EARTHWORM POSITIVELY INFLUENCES LARGE MACROPORES UNDER EXTREME DROUGHT CONDITIONS AND CONSERVATION TILLAGE IN A CHINESE MOLLISOL

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Abstract. It is often claimed that earthworms exert a huge influence on soil macroporosity. Nevertheless, gaps still exist in our knowledge of rangeability in soil macroporosity caused by earthworm activity. Earthworms are generally recognized as ecosystem engineers vital for soil ecosystem function and services, but the potential mechanism of earthworm response to the combination of extreme drought and conservation tillage has not yet been identified. To improve understanding of the effect of the earthworm on soil macroporosity and how earthworms, through soil macroporosity, respond to extreme drought and conservation tillage, a study was conducted to compare soil macroporosity under different tillage treatments with the same number of earthworms in incubation conditions and soil structural properties (soil penetration resistance, infiltration rate, saturated hydraulic conductivity) associated with macroporosity, together with crop yields under different tillage systems and drought stress in field conditions. The results show that earthworms only increase the volume of large macropores (>100 μm) rather than small macropores (30-100 μm) under different tillage systems. Earthworms played a positive role in the development of large macropores, as evidenced by the formation of paths of least resistance under high penetration resistance, higher infiltration rate and saturated hydraulic conductivity and no obvious yield loss under extreme drought and conservation tillage in a Chinese Mollisol.

Keywords: *soil macroporosity, soil penetration resistance, infiltration rate, saturated hydraulic conductivity, yield*

Introduction

Soil macroporosity plays an important role in the exchange of gases and water in soil (Francetto et al., 2016). One of the major ecological factors influencing soil porosity, especially macroporosity, is the burrowing activity of the earthworm (Maboeta et al., 2008). Numerous studies have emphasized that earthworms can greatly affect soil macroporosity (Bertrand et al., 2015). However, the extent of this effect on soil macroporosity is still somewhat vague, and very little is known.

Although earthworms are known to be affected by climate change (Eggleton et al., 2009), the impact of intense rainfall on earthworm behavior has up to now been given more attention than that of drought. Earthworms can increase the soil's ability to absorb water by creating macropores against intense rainfall disturbance and sustaining plant

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growth (Andriuzzi et al., 2015). During drought, current research indicates that earthworms become less active, and different species use different strategies to survive dry periods during the dry summer months (Bohlen et al., 1995) or when drought has a strong effect on earthworm communities (Mariotte et al., 2016). However, earthworm behavior under drought conditions is still unclear. In addition, conservation tillage has been recommended to improve resistance of crops to drought (Franzluebbers and Stuedemann, 2014), and many previous studies attribute the improved resistance to a heightened water retention capacity (Brouder and Gomez-Macpherson, 2014), whereas no further research has been evaluated. Thus, there is a need to explore the potential mechanisms of drought resistance under conservation tillage.

Modification of soil physical structure, especially macroporosity, by earthworms is well documented (Amossé et al., 2015), but most research has focused on studying the qualitative relationship between earthworm activity and macroporosity. In addition, the above qualitative relationship was obtained without considering the earthworm population. Earthworm population is largely influenced by tillage managements in different agricultural systems (van Schaik et al., 2016). Conservation tillage generally resulted in higher earthworm populations than conventional tillage (Johnston et al., 2015). Hence, in order to more accurately obtain the rangeability in soil macroporosity caused by earthworm activity, it is necessary to study the soil macroporosity changes caused by the same number of earthworms under different tillage practices. Nevertheless, it is difficult to uniformly distribute the same number of earthworms in field conditions. Sampled in situ soil columns, used with the addition of the same number of earthworms, can solve this problem, because sampled in situ soil columns are closest to and can simulate real field conditions. The question studied is thus how earthworms affect crop growth under drought and conservation tillage practices through soil macroporosity. Therefore, we hypothesized that (1) different tillage practices would lead to different effects on soil macroporosity under the same number of earthworms (sampled in situ soil columns with the addition of the same number of earthworms) in incubation conditions, and (2) soil structural properties (soil penetration resistance, infiltration rate, saturated hydraulic conductivity) associated with macroporosity would positively affect crop yields. Accordingly, the objectives of this study were to (1) compare the soil macroporosity under different tillage treatments and the same number of earthworms (sampled in situ soil column with the addition of the same number of earthworms) in incubation conditions, and (2) identify the soil structural properties (soil penetration resistance, infiltration rate, saturated hydraulic conductivity) associated with macroporosity and crop yields, to determine the influence of earthworms on macroporosity under extreme drought and tillage systems. The present study may not only improve understanding of the effect of earthworms on macroporosity and plant growth under conservation tillage and drought conditions but also deepen the comprehensive understanding of how earthworms affect soil macroporosity.

Materials and methods

Study area

The study area was established in 2001 at the Mollisols Experiment and Demonstration Base (44°12′ N, 125°33′ E) of the Northeast Institute of Geography and Agroecology, Chinese Academy of Sciences, in Dehui County, Jilin Province, northeast China (*Fig. 1)*. It is located in the north temperature zone with semi-humid temperate continental monsoon conditions. Annual average rainfall was 520 mm over the past 30 years with more than 70% occurring from June to August. Average annual temperature is 4.4 °C. The soil type in the current study is Typic Hapludoll with clay loam texture. Prior to this tillage experiment, the field had undergone more than ten years of conventional tillage management for continuous corn cultivation. The selected properties of the soil are reported in the references listed in *Table 1* (Liang et al., 2011).

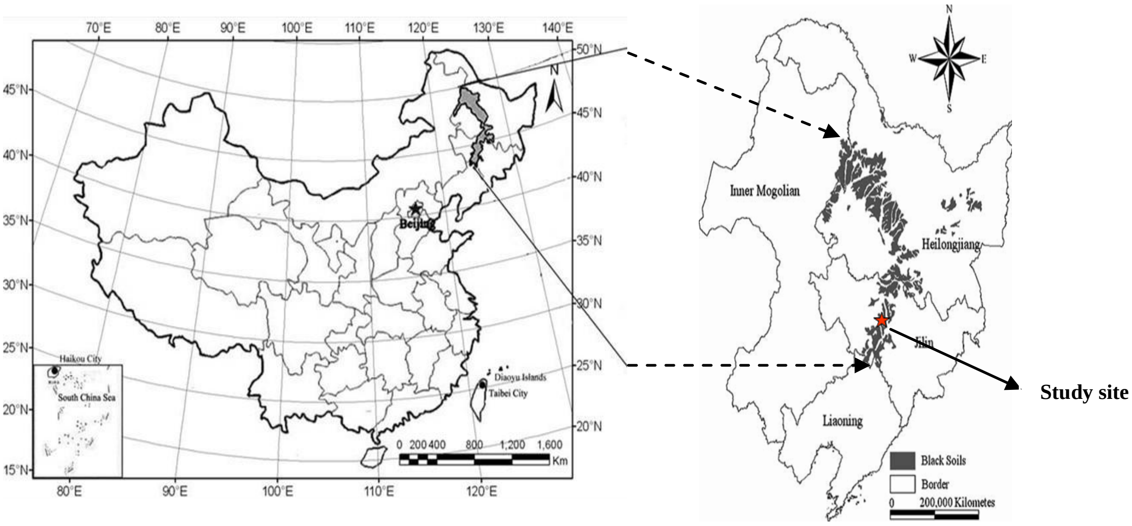

***Figure 1.** Location of study area (Left is map of China, right is Mollisols region of northeast China and study site)*

***Table 1.** Selected soil physical and chemical properties in 2001 prior to initiation of tillage treatments (Liang et al., 2011)*

Depth (cm)	**pH**	**Clay (%) (<2 µm)**	**Silt (%) (2-20 µm)**	**Sand (%) (20-200 µm)**	**Bulk density (g/cm^3)**	**Soil organic carbon (g/kg)**	**Total soil nitrogen (g/kg)**
0-5	6.48	36.03	24.00	39.97	1.24	16.48	1.42
5-10	6.45	35.83	23.78	40.39	1.38	16.29	1.39
10-20	6.51	35.68	24.35	39.98	1.36	16.08	1.37
20-30	7.03	36.56	25.00	38.72	1.38	14.22	1.16

Experimental design

The tillage treatments, including moldboard plow (MP), ridge tillage (RT) and no tillage (NT), were arranged in a completely randomized block design with four replicates. Each tillage plot was split into two sub-plots (5.2 m x 20 m). Corn-soybean rotation, with both crops present each year, was applied at the sub-plot level. Tillage for the MP treatment included one fall moldboard plowing (approximately 20 cm in depth) after harvest, one spring disking (7.5 to 10 cm in depth) and field cultivation. The RT treatment included ridging in June for corn and soybeans, chopping the crop stalk/roots in the fall (approximately 1/3 row width) and spring planting with an NT planter. For NT, no soil was disturbed except for spring planting by a KINZE-3000 NT planter (Kinze Manufacturing Inc., USA). Each year, 100 kg/ha of nitrogen (N), 45.5 kg/ha of phosphorus (P) and 78 kg/ha of potassium (K) were applied as starter fertilizer and an additional 50 kg/ha of N was applied as top dressing at the six-leaf (V6) stage for corn. All fertilizers including 40 kg/ha of N, 60 kg/ha of P and 80 kg/ha of K were used as starter fertilizer for soybeans.

Soil sampling and analysis

Laboratory experiment

On 22 April 2015 (before spring planting), 6 replications of in situ soil columns from each NT, MP and RT plot were collected down to a 10 cm depth using polyvinyl chloride (PVC) tubes (10 cm diameter, 15 cm height); the total number of PVC tubes was 36. These PVC tubes were then divided into two groups of 18 PVC tubes: one with added earthworms (3 specimens of *Eisenia fetida*) and the other without earthworms. Gas-permeable parafilm covered the tops and bottoms of PVC tubes. A MEMMERT HPP750 Constant Climate Chamber (Memmert Inc., Schwabach, Germany) was used to incubate the samples. Incubation time was from 15 June 2015 to 16 July 2015. On 30 June 2015 (15 days after incubation), 3 replications of soil samples from each NT, MP and RT treatment were collected using 5-cm-diameter cylinders from the above PVC tubes (including with and without earthworms, thus 18 PVC tubes were used to sample at this stage) to determine soil pore size distribution. The same procedure was used for sampling on 16 July 2015 (30 days after incubation). These 36 soil samples, from groups with and without earthworms at two stages, were saturated and submitted to a tension table within a pressure potential range of 0 to 1500 kPa (Soil Moisture Equipment Inc, USA). The relationship between capillary water retention and pore size was used to identify soil pore size distribution (Bhattacharyya et al., 2006). According to Bhattacharyya et al. (2006), at a given matric pressure (h), soil water potential (kPa), and equivalent pore diameter (EPD), the diameter of the smaller pores drained (μm) can be computed from the following equation:

$$EPD = \frac{300}{h} \qquad \text{(Eq.1)}$$

Soil pore volume occurring within a given size interval per unit soil (total) volume was used to present soil pore size distribution (Hayashi et al., 2006). According to the classification criteria defined by Chen et al. (2015), soil pore size distribution was classified as large macropores (>100 μm), small macropores (30-100 μm), mesopores (0.2-30 μm) and micropores (<0.2 μm) in this study.

Field experiment

On 29 July 2015, soil penetration resistance was determined in situ at 2.5 cm intervals down to 30 cm using a SC-900 handheld digital penetrometer (Spectrum Technologies Inc., USA) from each NT, MP and RT plot.

From 20 August 2015 to 22 August 2015, a Hood Infiltrometer IL 2700 (diameter 17.6 cm) (Umwelt-Geräte-Technik GmbH, Müncheberg, Germany) was used to determine the infiltration rate, saturated hydraulic conductivity, and macropore flow for each NT, MP and RT plot. Detailed information on the methods for Hood Infiltrometer IL 2700 measurement was given in Schwärzel and Punzel (2007).

2015 extreme drought in northeast China

Because of El Nino, our field site in 2015 experienced an extreme drought. Monthly precipitation during the growing season (April to September) decreased 48.82%, 52.53%, 27.79%, 170.31%, 57.23% and 134.88%, respectively compared to the 40-year (1975-2014) mean corresponding monthly precipitation (*Table 2*). Total precipitation decreased 80.91% compared to the 40-year mean value (*Table 2*). Precipitation in 2015 was also the lowest since the field's establishment in 2001 compared to the results obtained by Fan et al. (2012) and Zhang et al. (2015) in our field site. In addition, according to the evaluation standards for the variability and predictability of the northeast China climate through 1948-2012 in Gao et al. (2014), 2015 is still an extreme drought year. Thus, to better understand the effect of earthworms on macroporosity under drought and tillage systems, we selected 2015 as the studied year.

Table 2. *Monthly precipitation (mm) during the growing season in 2015 and 40-year mean monthly precipitation (mm) from 1975 to 2014 in Dehui County, Jilin Province, northeast China*

	April	**May**	**June**	**July**	**August**	**September**	**Total**
2015	12.7	31.6	68.0	57.6	83.7	32.4	286
40-yr mean (1975-2014)	18.9	48.2	86.9	155.7	131.6	76.1	517.4

Note: Data were taken from the National Climatic Center of China Meteorological Administration.

Statistical analysis

All statistical analysis was done by SPSS 13.0 software (SPSS Inc., USA). Since the main objectives of the present study were to compare the effects of tillage practices on soil pore size distribution, soil penetration resistance, infiltration rate, saturated hydraulic conductivity and crop yields, one-way analysis of variance (ANOVA) was conducted to test the treatments' main effects on soil pore size distribution, soil penetration resistance, infiltration rate, saturated hydraulic conductivity and crop yields. Means of soil pore size distribution, soil penetration resistance, infiltration rate, saturated hydraulic conductivity and crop yields among the treatments were compared using the least significant difference test at a 0.05 significance level.

Results

Soil macroporosity with and without earthworms under different tillage systems in incubation conditions

Earthworms greatly affected soil macroporosity (*Fig. 2*). NT, MP and RT had no significant ($p > 0.05$) effect on the volume of large macropores (>100 μm) after 15 and 30 days incubation without earthworms (*Fig. 2 a* and *Fig. 2 c*). However, with earthworms, NT and RT had significantly ($p < 0.05$) greater volume of large macropores (>100 μm) than MP after 15 and 30 day incubations (*Fig. 2 b* and *Fig. 2 d*). Concerning small macropores (30-100 μm), NT and RT led to significant ($p < 0.05$) volume reduction in contrast to MP after 15 and 30 day incubations both with and without earthworms (*Fig. 2*). There were no significant differences in the volume of mesopores (0.2-30 μm) and micropores (<0.2 μm) among NT, MP and RT after 15 and

30 days incubation either with and without earthworms (*Fig. 2*). Regarding incubation time, the volume of large macropores (>100 μm) in the NT, MP and RT plots with earthworms increased 16.73%, 1.69%, and 15.47%, respectively compared to those without earthworms after 15 days incubation (*Fig. 2 a* and *Fig. 2 b*). A similar result was found after 30 days incubation and the corresponding increasing ranges were 33.22%, 17.05%, and 31.35%, respectively (*Fig. 2 c* and *Fig. 2 d*).

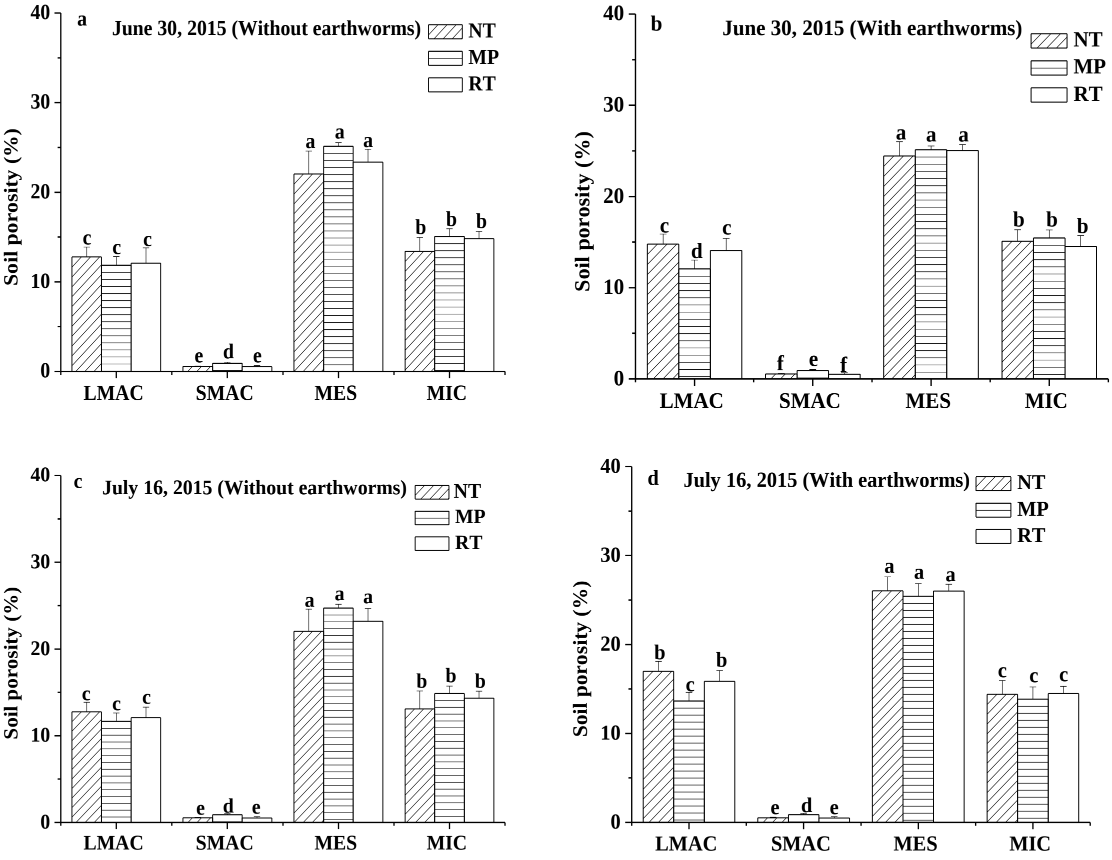

Figure 2. *Soil pore size distribution with and without earthworms on 30 June 2015 (15 days after incubation) and 16 July 2015 (30 days after incubation) under different tillage practices. NT: no tillage; MP: moldboard plow; RT: ridge tillage; LMAC: large macropores (>100 μm); SMAC: small macropores (30–100 μm); MES: mesopores (0.2–30 μm); MIC: micropores (<0.2 μm)*

Soil structural properties associated with macroporosity under extreme drought and different tillage systems in field conditions

Similar to 2013 (normal year), soil penetration resistance in NT and RT increased significantly ($p < 0.05$) compared to that of MP at 2.5-17.5 cm depth in 2015 (extreme drought year) (*Fig. 3*). Soil penetration resistance in all treatments reached the critical value of 2000 kPa at as shallow a depth as 12.5 cm in 2015 (extreme drought year), while the soil depth for this critical value in 2013 (normal year) was 20 cm (*Fig. 3*). Compared with 2013 (normal year), soil penetration resistance in NT, RT and MP soils increased an average of 31.99%, 66.05%, and 30.95%, respectively in 2015 (extreme drought year) (*Fig. 3*).

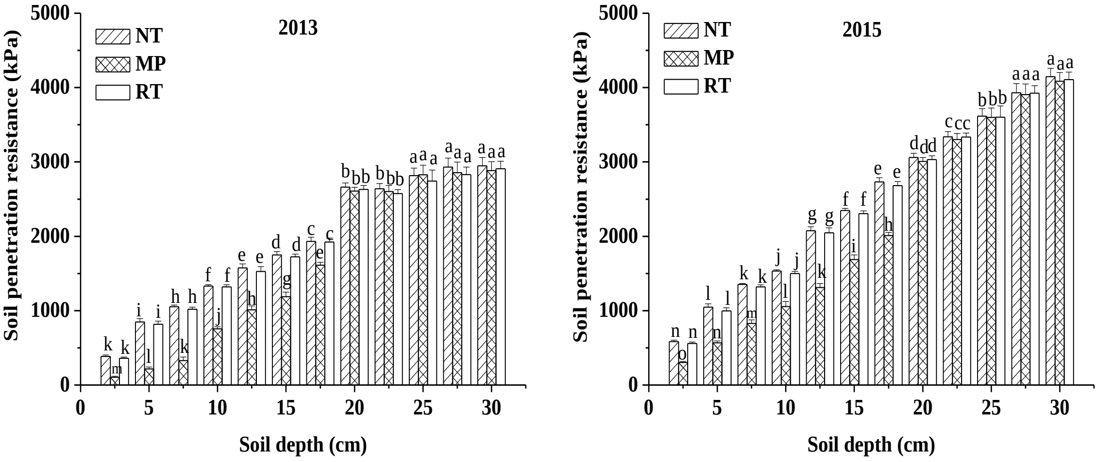

Figure 3. *Soil penetration resistance under no tillage (NT), moldboard plow (MP) and ridge tillage (RT) systems in 2013 (normal year) and 2015 (extreme drought year)*

In NT, MP and RT soils, variation trends of infiltration rates were high at first, gradually dropped, and then became close to a stable stage (*Fig. 4*). 20 min was the time to reach the stable stage for each tillage treatment, and the corresponding infiltration rates for NT, MP and RT plots were 5.2, 2.5 and 4.8 mm/min, respectively (*Fig. 4*).

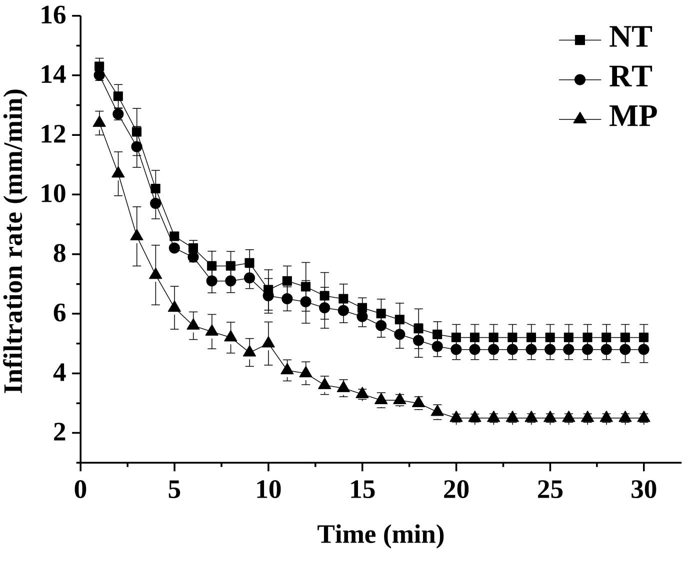

Figure 4. *Infiltration rate under no tillage (NT), moldboard plow (MP) and ridge tillage (RT) systems*

A significantly ($p < 0.05$) greater saturated hydraulic conductivity was found in NT and RT than in MP soils, and this was similar to the observations of contributions of macropores to total flow (*Table 3*).

Table 3. *Saturated hydraulic conductivity and contribution of macropores to total flow under different tillage practices*

	Saturated hydraulic conductivity (cm/min)	**Contribution of macropores to total flow (%)**
NT	1.92a	69.93a
MP	1.60b	41.48b
RT	1.83a	66.37a

Note: Contribution of macropores to total flow was calculated from the proportion of hydraulic conductivity corresponding to equivalent pore diameter for macroporosity of saturated hydraulic conductivity. NT: no tillage; MP: moldboard plow; RT: ridge tillage; values followed by the same letter within a column indicate no significant difference at the 0.05 level.

Comparison of crop yield in extreme drought year and long-term average

As in long-term average yields (2002-2014), MP had a significantly ($p < 0.05$) lower yield than NT and RT in the extreme drought year (2015) (*Table 4*). Although crop yield for NT and RT in the extreme drought year (2015) decreased 8.85% and 9.55%, respectively, compared with the long-term average (2002-2014), no significant differences in crop yield were found ($p > 0.05$) (*Table 4*). However, crop yield for MP in the extreme drought year (2015) significantly ($p < 0.05$) decreased by 51.74% compared to the long-term average (2002-2014) (*Table 4*).

Table 4. *Average crop yield from 2002 to 2014 and crop yield in 2015 (extreme drought year) under different tillage practices*

Crop yield (kg/ha)	**Tillage practices**		
	NT	**MP**	**RT**
2002-2014 average data	10769a(a)	10241b(a)	10822a(a)
2015 (extreme drought year)	9816a(a)	4942b(b)	9788a(a)

Note: NT: no tillage; MP: moldboard plow; RT: ridge tillage; values followed by the same letter inside and outside the brackets indicate no significant difference at the 0.05 level for vertical and horizontal comparisons, respectively.

Discussion

Soil macroporosity with and without earthworms under different tillage systems in incubation conditions

Earthworms resulted in a significant change in the volume of large macropores (>100 μm). On the surface, this is mostly due to the lower level of compression offered by NT, which protects the large macropores and keeps them intact. In addition, disturbance of soil by primary or secondary tillage would be expected to result in loosening of soil and thus an increase in macroporosity of the tilled zone (Kay and van den Bygaart, 2002). Moreover, traffic-induced compaction in MP intensified extrusion to large macropores. However, the essential reason was that earthworm activity, through bioturbation and egestion, influences soil porosity extrusion (Mariotte et al., 2016). This was agreed upon by previous research (Peigné et al., 2009; Amossé et al., 2015). While previous studies simply state earthworms can increase soil macroporosity (Blouin et al., 2007) or accelerate soil macroporosity turnover (Bottinelli et al., 2010), they do not clearly articulate the range of soil macroporosity for this change.

A significantly lower volume of small macropores was observed in NT and RT compared to MP after 15 and 30 day incubations, with and without earthworms. This means that earthworm activity has no effect on the small macropores. The reason for this situation was the partial destruction of large macropores by soil disturbance, resulting in the formation of a greater volume of small macropores under MP (Chen et al., 2016). Kay and van den Bygaart (2002) found that shifts from conventional to conservation tillage usually led to a reduction in the volume of small macropores. Considering the nonsignificant results concerning mesopores and micropores, our results again indicate that earthworms only affect large macropores (>100 μm) under different tillage systems. Greater differences between the large macropore volumes among the NT, MP and RT plots with and without earthworms under different incubation times indicate that the increasing range in the volume of large macropores (>100 μm) increased with time.

Soil structural properties associated with macroporosity under extreme drought and different tillage systems in field conditions

Both NT and RT increased soil penetration resistance in the 2.5-17.5 cm layer compared to MP ($p < 0.05$). This was mainly related to the fact that the accumulated soil consolidation with time occurred in NT and RT soils, in contrast to the tillage compaction in fall and spring of MP soil (Alvarez and Steinback, 2009). The results of different depths reaching 2000 kPa, which is the critical value for relatively unimpeded root growth (Reynolds et al., 2007), in 2015 (extreme drought year) and 2013 (normal year), together with the greater increment of soil penetration resistance in 2015 (extreme drought year) compared to 2013 (normal year), indicate that the actual resistance of the soil to root penetration is generally less than the average resistance measured by the penetrometer; this is because roots seek the path of least resistance during growth rather than penetrating straight through the soil (Olibone et al., 2010). The path of least resistance was associated with the large macropores burrowed by earthworms (Bengough, 2012; Bottinelli et al., 2015). In this study, it also means that earthworm activity, through the volume of large macropores, made a positive impact on soil penetration resistance under extreme drought conditions.

Higher infiltration rates for NT and RT than in MP soils in this study contrasted with the study of Lipiec et al. (2006) but were parallel to the results of other research (Abid and Lal, 2009; Kahlon et al., 2013). Previous studies have attributed this infiltration rate difference among tillage practices to soil texture, macropore quantity and continuity, soil organic matter content, soil moisture and soil bulk density (Ginting et al., 2003; Gajda et al., 2016; Zaibon et al., 2017), but all of these vary case by case. Ultimately, earthworm burrowing and root decomposition played an important role in increasing macropore quantity and thus made for preferential transport (Emmerling et al., 2015). This important role can also be verified by the results of saturated hydraulic conductivity values and contribution of macropores to total flow. These hydraulic property results indicate that the effect of earthworms on large macropores plays an important role in water infiltration and conservation under extreme drought conditions.

Comparison of crop yield in the extreme drought year to the long-term average

From the view of tillage effect on crop yield under long-term averages *versus* an extreme drought year (2015), these results were consistent with those reported by He et al. (2011), who found that conservation tillage practices were beneficial to crop yield. Crop yield variation under conservation tillage and extreme drought indicates that conservation tillage practices can make production highly resistant to climatic variables (Thierfelder et al., 2015). It also means that NT and RT can function as a buffer against the poor performance of crops in low-yield years, leading to better crop performance to counteract extreme adverse environmental effects compared to that of MP (Šíp et al., 2013). In other words, this could be explained by the fact that earthworm can buffer the soil-plant system against the effects of extreme drought events (Johnson et al., 2011). Bertrand et al. (2015) also noted that aboveground biomass generally increases in the presence of earthworms in extreme weather events.

No obvious yield losses were found under NT and RT in the extreme drought year (2015). This confirms that earthworms, through macroporosity and its related soil structural properties, affect crop growth positively under conservation tillage systems. Our results are in accordance with Spurgeon et al. (2013), who found positive effects of earthworms on soil macroporosity together with structural properties and plant growth. The possible explanation is that earthworm-induced changes and modification of soil macroporosity results in changes of water and oxygen availability to plants, and better soil structure conditions, which are beneficial for improving the carrying capacity of nutrients (van Groenigen et al., 2014).

Previous studies have emphasized the great effects of earthworms on soil macroporosity, but neglected the rangeability in soil macroporosity caused by earthworm activity and what range of soil macroporosity accounts for this change. On the other hand, earthworms' response to drought and different tillage is still inadequately characterized. To solve these problems, in the current study, research was conducted to create an in depth evaluation of the effect of earthworms on macroporosity under drought conditions and different tillage treatments, based on the comparison of soil macroporosity under different tillage treatments and the same number of earthworms in incubation conditions, soil structural properties (soil penetration resistance, infiltration rate, saturated hydraulic conductivity) associated with macroporosity under different tillage systems and drought in field conditions, and crop yields.

In summary, our study found that not only would earthworms increase the volume of large macropores (>100 μm), though not small macropores (30-100 μm), under different tillage systems, but that earthworm activity through large macropores also formed the path of least resistance under high penetration resistance, leading to higher infiltration rate and saturated hydraulic conductivity and affecting crop yield positively under conservation tillage and extreme drought conditions.

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APPENDIX

Appendix 1. *Soil bulk density (BD), soil water content (SWC) and soil air-filled porosity (AFP) under no tillage (NT), moldboard plow (MP) and ridge tillage (RT) systems in this study site*

		Treatments		
Soil properties	**Soil depth (cm)**	**NT**	**MP**	**RT**
BD (g/cm^3)	0-5	1.15a	1.01b	1.10a
	5-10	1.36a	1.21b	1.30a
	10-20	1.38a	1.23b	1.32a
	20-30	1.36a	1.35a	1.34a
SWC (weight, %)	0-5	30.20a	26.78b	31.65a
	5-10	24.78a	20.23b	25.99a
	10-20	16.12b	18.90a	16.87b
	20-30	12.36a	12.50a	12.47a
AFP (cm^3/cm^3)	0-5	1.24c	1.55a	1.35b
	5-10	0.90c	1.13a	0.98b
	10-20	0.87c	1.10a	0.95b
	20-30	0.90a	0.91a	0.93a

Values followed by the same letter within a row indicate no significant difference at the 0.05 level.