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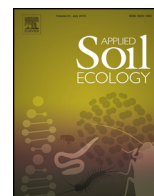
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Spatial Distribution of Earthworms in an East Texas Forest Ecosystem



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ABSTRACT

Earthworms were collected and identified in different ecological habitats of the Stephen F. Austin Experimental Forest (SFAEF) in the Piney Woods Ecoregion (PWE) of Texas. Earthworm spatial distribution data were collected over four distinct ecological habitats with a range of soil conditions and vegetative cover. A total of 128 sampling plots were surveyed in two different, broadly defined locations (mesic slope = 68 plots, dry-mesic upland = 60 plots). Using multivariate classification/ordination (TWINSPAN) and detrended correspondence analysis (DCA) of overstorey vegetation data, these two locations were further divided into four distinct habitats: dry-mesic mixed upland, transitional zone, mesic slope and wet forested seeps. By using TWINSPAN and principal component analysis (PCA), it was found that earthworm species assemblages and understory vegetation corresponded to these discrete ecological habitats. ArcGIS10 was then used to create spatial distribution maps of earthworm species throughout these delineated ecological habitats in the SFAEF. Six earthworm species from the native North American genus *Diplocardia* were collected at SFAEF: *D. caroliniana* Eisen (1899), *D. eiseni* Michaelsen (1894), *D. komareki* Gates (1977); *D. macdowellii* Murchie (1963), *D. mississippiensis* Smith (1924) and *D. ornata* Gates (1943). Two earthworm species from the native North American genus *Bimastos* were collected: *B. heimbürgeri* Smith 1928 and *B. longicinctus* Smith and Gittins 1915. The collection of *B. longicinctus* is a new state record for Texas. *Amyntas corticis* Kinberg (1867) specimens were collected, which represent a non-native genus from Southeast Asia. Ocneroдрilidae, a megadrile family first described by Beddard in 1891, were also collected likely representing non-native species originating from intertropical America and Africa (Christoffersen, 2008).

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1. Introduction

The spatial distribution of earthworms within ecosystems in the United States has been under researched in comparison to others regions of the world. While studies in Canada, Colombia and France have examined the spatial distribution of earthworms, little research has been done in the United States and particularly in the Piney Woods Ecoregion (PWE) of Texas. In recent decades earthworms have been identified as ecosystem engineers because of their ability to have long-term impacts on physical, chemical and biological soil properties (Lavelle, 2000).

While it is obvious that the actions of non-native earthworms may generally benefit soils in a manner similar to native earthworm species, from an ecosystem perspective non-native species may have an overall adverse effect on soil hydrology, food webs, seed bed dynamics and other key features either through increased activity or altering different soil characteristics. For

example, research in the Great Lakes Region of North America revealed that non-native earthworms altered hardwood forest ecosystems by reducing organic soil horizons causing a decrease in understory plant species and seedling densities (Hale et al., 2002).

Interactions between earthworms and their ecological habitat are poorly understood because of the difficult nature of studying belowground organisms. Spatial distribution mapping provides a visual representation of the information that correlates the environmental factors with earthworm species. Once these relationships are established, then above-ground plant community mapping can be used to predict below-ground earthworm communities and their potential effects on the soil. Whalen (2004) found that earthworm populations in a Canadian forest were spatially more stable than those in nearby agroecosystems and that variation in plant species and plant litter may influence earthworm spatial and temporal distribution in temperate regions. On a chalky slope of the Seine Valley of Upper Normandy, France, Margerie et al. (2001) found a significant spatial structure associated with alternating patches of particular earthworm species assemblages, but the locations of these patches did not

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seem to be clearly associated with specific vegetation. Thus these authors concluded that other factors must be influencing the earthworm communities in their study area. Many abiotic factors can affect distribution such as soil structural aggregation, porosity, nutrient runoff and leaching, decomposition and microbial activity (Bohlen et al., 1997; Ketterings et al., 1997). In a neotropical gallery forest in Colombia, it was found that soil heterogeneity contributed to the formation of population patches for some earthworm species. This variability of suitable sites, identified as resource availability patchiness, exerted an influence in the spatial distribution of earthworms (Jiménez et al., 2011). Understanding the spatial distribution of earthworm populations could facilitate predictions of specific earthworm species that affect processes such as organic matter decomposition, nutrient cycling and plant production (Whalen, 2004). The information gathered on earthworm diversity and densities through different ecological habitats may provide useful insight on the productivity and health of forest ecosystems. Earthworms and their ecology are an increasingly important research topic around the world, yet there remain many regions that are understudied, including the PWE where there are few spatial surveys and species descriptions. This study investigated the spatial distribution of earthworms within distinct ecological habitats in the Stephen F. Austin Experimental Forest (SFAEF), attempting to delineate associations between earthworm species, vegetative cover types and soil properties. Visual inspection of species distribution maps furthers understanding of species interaction and niche differentiation and increases insight into the role of earthworm communities in forested ecosystems.

2. Materials and methods

2.1. Sampling design

Sampling transects containing a total of 32 sites were set up in two broadly defined locations: a mesic slope and a dry-mesic mixed upland. A nearby underground utility pipeline and a service road were each used as reference points from which to create transects. At the mesic slope location, a set of three transects were located at distances of 80 m, 100 m and 120 m from the pipeline and at least 50 m away from areas disturbed by vehicle traffic. Each transect contained center points that were placed 20 m apart. A total of 17 center points were surveyed through a range of three ecological habitats: the transitional zone (TZ) at the top of the slope was covered in dense vegetation of *Ilex vomitoria* (yaupon), the lower mesic slope (MS) at the middle of the slope had a much more open canopy composed of a variety of large upland *Quercus* sp. (oaks) and the wet forested seeps (FS) at the bottom of the slope contained a mix of vegetation that is known to prefer wet or boggy soils, which included overstory species such as *Magnolia virginiana* (sweetbay magnolia) and *Acer rubrum* (red maple). An additional set of three transects containing a total of 15 center points also placed 20 m apart were surveyed in a dry-mesic mixed upland area located 200 m northeast of the mesic slope location. These sites represented the ecological habitat classified as dry-mesic mixed hardwood-pine upland (DU) having the highest elevation of all sites, and dominated by *Pinus taeda* (loblolly pine) and *Pinus echinata* (shortleaf pine).

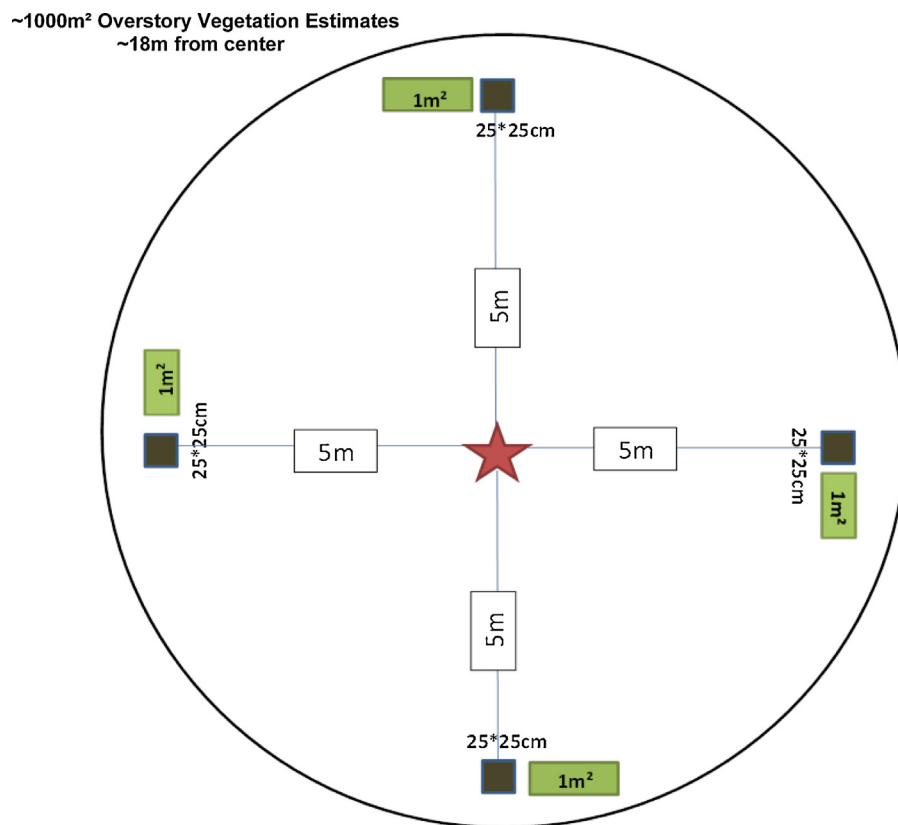


Fig. 1. A schematic diagram of sampling scheme. The star at the center represents the center point. The squares represent soil pits, while the rectangles represent understory cover plots. The circle surrounding the figure represents the area where overstory vegetation cover was estimated. *This image is not to scale.

2.2. Vegetation sampling

The overstory vegetation species and percent cover was recorded at all 32 center points at 18 m from the center plot for ~1000 m² estimates. Overstory plants were identified by having a height greater than 1 m and a diameter at breast height (DBH) greater than 10 cm. Soil pits and corresponding understory vegetation plots were sampled in each cardinal direction 5 m from each center point (Fig. 1). Understory plants included herbaceous non-woody species and small saplings with heights

less than 100 cm. The understory plant cover plots were immediately adjacent to the corresponding soil pit with dimensions of 1 m by 1 m (Fig. 1). There were a total of 128 sample sites, 68 for the mesic slope location and 60 for the dry-mesic mixed upland.

2.3. Soil sampling/analysis

The dimensions of the soil pits were 25 cm by 25 cm, dug by shovel to a depth of 30 cm. At each sample pit, the soil was placed

Table 1

An ordered two-way Table from TWINSpan showing classification of species and samples based on estimated percentage cover of overstory trees from 32 center points from SFAEF, Nacogdoches, Texas.

	TZ Sites		MS Sites		FS Sites		DU Sites	
<i>Quercus marilandica</i>	---	-----	-----		432222441	-----		
<i>Quercus sinuata</i>	---	-----	-----				2-	
<i>Pinus echinata</i>	---	-----	-----		435433443545533			
<i>Quercus stellata</i>	---	-----	-----				23---	2-
<i>Carya texana</i>	---	-----	-----		342--	1-	2122231-	
<i>Magnolia virginiana</i>	---	-----	53-33					
<i>Quercus michauxii</i>	---	-----1	---	242				
<i>Quercus laurifolia</i>	---	-----	---	534				
<i>Acer rubrum</i>	---	-----	5-	311				
<i>Pinus taeda</i>	-22	238331-4-	47755	323454544333355				
<i>Quercus falcata</i>	-31	--35365--	-----	-42333223334223				
<i>Liquidambar styraciflua</i>	---	7-25322--	43255	23--2111222-222				
<i>Ulmus alata</i>	432	3----4543	---	2-	122-12211-121--			
<i>Ilex opaca</i>	2--	-----2321	-----					
<i>Acer barbatum</i>	-1-	-----11---	-----					
<i>Celtis laevigata</i>	---	-----2-2--	-----					
<i>Morus rubra</i>	---	-----1--	-----					
<i>Carya alba</i>	---	---22-424	-----					
<i>Populus nigra</i>	---	-----1	-----					
<i>Quercus alba</i>	2-2	322771375	-3-32	-----				
<i>Nyssa sylvatica</i>	-21	53432----	-----					
<i>Prunus serotina</i>	--1	--33-----	1----	-----				
<i>Quercus nigra</i>	---	2-5-----1	---	2-	-----			
<i>Ilex vomitoria</i>	966	---2-2-2	-----					
<i>Sassafras albidum</i>	3--	-----	-----					
<i>Morella cerifera</i>	2-3	-----	-----					
<i>Ligustrum sinense</i>	23	-----	-----					

on a tarp and hand-sorted for earthworms (James, 1996). For each sample site, two soil samples were taken in the A-horizon: one for the physical analyses of soil moisture and texture and the other for chemical analyses of plant available soil nutrients. In addition, GPS coordinates were also taken at each sample pit location.

Soil samples from each of the 128 sample locations were analyzed for: moisture at the time of sampling, texture, pH, electrical conductivity, macronutrients, micronutrients, total carbon and total nitrogen. Soil moisture was determined by using the gravimetric method (Gardner, 1965) and soil texture was determined by the Bouyoucos method (Bouyoucos, 1962). Electrical conductivity and pH and were measured by appropriate probes in a 2:1 soil to water ratio (Thomas et al., 1996). Mehlich III extraction (Mehlich, 1984) was used to assess availability of phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg) and boron (B). The DTPA extraction method (Lindsay and Norvell, 1978) was used to determine availability of iron (Fe), zinc (Zn), copper (Cu) and manganese (Mn). All these elemental concentrations were determined using inductively coupled emission spectroscopy (ICP) (Sparks et al., 1996). Soil carbon (C) and nitrogen (N) were determined using a CN combustion analyzer (model Macro manufactured by Elementar Americas). Sample size for the CN analyzer was approximately 300 mg of soil, which was combusted with ultrapure oxygen (O₂) in a stream of helium (He) gas at a temperature of 960 °C with a weight adjusted concentration determined by a thermal conductivity detector (Yeomans and Bremner, 1991). These values were then used to calculate C/N ratios.

2.4. Earthworm preservation

Earthworms collected in the field were fixed and preserved by a regimen described in James (1990) and stored in 70% ethanol. Lumbricid specimens were identified to the species using external characteristics. Diplocardian specimens can only be identified to species following dissection and examination of internal morphology. Taxonomic determinations were made for diplocardian specimens using keys by Gates (1977) and James (1990), for lumbricids by Reynolds (1972) and Schwert (1990) and for ocerodrilids by Gates (1972).

Table 2

An ordered two-way Table from TWINSpan showing classification of species and samples based on presence of nine earthworm species, along with *Diplocardia* juveniles and ocerodrilid specimens at 128 sample sites in SFAEF, Nacogdoches, Texas. EW Group 1 were found in DU and TZ Sites, EW Group 2 were found MS sites, EW Group 3 were found in FS sites, EW. Group 4 contained no sites without earthworms presence.

	EW Group 1	EW Group 2	EW Group 3	EW Group 4
<i>D. mac.</i>	11-1222222222242421124131213132225333244-124222443653525234533	-----3-----	-----	-----
<i>D. caro.</i>	-----1-111-----2--2-----1-----	-----2-----	-----	-----
<i>D. ornata</i>	2111-----1--1-----12--11--11112-23--2-2--2-	-----3-1-----1-2--	-----	-----
<i>D. juvs.</i>	-----11111-1212-1-----2--2-11222221-1222455242-2	93763384412331322222344222111131341-	-----46--5272-----	-----
<i>B. long.</i>	-----1-1-----1-1-111---13241511122212333322--1----	-----2-----	-----1-----	-----
<i>D. kom.</i>	--1-1-----1-1-111---13241511122212333322--1----	-37645443--1-----322232-11-----21121-	-41657645253411142111	-----
<i>A. cort.</i>	-----11-----	-----2-----2-2-----2	1--1-222-1-----	-----
<i>D. eiseni</i>	-----	-----2-2-----	--52-3--3-----	-----
<i>B. heim.</i>	-----	2--1-----	-----	-----
<i>D. miss.</i>	-----	-----	---44-342374-----	-----
Ocner.	-----	-----	-----2-----44--	-----
Absent	-----	-----	-----	1111111

Table 3

Results of ANOVA for environmental parameters and plant available soil nutrients that were found to be significant across the four earthworm TWINSpan groups (DF = 3).

	Mean-Square	Pr > F
B	0.19	0.0052
Ca	632.155	0.0008
Cu	0.05	0.0116
Fe	160.848	<0.0001
Mn	993.79	<0.0001
P	284.78	0.0023
Soil pH	1.05	0.0011
Earthworm abundance	314.38	0.0002
Soil moisture	637.31	0.0003

2.5. Data interpretation

Multivariate statistical software PC-ORD used TWINSpan to hierarchically classify species data. Ordination was used to reveal relationships between ecological communities by ordering species data according to similarity or dissimilarity. These procedures assisted in finding associations between the overstory tree populations, understory plant species, earthworm species, soils and environmental parameters.

TWINSpan classification and detrended correspondence analysis (DCA) analyses were run on overstory data, while TWINSpan classification and principal component analysis (PCA) analyses were run on all understory plants and earthworm species data. Each contained joint-plots of selected environmental factors. In addition, selected understory plant species were used as joint-plots on the earthworm PCA to better describe the understory plant community in relation to the described ecological habitats and earthworm species.

The typical ecological habitat (overstory tree population, understory plant species and soil conditions) which contained unique earthworm assemblages were then described. All environmental parameters and plant available soil nutrients for TWINSpan earthworm groups were analyzed using analysis of variance (ANOVA) and Duncan's multiple range test. Environmental factors

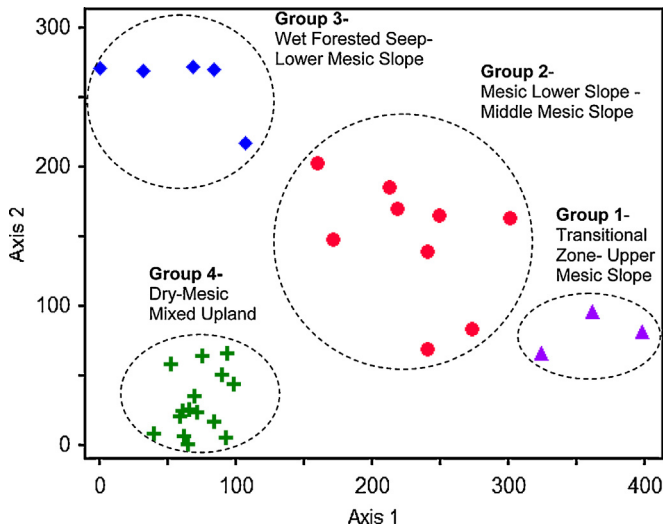


Fig. 2. A detrended correspondence analysis (DCA) ordination of 32 center points based on occurrence of 27 overstory tree species from the SFAEF, Nacogdoches, Texas. Dotted-lined circles represent cluster of sites derived from TWINSpan which form the basis of a community-type classification.

that had a significance of $p < 0.05$ and significantly different at the 0.05 alpha level were used in the final habitat descriptions. To visually portray these relationships, ArcGIS10 was used to create spatial distribution maps of the earthworm species found in the SFAEF.

3. Results

3.1. TWINSpan classification and DCA ordination of overstory vegetation

The overstory data collected at all 32 center points was classified using TWINSpan (Table 1): 60 sample sites around 15 center points were classified as dry-mesic mixed pine-hardwood upland (DU sites), 12 sample sites around 3 center points were classified as a transitional zone of a dry-mesic mixed uplands and lower mesic slopes sites (TZ sites), 36 sample sites around 9 center points were classified as lower mesic slope (MS sites) and the remaining 20 sample sites around 5 center points were classified as wet forested seeps (FS sites).

A DCA ordination (Fig. 2) analyzed overstory tree composition which identified distinct ecological habitat types previously classified by Diggs et al. (2006) and VanKley et al. (2007). The first axis of the DCA ordination (eigenvalue 0.5897) arranged the samples most consistently with TWINSpan overstory based classification: high scores given to the transitional zone (TZ) sites, intermediate values given to the dry-mesic mixed hardwood-pine upland (DU) and mesic lower slope (MS) sites and low values given to the wet forested seep (FS) sites. The second DCA axis had a lower eigenvalue (eigenvalue 0.3197). The second DCA axis was associated with the variation and diversity among mixed hardwood species composition. DU and TZ sites had the lowest scores, MS sites had intermediate scores, while the FS sites had the highest values. When comparing soil data with the ordering of samples, moving from the right side of axis one to the left, the

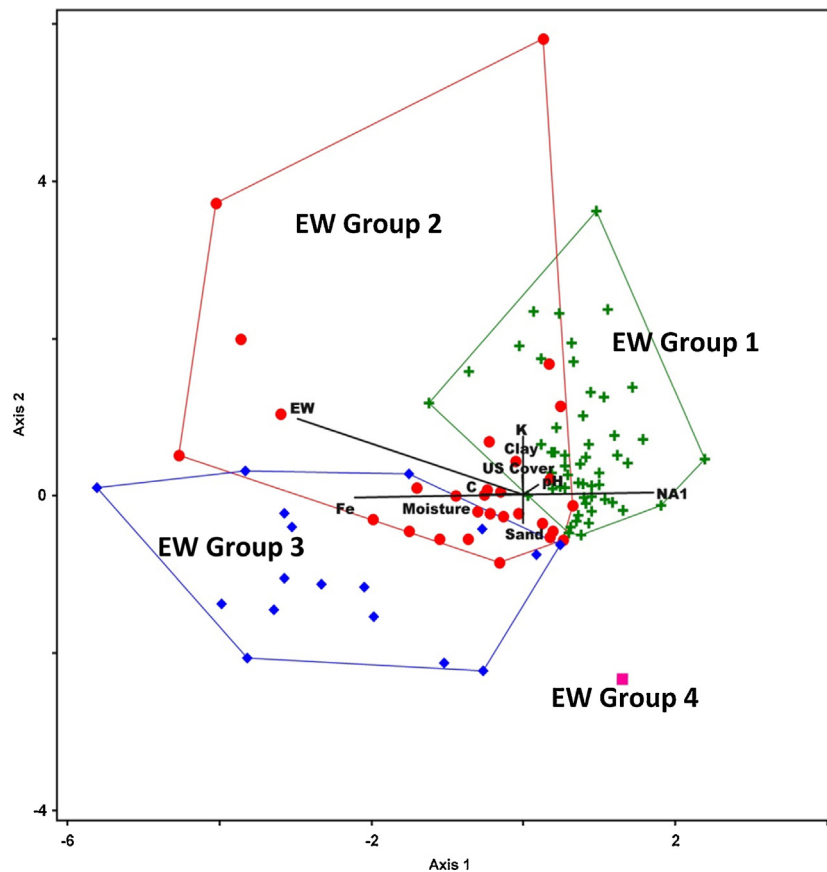


Fig. 3. A principal component analysis (PCA) ordination of 128 sample points based on presence of nine earthworm species, one earthworm family, and *Diplocardia* juveniles from sample locations in the SFAEF, Nacogdoches, Texas. Dotted-lined polygons represent clusters of sites derived from earthworm species TWINSpan groups which form the basis of a community-type classification. Joint-plot lines for environmental factors include: sand, clay, moisture, pH, K, C, NA1 (nutrient axis-1), Fe, understory plant cover (US Cover), and earthworm abundance (EW). The length of the joint-plot vector indicates the correlation with the ordination.

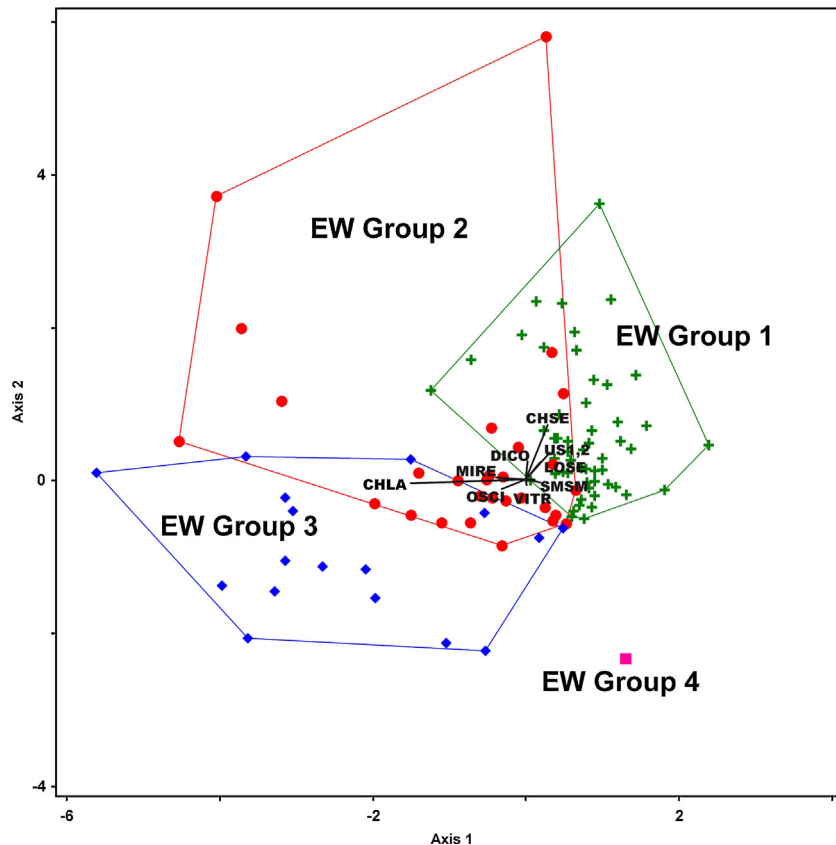


Fig. 4. A principal component analysis (PCA) ordination of 128 sample points based on presence of nine earthworm species, one earthworm family, and *Diplocardia* juveniles from sample locations in the SFAEF, Nacogdoches, Texas. Dotted-lined polygons represent cluster of sites derived from earthworm species TWINSpan groups which form the basis of a community-type classification. Joint-plot lines for understory plant species CHSE (*Chasmanthium sessiflorum*), LOSE (*Lonicera sempervirens*), SSM (*Smilax smallii*), VITR (*Viola triloba*), OSC (*Osmunda cinnamomea*), MIRE (*Mitchella repens*), CHLA (*Chasmanthium laxum*), DICO (*Dichantheium commutatum*) and understory PCA ordination scores for axis one and two (US 1, 2). The length of the joint-plot vector indicates the correlation with the ordination.

amount of sand and pH in the soil increased, while soil moisture levels decreased.

3.2. TWINSpan classification and PCA analysis of understory vegetation

A TWINSpan classification was performed on understory vegetation species along with a PCA analysis to see if understory species exhibited a similar trend to the overstory species. The data for percent cover of understory species data tended to have more plots overlap than overstory data, which may be due to some understory species being more sensitive to environmental conditions and gradients (Grace, 1999). Many understory plant species in this study were widespread on the landscape, while few species appeared to specialize in particular habitats. There appeared to be a trend, but nothing of note (data not shown). A joint-plot containing understory plant species was added to the earthworm species ordination and is described in more detail in the below section.

3.3. TWINSpan classification and PCA analysis of earthworm species

A TWINSpan classification was used to analyze earthworm species data. The abundance and distribution of the nine species of earthworms, *Diplocardia* juveniles and ocnerostrilid specimens were placed into a classification. TWINSpan created groups of

earthworm species that corresponded with overstory vegetation habitat classifications (Table 2).

The PCA ordination of earthworm species (EW) was used with a joint-plot of environmental conditions (Fig. 3) and understory plant species to explore potential understory plant and earthworm relationships (Fig. 4). The plant available soil nutrient trends were summarized by using the ordination scores of axis one from the PCA analysis. The nutrient ordination scores were used as joint-plots on other ordinations and are labeled as NA1 (nutrient axis one). Some of the understory plants added into the ordination may not have a statistically significant relationship but clearly exhibit some relationship and help describe the understory plant community. The first axis of PCA ordination (eigenvalue 2.162) arranged samples by high scores given to EW Group 1 which contained species found on the DU and TZ, intermediate values given to EW Group 2 that were found on the MS, and low values were given to EW Group 3 which were species found on the FS. The second PCA axis (eigenvalue 1.481) was associated with the variation and diversity among earthworm species composition. The TWINSpan groups in relation to axis two were in the same general order as axis one but with more overlap. An ANOVA and Duncan's test was performed on individual environmental parameters and plant available nutrients for TWINSpan earthworm groups. The parameters that were significantly different at the 0.05 alpha level are included as environmental factors in habitat descriptions (Table 3).

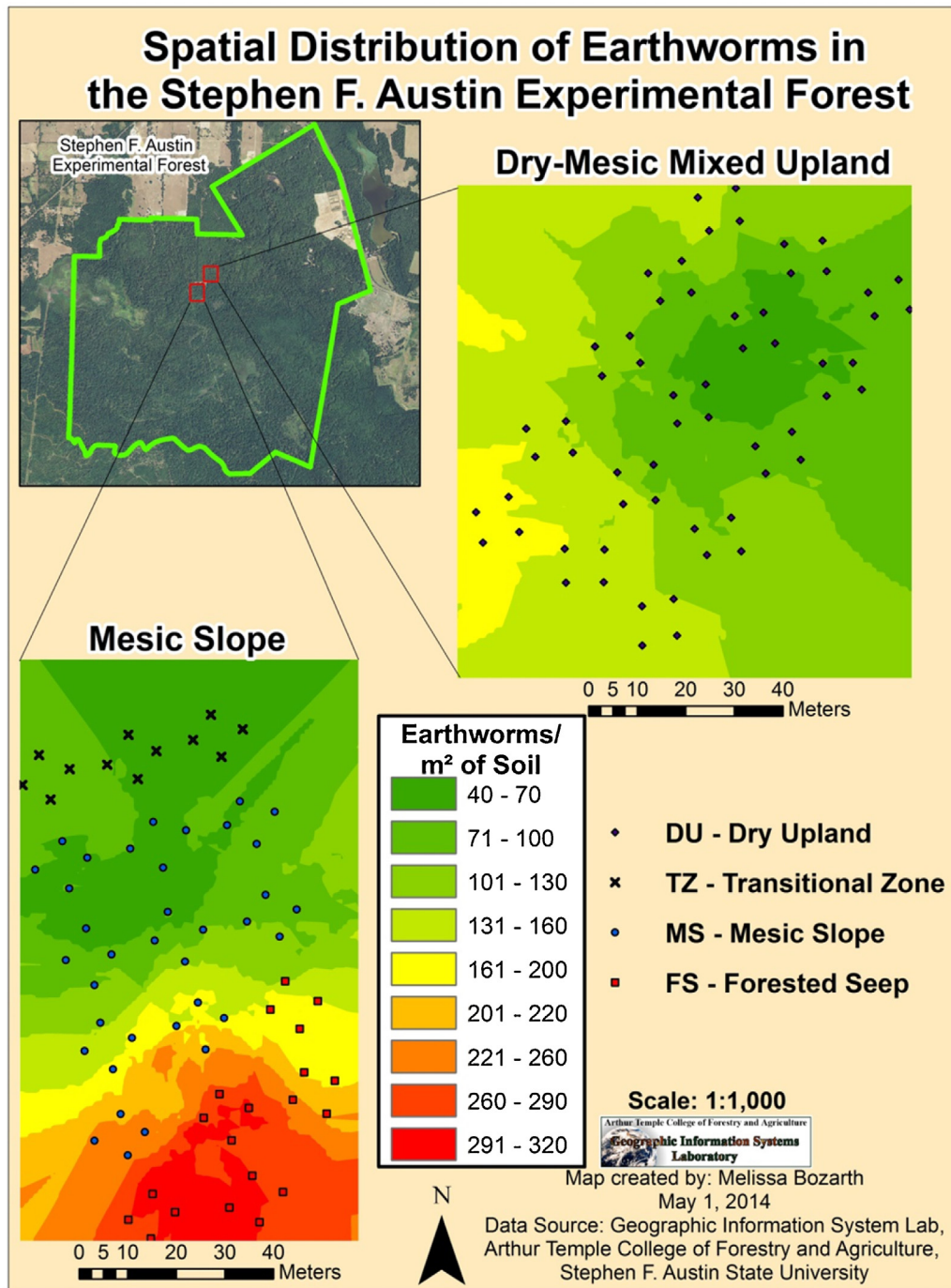


Fig. 5. Spatial distribution map of earthworm abundance in the SFAEF using ordinary kriging with variable search radius.

3.4. Habitat descriptions

The ordinations of overstory trees (Fig. 2) and earthworm communities with understory plant species (Figs. 3 and 4) revealed relationships between plant and earthworm communities. In addition, environmental factors from all of these ordinations helped describe environmental conditions present in these plant communities.

The DU and TZ habitats were dominated by the presence of *D. macdowellii*. In addition *D. ornata*, *D. caroliniana* and *B. longicinctus* were commonly found at these sites. As seen in Fig. 4, the understory had a higher presence of *Chasmanthium sessiliflorum* (longleaf woodoats) ($r = -0.548$, $p < 0.0001$), *Dichantheium*

commutatum (variable panic grass) ($r = 0.219$, $p < 0.05$), *Smilax smallii* (lanceleaf greenbrier) ($r = 0.196$, $p < 0.05$) and *Lonicera sempervirens* (coral honeysuckle) ($r = 0.151$, $p < 0.1$). Ordination scores from the understory PCA ordination correlated with axis two and confirmed that this habitat consists of upland vegetation ($r = 0.264, 0.216$, $p < 0.05$). Overstory vegetation in these areas consists of: *Pinus echinata* (shortleaf pine), *Pinus taeda* (loblolly pine), *Quercus falcata* (southern red oak) and *Ilex vomitoria* (yaupon). There were also numerous small *Ulmus alata* (winged elm) and *Liquidambar styraciflua* (sweetgum). Both the abundant and restricted earthworm species to this group can be described as upland species. Environmental factors of these sites include: lower soil moisture, Zn and Cu and higher pH, B, Ca, Mn and P.

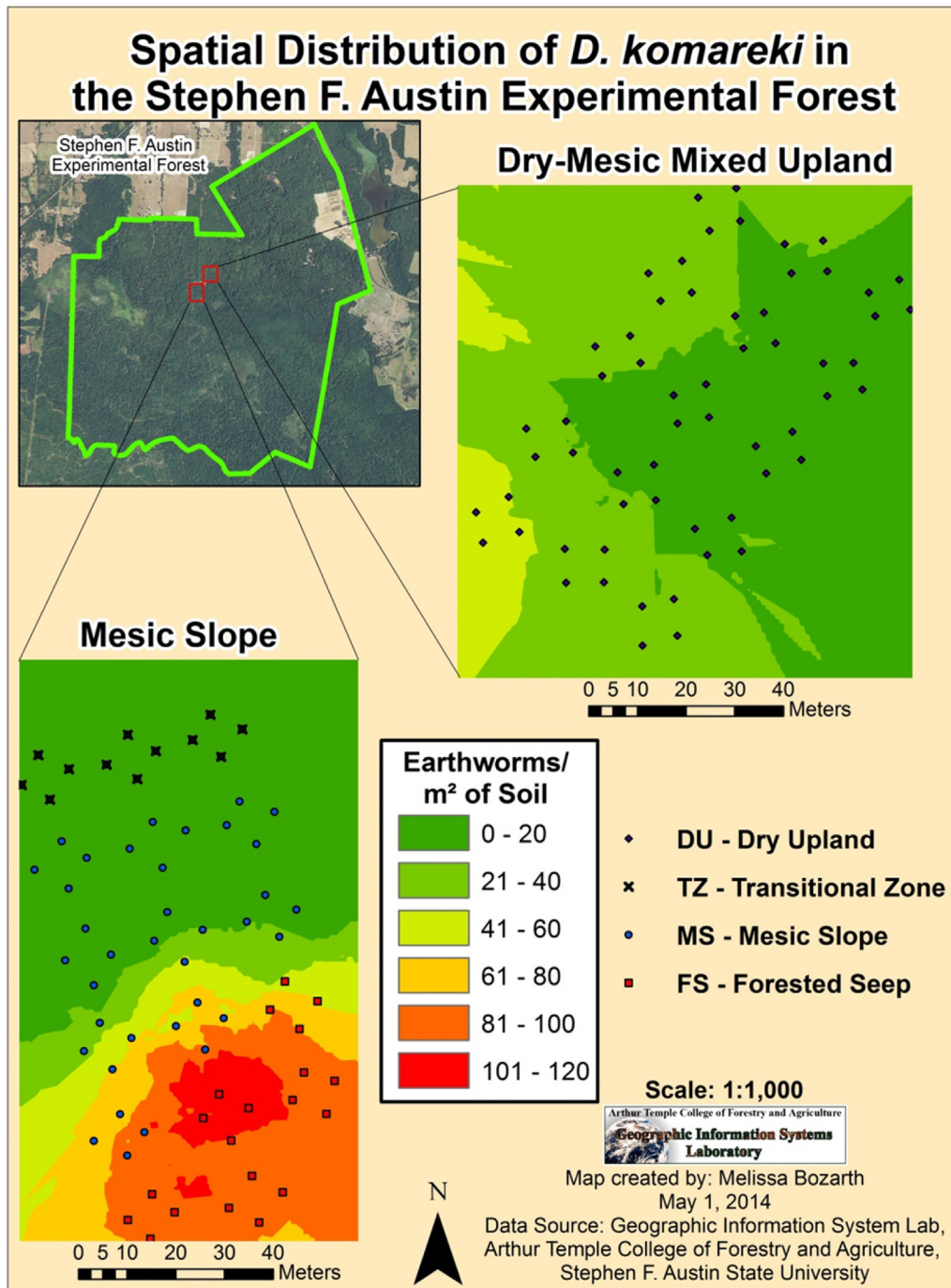


Fig. 6. Spatial distribution map of *Diplocardia komareki* species abundance in the SFAEF using ordinary kriging with variable search radius.

The MS habitat was dominated by *Diplocardia* juveniles. In addition, there were a low number of scattered EW individuals of: *B. heimbürgeri*, *D. caroliniana*, *D. macdowellii*, *D. ornata*. There was also a small presence of *D. komareki* and *A. corticis*. The earthworm species compositions in this group appear to be a transitional compilation of the DU habitat to the FS. As seen in Fig. 4, common understory species present at these sites were *Mitchella repens* (partridge berry) ($r = -0.277$, $p < 0.05$) and *Viola triloba* (three-lobbed violet) ($r = 0.125$, $p < 0.2$). Common overstory vegetation includes: *Quercus alba* (white oak), *Q. falcata* (southern red oak), *Carya alba* (mockernut hickory), *P. taeda* (loblolly pine), *L. styraciflua* (sweetgum) and *U. alata* (winged elm). Environmental

factors at these sites were intermediate compared to the other two TWINSpan groups (DU and FS).

The FS habitat consisted of higher number of *D. komareki*, *A. corticis* and *D. eiseni*. Furthermore, *D. mississippiensis* and ocnoderilid specimens were restricted to this area. As seen in Fig. 4, understory species indicators of these sites were *Chasmanthium laxum* (slender woodoats) ($r = -0.548$, $p < 0.0001$) and *Osmunda cinnamomea* (cinnamon fern) ($r = -0.252$, $p < 0.05$). Overstory vegetation that may indicate the presence of these earthworm species consisted of: *Magnolia virginiana* (sweetbay magnolia), *Quercus michauxii* (swamp chestnut), *Quercus laurifolia* (swamp laurel oak) and *Acer rubrum* (red maple). Environmental

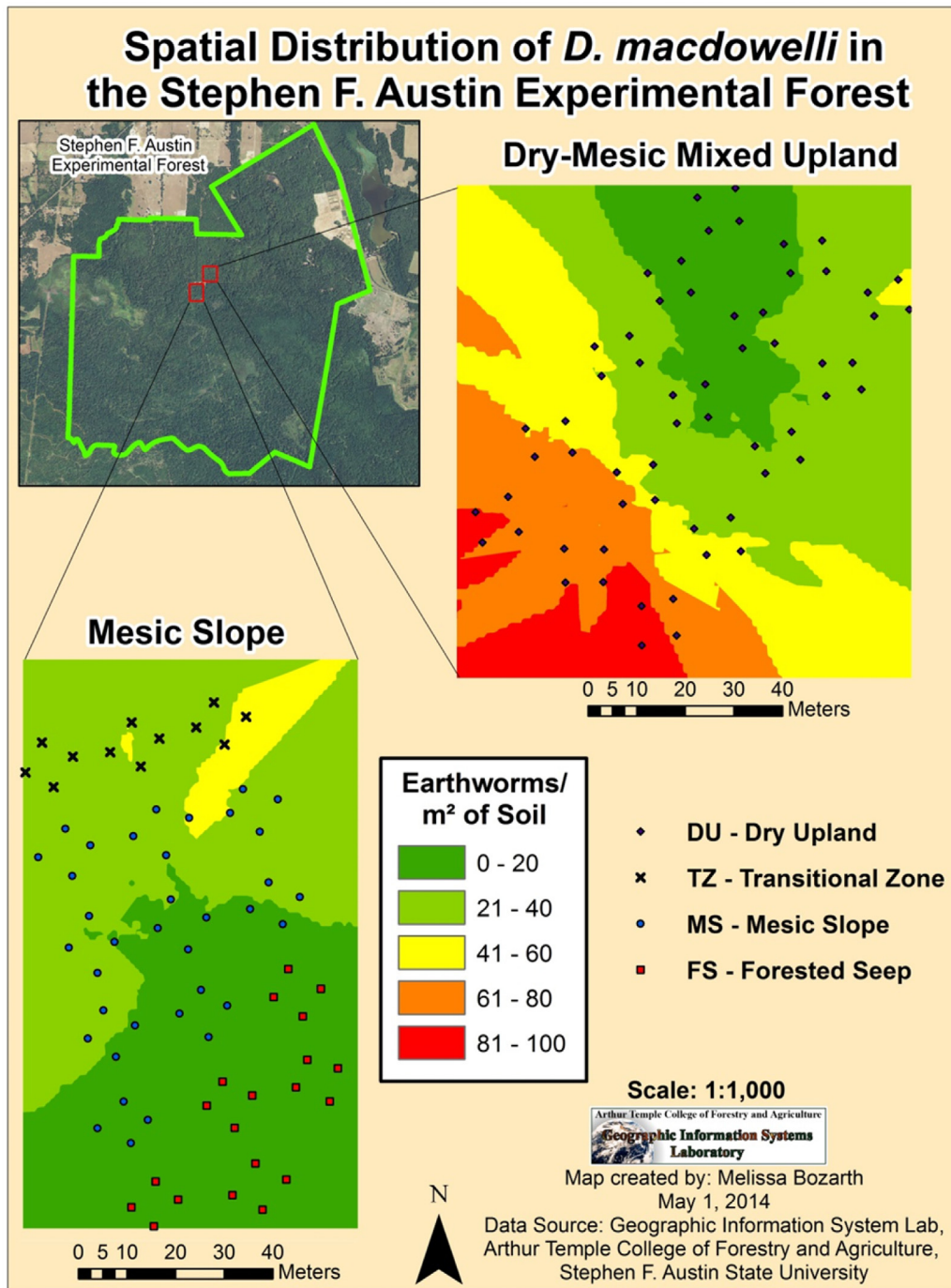


Fig. 7. Spatial distribution map of *Diplocardia macdowelli* species abundance in the SFAEF using ordinary kriging with variable search radius.

conditions of these sites include lower pH, B, Ca, Mn, and P and higher soil moisture, Cu and Fe.

3.5. Spatial distribution maps

The spatial distribution of earthworm species in the SFAEF were mapped using ArcGIS10. These spatial distribution maps help to visualize the distribution as well as the spatial relationships among earthworm species. The earthworm population density data was converted into earthworms per m² of soil by multiplying the number of specimens collected at each sample site by a factor of 16. The inputs for the Kriging analysis were determined by using semivariograms to find the model that best fits the data (lowest

root mean square error, RMSE) with the Geostatistical Analyst tool. Once the model was chosen, the semivariogram model, lag size, major range, partial sill and nugget values from the chosen models were input into the spatial analyst Kriging tool. For this study, the ordinary Kriging method and variable search radius was used on all earthworm density spatial interpolations.

A map of the distribution of earthworms was created, using data on the total amount of earthworms collected from each sample pit, to show distribution throughout the dry-mesic mixed upland and mesic slope (Fig. 5). Maps were created for the wide ranging species (existing in both dry-mesic mixed upland and mesic slope): *D. komareki* (Fig. 6), *D. macdowelli* (Fig. 7) and *D. ornata* (Fig. 8). An additional map displays the distribution of *B.*

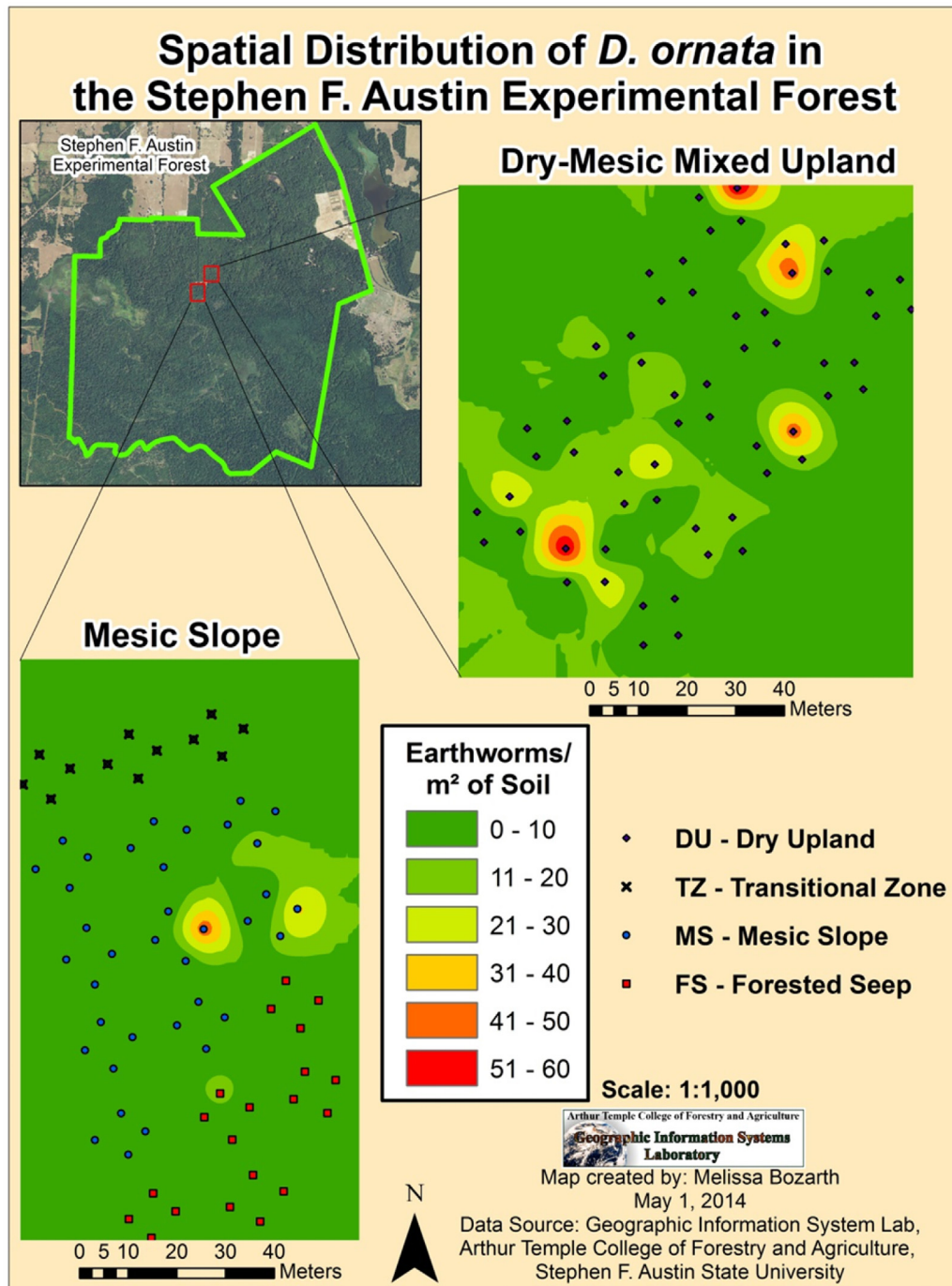


Fig. 8. Spatial distribution map of *Diplocardia ornata* species abundance in the SFAEF using ordinary kriging with variable search radius.

longicinctus in the dry-mesic mixed upland and *D. caroliniana* on the mesic slope for comparison (Fig. 9). A map displaying abundance of all the earthworm species that were collected on the lower mesic slope (wet forested seep) sites was created: *A. corticis*, *B. heimbürgeri*, *D. eiseni* and *D. mississippiensis* (Fig. 10). These maps can be examined separately to better understand the distribution of individual earthworm species or compared against each other to see if, and where, species coexist.

4. Discussion

This study confirms that the spatial distribution of earthworms can be influenced by plant cover, resulting in a horizontal mosaic of

areas with similar nutrient availability and microclimatic conditions (Lavelle, 1988). When Lavelle described this concept, earthworm communities were understood as a single entity. With the findings of this research, it appears that the same concept can be applied to specific populations or assemblages of earthworms. In some previous studies it has been shown that earthworm community distribution is irregular and aggregated in areas where resources and environmental conditions are relatively uniform (e.g. Guild, 1995; Satchell, 1955). While in mixed-species forests, a study by Boettcher and Kalisz (1990) found that the abundance and species composition of earthworm assemblages were spatially related to specific soil properties and the occurrence of particular overstory trees and understory plants. This study expands on the

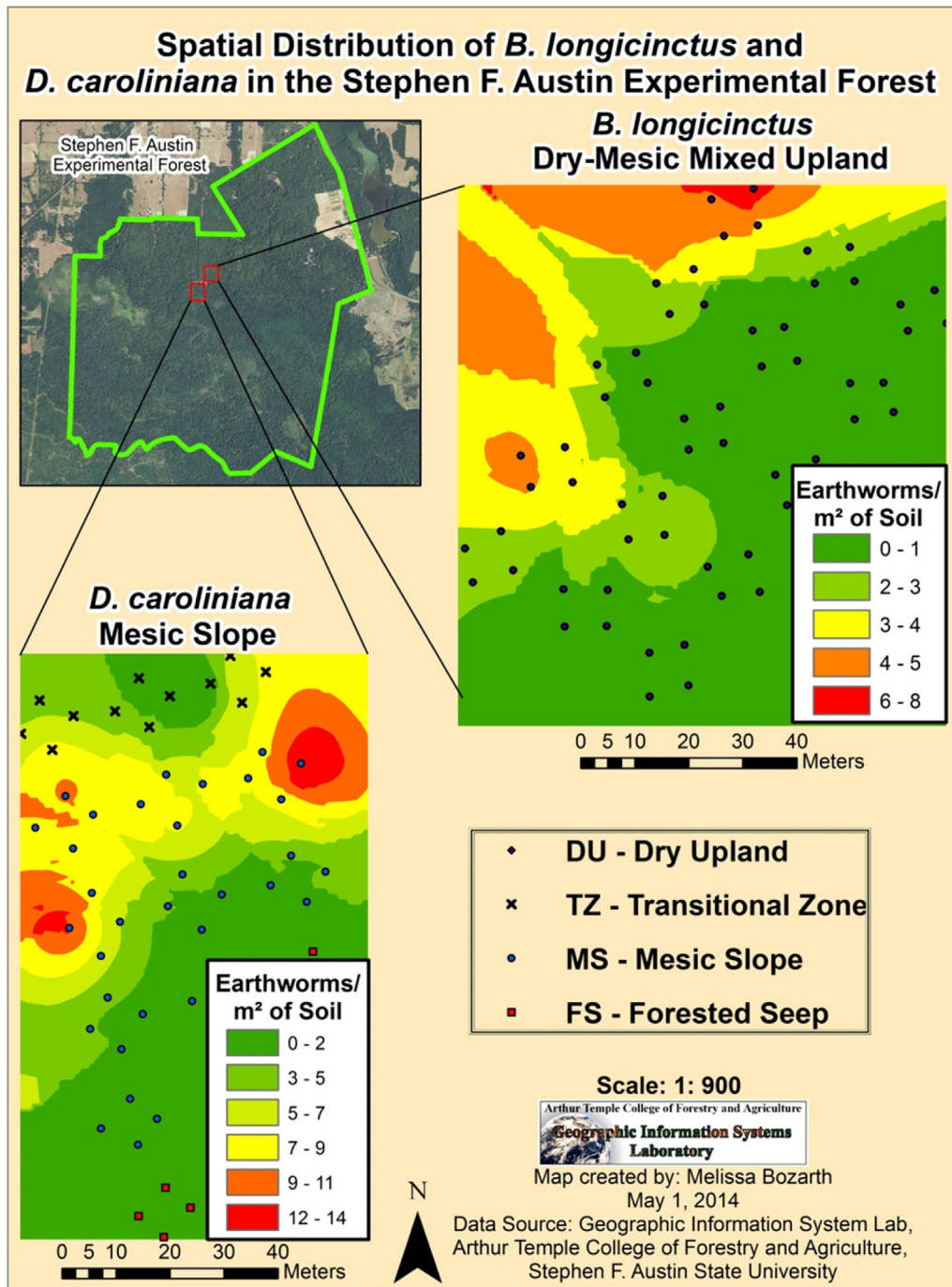


Fig. 9. Spatial distribution of species abundance of *Bimastos longicinctus* in the dry-mesic mixed upland and *D. caroliniana* in the mesic slope of SFAEF using ordinary kriging with variable search radius. Please note the differences in scales used for the densities of individual earthworm species represented in the different panels.

idea that earthworms, soil properties and vegetation are connected by associating defined earthworm assemblages to particular ecological habitats in the SFAEF.

Although many species of earthworm were found in the same pits, spatial distribution of earthworm species is likely related to competition. It can be seen in Fig. 4, that the larger more lively species, such as the non-native *A. corticis* and native *D. mississippiensis*, have the highest populations in the shady, moist, organic matter rich FS sites. These earthworms may have an advantage over the smaller less active native earthworms when competing for resources, thereby allowing them to proliferate in the FS sites.

This competition for resources may restrict some smaller native species to the less desirable sites where they can still live and thrive in harsher environmental conditions. Similar studies have shown that these environmental variables affect earthworm distribution. Cannavacciuolo et al. (1998) found that increased earthworm adults and biomass correlated with a hydromorphic gradient and Butt et al. (1997) saw that earthworm distribution increased in relation to the amount of soil organic matter in the mineral horizons.

It is also possible that particular earthworm species prefer ecological habitats based on environmental and soil properties. Varying soil properties have been known to affect distribution and

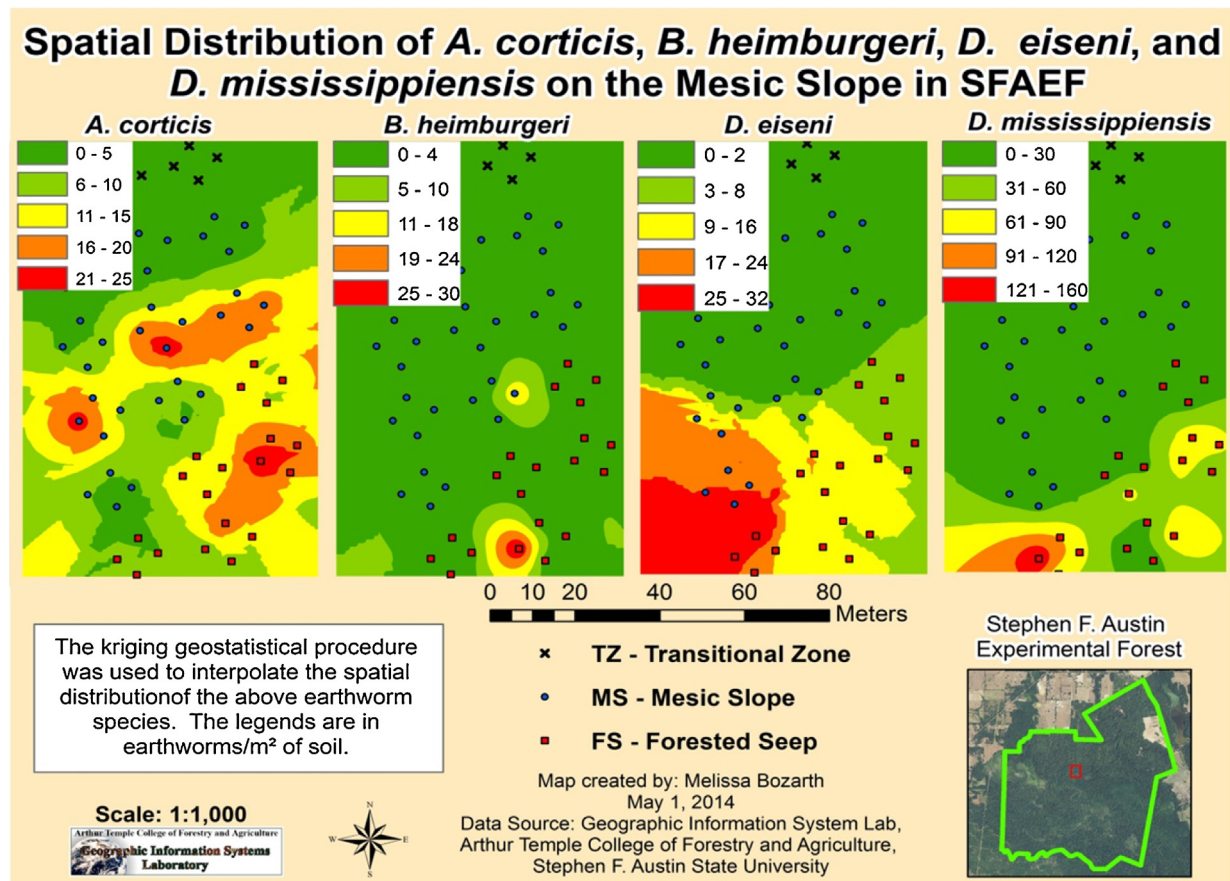


Fig. 10. The spatial distribution of *Amyntas corticis*, *Bimastos heimbürgeri*, *Diplocardia eiseni* and *Diplocardia mississippiensis* species abundance on the mesic slope using ordinary kriging with variable search radius. Please note the differences in scales used for the densities of individual earthworm species represented in the different panels.

may cause earthworms to be distributed with a spatial structure at multiple scales, depending on landscape and specific soil conditions (Whalen and Costa, 2003; Callahan et al., 2003). Some *Diplocardia* species have a positive response to burning vegetative cover, which raises soil pH (James, 1988), and many *Diplocardia* species can survive elevated temperatures (James and Hendrix, 2004) while many Lumbricidae species cannot. Spatial variation in earthworm populations has also been related to soil properties such as organic carbon content (Hendrix et al., 1992; Cannavacciuolo et al., 1998; Nuutinen et al., 1998). Even soil texture is known to have an influence on earthworm abundance, with medium textured soils generally preferred, but some species appear to favor finer textured soils (Hendrix et al., 1992; Curry, 2004). For this study, it is hypothesized that the earthworm species that were found in highest numbers at the DU sites, may favor loamy textured soils. While the species commonly found at the FS sites were associated with more coarse loamy soils.

Understory vegetation may also affect earthworm distribution due to its effect on the rhizosphere and microorganisms in the soil. For this study, understory vegetation helped describe ecological habitats and acted as a visual indicator of environmental conditions. The understory species found in the upland plots are known to be drought tolerant and are commonly found in these dry environments (Diggs et al., 2006). These include *C. sessiliflorum*, an extremely drought tolerant and a versatile grass, which was found most abundantly on upper slopes with open canopies. Other species, such as *D. commutatum* and *L. sempervirens* were limited to the DU sites, suggesting a need for direct sunlight. *S. smallii* is a wide-ranging species commonly found on coarse textured soils and was only found on the TZ sites (Diggs et al., 2006). *Mitchella*

repens and *Viola triloba* were two understory species commonly found at the mesic sites and are known to thrive in oak dominated woodlands, along stream banks, in dry or moist woods and on sandy slopes (Diggs et al., 2006). *Chasmanthium laxum* and *Osmunda cinnamomea* are adapted to shaded and moist environments with higher levels of organic matter, like the habitat of wet forested seeps (FS) where they were found (Diggs et al., 2006).

This study showed that unique earthworm communities are associated with distinct ecological habitats. These ecological habitats differed by soil conditions, overstory tree population and understory plant species, which allowed for specific vegetative cover to be broadly used as an earthworm species indicator. The data suggests that these different ecological habitats provide niches for unique earthworm assemblages, either by competition, environmental preferences or a combination of both. The study shows that native and non-native species are together in the same habitat but does not determine the long term effects of the non-native *A. corticis* presence in the SFAEF. Whether *A. corticis* will displace native earthworms and impact vegetation over time remains unclear.

5. Conclusions

This study was successful in finding relationships between ecological habitats, soil conditions and earthworm species that have never been revealed in this region. These results help to create a better understanding of earthworm communities and suggest that the different earthworms species collected at the SFAEF occupy specific ecological niches. The study suggests that there certainly are relationships between earthworms, overstory and

understory plant cover, but could not determine if understory cover affected earthworm abundance. Therefore, a study combining an intensive sampling design on a smaller area over multiple growing seasons, along with some assessment of soil microbial communities, could help further reveal these relationships. In addition, the first record of *B. longicinctus* and a second record of *D. mississippiensis* since the 1940s in Texas further indicates the undeveloped state of knowledge about such rudimentary things as species ranges for native North American earthworm species, and the need for more sampling in areas where native species are likely to be found. Furthermore, this study emphasizes the need for more research to better understand earthworm ecology in relation to plant communities and the complex interactions that take place below-ground.

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