatively, invasive earthworms may decrease salamander populations by lowering the biomass of native soil invertebrates such as collembolans, which serve as food for salamanders (Maerz et al. 2009). Earthworms also create burrows within the soil that salamanders use as refugia, especially during winters (Cáceres-Charneco and Ransom 2010; Ransom 2011; 2012; 2017).

The effect of earthworm leaf-litter feeding on salamander abundance and that of other litter-dwelling organisms have been variable, with studies showing both positive effects by providing food and protective burrows (Ransom 2012), and negative effects by removal of the leaf-litter layer and a decline in native invertebrates (Maerz et al. 2009). Probable factors influencing this variability include geographic location and climate (Ransom 2012), seasonal temperature and rainfall conditions (Maerz et al. 2005), forest type (Maerz et al. 2009), time since first earthworm invasion (Hale et al. 2006), forests with native and invasive earthworms (Ransom 2012), and site physiographic conditions. Among these, there is a paucity of information as to how physiographic factors such as soil, elevation, aspect, and slope (Peterman and Semlitsch 2013; Costa et al. 2016), and their interactions with different earthworm guilds influence woodland salamanders.

Earthworm species are generally divided into ecological guilds based on their feeding habits and the depth at which they inhabit the soil, which could have different effects on forest communities (Blair et al. 1995). For example, the epigeic earthworms that are leaf-litter dwellers and feeders may be more detrimental to litter-dwelling plethodontid salamanders because these salamanders are lungless and breath through their skin and oral mucosa, and a loss of a moist protective cover could lead to death (Peterman and Semlitsch 2014). This loss of cover could also decrease dispersal of salamanders, especially by juveniles, which is important in maintaining populations and genetic diversity (Peterman and Semlitsch 2014). By contrast, endogeic earthworm species only burrow within the shallow mineral soil and digest microorganisms and organic materials, making them less detrimental to salamanders (Maerz et al. 2009). Anecic earthworms, however, also have the potential to feed on large amounts of leaf-litter and reduce salamander populations, and also tunnel deeply into the mineral soil and return to the surface to deposit nutrient rich castes (feces and leaf particles), which may benefit soil invertebrates and salamanders (Edwards and Bohlen 1996).

The focus of this study was to investigate the influence of site physiographic and soil factors on the distribution of earthworm species and to determine the effect of different earthworm guilds on terrestrial salamander relative abundance in a ravine deciduous forest in southwest Michigan. We think that higher populations of epigeic earthworms would be associated with lower salamander counts by causing a decline in leaf-litter and subsequently lead to a decline in soil moisture, which is important for the survival of salamanders. We also think that aspect and slope position would play a significant role in influencing the impact of earthworms on salamanders, as the south facing upper slopes would be drier, and as a consequence, the effect on salamander would be greater there than on north facing and lower elevation slopes. While there has been recent research of the impact of earthworms on vegetation and soil in northern hardwood forests (Bohlen et al. 2004b; Hale et al. 2005; Fahey et al. 2013), there have been few studies investigating the effect of invasive earthworms on salamander distributions and abundance (Maerz et al. 2009; Ziemba et al. 2016). In addition, there have been no studies looking at the interactive effects of slope position, aspect, and soil moisture on earthworm populations and subsequently salamander abundance among forest sites with varying environmental conditions.

### MATERIALS AND METHODS

**Study site.**—Our study took place in a previously glaciated, but never plowed, mature secondary growth forest in the Ravine Natural Area (42°57'49.38"N; 85°52'49.43"W) of Grand Valley State University, situated along the Grand River, Ottawa County, Michigan, USA. Seasonal temperatures range from -15.2 to 31.4° C, the average annual precipitation for rainfall is 91.5 cm, and snowfall is 201 cm. Open ridges do not occur in this site, as the ravines are mostly closed-canopy forest from the top of the slope to the base, ending at a narrow floodplain. The overstories of the forests are dominated by oak (*Quercus* spp.) and hickory (*Carya* spp.), with scattered ash (*Fraxinus* spp.), Black Cherry (*Prunus serotina*), American Beech (*Fagus grandifolia*), maple (*Acer* spp.), and Eastern Hemlock (*Tsuga canadensis*). The overall ravine runs north-south along the Grand River and is bisected by four eastwardly flowing first-order streams, each of which has formed steep cut ravines with 25–45° slopes and narrow flood plains, before entering into the larger Grand River. Elevation differences from the top to the bottom of each slope ranges from 183–213 m, and soils are clay loam with an A horizon of 10–18 cm and subsoil of 45 cm in thickness to an underlying bedrock of limestone.

We used stratified random sampling to estimate earthworm and salamander relative abundance within three east to west running ravines. We divided each of four ravines into three equivalent strata, starting at the eastern end of the ravine, approximately 300 m from the Grand River and running sites westerly up the streams. Within each stratum, we randomly selected a point on both the north and south facing slopes of the ravine. We measured 5 m from the top of the ravine slope at the chosen points and set up a 20 × 5 m plot. Then we measured 5 m from the bottom up the ravine slope and set up another 20 × 5 m plot, resulting in a total of 36 plots. All sample plots were within mature closed-canopy forest (percentage canopy coverage; mean = 94.15, standard error = 0.64, range 79.5% to 100%).

**Earthworm sampling.**—We sampled earthworms from late May into early June 2015. We divided each of the 20 × 5 m plots into two 10 × 5 m sections. We randomly chose one side to sample for earthworms, while we used the other side to sample only salamanders, to minimize their disturbance. Within the 10 × 5 m section, we chose two points, and placed a 35 × 35 cm metal frame at each point. We removed all leaf-litter to the mineral layer within the frame and searched for any residual earthworms within. We used a standard mustard extraction method (Gunn 1992; Tim McCay, unpubl. report) to sample earthworms, in which we dissolved 40 g of mustard powder in a gallon of water and poured it on the soil. We sampled all earthworms that came to the surface within 15 min and put them into cups of 93% isopropyl alcohol. We then preserved all earthworms in formalin for 2 d, identified them to species using the Great Lakes Worm Watch dichotomous key (<http://greatlakeswormwatch.org>), and put them into vials of 93% isopropyl alcohol for long-term storage.

**Salamander sampling.**—We sampled salamanders at each of the 36 sites using eight 35 × 35 cm cover boards of rough cut untreated Northern Red Oak (*Quercus rubra*) that were 2.54 cm thick. We divided each of the 10 × 5 m sections into a 2 × 4 grid pattern and placed a cover board near the center of each square of the grid. We cleared the leaf-litter under each board so they were flush with the soil (Hyde and Simons 2001; Marsh and Goicochea 2003). We placed cover boards in early May and checked them for salamanders during the day (0900–1600) once in mid-June. We lifted each cover board and counted all salamanders found underneath. We then identified them to species and released them carefully next to the cover board they were found under, allowing them to crawl back underneath.

**Physical factors.**—At each site, we measured the percentage of soil moisture using a FIELDSCOUT

TDR 300 soil moisture meter (Spectrum Technologies Inc., Aurora, Illinois, USA) at eight points to a depth of approximately 18 cm (lower A horizon) in a 2 × 4 grid pattern. We visually estimated leaf-litter ground coverage to the nearest 5% by randomly selecting two areas within the earthworm side of the plot and estimating coverage of each type with a 1 × 1 m graduated plant frame. We measured canopy cover with a spherical densiometer by taking four measurements at the cardinal directions in the center of each plot. We measured coarse woody debris (CWD) in each site by recording the number, length, and diameter of all pieces of CWD that were at least partially within a site (Gove and Van Deusen 2011). We omitted sections of CWD that were elevated off the ground because they would not provide suitable cover for a salamander. We used the diameter and length of each piece of CWD to find the total volume in each site. To determine pH and soil organic content, we randomly selected two points at each site and collected soil using a soil probe. The probe was inserted 20 cm into the soil, and we divided the samples into a top 10 cm and a bottom 10 cm. To measure pH, we air dried, pulverized, and sifted the soil samples. We then mixed a tablespoon of the soil with 25 ml of deionized water on a stir plate and used a standard pH meter to determine pH. To determine soil organic content, we put approximately 3 g of air-dried soil into crucibles and heated the soil in a combustion oven at 500° C (Ellert et al. 2008). We calculated percentage organic matter by dividing post combustion soil weight by pre-combustion soil weight.

**Statistical analysis.**—We used the SAS FMM (SAS software, SAS Institute, Cary, North Carolina, USA) procedure that compared alternative regression distribution models to determine the distribution that best fit our data. We compared the Akaike Information Criterion (AIC) and Pearson statistics among alternative distributions (Nielson and Sawyer 2013), and looked for which had an over-dispersion value (Chi-Square/df ratio) closest to one (Anderson et al. 1994). Based on these factors, along with our type of count data and with the number of zero counts and sample size (Nielson and Sawyer 2013), we determined that the best fitting model was the zero-inflated Gaussian distribution as used by Peterman and Semlitsch (2013), who also investigated physiographic effects on salamander counts in ravines. We further investigated soil factor differences among aspect and elevation, independent of each other, to see if they may have affected the distribution of earthworms and salamanders by independent sample *t*-tests. Furthermore, we used linear regression ( $\alpha = 0.05$ ) to investigate the relationship between epigeic earthworms and salamander counts among all 36 sites.

## RESULTS

We sampled 1,183 invasive earthworms from 11 species among the three feeding guilds. Epigeic earthworms accounted for 56.2% of those sampled, which included *Lumbricus rubellus* (Red Earthworm) and *Dendrobaena octaedra* (Octagonal-tail Worm). Endogeic earthworm counts were at their highest in south facing, low elevation sites, and lowest in south facing, high elevation sites (Table 1). Endogeic earthworms were 38.9% of those sampled, and included *Allolobophora chlorotica* (Green Worm), *Aporrectodia calignosa* (Grey Worm), *Aporrectodia longa* (Black-headed Worm), *Aporrectodia trapezoides* (Southern Worm), *Aporrectodia tuberculata* (Canadian Worm), *Aporrectodia rosea* (Rosy-tipped Worm), *Octolasion cyaneum* (Blue-grey Worm), and *Octolasion tyrtaeum* (Woodland White Worm). Endogeic earthworm counts were also highest in south facing, low elevation sites, but were at their lowest in north facing, high elevation sites (Table 1). The only anecic species of earthworm sampled was *Lumbricus terrestris* (Common Earthworm). We did not include anecic earthworms as a variable in our statistical models due to low counts ( $n = 46$ ), which we attribute to possible sampling error, as these deep burrowing earthworms tend to stay deep in areas of the soil during the drier conditions of summer and may not have surfaced using the methods we employed of 15 min extractions. We did not collect any native earthworm species. We also found 121 salamanders from two species; five *Ambystoma laterale* (Blue-spotted Salamander) and 116 *Plethodon cinereus* (Red-backed Salamander). Salamander counts were at

their highest in north facing, high elevation sites, but they were at their lowest in south facing, low elevation sites, where both earthworm feeding guilds were most abundant (Table 1).

**SAS FMM procedure models.**—Model I shows how epigeic earthworms, physiographic conditions (aspect and elevation), and soil conditions related to salamander counts. Salamander counts were negatively associated with increasing epigeic earthworm counts, while increasing CWD and soil moisture had a positive association with salamander counts (Table 2). Moreover, salamanders had a negative association with increasing soil pH; whereas, leaf-litter cover and organic content of the A horizon had no significant effects on salamanders. Aspect and elevation had no effect on salamander counts. Model I had an AIC of 186.1, Pearson statistic of 31.2, and an overdispersion value of 1.25. The model equation for Model I is

$$\log(\mu) = 8.992 \text{ (intercept)} + 2.140 + 1.599 + 2.602 - 0.135 \text{ (epigeic)} - 0.007 \text{ (litter)} + 0.881 \text{ (CWD)} + 0.158 \text{ (soil moisture)} + -0.816 \text{ (soil pH)} - 0.045 \text{ (A horizon organic matter)} + 4.282 \text{ (variance)}.$$

Salamander counts were negatively related to epigeic earthworm counts ( $F_{1,36} = 6.69$ ,  $P = 0.014$ ;  $n = 36$ ,  $r^2 = 0.12$ , slope =  $-0.129 \pm 0.049$ ; Fig. 1).

Model II shows how endogeic earthworm counts, physiographic conditions, and soil conditions related to salamander counts. Endogeic earthworms did not have a significant effect on salamander counts, whereas, increasing CWD, soil moisture, and soil pH had a positive

TABLE 1. Earthworm and salamander counts and physical factor data ( $\bar{x} \pm 1$  standard error and minimum-maximum) by north and south aspect and high and low elevation in the forested ravines of southwest Michigan, USA, 2015.

Site	North/Low n = 9	North/High n = 9	South/Low n = 9	South/High n = 9
Epigeic Counts	19.44 $\pm$ 1.09 16.00–24.00 n = 175	19.22 $\pm$ 3.77 6.00–40.00 n = 173	20.33 $\pm$ 3.27 8.00–39.00 n = 183	14.89 $\pm$ 2.86 4.00–34.00 n = 134
Endogeic Counts	13.56 $\pm$ 3.07 4.00–30.00 n = 122	9.22 $\pm$ 2.51 0.00–22.00 n = 83	15.22 $\pm$ 2.16 9.00–29.00 n = 137	13.11 $\pm$ 1.93 3.00–20.00 n = 118
Salamander Counts	3.78 $\pm$ 1.01 1.00–11.00 n = 34	4.00 $\pm$ 1.13 0.00–10.00 n = 36	2.56 $\pm$ 0.82 0.00–7.00 n = 19	3.56 $\pm$ 0.78 0.00–7.00 n = 32
Leaf-litter Cover (%)	91.33 $\pm$ 1.66 84.00–97.50	73.39 $\pm$ 6.86 42.50–98.00	95.50 $\pm$ 1.13 90.00–100.00	84.11 $\pm$ 3.61 60.00–96.50
Soil Moisture (%)	33.44 $\pm$ 3.87 19.03–53.18	27.11 $\pm$ 2.33 13.84–37.45	37.00 $\pm$ 3.45 22.31–55.71	26.80 $\pm$ 2.55 15.41–38.71
CWD (m <sup>3</sup> )	1.86 $\pm$ 0.73 0.03–6.43	0.84 $\pm$ 0.19 0.00–1.81	1.52 $\pm$ 0.53 0.00–4.95	0.36 $\pm$ 0.12 0.00–1.94
A Horizon Organic Matter (%)	6.38 $\pm$ 0.49 4.35–8.27	7.38 $\pm$ 0.92 4.59–13.11	7.00 $\pm$ 0.76 4.82–11.30	6.49 $\pm$ 0.60 3.93–8.64
A Horizon pH	5.93 $\pm$ 0.20 5.15–7.03	4.99 $\pm$ 0.19 4.33–5.93	5.67 $\pm$ 0.34 3.95–7.63	5.13 $\pm$ 0.26 4.37–6.86



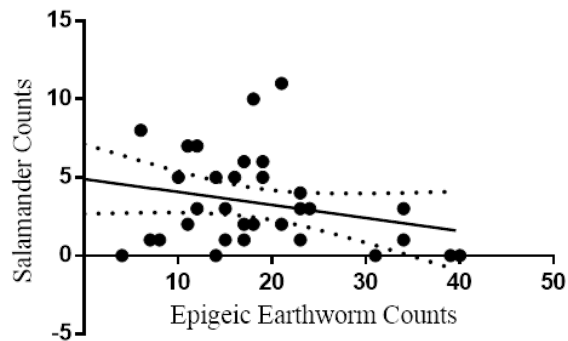


FIGURE 1. Relationship between total epigeic earthworm counts and total salamander counts among 36 sites from southwest Michigan, USA, in 2015. ( $r^2 = 0.164$ ,  $F_{1,36} = 6.69$ ,  $n = 36$ ,  $P = 0.014$ ).

association with salamander counts (Table 3). Model II had an AIC of 189.8, Pearson statistic of 36.13, and an overdispersion value of 1.45. The model equation for Model II is

$$\log(\mu) = 2.430 \text{ (intercept)} + 2.763 + 1.148 + 2.838 + 0.034 \text{ (endogeic)} + 0.009 \text{ (litter)} + 0.808 \text{ (CWD)} + 0.138 \text{ (soil moisture)} - 1.291 \text{ (soil pH)} + 0.067 \text{ (organic matter)} + 4.823 \text{ (variance)}.$$

The interactive effects of aspect and elevation had no effect on salamander counts (Table 2 and 3). There were no significant differences among north and south facing slopes for soil moisture, leaf-litter cover, coarse woody debris, organic content and pH of the A horizon, or earthworm and salamander counts. There were, however, significant differences in soil moisture between lower elevation (mean =  $35.2 \pm 2.55$ ) and higher eleva-

tion sites (mean =  $26.96 \pm 1.67$ ;  $t = 271$ ,  $df = 34$ ,  $P = 0.0105$ ). Leaf-litter cover was significantly higher at low elevation sites (mean =  $93.42 \pm 1.09$ ), compared to higher elevation sites (mean =  $78.75 \pm 3.98$ ;  $t = 3.55$ ,  $df = 34$ ,  $P = 0.001$ ). Coarse woody debris was also significantly higher at the low elevation sites (mean =  $1.69 \pm 0.44$ ) compared to higher elevation sites; (mean =  $0.60 \pm 0.15$ ;  $t = 2.35$ ,  $df = 34$ ,  $P = 0.025$ ). Soil pH of the A horizon was significantly more acidic in higher elevation sites (mean =  $5.07 \pm 0.17$ ) compared to low elevation sites (mean =  $5.81 \pm 0.19$ ;  $t = 2.85$ ,  $df = 34$ ,  $P = 0.007$ ). Elevation had no effect on soil organic content, however, and as an independent factor, had no significant effect on salamander counts (Table 2 and 3).

## DISCUSSION

Our results support our hypothesis and those of others (Maerz et al. 2009; Ziembra et al. 2016; Ransom 2017) that invasive earthworms affect salamander populations and play a role in the reduction of the relative abundance of woodland salamanders in deciduous forests. We found evidence of a negative relationship of epigeic earthworms, but not endogeic earthworms, on woodland salamander counts. This is likely due to the different feeding habits of epigeic and endogeic earthworms (Maerz et al. 2005). Epigeic earthworms consume leaf-litter (O horizon) that salamanders and their arthropod prey depend on for moisture and protection; whereas, endogeic earthworms primarily consume microorganisms and soil invertebrates in the underlying mineral soil of the A horizon (Maerz et al. 2005; Ransom 2012). Maerz et al. (2009) found that

TABLE 2. Parameter estimate, standard error (SE), z score (z), P value ( $Pr > |z|$ ), and lower and upper confidence limits (LCL and UCL) with 95% confidence for zero-inflated Gaussian model for salamander counts in relation to epigeic earthworm counts and physiographic conditions (CWD = coarse woody debris) in the forested ravines of southwest Michigan, USA, in 2015.

Component Effect	Estimate	SE	z	$Pr >  z $	LCL	UCL
Intercept	8.9920	5.09	1.77	0.0772	-0.980	18.964
Aspect/Elevation (North/High)	2.1398	1.58	1.36	0.1746	-0.950	5.230
Aspect/Elevation (North/Low)	1.5991	1.17	1.37	0.1720	-0.696	3.894
Aspect/Elevation (South/High)	2.6015	1.36	1.91	<b>0.0557</b>	-0.064	5.267
Aspect/Elevation (South/Low)	0.0000	—	—	—	—	—
Epigeic Earthworms	-0.1354	0.07	-2.08	<b>0.0379</b>	-0.263	-0.008
Litter Cover	-0.0066	0.04	-0.18	0.8571	-0.079	0.066
CWD	0.8809	0.32	2.74	<b>0.0062</b>	0.251	1.511
Soil Moisture	0.1576	0.05	2.98	<b>0.0029</b>	0.054	0.261
A Horizon pH	-1.8160	0.65	-2.81	<b>0.0049</b>	-3.082	-0.550
A Horizon Organic Matter	0.0454	0.28	0.16	0.8722	-0.508	0.599
Variance	4.2823	1.09	—	—	—	—

**TABLE 3.** Parameter estimate, standard error (SE), z score (z), *P* value ( $\text{Pr} > |z|$ ), and lower and upper confidence limits (LCL and UCL) with 95% confidence for zero-inflated Gaussian model for salamander counts in relation to endogeic earthworm counts and physiographic conditions (CWD = coarse woody debris) in the forested ravines of southwest Michigan, USA 2015.

Component Effect	Estimate	SE	z	Pr >  z	LCL	UCL
Intercept	2.4301	4.64	0.52	0.6003	-6.661	11.521
Aspect/Elevation (North/High)	2.7629	1.69	1.63	0.1021	-0.550	6.075
Aspect/Elevation (North/Low)	1.1477	1.22	0.94	0.3466	-1.242	3.538
Aspect/Elevation (South/High)	2.8375	1.46	1.94	0.0521	-0.025	5.700
Aspect/Elevation (South/Low)	0.0000	—	—	—	—	—
Endogeic Earthworms	0.0336	0.06	0.59	0.5520	-0.077	0.144
Litter Cover	0.0093	0.04	0.24	0.8077	-0.065	0.084
CWD	0.8084	0.34	2.38	<b>0.0175</b>	0.141	1.475
Soil Moisture	0.1383	0.06	2.50	<b>0.0125</b>	0.030	0.247
A Horizon pH	-1.2909	0.64	-2.02	<b>0.0434</b>	-2.543	-0.038
A Horizon Organic Matter	0.0674	0.30	0.22	0.8226	-0.522	0.657
Variance	4.8228	1.23	—	—	—	—

invasive epigeic earthworm species had a significant negative impact on leaf-litter volume and associated invertebrate populations, potentially leading to lower salamander abundance. Bohlen et al. (2004a) also found that epigeic earthworm invasions led to significant decreases in leaf-litter mass. In contrast, we did not find a negative relationship between increasing earthworm counts and leaf-litter and this may be due to differences in methods used to quantify leaf-litter. We measured the difference in the percentage of mineral soil covered by leaf-litter, as opposed to differences in mass or thickness, which was done in these previous studies. Another potential factor is that earthworm abundance or the impact of earthworms at our sites could have been lower than those from previous studies, as we did not observe large areas of bare soil with exposed roots, or a notable invasion by exotic plants or graminoids, which was reported by past studies (Hale et al. 2006; Maerz et al. 2009; Ziemba et al. 2016). Ransom (2017) found that in areas with resident native earthworms that were recently colonized by low densities of invasive epigeic earthworms, however, that leaf-litter thickness and associated invertebrates were reduced.

Differences in invasion history of earthworms among study locations could have had an effect on the severity of their ecological impact. Although knowledge of earthworm invasion history of our site is unknown, there is reason to believe that the initial colonization occurred much earlier than those reported in studies in more northern and human-isolated forests (James 1995; Hale et al. 2005; Frelich et al. 2006; Maerz et al. 2009). Our site is immediately bounded by a college campus, a river that is heavily sport-fished, and in an area of intensive

agriculture since the 1930s, with significant densities of European settlers since the 1850s (Moore 1974). All of these factors likely led to a much earlier and constant invasion by exotic earthworms. There is also evidence that the detrimental effects to an ecosystem caused by invasive species can decline over time. Strayer et al. (2006) proposed that invasions generally have an acute phase immediately after invasion, followed by a chronic phase with a damping of ecological effects after various ecological and evolutionary processes come into play. An example of this damping of effects is with the invasive soil dwelling Fire Ant, *Solenopsis invicta*. As it spread into new areas, *S. invicta* became very abundant and greatly reduced the population of native soil insects, but after a decade or more of establishment, local populations of the insects returned to near pre-invasion levels with less reported ecological disruption (Porter and Savignano 1990; Morrison 2002). This may have played a role in our study, but notwithstanding, we found a significant negative relationship between epigeic earthworms and salamander counts.

Elevation and aspect of a slope can create subtle, but important differences in the characteristics of an environment that can lead to differences in the population distribution of species, including salamanders (Heatwole 1962; Suggitt et al. 2011; Peterman and Semlitsch 2013; Costa et al. 2016). We found no significant effect of slope or aspect on the distribution of epigeic or endogeic earthworms or salamanders, though south facing sites at low elevations were trending toward a significant association with salamanders. This was unexpected, as north-facing slopes tend to be cooler and moister than south-facing slopes, making them more ideal for

salamander habitats (Heatwole 1960; Sugalski and Claussen 1997; Peterman and Semlitsch 2013; Costa et al. 2016). We found no significant difference in moisture levels among north and south facing slopes in late spring/early summer; however, we did find significantly wetter soils at the lower elevational sites, but these differences did not explain the distribution of earthworms or salamanders throughout our ravines. Diurnal temperature differences, which we did not measure, or some other untested environmental or biological factor, could potentially be a contributing factor. At our study site, the width of the ravines are relatively narrow with a dense closed forest canopy (mean 94%), covering both the north and south slopes from top to bottom for much of the day. This suggests that solar exposure and related soil temperature differences among sites would have been minimal at least during the growing season, which would explain our lack of finding an effect of aspect. Although independent of a slope and aspect interaction, our results did show that soil moisture as well as CWD did have a positive correlation with salamander counts, and that epigeic earthworms had a negative association with salamanders among our sites.

Our results add to the growing body of evidence of the impact of non-native earthworms on the ecological disruption of forest ecosystems (Bohlen et al. 2004a, b; Migge-Kleian et al. 2006). We found significantly lower salamander counts in areas with more epigeic earthworms, which feed on and within the leaf-litter layer. A reduced leaf-litter layer could lead to higher rates of salamander desiccation and less time to forage for prey (Peterman and Semlitsch 2014), along with a decline in soil-litter invertebrates that serve as the primary prey of salamanders (Burtis et al. 2014; McCay and Scull 2019). This potential impact could be a part of an already suggested complex decline syndrome of woodland salamanders (Highton 2005; Beebee and Griffiths 2005), which are considered a detrital keystone species in deciduous forests, ideal biological indicators of forest ecological integrity, and can be used as a proxy measure of change in species richness (Welsh and Droege 2001; Best and Welsh 2014). Stricter regulations on human activities that promote earthworm introductions of non-native earthworms into natural area previously devoid and public educational programs, such as those by Great Lakes Worm Watch should be implemented to reduce invasion and ecosystem impact.

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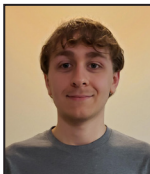
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**HUNTER J. BRUNGES** received his B.S. in Biology from Grand Valley State University, Allendale, Michigan, USA, in 2017. In addition to herpetology, his interests include ornithology and community ecology. He currently works as a Senior Production Specialist in the testing lab of Versiti Blood Center of Michigan. (Photographed by Amy Brunges).



**JAMES P. DUNN** is a graduate of the University of Michigan, Ann Arbor, USA, and the University of Kentucky, Lexington, USA, who is a Professor of Biology at Grand Valley State University, Allendale, Michigan, USA. His passion is teaching ecology courses and working with students on conservation-based research projects involving varied taxa from soil invertebrates, butterflies, beetles, aquatic macroinvertebrates, and plants to salamanders. He has worked on conservation projects in forests, prairies, savanna, farms, and streams. (Photographed by Daryl Melpolder).



**DAVID R. HELDER** completed his B.S. in Natural Resource Management and Biology at Grand Valley State University, Allendale, Michigan, USA. Aside from studying interactions in forest ecology, his interests also include protecting and restoring natural aquatic systems. He currently works for Streamside Ecological Services, where he manages water resources and works on ecological restoration projects. He is also an active board member of Schrems Trout Unlimited in Grand Rapids, Michigan, USA. (Photographed by Aaron Snell).



**SANGO OTIEMO** is an Associate Professor of Statistics and Director of the Statistical Consulting Center at Grand Valley State University in Allendale, Michigan, USA. He earned his Ph.D. in Statistics from Virginia Tech in Blacksburg, Virginia, USA, and has been exposed to a variety of statistical methodology, data analysis, and statistical computing. Sango's research focuses on directional data. (Photographed by Sarah McCauley).