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Distribution of earthworm growth stages along a naturally occurring soil salinity gradient

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Abstract

One major soil health challenge of the Northern Great Plains of North America is the natural occurrence of soluble salts in the soil. Salinity impacts on plant production are well understood, but we stand to learn more about the effects of salinity on soil biological characteristics, especially for sulfate-based salts. We conducted a field survey for three years to quantify the abundance and growth stages of earthworms across a naturally occurring salinity gradient. The gradient was approximately 150 m in length and traversed electrical conductivity values < 1 to 6 mS/cm and associated organic matter content from 6.9 to 4.8 %. We recovered earthworms and cocoons from intact soil cores (20 cm diameter) to 15 cm depth. Most of the recovered earthworms were *Aporrectodea trapezoides* (Dugés, 1828); however, *Aporrectodea tuberculata* (Eisen, 1874) and *Octolasion tyrtaeum* (Savigny, 1826) were also observed in low abundance. Juvenile earthworms were abundant and contributed the most individuals to the total counts in non-saline soils; they were also the group that exhibited the steepest decline in response to increasing salinity levels. Mature earthworm and cocoon counts were generally stable across salinity levels. Though we observed some inter-year variability in earthworm observations, we conclude that the occurrence of juvenile earthworms may serve as a soil health indicator in a salinity context.

Keywords dryland salinity | endogeic | mollisol | soil health | sulfate salts

1. Introduction

Due to a glaciated history, earthworms are not native to the Northern Great Plains of North America, but European species that can tolerate cold climates have migrated into the region and they are now commonplace (summarized by Hendrix & Bohlen 2002, Reynolds 2004, Reynolds 2020). Despite their historical absence, most earthworms are considered favorable immigrants in annual row-crop, forage production, and rangeland systems, which occupy approximately 87 % of North Dakota's land area (National Agricultural Statistics Service 2020). Earthworm distribution around the state of North Dakota has been associated with organic matter content of the soil greater than 2.5 %, fine textured soils, and close proximity to source locations or earthworm introduction activities (Deibert & Utter 2003). Currently, multiple species of *Apporrectodea* sp. and *Octolasion tyrtaeum* (Savigny, 1826) commonly occupy agricultural soils throughout the region, and a few other species, all belonging to the Lumbricidae family, may be found less frequently (Reynolds 1978, Deibert & Utter 2003, Reynolds 2015, Reynolds 2020).

In Northern Great Plains agricultural soils, earthworms provide services that improve soil condition in multiple ways (see sumary by Tomlin & Fox 2003). Specifically, earthworms re-arrange soil particles to improve aggregation (Bossuyt et al. 2004, Hallam & Hodson 2020), which increases porosity and facilitates infiltration and air exchange (Francis & Fraser 1998, Hallam et al. 2020). Their activities increase plant-available nitrogen (van Vliet et al. 2007, Lubbers et al. 2011) and phosphorus (Vos et al. 2014, Vos et al. 2019) and their castings stabilize organic carbon (Bossuyt et al. 2004, Lubbers et al. 2017).

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Earthworms facilitate decomposition and incorporation of plant residues into soil (Marhan & Scheu 2006, Lubbers et al. 2017), they create and modify habitats for other soil organisms (Mummey et al. 2006, Eisenhauer 2010), and they occupy multiple roles within the soil food web (reviewed by Curry & Schmidt 2007). Because of these benefits, earthworms are popular in-field indicators of soil health (Stroud 2019). Many research methods and commercial indicators of soil biological activity aim to quantify microbial activity, so they require lab analyses involving chemical extractions, incubations, or instrumental analysis. However, earthworm bodies, cocoons, and evidence of burrowing activity are easy to see. Their presence reflects generally hospitable soil conditions—absence of severe chemical or physical disturbance—which may imply that an abundance of earthworms is associated with a variety of other soil organisms and their functions in the soil.

A major, persistent soil health problem and plant production barrier in the Northern Great Plains is the natural occurrence of soluble salts in the soil (Sandoval et al. 1964, George 1978). Salinity negatively affects crop and forage growth, through osmotic regulation and direct ion toxicity (Munns 2002). Crop losses due to salinity in the Red River Valley of eastern North Dakota alone have been estimated to reach 150 million U.S. dollars annually (Hadrich 2012), yet salt-affected yield impacts extend across the entire Northern Great Plains region. Salinity in the region originates from the underlying parent material (ancient lakebed sediments), and salts are primarily sulfate-based (Sandoval et al. 1964, Keller et al. 1986). Salts migrate to the soil surface as evaporation pulls water from shallow water tables and surface waters into the atmosphere, and as seep drainage carries soluble salts to the surface (Doering & Sandoval 1981, Skarie et al. 1986). Dryland cultivation and annual crop production practices exacerbate the problem by facilitating salt migration to the surface (Clarke et al. 2002). Thus, the source and chemical nature of salts in these dryland Northern Great Plains soils are starkly different than soil salinization due to poor quality irrigation water or seawater intrusion, which are typically dominated by chloride-based salts. Saline patches in the region also experience frequent and prolonged water inundation and poor drainage (Arndt & Richardson 1988, Arndt & Richardson 1989), effectively altering the physical and chemical properties of the soil and degrading soil health.

Soluble salts in the soil deter earthworms through both direct and indirect means. Field surveys and laboratory and greenhouse experiments indicate that many species of earthworms are sensitive to high ion concentrations as evidenced by their avoidance, declining densities, or suppressed growth and reproduction as salt concentrations increase (Khalaf El-Duweini & Ghabbour 1965, Owojori & Reinecke 2009, Owojori et al. 2009, Ivask et al. 2012, Jun et al. 2012, Owojori & Reinecke 2014, Wu et al. 2015, Sharif et al. 2016, Karimi et al. 2020). Indirectly, poor soil structure, high water content, reduced vegetation, low organic matter inputs, and co-occurrence of toxic metals of saline patches further reduce habitat quality and reinforce earthworm avoidance of affected soils. Largely, the salts used and observed in existing studies are chloride-based soluble salts, especially sodium chloride (NaCl), but salinity toxicity to earthworms varies by ion composition (Owojori & Reinecke 2014). In these reports, the threshold values of electrical conductivity that cause earthworm declines are often above 2 mS/cm, but many studies don't specify the method used to measure conductivity, which precludes direct comparisons across studies. We do not know if the salt composition, and sulfate-based salts in particular, would result in a different degree of earthworm tolerance. Furthermore, there is a paucity of studies that evaluate the responses of the common endogeic earthworm species present in the Northern Great Plains.

The management of saline soils aims to limit the spread and severity of salinity, and possibly reverse salinization, through hydrological regulation. Management strategies for achieving this include reducing evaporative demand at the soil surface by reducing tillage, maximizing surface residues, and maximizing vegetation that will intercept surface-bound waters and lower the water table (Clarke et al. 2002, Pannell & Ewing 2006). These salinity-related management strategies are assumed to concurrently improve earthworm habitat by dampening soil water and temperature fluctuations and by increasing organic matter inputs. In turn, earthworms may then provide a positive feedback as they further facilitate the recovery of soil health through improved porosity and fertility (Curry & Cotton 1983, Lee & Foster 1991), which would directly and indirectly assist in leaching salts through the zone of activity and disrupting capillary rise (McDaniel et al. 2015). Earthworms have been observed to improve soil structure in agricultural fields that suffer from compaction (Capowiez et al. 2009, Capowiez et al. 2012) and in restoration scenarios following industrial disturbance, such as mining (Curry & Cotton 1983, Curry 1988, Butt et al. 1995). The extent to which earthworms can contribute to this recovery process, and their utility as soil health indicators in dryland saline soils relies on their tolerance to salinity levels, and our understanding of their threshold salinity levels.

We understand that salinity decreases plant production and soil health, and we know it affects earthworm activity in soils; however, we do not know specifically how earthworm communities change in soils with increasing levels of naturally occurring sulfate-based salinity. To address this knowledge gap, we conducted field surveys of earthworm communities and soil properties for three years. We expected that earthworm abundance would decline as the salinity concentration increased, and that this response might be accompanied by shifts in the earthworm communities based on growth stage. This survey helps us understand the utility of earthworms as indicators of soil salinity.

2. Materials and Methods

2.1 Site description

Earthworm surveys were conducted at an on-farm salinity management demonstration site in Sargent County in North Dakota, U.S.A. (46°15’19.44”, -97°18’41.04”, Fig. 1). This site hosts ditch-effect salinity, where salts originating from subsoil are driven to the soil surface by evaporation adjacent to roadside ditches (Skarie et al. 1986). Salts in this region are primarily sodium- and magnesium-sulfates and calcium-carbonates (Keller et al. 1986). Soils at the study site are mapped according to the U.S. soil taxonomy system as fine-silty, mixed, superactive, frigid Aeric Calciaquolls (Soil Survey Staff 2020). The area experiences a continental climate; the daily average air temperature (30-year average) is 6°C (average daily range: 0–12°C) (NDAWN 2020). The average annual cumulative rainfall is 46 cm, while the average annual total Penman potential evapotranspiration is 116 cm (NDAWN 2020).

The demonstration site was approximately 10 hectares in size, and it was removed from row crop production in 2014. For the duration of the survey, the site followed a management plan to reduce salinity, which includes maximizing vegetative cover and surface residue. In 2014, the site was planted to alfalfa (*Medicago sativa* L.), AC Saltlander green wheatgrass (*Elymus hoffmannii* K. B. Jensen & K. H. Asay), and Garrison creeping meadow foxtail (*Alopecurus arundinaceus* Poir.) and was annually overseeded with a full-season annual cover crop mix (*Hordeum vulgare* L., *Secale cereale* L., *Raphanus sativus* L. var. *longipinnatus* L. H. Bailey, and *Brassica napus* L.). Once or twice per year, the site was mowed, and residue was left on the surface. The site was colonized by weedy species (including *Hordeum jubatum* L. and *Kochia scoparia* (L.) Schrad.), but much of the area lacked vegetation where high salt concentrations occurred in the soil.

During the 2015 growing season, we surveyed the site with a Soil EC 3100 sensor (Veris Technologies, Salinas, Kansas), which provides measurements of

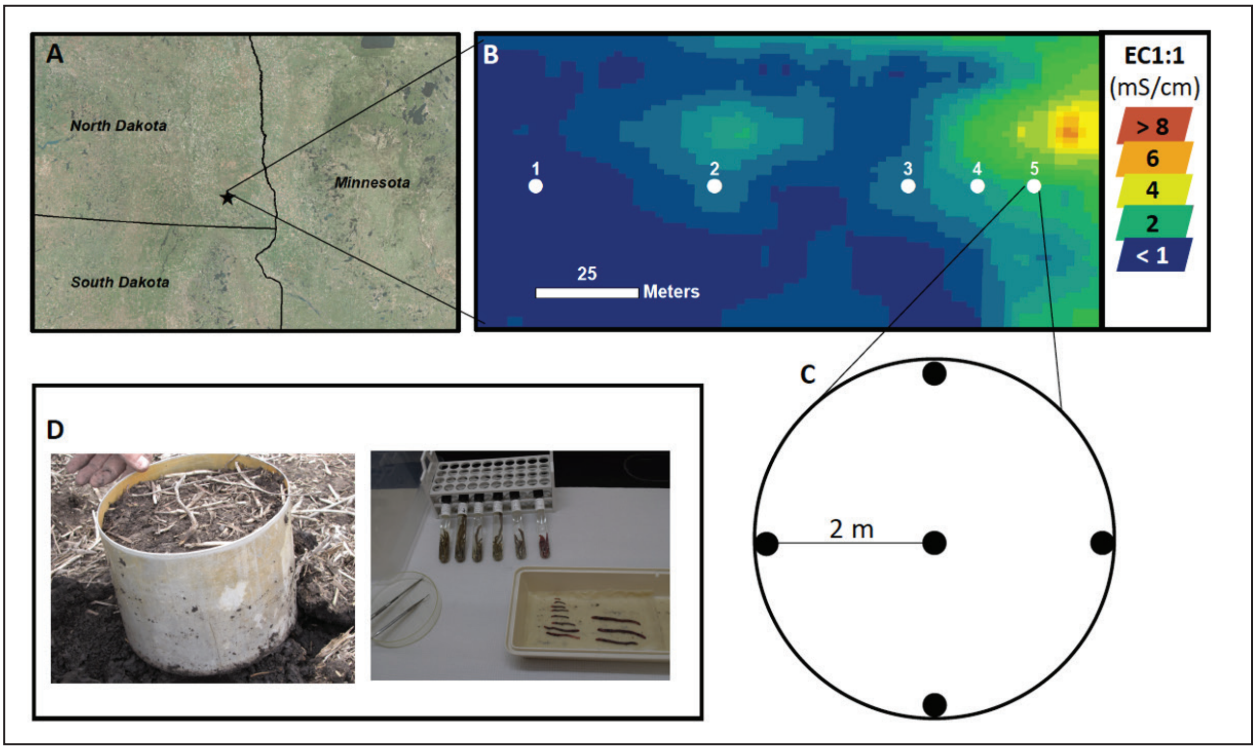

Figure 1. (**A**) Field sampling location for earthworm and soil collections in southeast North Dakota, USA. (**B**) Five plots were established along a salinity gradient based on an electrical conductivity ($EC_{1:1}$) survey. (**C**) Within each plot, five sub-plot locations were sampled. (**D**) Intact soil cores (20 cm in diameter, 15 cm deep) were hand-sorted for earthworms, which were counted and classified based on growth stage. Soils from each core were analyzed for physical and chemical soil properties.

apparent electrical conductivity (EC_a). During the survey, we collected soil samples (0–30 cm) from locations corresponding to a range of EC_a readings and measured electrical conductivity on a 1:1 soil-to-water slurry ($EC_{1:1}$) in the laboratory (Rhoades 1996). We converted the survey readings to $EC_{1:1}$; the readings were then interpolated using inverse-distance weighting in ArcGIS (ESRI Redlands, California). Based on the conductivity map for the 0–30 cm depth, we identified an area that had clear changes in $EC_{1:1}$. In the field, we used a handheld electrical conductivity meter (HI98331, Hanna Instruments, Inc., Woonsocket, Rhode Island) to navigate the conductivity gradient and locate five zones of increasing electrical conductivity. A plot was established in each of the five zones, which consisted of a center point and a point in each of the four cardinal directions, located two meters from the center point (Fig. 1). Cores were taken within a half meter of the previous year's cores. Plot 1 represented the non-saline condition, and the salinity level increased as the plot number increased.

2.2 Soil Sampling

Earthworm and soil samples were collected annually in late May of 2016–2018. There was no vegetation growing in the plots at the time of sampling, as vegetation had not yet emerged for the growing season. Based on our observations, earthworm activity in this region coincides with periods of hospitable soil moisture (near 30% by mass) and temperature (near 10–15°C), which typically occur in spring and fall, rather than the dry and hot period of the growing season. Our sample timing aimed to capture a period of high earthworm abundance and activity. At each sample point, we collected a soil core that measured 20.3 cm in diameter and 15.2 cm deep (total volume of 4,940 cm^3). Intact cores were placed in a fine mesh bag and transported in pails to a storage facility, where they were kept at 4°C until processing. At each core sample location, additional soil samples were collected (0–15 cm depth) for chemical and physical analyses.

2.3 Earthworm and Soil Processing

Soil cores were hand sorted for live earthworms. Recovered earthworms were anesthetized in dilute absolute alcohol, fixed, and stored in 10% formalin for later identification. Adult earthworm species were identified using a taxonomic key developed by Schwert (1990), and classified based on growth stage (juvenile, aclitellate adult, clitellate adult, and post clitellate adult) according to definitions by Reynolds (1977). Briefly, juveniles lacked genital markings, aclitellate adults lacked a clitellum or any markings indicating a past clitellum, clitellate adults had a fully formed clitellum, and post-clitellate adults displayed a band of discoloration where a clitellum had abated. From core earthworm counts, we estimated earthworm densities for a 1-m^2 area to a depth of 15 cm, based on the core dimensions.

Soil remaining after hand sorting was stored in the fine mesh bag at 4°C until processed for cocoons. A nematode flotation tank was used for cocoon recovery. Water from the flotation tank passed through a series of sieves: 4 mm sieve to collect large debris, 2 mm sieve to collect cocoons, and 1 mm sieve to collect any remaining cocoon or juvenile earthworms. Soil remaining in the flotation tank was removed and washed through the 2 mm and 1 mm sieves. Earthworms recovered at this step were fixed in 10% formalin for classification as described. We recorded cocoon count for each core, and estimated density per m^2 as described.

Soil gravimetric water content was measured on fresh field subsamples (Gardner 1986). Remaining soil was air dried and ground to pass through a 2 mm sieve. Organic matter content of the soil was estimated via mass loss on ignition, by heating the soil to 360°C for two hours (Combs & Nathan 2011). We measured electrical conductivity of a saturated paste extract (EC_e) (Rhoades 1996) and pH (10 g soil in 10 ml water according to Thomas 1996) on each soil sample with a combination conductivity/pH meter (HQ40d, Hach, Loveland, Colorado).

In 2016, we analyzed each soil sample for additional physical and chemical properties; we repeated chemical analyses in 2017 and 2018 but did not observe changes, so we only present results from 2016. We measured $EC_{1:1}$ on each sample, and submitted saturated paste extracts (Rhoades 1996) to the North Dakota State University Soil Testing Lab for soluble ion concentration analysis: potassium (K^+), sodium (Na^+), magnesium (Mg^{+2}), calcium (Ca^{+2}) (measured with a Buck Scientific Model 210VGP atomic absorption spectrophotometer, Norwalk, CT), bicarbonate (HCO_3^-) (measured by titration, Loeppert & Suarez 1996), chloride (Cl^-), and sulfate (SO_4^{-2}) (measured with a FIAlab flow injection analyzer, Seattle, WA). We analyzed one soil sample per plot for particle size distribution using the hydrometer method (Gee & Or 2002).

2.4 Data Analysis

Mean earthworm counts at each growth stage, cocoon counts, and soil properties at the plot-level were compared using a Kruskal-Wallis test and post-hoc pairwise comparisons using a Wilcox test to distinguish differences in values across the salinity gradient. These tests accommodated the lack of dependency between observations (due to close proximity) and the lack of true replication across salinity levels. Results are presented for each year individually, since earthworm and soil variables did not differ across years as indicated by across year means comparison tests. We also obtained Spearman correlation coefficients (rho) between EC$_e$, soil organic matter, and total earthworm counts for all observations (years and plots pooled), as well as for growth stages within each year. Specific ion concentrations (except K^+ and HCO_3^-) were strongly positively correlated with EC$_e$ (rho > 0.8), so pairwise correlation coefficients for ion concentrations and other soil properties are not reported. All analyses and data visualizations were conducted in R (R Core Team 2020) with assistance from 'dplyr' (Wickham et al. 2020), 'fields' (Nychka et al. 2020), 'Hmisc' (Harrell & Dupont 2020), and 'scales' (Wickham 2020) packages.

3. Results

Our intention was to understand earthworm distribution along naturally increasing levels of salinity. Soil properties along this transect from low salinity (plot 1) to high salinity (plot 5) are listed in Tab. 1. Soil textures for all plots were consistently silt loam or loam (approximately 25 % sand, 50 % silt, 25 % clay). At the time of sampling, soil temperatures (0–15 cm) averaged 16.7°C in 2016, 9.6°C in 2017, and 13.5°C in 2018 and gravimetric water content averaged 33 % in 2016, 32 % in 2017, and 28 % in 2018.

The measures of electrical conductivity confirmed a steady increase in salinity along the transect from a mean EC$_{1:1}$ of 0.51 mS/cm in plot 1 to a mean of 2.69 mS/cm in plot 5 (Tab. 1). These values coincide with mean EC$_e$ values that ranged from 0.79 mS/cm in plot 1 to 5.93 mS/cm in plot 5. The local relationship between the two conductivity measures is described by Matthees et al. (2017) and supported with our empirical data. Soil is considered saline if the EC$_e$ is greater than 4 dS/m (or 4 mS/cm) (Weil & Brady 2017); therefore, soil in plots 4 and 5 meet this formal criterion.

This salinity gradient was accompanied by a slight decrease in pH in plots 4 and 5 and a steady decrease of soil organic matter content along the transect by about 2 % (Tab. 1). Though plants were not growing at the time of sampling, this transect spans the range of salinity levels that reduce local cash crop yields (Butcher et al. 2018). We would expect that plant productivity decreases substantially along this transect in response to increasing salinity levels.

Ion analysis of the saturated paste extracts indicated that sodium- and magnesium-sulfates were primarily responsible for the increasing conductivity (Tab. 1). The sodium adsorption ratio (an indicator for the relative proportion of sodium adsorbed to clays), is used to identify sodic soils, which suffer from soil particle dispersion and development of dense structure (Rengasamy & Olsson 1991). We observed sodium adsorption ratios greater than 13 for plots 2 through 5 (calculations not shown), which indicates that these soils are considered saline-sodic (Weil & Brady 2017). The effects of high ion concentrations on soil organisms in these soils likely overwhelms the physical effects of the high sodium concentrations (dense and massive soil structure with low porosity).

Earthworm abundance decreased as salinity increased across the plots (Fig. 2, Tab. 2), but the response differed across the earthworm maturity stages. The total earthworm density significantly declined as salinity increased from plot 1 to plot 5 in 2016 and 2017. Earthworm counts were lowest and most variable within and across plots in 2018, contributing to a declining, but statistically insignificant trend with increasing salinity. Few live earthworms were recovered from plot 5 in any year. The total earthworm densities were primarily comprised of juvenile-stage earthworms (often over 50 % of the total counts), followed by clitellate adults, aclitellate adults, and finally post-clitellate adults. Aside from the significant decline in juveniles along the salinity gradient in 2016 and 2017, the only other significant decline was observed for aclitellate

Table 1. Mean soil chemical characteristics from 0–15 cm depth for each earthworm collection plot (n = 5) in 2016. Electrical conductivity and pH were determined on a 1:1 soil:water slurry (EC$_{1:1}$); conductivity (EC$_e$) and ion concentrations were determined on a saturated paste extract. Different superscript letters indicate statistically significant differences ($p \leq 0.05$) across plots.

Plot	EC$_{1:1}$ (mS/cm)	EC$_e$ (mS/cm)	pH	Soil Organic Matter (%)	K^+ (mg/kg)	Na^+ (mg/kg)	Mg^{+2} (mg/kg)	Ca^{+2} (mg/kg)	HCO_3^- (mg/kg)	Cl^- (mg/kg)	SO_4^{-2} (mg/kg)
1	0.51^{C}	0.79^{C}	7.58^{A}	6.9^{A}	65^{AB}	21^{B}	21^{D}	99^{B}	396^{A}	38^{A}	33^{C}
2	1.63^{B}	3.10^{B}	7.66^{A}	5.9^{B}	70^{A}	281^{A}	138^{C}	502^{A}	332^{A}	57^{A}	$1{,}695^{B}$
3	1.84^{AB}	3.69^{B}	7.63^{A}	5.8^{B}	72^{A}	270^{A}	208^{BC}	521^{A}	379^{A}	100^{A}	$1{,}989^{B}$
4	2.67^{A}	5.88^{AB}	7.36^{B}	5.4^{C}	69^{A}	689^{A}	461^{AB}	490^{A}	414^{A}	111^{A}	$3{,}720^{AB}$
5	2.69^{A}	5.93^{A}	7.03^{C}	4.8^{D}	45^{B}	513^{A}	$1{,}368^{A}$	569^{A}	241^{B}	188^{A}	$3{,}774^{A}$

adults in 2016 (Tab. 2). We recovered cocoons from nearly all plots in all years, with plot 5 in 2016 and 2018 being the exceptions (Tab. 2). The cocoon counts largely mirrored adult earthworm count numbers and did not appear to decline with increasing salinity.

We only found three earthworm species in these plots (based on adult characteristics). The most common species (> 85 % of adults each year) was *Aporrectodea trapezoides* (Dugés, 1828) in all years, with only a few individuals (11–12 % of adults across years) of *Aporrectodea tuberculata* (Eisen, 1874) each year, and even fewer *Octolasion tyrtaeum* (0–2 % of adults across years). With such low abundance of some species, we cannot form conclusions about how the different species responded differently to salinity, and one could argue that our results reflect behavior of the *Aporrectodea* complex to salinity, rather than the behavior of a diverse community of earthworms. All three of these species are considered endogeic, residing primarily in the top 20 cm of the soil and occasionally feeding on the soil surface (see Hendrix & Bohlen 2002).

We observed a strong overall negative relationship between individual observations of earthworms and electrical conductivity (rho = -0.78, p-value < 0.001,

Table 2. Mean earthworm and cocoon densities for each plot (counts/m^2 to 15 cm depth), separated by growth stage (n = 5). Different superscript letters indicate statistically significant differences ($p \leq 0.05$) across plots, within each year. There were no statistically significant differences observed across years and within plots.

Plot	Total live worms	Cocoon	Juvenile	Aclitellate	Clitellate	Post-clitellate
	2016					
1	58^{A}	5^{A}	45^{A}	8^{A}	4^{A}	2^{A}
2	20^{B}	3^{A}	12^{B}	3^{AB}	3^{A}	1^{A}
3	13^{BC}	4^{A}	4^{BC}	0^{BC}	8^{A}	1^{A}
4	4^{CD}	6^{A}	1^{CD}	0^{C}	4^{A}	0^{A}
5	0^{D}	0^{A}	0^{D}	0^{C}	0^{A}	0^{A}
	2017					
1	48^{A}	1^{A}	43^{A}	3^{A}	1^{A}	1^{A}
2	28^{AB}	4^{A}	16^{B}	2^{A}	11^{A}	0^{A}
3	19^{BC}	8^{A}	10^{BC}	1^{A}	9^{A}	0^{A}
4	4^{CD}	5^{A}	3^{CD}	0^{A}	1^{A}	0^{A}
5	1^{D}	4^{A}	0^{D}	0^{A}	1^{A}	0^{A}
	2018					
1	33^{A}	3^{A}	24^{A}	4^{A}	5^{A}	0^{A}
2	38^{A}	3^{A}	24^{A}	9^{A}	3^{A}	3^{A}
3	25^{A}	5^{A}	14^{A}	4^{A}	3^{A}	3^{A}
4	12^{A}	3^{A}	5^{A}	3^{A}	4^{A}	1^{A}
5	2^{A}	0^{A}	2^{A}	0^{A}	0^{A}	0^{A}

Table 3. Spearman correlation coefficients (rho) between earthworm densities for each growth stage and electrical conductivity obtained from saturated paste (EC_e) and soil organic matter content (SOM) of soils collected in each year (n = 25). Asterisks indicate the significance level of the relationship†.

	2016		**2017**		**2018**	
	EC_e (mS/cm)	SOM (%)	EC_e (mS/cm)	SOM (%)	EC_e (mS/cm)	SOM (%)
Total live worms	-0.88 ***†	0.88***	-0.87***	0.83***	-0.72***	0.49*
Cocoon	-0.38	0.26	0.22	-0.16	-0.51**	0.3
Juvenile	-0.87***	0.83***	-0.84***	0.81***	-0.71***	0.48*
Aclitellate	-0.70***	0.81***	-0.39	0.38	-0.51**	0.29
Clitellate	-0.33	0.26	-0.21	0.22	-0.50*	0.17
Post-clitellate	-0.52**	0.49*	-0.41*	0.45*	-0.14	0.08

† Significance levels for correlations: *** for $p < 0.001$, ** for $p \leq 0.01$, and * for $p \leq 0.05$.

Fig. 3A). Beyond an EC_e of 6 mS/cm, earthworm densities were very low and primarily comprised of mature adults. In fact, clitellate adults were only weakly associated with EC_e (rho = -0.21 to -0.5), while juveniles were particularly strongly negatively correlated with EC_e in all years (rho = -0.72 to -0.88, Tab. 3).

The salinity level of the soil also greatly impacts plant productivity, and though we did not measure plant characteristics in this survey, soil organic matter content declined along the salinity gradient (Tab. 1). Earthworms feed on particulate organic residues; as such, we observed a positive association between earthworm density and organic matter content (Fig. 3, Tab. 3). Overall, total earthworm counts had a strong positive correlation coefficient with soil organic matter content (rho = 0.71, $p < 0.001$, Fig. 3B), and this relationship held true for juvenile earthworms in all years, aclitellate and post-clitellate earthworms in 2016, and post-clitellate earthworms in 2017 (Tab. 3).

4. Discussion

Our purpose in conducting this survey was to quantify the relationship between earthworm abundance and distribution with increases in naturally occurring sulfate-based salinity. While we expected earthworms to decline as salt concentration in soils increased, we were unsure if different earthworm growth stages would respond to the salts differently. Indeed, we observed that juvenile earthworms were less abundant as salinity increased, compared to more mature earthworms.

We successfully located a transect that spanned a gradient of soil properties, including increasing concentrations of salts and decreasing organic matter content. The soil properties that we measured along this transect were stable for the three years of the survey, and the transect provided a means for observing associated earthworm abundance. The earthworm observations in the first two years of the study were consistent, while

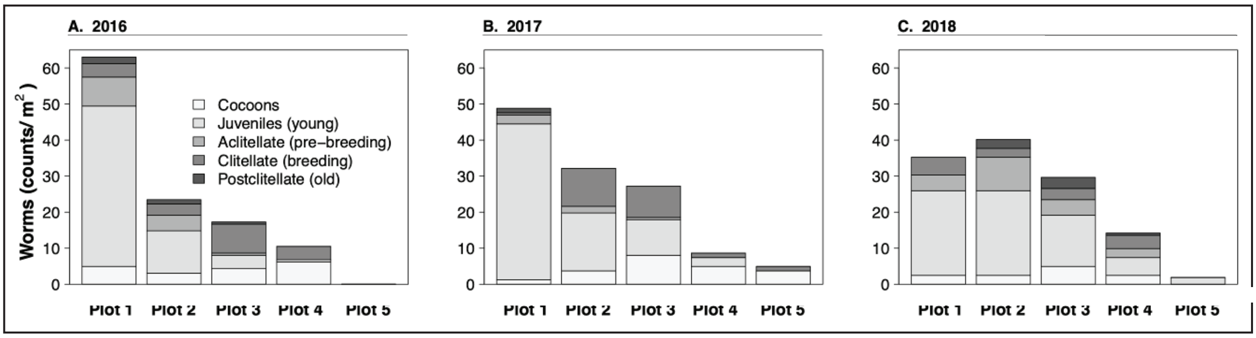

Figure 2. Mean live earthworm and cocoon densities to 15 cm depth for each growth stage recovered from each plot in each year (n = 5). Salinity levels increase with plot number.

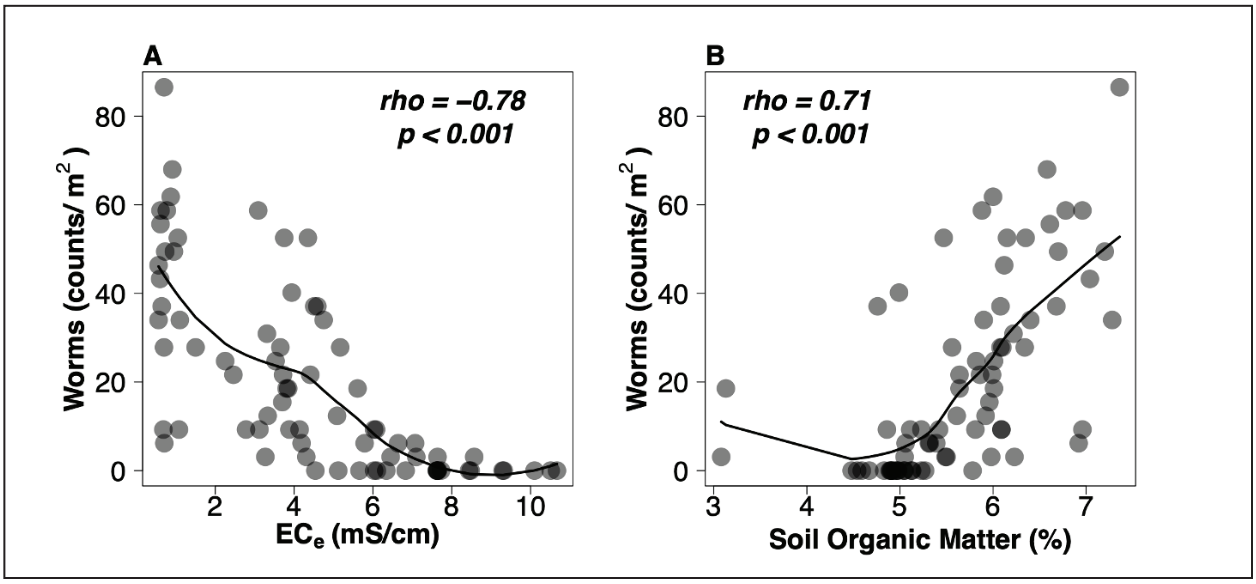

Figure 3. Scatterplot with locally weighted regression lines for observations of total earthworm densities and (**A**) electrical conductivity of saturated paste (EC_e), and (**B**) soil organic matter for all observations for all years (n = 75). Spearman correlation coefficients (rho) and associated p-values indicate strong relationships in opposite directions.

the third year was unique in its lack of apparent declines in earthworm abundance across the salinity gradient. The soils were 4-5% drier, on average, in 2018 at the time of sampling, following a much drier fall and winter compared to previous years. Total rainfall received during the preceding October 1 to May 31 was approximately 15 cm in 2016, 12 cm in 2017, and 6 cm in 2018 (NDAWN 2020). The lower precipitation received during the winter before sampling in 2018 may explain why we observed lower total earthworm counts, particularly in the juvenile category. We also observed a more uniform distribution of earthworms across plots in 2018 compared to previous years, represented by the increased abundance of juvenile earthworms in plots 2–4 in that year. We do not know the mechanism behind this observation or if the soil in saline plots became more hospitable temporarily in 2018 due to seasonal conditions, or if the saline soils are on a longer-term trajectory to being more habitable, particularly by juveniles.

Perhaps the most enlightening observation was that the juvenile growth stage was most sensitive to the increasing salts and decreasing organic matter content. From this, we conclude that as earthworms mature, they grow more tolerant to the salts (and perhaps other sub-optimal habitat conditions), as evidenced by adult earthworm and cocoon presence across salinity levels. Both aclitellate and clitellate earthworms appear to be most tolerant to changes in ion concentrations and organic matter content. This observation may indicate that during early growth, juveniles either die or move out of the saline soils (possibly using existing burrows created by adults), but that as juvenile worms mature, they may become more active in soils that have higher salt concentrations and lower organic matter. In general, the earthworm distribution across the gradient coincides with plant salt tolerance, where biomass production decreases steadily when the salinity concentration exceeds 2 mS/cm (based on $EC_{1:1}$, Butcher et al. 2018).

We recovered cocoons from soils at all salinity levels. Owojori et al. (2009) evaluated *Eisenia fetida* (Savigny, 1826) and *Aporrectodea caliginosa* (Savigny, 1826) survival and reproduction in a range of saline soils for 28 days and did not observe cocoons produced in soils with an electrical conductivity greater than 0.08 mS/cm (1:5 soil-to-water extract) during the duration of the study. The number of cocoons recovered in our study was fairly low, but in 2018, we allowed the cocoons to incubate in salt solutions similar to the soils from which they originated. A small proportion of the cocoons originating from saline plots successfully hatched. The low number of cocoons obtained and the lack of replication of this screening precludes us from stating conclusions on the effects of salt concentration on cocoon viability, or baby earthworm survival in saline soils, but cocoon viability in saline soils would be worth investigating further.

Overall, our observations indicate that despite salinity levels prohibitive of plant growth, mature earthworms are able to occupy saline soils up to 3 mS/cm (based on a 1:1 soil-to-water slurry). As salinity decreases, following periods of steady water infiltration (upon spring thaw or with regular precipitation), or in response to management, earthworm activity may serve as an early indicator of soil recovery at higher levels of salinity than tolerated by salt-sensitive plants. While other studies have examined earthworm-salinity relationships, we've learned that the abundance of earthworms in saline soils likely depends on the earthworm growth stage and other soil conditions (such as organic matter content and water content).

In a salinity management context, adult earthworms may have the capacity to assist in soil health improvement, and they may work synergistically with organic matter amendments to alleviate salt stress in plants. Earthworm feeding and bioturbation actions re-arrange soil particles and modify water-holding capacities and infiltration pathways which further influences the behavior of soil solutes (McDaniel et al. 2015). Wang et al. (2016) demonstrated that soils conditioned by earthworms increased leachate volume and dissolved salt concentration compared to soils without earthworms.

While adult earthworm activity alone may play a role in improving the structural characteristics of a saline soil, the addition of organic amendments may further facilitate these activities and provide benefits to living plants. For example, earthworm survival, cocoon production, and juvenile abundance in saline soils (5.26 mS/cm, water-to-soil ratio unreported) increased in response to additions of maize residue in a microcosm study (Jun et al. 2012). In another study (Oo et al. 2015), cassava waste compost and vermicompost were applied to saline (4.26 mS/cm, saturated paste) and non-saline soils in pots, either with or without earthworms. The amendments alone increased soil organic carbon, plant nutrient availability, and microbial activity of saline soils to levels above non-saline soils, and the addition of earthworms reduced the electrical conductivity of saline soils and shifted the distribution of anions associating with the cation exchange complex. While none of these changes resulted in increased maize production observed during the study, there may be long term benefits to crop production. In yet another study (Sharif et al. 2016), sorghum growth was improved in saline (13.72 mS/cm, saturated paste) soils when treated with vermicompost and vermi-wash, and the authors conclude that vermi-amendments may be used to enhance crop production at salinity levels that prohibit earthworm survival. In addition to providing substrates for earthworms (Hurisso et al. 2011, Miller et al. 2019), surface-applied organic amendments can restrict evaporation and moderate soil temperature, which are important conditions for attracting earthworms and maintaining their populations. This body of work suggests that earthworms may play a role in improving saline soils for plant production.

In conclusion, we observed that sulfate-based saline soils have lower earthworm abundance, and that juvenile abundance steadily declines as salinity increases. Therefore, earthworms that commonly inhabit the soils of the Northern Great Plains have the potential to serve as indicators of soil health in a salinity context. We suggest that both total earthworm abundance and abundance (or presence) of juvenile earthworms may be used to monitor salinity levels in susceptible soils and in soils undergoing salt remediation.

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6. References

Arndt, J. L. & J. L. Richardson (1988): Hydrology, salinity and hydric soil development in a North Dakota prairie-pothole wetland system. – Wetlands **8**: 93–108.

Arndt, J. L. & J. L. Richardson (1989): Geochemistry of hydric soil salinity in a recharge-throughflow-discharge prairie-pothole wetland system. – Soil Science Society of America Journal **53**: 848–855.

Bossuyt, H., J. Six & P. F. Hendrix (2004): Rapid incorporation of carbon from fresh residues into newly formed stable microaggregates within earthworm casts. – European Journal of Soil Science **55**: 393–399.

Butcher, K., A. F. Wick, T. DeSutter, A. Chatterjee & J. Harmon (2018): Corn and soybean yield response to salinity influenced by soil texture. – Agronomy Journal **110**: 1243–1253.

Butt, K. R., J. Frederickson & R. M. Morris (1995): An earthworm cultivation and soil inoculation technique for land restoration. – Ecological Engineering **4**: 1–9.

Capowiez, Y., S. Cadoux, P. Bouchand, J. Roger-Strade, G. Richard & H. Boizard (2009): Experimental evidence for the role of earthworms in compacted soil regeneration based on field observations and results from a semi-field experiment. – Soil Biology and Biochemistry **41**: 711–717.

Capowiez, Y., S. Samartino, S. Cadoux, P. Bouchant, G. Richard & H. Boizard (2012): Role of earthworms in regenerating soil structure after compaction in reduced tillage systems. – Soil Biology and Biochemistry **55**: 93–103.

Clarke, C. J., R. J. George, R. W. Bell & T. J. Hatton (2002): Dryland salinity in south-western Australia: its origins, remedies, and future research directions. – Australian Journal of Soil Research **40**: 93–113.

Combs, S. M. & M. V. Nathan (2011): Soil Organic Matter. – In: Nathan, M. & R. Gelderman (eds): Recommended Chemical Soil Test Procedures for the North Central Region. North Central Region Research Publication No. 221 (Revised) [https://extension.missouri.edu/publications/sb1001].

Curry, J. P. (1988): The ecology of earthworms in reclaimed soils and their influence on soil fertility. – In: Edwards, C. A. & E. F. Neuhauser (eds): Earthworms in Waste and Environmental Management. – SPB Academic, The Haugue: 251–261.

Curry, J. P. & D. C. F. Cotton (1983): Earthworms and land reclamation. – In: Satchell, J. E. (ed): Earthworm Ecology; From Darwin to Vermiculture. – Chapman and Hall, London: 215–228.

Curry, J. P. & O. Schmidt (2007): The feeding ecology of earthworms – A review. – Pedobiologia **50**: 463–477.

Deibert, E. J. & R. A. Utter (2003): Earthworm (Lumbricidae) survey of North Dakota fields placed in the U.S. Conservation Reserve Program. – Journal of Soil and Water Conservation **58**: 39–45.

Doering, E. J. & F. M. Sandoval (1981): Chemistry of seep drainage in southwestern North Dakota. – Soil Science **132**: 142–149.

Eisenhauer, N. (2010): The action of an animal ecosystem engineer: Identification of the main mechanisms of earthworm impacts on soil microarthropods. – Pedobiologia **53**: 343–352.

Francis, G. S. & P. M. Fraser (1998): The effects of three earthworm species on soil macroporosity and hydraulic conductivity. – Applied Soil Ecology **10**: 11–19.

Gardner, W. H. (1986): Water Content. – In: Klute, A. (ed): Methods of Soil Analysis, Part 1. Physical and Mineralogical Methods. – American Society of Agronomy & Soil Science Society of America, Madison: 493–544.

Gee, G. W. & D. Or (2002): Particle-Size Analysis – In: Dane, J.H. & G.C. Topp (eds): Methods of Soil Analysis, Part 4. Physical Methods. – Soil Science Society of America, Madison: 255–293.

George, P. R. (1978): The dryland salinity problem in North America. – Journal of the Department of Agriculture, Western Australia, Series 4 3.

Hadrich, J. C. (2012): Managing the economics of soil salinity in the Red River Valley of North Dakota. – Journal of ASFMRA 80–88.

Hallam, J., D. Berdeni, R. Grayson, E. J. Guest, J. Holden, M.G. Lappage, M.T. Prendergast-Miller, D. A. Robinson, A. Turner, J. R. Leake & M. E. Hodson (2020): Effect of earthworms on soil physico-hydraulic and chemical properties, herbage production, and wheat growth on arable land converted to ley. – Science of The Total Environment **713**: 136491.

Hallam, J. & M. E. Hodson (2020): Impact of different earthworm ecotypes on water stable aggregates and soil water holding capacity. – Biology and Fertility of Soils **56**: 607–617.

Harrell, F. E. J. & C. Dupont (2020): Hmisc: Harrell Miscellaneous. – R package version 4.4-1 [https://cran.r-project.org/web/packages/Hmisc/index.html].

Hendrix, P. F. & P. J. Bohlen (2002): Exotic earthworm invasions in North America: ecological and policy implications. – Bioscience **52**: 801–811.

Hurisso, T. T., J. G. Davis, J. E. Brummer, M. E. Stromberger, F. H. Stonaker, B. C. Kondratieff, M. R. Booher & D. A. Goldhamer (2011): Earthworm abundance and species composition in organic forage production systems of northern Colorado receiving different soil amendments. – Applied Soil Ecology **48**: 219–226.

Ivask, M., M. Meriste, A. Kuu, S. Kutti & E. Sizov (2012): Effect of flooding by fresh and brackish water on earthworm communities along Matsalu Bay and the Kasari River. – European Journal of Soil Biology **53**:11–15.

Jun, T., G. Wei, B. Griffiths, L. Xiaojing, X. Yingjun & Z. Hua (2012): Maize residue application reduces negative effects of soil salinity on the growth and reproduction of the earthworm *Aporrectodea trapezoides*, in a soil mesocosm experiment. – Soil Biology and Biochemistry **49**: 46–51.

Karimi, F., G. Rahimi & Z. Kolahchi (2020): Interaction effects of salinity, sewage sludge, and earthworms on the fractionations of Zn and Cu, and the metals uptake by the earthworms in a Zn- and Cu-contaminated calcareous soil. – Environmental Science and Pollution Research **27**: 10565–10580.

Keller, L. P., G. J. McCarthy & J. L. Richardson (1986) Mineralogy and stability of soil evaporites in North Dakota. – Soil Science Society of America Journal **50**: 1069–1071.

Khalaf El-Duweini, A. & S. I. Ghabbour (1965): Population density and biomass of earthworms in different types of Egyptian soil. – Journal of Applied Ecology **2**: 271–287.

Lee, K. E. & R. C. Foster (1991): Soil fauna and soil structure. – Australian Journal of Soil Research **29**: 745–775.

Loeppert, R. H. & D. L. Suarez (1996): Carbonate and Gypsum. –In: Sparks, D. L., A. L. Page, P. A. Helmke, R. H. Loeppert, P. N. Soltanpour, M. A. Tabatabai, C. T. Johnson & M. E. Sumner (eds): Methods of Soil Analysis, Part 3. Chemical Methods. – American Society of Agronomy & Soil Science Society of America, Madison: 437–474.

Lubbers, I. M., L. Brussaard, W. Otten & J. W. Van Groenigen (2011): Earthworm-induced N mineralization in fertilized grassland increases both N_2O emission and crop-N uptake. – European Journal of Soil Science **62**: 152–161.

Lubbers, I. M., M. M. Pulleman & J. W. Van Groenigen (2017): Can earthworms simultaneously enhance decomposition and stabilization of plant residue carbon? – Soil Biology and Biochemistry **105**: 12–24.

Marhan, S. & S. Scheu (2006): Mixing of different mineral soil layers by endogeic earthworms affects carbon and nitrogen mineralization. – Biology and Fertility of Soils **42**: 308–314.

Matthees, H. L., Y. He, R. K. Owen, D. Hopkins, B. Deutsch, J. Lee, D. E. Clay, C. Reese, D. D. Malo & T. M. DeSutter (2017): Predicting soil electrical conductivity of the saturation extract from a 1:1 soil to water ratio. – Communications in Soil Science and Plant Analysis **48**: 2148–2154.

McDaniel, J. P., G. Butters, K. A. Barbarick & M. E. Stromberger (2015): Effects of *Aporrectodea caliginosa* on soil hydraulic properties and solute dispersivity. – Soil Science Society of America Journal **79**: 838–847.

Miller, J. J., M. L. Owen, C. F. Drury & D. S. Chanasyk (2019): Short-term legacy effects of feedlot manure amendments on earthworm abundance in a clay loam soil. – Canadian Journal of Soil Science **99**: 447–457.

Mummey, D. L., M. C. Rillig & J. Six (2006): Endogeic earthworms differentially influence bacterial communities associated with different soil aggregate size fractions. – Soil Biology and Biochemistry **38**: 1608–1614.

Munns, R. (2002): Comparative physiology of salt and water stress. – Plant, Cell and Environment **25**: 239–250.

National Agricultural Statistics Survey (2020): North Dakota Agricultural Overview for 2019. – United States Department of Agriculture [https://www.nass.usda.gov/Statistics_by_State/North_Dakota/index.php].

NDAWN (2020): North Dakota Agricultural Weather Network, Mooreton Station. – North Dakota State University [https://ndawn.ndsu.nodak.edu/].

Nychka, D., R. Furrer, J. Paige, F. Gerber, M. Iverson & University Corporation for Atmospheric Research (2020): fields: Tools for spatial data. – R package version 11.4 [http://CRAN.R-project.org/package=fields].

Oo, A. N., C. B. Iwai & P. Saenjan (2015): Soil properties and maize growth in saline and nonsaline soils using cassava-industrial waste compost and vermicompost with or without earthworms. – Land Degradation & Development **26**: 300–310.

Owojori, O. J. & A. J. Reinecke (2009): Avoidance behaviour of two eco-physiologically different earthworms (*Eisenia fetida* and *Aporrectodea caliginosa*) in natural and artificial saline soils. – Chemosphere **75**: 279–283.

Owojori, O. J., A. J. Reinecke, P. Voua-Otomo & S. A. Reinecke (2009): Comparative study of the effects of salinity on life-cycle parameters of four soil-dwelling species (*Folsomia candida*, *Enchytraeus doerjesi*, *Eisenia fetida* and *Aporrectodea caliginosa*). – Pedobiologia **52**: 351–360.

Owojori, O. J. & A. J. Reinecke (2014): Differences in ionic properties of salts affect saline toxicity to the earthworm *Eisenia fetida*. – Applied Soil Ecology **83**: 247–252.

Pannell, D. J. & M. A. Ewing (2006): Managing secondary dryland salinity: options and challenges. – Agricultural Water Management **80**: 41–56.

R Core Team (2020): R: A language and environment for statistical computing. – R Foundation for Statistical Computing [https://www.R-project.org/].

Rengasamy, P. & K. A. Olsson (1991): Sodicity and soil structure. – Australian Journal of Soil Research **29**: 935–952.

Reynolds, J. W. (1977): The Earthworms (Lumbricidae and Sparganophilidae) of Ontario. – Royal Ontario Museum, Toronto: 141 pp.

Reynolds, J. W. (1978): A contribution to our knowledge of the earthworm fauna of North Dakota. – Megadrilogica **3**: 148–149.

Reynolds, J. W. (2004): The status of earthworm biogeography, diversity, and taxonomy in North America revisited with glimpses into the future. – In: Edwards, C. A. (ed.): Earthworm Ecology, second edition. – CRC Press, Boca Raton: 63–74.

Reynolds, J. W. (2015): A checklist by counties of earthworms (Oligochaeta: Lumbricidae) in North and South Dakota, USA. – Megadrilogica **17**: 157–178.

Reynolds, J. W. (2020): Earthworms in American Ecoregions. – OmniScriptum, Republic of Moldova: 430 pp.

Rhoades, J. D. (1996): Salinity: Electrical conductivity and total dissolved solids. –In: Sparks, D. L., A. L. Page, P. A. Helmke, R. H. Loeppert, P. N. Soltanpour, M. A. Tabatabai, C. T. Johnson & M. E. Sumner (eds): Methods of Soil Analysis, Part 3. Chemical Methods. American Society of Agronomy & Soil Science Society of America, Madison: 417–435.

Sandoval, F. M., L. C. Benz & R. H. Mickelson (1964): Chemical and physical properties of soils in a wet saline area in eastern North Dakota. – Soil Science Society of America Journal **28**: 195–199.

Schwert, D. P. (1990): Oligochaeta: Lumbricidae. – In: Dindal, D. L. (ed.): Soil Biology Guide. – John Wiley & Sons Inc., New York: 341–356.

Sharif, F., M. U. Danish, A. S. Ali, A. U. Khan, L. Shahzad, H. Ali & A. Ghafoor (2016): Salinity tolerance of earthworms and effects of salinity and vermi amendments on growth of *Sorghum bicolor*. – Archives of Agronomy and Soil Science **62**: 1169–1181.

Skarie, R. L., J. L. Richardson, A. Maianu & G. K. Clambey (1986): Soil and groundwater salinity along drainage ditches in eastern North Dakota. – Journal of Environmental Quality **15**: 335–340.

Soil Survey Staff (2020): Web Soil Survey. – Natural Resources Conservation Service, United States Department of Agriculture [https://websoilsurvey.nrcs.usda.gov/].

Stroud, J. L. (2019): Soil health pilot study in England: Outcomes from an on-farm earthworm survey. – PLOS ONE **14**: e0203909.

Thomas, G. W. (1996): Soil pH and soil acidity. – In: Sparks, D. L., A. L. Page, P. A. Helmke, R. H. Loeppert, P. N. Soltanpour, M. A. Tabatabai, C. T. Johnson & M. E. Sumner (eds): Methods of Soil Analysis, Part 3. Chemical Methods. – American Society of Agronomy & Soil Science Society of America, Madison: 475–490.

Tomlin, A. D. & C. A. Fox (2003): Earthworms and agricultural systems: Status of knowledge and research in Canada. – Canadian Journal of Soil Science **83**: 265 – 278.

van Vliet, P. C. J., B. van der Stelt, P. I. Rietberg & R. G. M. de Goede (2007) Effects of organic matter content on earthworms and nitrogen mineralization in grassland soils. – European Journal of Soil Biology **43**: S222–S229.

Vos, H. M. J., G. F. Koopmans, L. Beezemer, R. G. M. de Goede, T. Hiemstra & J. W. van Groenigen (2019): Large variations in readily-available phosphorus in casts of eight earthworm species are linked to cast properties. – Soil Biology and Biochemistry **138**: 107583.

Vos, H. M. J., M. B. H. Ros, G. F. Koopmans & J. W. van Groenigen (2014): Do earthworms affect phosphorus availability to grass? A pot experiment. – Soil Biology and Biochemistry **79**: 34–42.

Wang, Y., J. Chen, W. Gu, Y. Xu, J. Gu & J. Tao (2016): Earthworm activities increase the leaching of salt and water from salt-affected agricultural soil during the wet–dry process under simulated rainfall conditions. – Biology and Fertility of Soils **52**: 323–330.

Weil, R. R. & N. C. Brady (2017): The Nature and Properties of Soils. – Pearson, Upper Saddle River: 1104 pp.

Wickham, H. (2020): scales: Scale functions for visualizations. – R package version 1.1.1 [http://CRAN.R-project.org/package=scales].

Wickham, H., R. Francois, L. Henry, K. Muller & RStudio (2020): dplyr: A grammar of data manipulation. – R package version 1.0.2. [https://cran.r-project.org/web/packages/dplyr/index.html].

Wu, Y. P., Y. Zhang, Y. M. Bi & Z. J. Sun (2015): Biodiversity in saline and non-saline soils along the Bohai Sea coast, China. – Pedosphere **25**: 307–315.