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# Earthworm populations in a wheat-soybean double-crop system under seven years of established residue management practices

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## ABSTRACT

Earthworms improve soil structure, distribute litter and microbes, stimulate microbial activity, facilitate decomposition, and increase nitrogen (N) availability for plant growth. Earthworm density is often reduced in low organic matter soils that are intensively managed to grow row crops. This study was designed to relate earthworm density and community composition to residue management after seven years of established management practices in a wheat (*Triticum aestivum* L.)-soybean (*Glycine max* (L.) Merr.) double-crop system maintained in Marianna, Ark. Residue management practices included conventional tillage (CT) and no-tillage (NT), N fertilization to produce high and low wheat residue amounts left in the field, and burning and non-burning of residue after wheat harvest. Total earthworm densities ranged from 271 to 508 m<sup>-2</sup> across treatments. Both exotic *Aporrectodea trapezoides* (Duges) and native *Diplocardia sylvicola* (Gates) adult earthworms were present with very little difference in diversity among sampled communities; however, more than 50 % of adults were *D. sylvicola* in all treatments. Residue level and burning influenced total, juvenile, and native earthworm densities differently in CT and NT. Adult native earthworms predominated over a common exotic species in a wheat-soybean double-crop system in Arkansas with residue management practices interacting to impact the density of earthworms.

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§ Kristofor Brye is a professor in the Department of Crop, Soil, and Environmental Sciences.

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## MEET THE STUDENT-AUTHOR



*Jill Thomason*

I am from Fayetteville, Arkansas and graduated from Fayetteville High School in 2011. I graduated in May 2015 from the Dale Bumpers College of Agricultural, Food and Life Sciences at the University of Arkansas with a degree in Animal Science, a concentration in Pre-Veterinary Medicine and a minor in German. In August 2015, I plan to begin pursuit of a Doctor of Veterinary Medicine degree at the Louisiana State University School of Veterinary Medicine in Baton Rouge, La. As a student at the University of Arkansas, I was a member of the Pre-Veterinary club, as well as the German Honors Society, Delta Phi Alpha, while also working at a local veterinary clinic, Stanton Animal Hospital.

I would like to thank my faculty advisor, Dr. Mary Savin, for all of the help and insight she provided throughout the completion of this project. I would also like to thank the other members of my committee, Dr. Kristofor Brye and Dr. Donn T. Johnson, for the additional assistance they provided for completion of this project. Special thanks are also given to Dr. Bruce Snyder at Kansas State University for assistance in identification of the earthworms found in this study and Dr. Edward Gbur at the University of Arkansas for performing much of the statistical analyses necessary for completion of this project.

## INTRODUCTION

Understanding the factors affecting earthworm population densities is important because earthworms are ecological engineers and keystone species (Lavelle et al., 1989). Earthworms play a number of important roles in improving soil quality. Their presence impacts nutrient cycling as well as many soil characteristics, such as soil aggregate structure, litter and microorganism distribution, microbial activity, decomposition rates and extent, and timing and amount of available N (Lavelle et al., 1989).

Typically, agricultural management practices that decrease residue, such as conventional tillage (CT), largely affect invertebrate community composition in the southern United States, causing less diversity and resulting in communities made mostly of a few, often exotic species (Callahan et al., 2006). Increased residue increases food for earthworms, so earthworm densities are expected to increase. A previous study by Eriksen-Hamel et al. (2009) shows that CT and practices that increase crop residue amount can increase organic carbon in soil, providing earthworms with more organic resources for growth. However, this study also showed that earthworm movement might be physically restricted due to barriers created as a result of tillage in fields subjected to repeated CT. Tillage practices that reduce soil disruption and return more

crop residues to the soil tend to increase earthworm population densities (Eriksen-Hamel et al., 2009).

In the southern United States, there are native and exotic earthworms. Previous research shows that native and exotic earthworms interact in a number of ways, primarily competing for soil nutrients (Kalisz and Wood, 1995; Winsome et al., 2006). Often, the introduction of exotic earthworms coincides with increased disturbance and reductions in native earthworm densities, especially in disturbed soils characteristic of urban and rural areas (Kalisz and Wood, 1995; Winsome et al., 2006). Winsome et al., 2006 found that an exotic species, *Aporrectodea trapezoides* (Duges), consistently had greater relative increase in density than a native species, *Argilophilus marmoratus* (Eisen) in a California grassland, but differences in densities declined with decreasing habitat quality. However, research is not definitive as to whether exotic earthworms will replace native earthworms in managed agroecosystems and to what extent species predominate or coexist with differences in residue management in row crop systems.

Earthworm abundance observationally appeared to increase over time in plots established to investigate residue management (CT versus NT; fertilization to produce HIGH amount versus LOW amount of wheat residue; and burning (BURN) versus not burning (NO BURN) residue) in a wheat-soybean double-crop system in east-

ern Arkansas. Thus, the goal of this research was to determine if seven consecutive years of consistent residue management treatment combinations resulted in distinguishable differences in density, diversity, or species identity of earthworms. The null hypothesis for this research was that there would be no significant differences in densities, diversity, or identities of earthworm species after seven years of different residue management treatments.

## MATERIALS AND METHODS

The field experiment evaluating residue management practices in a wheat-soybean double-crop system was established at the University of Arkansas System Division of Agriculture's Lon Mann Cotton Research Station in the spring of 2002 in Marianna, Arkansas as described in Brye et al. (2007). Plots (48, 3 × 6 m<sup>2</sup>) have been managed each year according to the original timeline as briefly described below. Prior to the establishment of the site, soybean was grown in a non-double-cropped system under CT management. Land was prepared by disking twice followed by field cultivation and broadcast application of 20 kg N ha<sup>-1</sup>, 22.5 kg P ha<sup>-1</sup>, 56 kg K ha<sup>-1</sup> and 1120 kg ha<sup>-1</sup> of pelletized limestone to adjust pH levels prior to planting of the first wheat crop in Fall 2001. All plots received 101 kg N ha<sup>-1</sup> as urea in early March beginning in 2002, while the HIGH plots were fertilized with an additional 101 kg N ha<sup>-1</sup> broadcast application of urea in late March for the first 3 years. After the first 3 years, LOW plots received 0 kg N ha<sup>-1</sup> and HIGH plots received 101 kg N ha<sup>-1</sup>. Wheat was harvested in June, with the aboveground wheat residue mowed to the soil surface. Mowed residue was raked uniformly back onto its respective plot, and the burn treatment imposed on the BURN plots only. Mowed residue was then raked uniformly back onto its respective unburned plot. Following the burning, CT consisting of disking twice, and seedbed smoothing with a soil conditioner was imposed before planting of a glyphosate-resistant soybean seed in mid-June. All 48 plots were furrow-irrigated as needed throughout the season for the first 3 years of the study, after which only the first 24 plots were furrow-irrigated and the last 24 plots were not irrigated. In early November, soybean was harvested by hand, and the remaining soybean stubble was left standing in the plots before replanting of the wheat crop.

Earthworms for this study were collected in March 2009. Earthworms were sampled by the hand-sorting dig-and-sort method from a randomly located 30 × 30 × 20 cm<sup>3</sup> sub-plot within 24 non-irrigated treatment plots. All possible combinations of tillage (CT versus NT), wheat residue (HIGH versus LOW) and burn (BURN versus NO BURN) treatments were sampled (n = 3). Earthworms were

boiled at the collection site, preserved in 5% formalin solution, and stored in vials. Formalin was increased to a 10% concentration to maintain preservation for long-term storage. Juvenile, adult and total earthworms were counted in February 2014 after 5 years of storage in formalin. Adult earthworms were identified in spring and summer 2014 using relevant identification keys (Causey, 1958; Dindal, 1990; Eisen, 1899; Gates and Reynolds, 1968). Juvenile earthworms were considered unidentifiable due to lack of reproductive structures which are necessary for identification.

A Poisson distribution was assumed for the earthworm counts, which were log transformed, and evaluated by analysis of variance as a split-split plot in which the whole plot portion of the design was a randomized complete block with three blocks and tillage as the main treatment factor. The split plot factor was the burn treatments and the split-split plot factor was residue level. Least square means for significant effects ( $P < 0.05$ ) were compared using a protected least significant difference (LSD) procedure where appropriate. Statistical analyses were carried out using SAS<sup>®</sup> v. 9.4 (SAS Institute Inc., Cary, N.C.).

## RESULTS AND DISCUSSION

Mean total and juvenile earthworm abundances were significantly affected by the three-way interaction of tillage by burn treatment by residue level ( $P < 0.0001$  for both total and juvenile abundances; Tables 1 and 2). There were no significant differences in densities between tillage treatments; however, the effect of burning and residue level was significantly different within a tillage treatment for both mean total and juvenile earthworm densities. Within CT, the highest mean total (Table 1) and juvenile earthworm (Table 2) densities occurred in the absence of burning (NO BURN) and presence of HIGH residue level and were similar to densities in the BURN treatment with LOW residue level. Mean total and juvenile earthworm densities were lower and similar when burned (BURN) with high residue level (HIGH) and juvenile earthworms were lower in density when residue was unburned (NO BURN) and LOW. In contrast, in the NT, the highest total density compared to the other treatments was in the HIGH residue, BURN treatment (Table 1). Density was also lower in the NO BURN, HIGH residue compared to the NO BURN, LOW residue. For juveniles in the NT, the highest density was in the HIGH residue, BURN treatment which was not different than in LOW residue, NO BURN (Table 2). Juvenile densities were similar across the two LOW residue levels and the HIGH residue, NO BURN treatments. Juvenile earthworms accounted for more than half (56-74%) of the mean total earthworm densities (Tables 1 and 2).

**Table 1. Mean total earthworm densities (# m<sup>-2</sup> ± standard error) collected in March 2009 from non-irrigated plots in a wheat-soybean double-crop system subjected to a main effects of conventional tillage (CT) or no till (NT), split plot effects of burning (BURN) or leaving (NO BURN) wheat residue to decompose on the soil surface, and split-split plot effects of LOW or HIGH residue amount† at the Lon Mann Cotton Research Station in eastern Arkansas.**

	CT			NT	
	High	Low		High	Low
<b>Burn</b>	271 (50)d <sup>‡</sup>	428 (79)bc	<b>Burn</b>	497 (92)a	281 (52)bc
<b>No Burn</b>	508 (94)ab	320 (59)cd	<b>No Burn</b>	275 (51)c	325 (60)b

† For the first 3 years, 101 kg N ha<sup>-1</sup> was applied to the LOW residue plots and 202 kg N ha<sup>-1</sup> was applied to the HIGH residue plots. After the first 3 years, 0 kg N ha<sup>-1</sup> was applied to the LOW residue plots and 101 kg N ha<sup>-1</sup> was applied to the HIGH residue plots.

‡ Means followed by a similar letter within a tillage treatment are not statistically different ( $P < 0.0001$ ). There are no significant differences between CT and NT.

The predominance of juveniles in many samples may be explained by the March sampling time. Spurgeon and Hopkin (1999) found juveniles were more abundant than adults in spring and summer; whereas adults outnumbered juveniles in fall and winter at a site in southwest England. A potential reason for the juveniles being more numerous than adults at the time of sampling in March is that *Ap. trapezoides* and *Diplocardia sylvicola* hatch in the winter and spring, and mature to adulthood later in the year; however, little previous research has been done on the reproductive cycling of either species in the southeastern United States (Spurgeon and Hopkin, 1999). It is expected that during the dry, hot summer, very few earthworms will be active in a non-irrigated system, so another possible explanation for the large densities at the March sampling is that samples were taken in the cool, wet spring (Spurgeon and Hopkin, 1999).

Only two species, one native and one exotic, were identified among the adult earthworms from the 24 plots. This seems to be relatively low number of species compared to similar studies performed in managed systems

(Callaham et al., 2003; Callaham et al., 2006; Emmerling, 2001). All native earthworms were identified as *D. sylvicola*, and all exotic species were identified as *Ap. trapezoides*. Native earthworms accounted for 65–100% of adult earthworms collected across treatments. Furthermore, except for three samples in NO BURN treatments where native earthworms accounted for 19%, 54%, and 60% of the adult population, native earthworms accounted for 88–100% of adult earthworms collected in individual samples (data not shown). Adult earthworms consisted solely of *D. sylvicola* in the CT, LOW residue, BURN and the NT, LOW residue, BURN treatments (data not shown). The only treatment that had exotic earthworms in all three replicate samples was the CT, HIGH residue, NO BURN (data not shown).

Because of the infrequent detection of exotic species, statistical analysis of adult earthworms was conducted only on native earthworm densities. The three-way interaction among treatments (tillage × burn × residue level) had a significant effect on native earthworm density ( $P < 0.0001$ ; Table 3). Similar to total and juvenile earthworm

**Table 2. Mean juvenile earthworm densities (# m<sup>-2</sup> ± standard error) collected in March 2009 from non-irrigated plots in a wheat-soybean double-crop system subjected to main effects of conventional tillage (CT) or no till (NT), split plot effects of burning (BURN) or leaving (NO BURN) wheat residue to decompose on the soil surface, and split-split plot effects of LOW or HIGH residue amount† at the Lon Mann Cotton Research Station in eastern Arkansas.**

	CT			NT	
	High	Low		High	Low
<b>Burn</b>	181 (36)b <sup>‡</sup>	271 (54)a	<b>Burn</b>	301 (59)a	200 (40)b
<b>No Burn</b>	371 (73)a	176 (35)b	<b>No Burn</b>	195 (39)b	217 (43)ab

† For the first 3 years, 101 kg N ha<sup>-1</sup> was applied to the LOW residue plots and 202 kg N ha<sup>-1</sup> was applied to the HIGH residue plots. After the first 3 years, 0 kg N ha<sup>-1</sup> was applied to the LOW residue plots and 101 kg N ha<sup>-1</sup> was applied to the HIGH residue plots.

‡ Means followed by a similar letter within a tillage treatment are not statistically different ( $P < 0.0001$ ). There are no significant differences between CT and NT.

**Table 3. Mean native earthworm densities (# m<sup>-2</sup> ± standard error) collected in March 2009 from non-irrigated plots in a wheat-soybean double-crop system subjected to main effects of conventional tillage (CT) or no till (NT), split plot effects of burning (BURN) or leaving (NO BURN) wheat residue to decompose on the soil surface, and split-split plot effects of LOW or HIGH residue amount<sup>†</sup> at the Lon Mann Cotton Research Station in eastern Arkansas.**

	CT			NT	
	High	Low		High	Low
<b>Burn</b>	84 (25) <sup>b‡</sup>	152 (45) <sup>a</sup>	<b>Burn</b>	185 (55) <sup>a</sup>	80 (24) <sup>b</sup>
<b>No Burn</b>	86 (26) <sup>b</sup>	106 (32) <sup>a</sup>	<b>No Burn</b>	71 (21) <sup>b</sup>	98 (29) <sup>a</sup>

<sup>†</sup> For the first 3 years, 101 kg N ha<sup>-1</sup> was applied to the LOW residue plots and 202 kg N ha<sup>-1</sup> was applied to the HIGH residue plots. After the first 3 years, 0 kg N ha<sup>-1</sup> was applied to the LOW residue plots and 101 kg N ha<sup>-1</sup> was applied to the HIGH residue plots.

<sup>‡</sup> Means followed by a similar letter within a tillage and burn treatment are not statistically different ( $P < 0.0001$ ). There are no significant differences between CT and NT, and there are no significant differences between burn treatments.

density, the effect of residue level was different within tillage treatment, but unlike total and juvenile earthworm densities, the native earthworm densities in CT were similar and higher with LOW compared to HIGH residue level within a burn treatment regardless of BURN or NO BURN treatment. The effect of residue level within NT was also different for native earthworms than it was for total and juvenile earthworm densities. In NT, native earthworm densities were higher in BURN and HIGH. Lower abundances were obtained in NO BURN, HIGH residue level compared to the BURN, LOW residue treatment. There were no differences within NT between burn treatments for native earthworms.

Many studies indicate that earthworm populations are larger in NT than other tillage systems (Eriksen-Hamel et al., 2009; Ernst and Emmerling, 2009). Chan (2001) compared several studies that revealed earthworm densities in various NT systems ranged between 137 and 467 m<sup>-2</sup> and between 52 and 213 m<sup>-2</sup> in CT systems. Total earthworm densities in this study were similar between CT and NT with densities under CT greater than those reported in Chan (2001). Densities in NT were within range or greater than previously reported (Chan, 2001). Both *D. sylvicola* and *Ap. trapezoides* are endogeic species. Endogeic, or soil-dwelling, earthworms have been known to benefit under tillage, likely from increased nutrient availability resulting from decomposition of plowed-in organic matter (Chan, 2001).

In general, cultivation may provide a competitive advantage for exotic species. In the midwestern and south-eastern United States, exotic species have been found in greater densities than native species in cultivated systems (Callaham et al., 2003; Callaham et al., 2006). It is often assumed that this is due to the exotic species' ability to tolerate disturbances, such as tillage or fertilization, to a greater extent than the native species (Callaham et al., 2006).

Although species were not identified among juvenile earthworms due to the lack of clitellum—the location of which is vital to identifying earthworms—in this experiment, native adults outnumbered exotic earthworms.

Although residue management within a tillage treatment impacted earthworm densities differently, earthworms were not more abundant in NT compared to CT treatment combinations. Rather, the interaction between residue amount and burning impacted earthworm densities and relative abundances differently within tillage type. Prior to European settlement and subsequent introduction of exotic earthworm species, soils were burned frequently with aboveground biomass removal, thus native earthworms may be better adapted to temperature and resource availability changes that occur with aboveground burns than exotic earthworms (Callaham et al., 2003).

## SUMMARY AND CONCLUSIONS

Earthworms were collected in March 2009 in eastern Arkansas from non-irrigated plots in a wheat-soybean double-crop system subjected to CT or NT, BURN or NO BURN, and fertilization to produce LOW or HIGH wheat residue amounts. Earthworms were abundant with total densities ranging from 271–508 m<sup>-2</sup>. Native adult earthworms (*D. sylvicola*) outnumbered an exotic species (*Ap. trapezoides*), and juveniles outnumbered adult earthworms in all treatments. Residue management practices of tillage, fertilization impacting residue amount, and burning interacted to impact the density of total, juvenile and native earthworms.

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