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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, AR. 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^{-2} 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. Native, total, or juvenile earthworm densities in different treatment combinations were related to soil properties, including pH, electrical conductivity, and Mehlich-III- K, Mn, Mg, Cu, S and Ca concentrations. Native earthworms predominated with a common exotic species in a wheat-soybean double-crop system in Arkansas with residue management practices interacting to impact the density of earthworms.

Significance:

The significance of this study is two-fold. First, there is very little published research on Arkansas earthworms. Second, there is less research in intensively managed row crop systems as compared to forested and grassland ecosystems investigating effects of residue management on composition of earthworm populations, in particular with regard to native and exotic population distributions.

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).

Introduction:

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 (Callaham 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 with CT in fields earthworm movement might be physically restricted due to barriers created as a result of tillage. The extent of restriction depends on the frequency of tillage, plow depth and amount of crop residue returned to soils (Eriksen-Hamel et al., 2009). Frequent tillage can cause physical harm to earthworms, whereas deep tillage can destroy burrows, causing earthworms to spend energy rebuilding soil structures instead of reproducing, resulting in a decrease in density (Eriksen-Hamel et al., 2009). 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. Some common native earthworm families found in North America are *Megascolecidae* (common genera: *Diplocardia* and *Argilophilus), Lumbricidae (*common genera: *Bimastos* and *Eisenoides),* and *Sparganophilidae* (common genera: *Sparganophilus)* (Kalisz and Wood, 1995). *Diplocardia meansi* (Gates) and *Diplocardia sylvicola* (Gates) are two earthworm species that are endemic to Arkansas (Robison and Allen, 1995). The most common exotic earthworm family found in North America is *Lumbricidae* (common genera: *Apporectodea, Eisenia, Eiseniella, Lumbricus*, and *Octolasion*) (Kalisz and Wood, 1995).

Previous research shows that native and exotic earthworms interact in a number of ways, primarily competing for soil nutrients. 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), but that 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 co-exist with differences in residue management in row crop systems.

Brye et al. (2007) showed that, in a wheat (*Triticum aestivum* L.)-soybean (*Glycine max* (L.) Merr.) double-crop production system, several soil properties could be improved when no till (NT) is used rather than CT and when residues are left on the soil surface (unburned) rather than burned after harvest. Specifically, soil organic matter (OM), total nitrogen and carbon and extractable Zn increased more under NT than CT over a two-year study period in collection of surface 10 cm of soil (Brye et al., 2007).

Null Hypothesis:

There are no significant differences in densities and diversity of earthworm species after seven years of different residue management treatments in a wheat-soybean double-crop system. Residue was managed through tillage: conventional tillage (CT) versus no till (NT); fertilization of wheat (to produce HIGH versus LOW amount of wheat residue); and burning (BURN versus NO BURN).

Objectives:

The goal of this research is to determine if seven consecutive years of consistent residue management treatment combinations (CT versus NT; HIGH amount versus LOW amount of wheat residue; and burning (BURN) versus not burning (NO BURN) residue) alter total density and/or the diversity of earthworm species.

Methods:

The field experiment evaluating residue management practices in a wheat-soybean double-crop system was established at the Lon Mann Cotton Research Station in the spring 2002 in Mariana, Arkansas as described in Brye et al. (2007). Plots (48, 3 x 6 m^2) have been managed each year according to the original timeline as briefly described below. A representation of the layout of the plot plan is shown in Figure 1. 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^{-1}, 22.5 kg P ha^{-1}, 56 kg K ha^{-1} and 1120 kg ha^{-1} 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^{-1} as urea in early March beginning in 2002, while the HIGH plots were fertilized with an additional 101 kg N ha^{-1} broadcast application of urea in late March for the first 3 years. After the first 3 years, LOW plots received 0 kg N ha^{-1} and HIGH plots received 101 kg N ha^{-1}. 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 dig-and-sort method from a randomly located 30 x 30 x 20 cm^3 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 to kill, 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 an identification key (Dindal, 1990) and other relevant taxonomic keys (Causey, 1958; Eisen, 1899; Gates and Reynolds, 1968).

In June 2009, within two weeks prior to soybean planting, soil was combined from 10 cores collected from the surface 10 cm from each plot and a portion was analyzed as described in Verkler et al. (2009). Dried (70 °C for 48 hours) and sieved (2-mm mesh screen) soil samples were used to measure pH and electrical conductivity (EC) potentiometrically in a 1:2 (w/v) soil-to-water solution, and total carbon (TC) and total nitrogen (TN) concentrations were determined by high-temperature combustion (LECO CN-2000, St. Joseph, MI). Mehlich-III extractable soil P, K, Ca, Mg, S, Na, Fe, Mn, Zn, Cu, and B concentrations ((1:10 (w/v) soil-to-extract ratio) were determined by inductively coupled argon-plasma spectrophotometry (ICAP; CIROS CCD model, Spectro Analytical Instruments, MA).

A Poisson distribution was assumed for the earthworm counts, which were log transformed, and analyzed by ANOVA 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® Version 9.4 (SAS Institute, 2013.). Pearson product moment correlation analysis was conducted for total, juvenile and native earthworm density with each of the fifteen measured soil properties (SigmaPlot 13.0, Systat Software, Inc., 2014).

Results:

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 in unburned (NO BURN) with low residue level (LOW). In contrast, in the NT, the significantly highest total and juvenile densities were in the HIGH residue, BURN treatment (Tables 1 and 2).

Total density was significantly lowest in the NO BURN, HIGH residue compared to the other treatments (Table 1), while juvenile densities were similar across the two LOW residue level and the HIGH residue, NO BURN treatments (Table 2). In all but three of the twenty-four plots, juveniles were more abundant than adults (data not shown). Likewise, in all treatments, juvenile earthworms accounted for more than half (56 - 74%) of the mean total earthworm densities (Table 1 and 2).

Only two species, one native and one exotic, were identified among the adult earthworms from the twenty-four plots. All native earthworms were identified in the species *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 the native species was found in greater density than the exotic species, in all treatments, statistical analysis was conducted on native earthworm densities. The three-way interaction among treatments (tillage x burn x residue level) had a significant effect on native earthworm density ($P < 0.0001$; Table 3). Similar to total and juvenile earthworm density, the effect of burning and 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 regardless of burn treatment. The effect of burning and 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 and similar to NO BURN and LOW residue level treatments. Lower abundances were obtained in both NO BURN, HIGH residue level and BURN, LOW residue treatments.

Several relationships between specific soil properties and earthworm densities in specific treatments were found to be significant (Table 4). All significant relationships between soil property and earthworm density in specific treatments were inversely related. No significant relationships were found between TC, TN, LOI, or Mehlich-III- Na, Fe, Zn, or B and earthworm densities.

Discussion:

In agriculturally managed row crop systems, one might expect to find a relatively low number of species (Callaham et al., 2006). In a previous study performed in Southern Piedmont soils, four different earthworm species, two native and two exotic, (*Ap. trapezoides*, *Ap. caligonosa* (Savigny), *Bimastos tumidus* (Eisen), and *B. longicinctus* (Smtih and Gittins)) were found in samples from cultivated soils under CT used to produce wheat, sorghum, and corn (Callaham et al., 2006). In another study performed at a managed tall grass prairie in eastern Kansas, two exotic species (*Ap. trapezoides* and *Octolasion* spp.) and one native species (*Diplocardia* spp.) were present (Callaham et al., 2003). In yet another study performed at a five-crop rotation location in Germany which employed three different tillage types (ploughing, two-layer ploughing, and layer cultivation), eight different species were found (Emmerling, 2001). Two species appears to be somewhat low compared to other managed systems.

In addition to impacting species number, cultivation may provide a competitive advantage for exotic species. In the midwestern and southeastern 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 generally assumed that this is due to the exotic species' ability to tolerate disturbances such as disturbed soils from tilling or fertilization to a greater extent than the native species (Callaham et al., 2006). In this study, the native species had higher densities in all treatments. This is possibly because exotic earthworms are not surviving to adulthood rather than not being present, although species were not distinguishable among juvenile earthworms due to the lack of clitellum, the location of which is vital to identifying earthworms.

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 south-west England. Several species of *Lumbricus*, *Allolobophora*, and *Aporrectodea* were found in that study (Spurgeon & Hopkin, 1999). A potential reason for the juveniles being more numerous than adults at the time of sampling in March is that *Ap. trapezoides* and *D. 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). Juvenile earthworm density was high in the winter and late spring in tall fescue fields in Fayetteville, AR (Rashé and Savin, 2007).

Iordache and Borza (2010) showed that inorganic fertilizers potentially influenced the reproduction rate of earthworms, so it is also possible that the N fertilization used in this study affected the reproduction rate of the earthworms sampled. 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). The fact that most of the adults were a native species could be attributed to exotic abundances being skewed by either sampling time, method of collection, or lack of survival of exotic species to adulthood and reproductive age.

Many studies indicate that earthworm populations are larger in NT than other tillage systems (Eriksen-Hamel et al., 2009; Ernst and Emmerling, 2009). While the NT and CT results of earthworm abundance were different in this study, earthworms were not more abundant in NT treatment combinations. This is possibly due to the fact that before this study, the location was cropped under CT methods, potentially leaving a legacy effect; however, residue management treatments had been in place for seven years.

Several studies indicate BURN or NO BURN treatments may affect earthworm densities or species composition. Callaham et al. (2003) found that NO BURN treatments increased total invertebrate density and biomass, and, in combination with mowing and fertilization treatments, altered the proportions of native to exotic earthworm species densities. For example, BURN, mowing, and N addition combinations increased relative density of *Diplocardia*, a native genera, to more than 75%, but in NO BURN plots, *Diplocardia* biomass was highly variable (Callaham et al., 2003). This is likely due to the fact that before European settlement, soils were burned frequently with aboveground biomass removal (Callaham et al., 2003).

Several studies have been done to relate earthworm density and species composition to specific soil properties. Eriksen-Hamel et al. (2009) revealed that earthworm growth rate is positively affected by increased soil organic carbon; however, other soil properties had little effect on earthworm growth rates. Similarly, De Oliveira et al. (2012) also showed that earthworm densities increased with increased soil organic carbon. A study by Iordache and Borza (2010) found that humus, TN, and phosphorus had the greatest positive effect on earthworm density and biomass, whereas lower pH actually decreased earthworm density and biomass. It is clear that there is no one clear-cut relationship between earthworm density and species composition and any one soil property, so it is difficult to tell why certain soil properties measured in this study were inversely related to earthworm density per treatment type, while others such as TC and TN were not related to earthworm densities. It is also important to note that soils were sampled in June after earthworm sampling in March, so there were temporal differences between the two samples that could have affected these results.

Summary and Conclusion

Earthworms were collected in March 2009 from non-irrigated plots in a wheat-soybean double-crop system subjected to conventional tillage (CT) or no till (NT), burning (BURN) or leaving (NO BURN) wheat residue to decompose on the soil surface, and 101 kg urea-N ha^{-1} fertilization (LOW) or 202 kg urea-N ha^{-1} (HIGH) for the first 3 years and 0 kg urea-N ha^{-1} (LOW) or 101 kg urea-N ha^{-1} (HIGH) thereafter to produce low or high residue amount, respectively, at the Lon

Mann Cotton Research Station in eastern Arkansas. Adult earthworms were identified to species. Native earthworms (*D. sylvicola*) outnumbered an exotic species (*Ap. trapezoides*) in all treatments. Juveniles outnumbered adult earthworms in the majority of plots at the March sampling. Juvenile, native, and total earthworm densities resulted in significant differences among the eight treatment combinations. Earthworms were abundant in a wheat-soybean double-crop system in Arkansas with total abundance ranging from 271 – 508 m^{-2}, and 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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Table 1. Mean total earthworm densities (# m^{-2} ± 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**
Burn	271 (50)d[†]	428 (79)bc	**Burn**	497 (92)a	281 (52)bc
No Burn	508 (94)ab	320 (59)cd	**No Burn**	275 (51)c	325 (60)b

[§]For the first 3 years, 101 kg N ha^{-1} were applied to the LOW residue plots and 202 kg N ha^{-1} were applied to the HIGH residue plots. After the first 3 years, 0 kg N ha^{-1} were applied to the LOW residue plots and 101 kg N ha^{-1} were 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 2. Mean juvenile earthworm densities (# m^{-2} ± 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**
Burn	181 (36)b[†]	271 (54)a	**Burn**	301 (59)a	200 (40)b
No Burn	371 (73)a	176 (35)b	**No Burn**	195 (39)b	217 (43)ab

[§]For the first 3 years, 101 kg N ha^{-1} were applied to the LOW residue plots and 202 kg N ha^{-1} were applied to the HIGH residue plots. After the first 3 years, 0 kg N ha^{-1} were applied to the LOW residue plots and 101 kg N ha^{-1} were 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^{-2} ± 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**
Burn	84 (25)b[†]	152 (45)a	**Burn**	185 (55)a	80 (24)c
No Burn	86 (26)b	106 (32)a	**No Burn**	71 (21)c	98 (29)a

[§]For the first 3 years, 101 kg N ha^{-1} were applied to the LOW residue plots and 202 kg N ha^{-1} were applied to the HIGH residue plots. After the first 3 years, 0 kg N ha^{-1} were applied to the LOW residue plots and 101 kg N ha^{-1} were 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, and there were no significant differences between burn treatments.

Table 4. Significant correlations between specific soil properties and total, juvenile or native earthworm density per treatment (n = 12). All soil properties were inversely related to earthworm density. Earthworms were sampled in March 2009 and soils were sampled from the surface 10 cm in June 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.

Soil property	Earthworm density	Treatment	r value	P value
pH	Native	NT	-0.678	0.015
Electrical conductivity	Juvenile	BURN	-0.612	0.035
	Juvenile	LOW	-0.586	0.045
Mehlich –III K	Total	NO BURN	-0.665	0.018
	Juvenile	NO BURN	-0.631	0.028
	Native	NT	-0.716	0.009
	Native	HIGH	-0.683	0.014
Mehlich –III Ca	Native	NT	-0.673	0.016
Mehlich –III Mg	Native	NT	-0.581	0.048
Mehlich –III S	Native	NT	-0.669	0.017
Mehlich –III Mn	Native	NT	-0.578	0.049
	Native	HIGH	-0.597	0.040
Mehlich –III Cu	Native	HIGH	-0.777	0.003

§For the first 3 years, 101 kg N ha^{-1} were applied to the LOW residue plots and 202 kg N ha^{-1} were applied to the HIGH residue plots. After the first 3 years, 0 kg N ha^{-1} were applied to the LOW residue plots and 101 kg N ha^{-1} were applied to the HIGH residue plots

Figure 1. Representation of the plot plan of 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.

		BURN ↓			NO BURN ↓	
Block 1	CT →	HIGH	LOW		HIGH	LOW
	NT →	HIGH	LOW		LOW	HIGH
Block 2	CT →	HIGH	LOW		HIGH	LOW
	NT →	LOW	HIGH		HIGH	LOW
Block 3	CT →	HIGH	LOW		LOW	HIGH
	NT →	LOW	HIGH		HIGH	LOW

§For the first 3 years, 101 kg N ha^{-1} were applied to the LOW residue plots and 202 kg N ha^{-1} were applied to the HIGH residue plots. After the first 3 years, 0 kg N ha^{-1} were applied to the LOW residue plots and 101 kg N ha^{-1} were applied to the HIGH residue plots