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# Earthworm Burrowing Activity and Its Effects on Soil Hydraulic Properties under Different Soil Moisture Conditions from the Loess Plateau, China

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**Abstract:** Earthworm activity has become more important in the Loess Plateau, where hydrological processes are crucial for ecosystem sustainability. In this study, we conducted a laboratory microcosm experiment to determine the various burrowing activities of *Eisenia fetida* and their impact on the soil hydraulic properties in response to different levels of soil moisture (50%, 70%, 90% of field capacity) in two common soil types (loessial and Lou soil) obtained from the Loess Plateau. Burrowing activity of *E. fetida* increased with higher soil moisture and was greater in loessial than in Lou soil. Most burrowing activities occurred within the top 5 cm and decreased with increasing soil depth. Macropores and burrow branching, which are highly related to the earthworm burrowing, were more prevalent in wetter soil. Earthworms significantly altered the formation of large soil aggregates ($AG_L$, diameter >2 mm) under different soil moistures and depths. Distinct earthworm burrowing activities, controlled by soil moisture, altered soil hydraulic properties. However, soil saturated hydraulic conductivity (Ks) showed little differences between different treatments due to the horizontal and high–branched burrows of *E. fetida*, although higher burrowing activities were found in wetter soil. Soil field capacity was highest in drier soil due to the less macropores and burrowing activities.

**Keywords:** earthworm burrows; soil moisture; soil aggregates; hydraulic properties; Loess Plateau

## 1. Introduction

Earthworms play an important role in the regulation of soil physicochemical processes and ecosystem services, such as the maintenance of soil structure, water regulation, and nutrient inputs for plants [1,2]. Their burrowing activities increase the proportion of macropores in the soil, which have important hydrological functions: (1) preferential flow through macropores could improve water movement to the deep soil, enhance water infiltration and drainage, reduce surface runoff, and subsequently, decrease soil erosion [3,4]; (2) increased soil aeration in the macropores enhances water loss through evaporation, decreasing soil moisture content [5]. However, the variability in earthworm burrowing activities is strongly related to soil properties (e.g., soil type, soil texture, organic matter) and soil temperature and is particularly sensitive to soil moisture [6]. Several studies have shown that earthworms were often inactive, entered diapause, and lost weight when soil moisture was low [7,8]. Limited burrowing activities were observed in some laboratory experiments when the soil water potential or water content decreased [9–11]. A wet habitat is well–known to be essential for earthworm activity; however, Perreault and Whalen [12] found greater earthworm burrowing activity in drier soil than wetter soil, as earthworms explore a larger volume of soil for their survival when the soil is dry. Earthworm activity is based on complex interactions between soil conditions, climate, and earthworm species and has been well documented in Europe and North America for decades [13,14]. Thus, more basic studies evaluating the effect of soil conditions (e.g., moisture) on burrowing activity are critically needed in soils of Asia, which has received little attention.

Earthworms form a mass of soil aggregates through casting and burrowing, which could account for 50% of the topsoil aggregates in temperate regions [6,15]. Earthworms redistribute fine particles within the soil by aggregation, wherein the advanced large pores between aggregates and inner pore structure of casts (bio–aggregates) can improve infiltration, water retention, and discharge [16–19]. Some studies have shown that aggregation due to casting activities is related to soil moisture [6,20]. A model developed based on earthworm biomass, soil moisture, and temperature estimated that the soil aggregate formation rate by earthworms increased with higher soil water potential [21]. Hindell et al. [22] found that earthworm casting activity was greatest at a high soil water potential of 0 to −2 kPa in a gradient of low to high (from −25 kPa to −2 kPa) water potential.

The impact of earthworms on soil hydraulic properties is well–studied by describing the burrowing activity and burrow characteristics [23–25]. Higher permeation rates and faster water discharge were observed resulting from the high burrowing activities of earthworms. In addition, soil Ks increased as more surficial, connected, and continuous burrows are created by earthworms [23,24,26]. However, horizontally created earthworm burrows with many branches have been found to limit or reduce the water conductivity [27,28], indicating a variety of water–based processes determined by earthworm burrowing activities. Water retention is therefore closely related to the earthworm activities. Indeed, water holding capacity can decrease with increasingly efficient water drainage or faster evaporation through earthworm burrows [2]; conversely, it can increase following the increased content of water–stable aggregates also formed by earthworms [29]. Since soil moisture is one of the most important factors influencing the earthworm activities, the impact of earthworms on soil hydraulic properties may vary depending on soil moisture. Therefore, the relationship between the various burrowing activities and their effects on water transfer, water–retention properties under different soil moisture levels merit investigation.

The Loess Plateau is widely known for having severe soil erosion and hydrological problems; hence, it is urgent to evaluate and improve the efficiency of water infiltration and conservation in this area [30]. Soil macrofauna has already been shown to play an important role on soil hydrological processes in the region. A series of studies by Li et al. [31,32] reported the importance of crickets and ants on regulating soil structure and water movement on the Loess Plateau. The burrows of mole crickets effectively intercepted rainfall, improved water infiltration, and reduced runoff; the preferential flow into the burrows and nests also effectively increased soil moisture content. There is a surprising lack of research on earthworms on the Loess Plateau, even though earthworms are one of the major soil engineers and are used frequently in ecological farming in recent years. *E. fetida* is a common species used in ecological farming for its prominent capacity for organic matter decomposition and vermicomposting [33]. Therefore, most studies on *E. fetida* have been carried out to investigate biodegradation and nutrient recycling. Despite the species' intense burrowing activity within the topsoil layer, impacts on soil's physical and hydraulic properties are less considered and therefore necessary to evaluate the role of earthworms on ecological sustainability on the Loess Plateau.

Here, we conducted a microcosm experiment to quantitatively characterize the burrows of *E. fetida* under three different levels of soil moisture in two common soil types from the Loess Plateau. In addition, soil aggregation and hydraulic properties were determined to evaluate the effects of the burrowing activity of *E. fetida* on soil physical–hydrological functions.

## 2. Materials and Methods

### *2.1. Experimental Design*

The study was conducted based on microcosm experiment using a completely randomized factorial design with two soil types: Lou soil and loessial soil, and under three soil moisture conditions: 50%, 70%, and 90% of field capacity. A native and dominant epigeic earthworm species *E. fetida* was used for the study.

Lou soil and loessial soil are two common soil types occurring on the central and southern part of the Loess Plateau, respectively. Lou soil is clay loam, described as a Cumulic Anthrosol and loessial soil is silty clay loam (FAO/UNESCO); physical and chemical properties of the soil types are shown in Table S1. Lou soil was obtained from the upper 20 cm of a wheat–fallow field in Yangling (108°04′ E, 34°17′ N), while loessial soil was obtained from the upper 20 cm of an apple orchard in Changwu (107°41′ E, 35°14′ N). The soil was air–dried to a soil moisture content of less than 5% and sieved at 2 mm. The microcosms used in this study were plexiglass columns with diameter of 20.4 cm and height of 25 cm. Soil was packed to a height of 20 cm at a dry bulk density of 1.19 g cm$^{-3}$ and 1.16 g cm$^{-3}$ for Lou and loessial soil, respectively; the density of each soil type was established by a field test conducted at each study site. In this process, the soil in each column was compacted stepwise in three layers to minimize the variation in the bulk density within the soil column. Mesh fabric was placed at the top and bottom of the column to prevent earthworms escaping and soil loss. A plastic tray was placed at the bottom of the column for water supplementation.

*E. fetida*, a widely distributed species in both soil types, was collected from the study sites. The average weight of an individual was about 0.5 g. Six adult earthworms were introduced into each microcosm, and 5 g of litter and 100 g of cow manure were added to the soil surface as the food supply. After the earthworms were added, soil moisture was supplied at three different levels. The optimum soil moisture condition for *Eisenia* earthworms is 60–70% of the water holding capacity [21]. In this study, 70% of the field water capacity was set as the "Medium" water treatment, while 90% and 50% were set as the "High" and "Low" water treatments, respectively. The field capacity on average was 40% of soil mass in both the Lou and loessial soils. Each treatment with earthworms had four replicates. Besides, we also set one microcosm without earthworms for every soil types and soil moisture levels.

All microcosms were randomly placed in the laboratory at 20–25 °C to allow the earthworms to burrow for four weeks. During the incubation period, the weight of water loss was measured every two days using a weighing machine (precision = 0.01 kg) followed by water addition (as necessary) to maintain the initial water level in each column.

### *2.2. Characterization of Earthworm Burrows*

A simple and effective method reported by Capowiez et al. [34] was used to quantitively characterize the earthworm burrows. The method takes advantage of earthworm preference to burrow at the soil–column interface, and thus, most of the burrows were created along the microcosm walls. Before packing soil, a transparent plastic sheet (64 cm long, 20 cm wide) was placed along the inner wall of each microcosm. At the end of the incubation experiment, burrow traces on the sheet were marked with a black pen to represent the earthworm burrows (see samples in Figure 1). The number of continuous burrows and branches were counted, respectively. Each transparent sheet was marked with a scale and then digitized (scanner resolution: 300 dpi). The scan images were processed using Image J 1.8.0 to calculate the percentage of burrow area (%) and macropores (pore diameter > 2 mm) area (%), respectively.

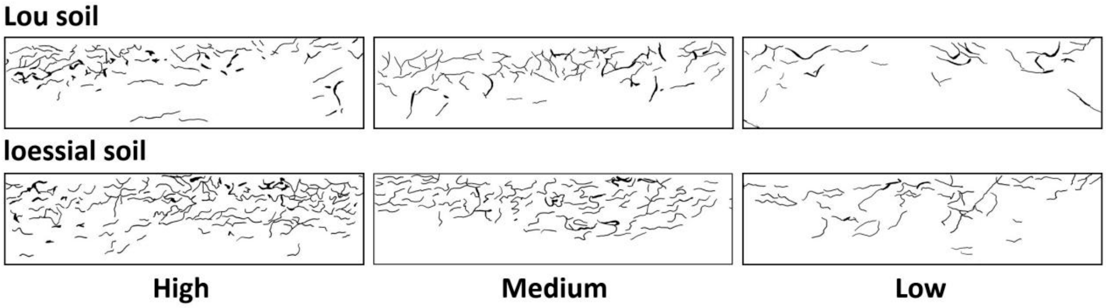


**Figure 1.** Scannogram of the earthworm burrow systems along the soil column wall (0–15 cm of soil layer) in two soil types for the three soil moisture treatments (High, Medium, and Low).

### 2.3. Soil Sampling and Analysis

At the end of the incubation, undisturbed soil samples were collected using 100 $cm^3$ density rings at 5 cm intervals (0–5 cm, 5–10 cm, and 10–15 cm) from each soil column. At each depth, four undisturbed soil samples including two from the center and two from the edge were randomly collected. Of these, two samples, one from central area and the other from edge, were used for bulk density determination (oven drying at 105 °C); the other two were used for measuring soil saturated hydraulic conductivity (Ks) and field capacity. Ks was measured by the constant hydraulic head method in saturated soil samples, as in Klute and Dirksen [35] and Wang et al. [36]. The field capacity was measured as the water content of saturated soil samples after discharging for 8 h. Soil aggregate content (AG), particle size distribution (PSD), and soil organic carbon (SOC) were also measured in 200 g of disturbed soil, collected at each depth and air–dried. AG ($AG_L$: AG > 2 mm, $AG_S$: 0.25 mm < AG < 2 mm) was determined by sieving [37], whereby samples of 50 g of air–dried soil were soaked for 30 min in filter paper and placed on a wet sieving apparatus with a series of five sieves of 5 mm, 2 mm, 1 mm, 0.5 mm, and 0.25 mm. The sieving procedure was conducted 30 times within 1 min. After this procedure, the aggregates in each sieve type were oven–dried at 50 °C and weighed to calculate the AG content (%). PSD was measured using a laser diffraction apparatus (Mastersizer 2000, Malvern Instruments, Malvern, UK) with 5 g of soil (particle size < 2 mm). Organic matter and calcium carbonate ($CaCO_3$) were removed beforehand. Soil organic carbon (SOC) was measured using dichromate oxidation [38] with 0.5 g of soil (particle size < 2 mm).

The multi–factor analysis of variance (ANOVA) followed by a Duncan's test was used to analyze significant differences of earthworm burrowing activity between soil types, soil moisture content, soil depth, and their interactions. Statistical analyses were performed using the SPSS 21.0 software package (SPSS Inc., Chicago, IL, USA). Significant differences were tested at $p < 0.05$. Data are expressed as the mean ± SE (as shown in figures and tables).

## 3. Results

### 3.1. Characteristics of Earthworm Burrows

The number of burrows was 37, 54, and 64 in Low, Medium, and High treatments in Lou soil, and 34, 59, and 65 in loessial soil for the total soil layer (0–15 cm) (Figure 1, Table 1). Burrow numbers were 1.7 times and 1.9 times higher in the High than in the Low treatment for Lou soil and loessial soil, respectively. Burrow area at High and Medium treatments ranged from 4.4% to 7.7% and was 1.7–2 times higher in these treatments than at Low. The burrow area was significantly different between the two soil types ($p < 0.001$), as the burrow area in loessial soil was 1.6, 1.4, and 1.5 times higher compared to those in Lou soil at High, Medium, and Low moisture, respectively. The area of macropores ranged from 0% to 0.25%, showing little difference between two soil types but was significantly higher at High than at Low soil moisture for the total 0–15 cm of the soil. Both the area of burrows and macropores were significantly different between soil depths ($p < 0.001$, Table 2), which decreased with increasing soil depth. Burrow area ranged between 1.8–3.4%, 0.6–3.5% at the 0–5 cm and 5–10 cm soil depths, respectively, but dramatically decreased at the 10–15 cm soil depth to a range of 0.2% to 1.2% (Figure 2). In addition, the area of burrows was greatly affected by soil moisture at 0–5 cm and 5–10 cm; however, burrow area was hardly affected at 10–15 cm depth. The macropore area also decreased greatly from a range of 0–0.2% at 0–5 cm to a range of 0% to 0.02% at 10–15 cm. The number of burrow branches was 23, 29, and 8 in Low, Medium, and High treatments in Lou soil, and 58, 31, and 19 in loessial soil for all depths 0–15 cm within the soil, indicating significant differences in the number of branches between soil moisture and soil types. The number of branches significantly decreased with increasing soil depth ($p < 0.05$), i.e., from a range of 6–30 at 0–5 cm to a range of 0.3–5 at 10–15 cm soil depth.

**Table 1.** Earthworm burrowing activity under three soil moisture treatments (High, Medium, and Low) and two soil types (Lou and loessial soil) after a four–week earthworm incubation period. Lower–case letters indicate significant differences between the three soil moisture conditions in each soil type. Burrow and macropore areas are mean ± SE ($n$ = 4) and numbers of burrows and branches are means ($n$ = 4).

| | **Lou Soil** | | | **Loessial Soil** | | |
|---|---|---|---|---|---|---|
| | **High** | **Medium** | **Low** | **High** | **Medium** | **Low** |
| 0–5 cm | | | | | | |
| Area of burrows (%) | 2.96 ± 0.26 $^{a}$ | 2.54 ± 0.32 $^{ab}$ | 1.79 ± 0.10 $^{b}$ | 3.38 ± 0.51 $^{a}$ | 2.67 ± 0.39 $^{ab}$ | 2.04 ± 0.17 $^{b}$ |
| Area of macropores (%) | 0.15 ± 0.06 | 0.11 ± 0.06 | 0.02 ± 0.01 | 0.17 ± 0.06 $^{a}$ | 0.05 ± 0.02 $^{ab}$ | 0 $^{b}$ |
| Number of branches | 15.75 $^{ab}$ | 20.25 $^{a}$ | 6 $^{b}$ | 29.75 $^{a}$ | 16.25 $^{b}$ | 11.75 $^{b}$ |
| 5–10 cm | | | | | | |
| Area of burrows (%) | 1.66 ± 0.17 $^{a}$ | 1.84 ± 0.32 $^{a}$ | 0.62 ± 0.11 $^{b}$ | 3.52 ± 0.37 $^{a}$ | 2.52 ± 0.20 $^{a}$ | 1.24 ± 0.34 $^{b}$ |
| Area of macropores (%) | 0.10 ± 0.05 | 0.04 ± 0.02 | 0.01 ± 0.01 | 0.07 ± 0.05 | 0.02 ± 0.01 | 0 |
| Number of branches | 6.50 $^{a}$ | 7.75 $^{a}$ | 1.50 $^{b}$ | 25.50 $^{a}$ | 10.25 $^{b}$ | 6 $^{b}$ |
| 10–15 cm | | | | | | |
| Area of burrows (%) | 0.16 ± 0.07 | 0.32 ± 0.12 | 0.22 ± 0.09 | 0.76 ± 0.18 | 1.24 ± 0.16 | 0.57 ± 0.35 |
| Area of macropores (%) | 0 | 0 | 0 | 0.01 ± 0.01 | 0.02 ± 0.01 | 0 |
| Number of branches | 0.25 | 0.5 | 0.25 | 2.25 | 4.50 | 1.50 |
| Total layer (0–15 cm) | | | | | | |
| Number of burrows | 64 $^{a}$ | 54 $^{ab}$ | 37 $^{b}$ | 64.5 $^{a}$ | 58.75 $^{ab}$ | 34 $^{b}$ |
| Area of burrows (%) | 4.78 ± 0.30 $^{a}$ | 4.70 ± 0.30 $^{a}$ | 2.63 ± 0.20 $^{b}$ | 7.66 ± 0.81 $^{a}$ | 6.43 ± 0.60 $^{a}$ | 3.84 ± 0.81 $^{b}$ |
| Area of macropores (%) | 0.25 ± 0.04 $^{a}$ | 0.15 ± 0.08 $^{ab}$ | 0.03 ± 0.01 $^{a}$ | 0.25 ± 0.10 $^{a}$ | 0.08 ± 0.01 $^{ab}$ | 0 $^{b}$ |
| Number of branches | 22.50 $^{a}$ | 28.50 $^{a}$ | 7.75 $^{b}$ | 57.50 $^{a}$ | 31 $^{b}$ | 19.25 $^{b}$ |

### 3.2. Soil Aggregate Formation

$AG_L$ formation following earthworm activity significantly varied across soil moisture and depth ($p < 0.05$, Table 2). $AG_L$ content ranged from 1.1% to 8.5% in High, 1.7% to 6.6% in Medium, and 2.3% to 6.2% in Low treatments and was significantly higher at the 0–5 cm soil depth than the other two soil depths ($p < 0.05$, Figure 2). At 0–5 cm soil depth, maximum $AG_L$ content was found in the High treatment, along with the greatest earthworm burrowing activity in terms of burrow area. However, $AG_L$ content in the High treatment dramatically decreased by 77.2–85.3% and 64.9–72.7% in Lou and loessial soil, respectively, at 5–10 cm and 10–15 cm, respectively, and $AG_L$ content in the Low treatments were highest. Small soil aggregates ($AG_S$) were independent of soil moisture and depth ($p > 0.05$), indicating that earthworm activities mainly affected soil $AG_L$ formation.

### 3.3. Soil Hydraulic Characteristics

Soil hydraulic conductivity (Ks) under earthworm activities was independent of soil moisture content and soil type ($p > 0.05$, Table 2). The average Ks were 0.13 mm min$^{-1}$, 0.18 mm min$^{-1}$, and 0.14 mm min$^{-1}$ in High, Medium, Low treatments, respectively. Ks in Lou soil ranged from 0.017 mm min$^{-1}$ to 0.723 mm min$^{-1}$ in all three moisture treatments for the total 0–15 cm soil depth, which was generally higher than in loessial soil, ranging from 0.004 mm min$^{-1}$ to 0.304 mm min$^{-1}$ (Figure 3). Ks showed significant differences between soil depths; Ks was highest at 0–5 cm soil depth, decreasing by 79% on average at 5–10 cm and showing little change at 10–15 cm. At 0–5 cm, Ks was highest in the Medium treatment in Lou and loessial soil, respectively (Figure 3). Ks in High and Medium treatments greatly decreased at deeper soil depths, and the highest Ks occurred in Low treatments at depths of 5–10 and 10–15 cm. Although Ks was independent of soil type (Table 2), Ks at 0–5 cm soil depth in Lou soil was up to 7.6 times higher than in loessial soil.

Field capacity showed significant differences across soil moisture treatments and highest values in Low treatments ($p < 0.05$, Figure 4). The field capacity in Low treatments on average was 36.0%, approximately 1.2 times higher than in High and Medium treatments. Field capacity was 31.5% and 33.3% in Lou and loessial soil, respectively, showing little difference between soil types ($p > 0.05$). However, field capacity was significantly different between soil depths ($p < 0.05$), decreasing with increasing depth except in the Low treatment in Lou soil (higher at 10–15 cm than at 5–10 cm).

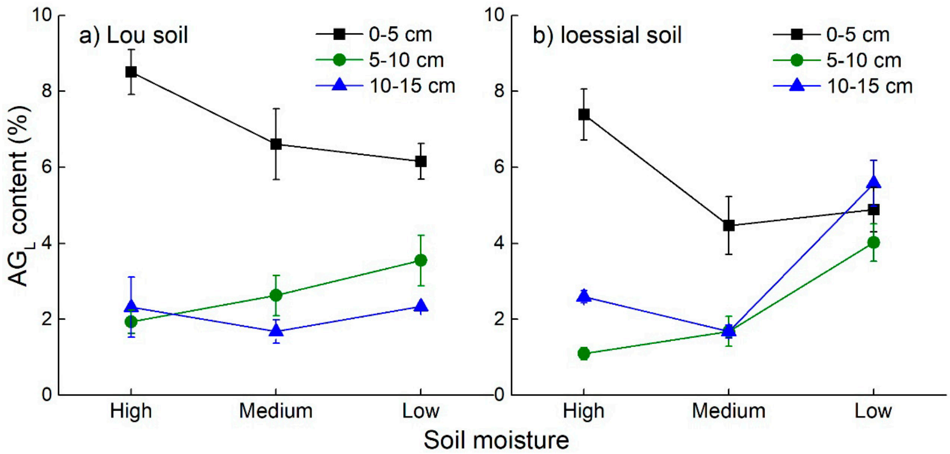


**Figure 2.** Large soil aggregate ($AG_L$: AG > 2 mm) content following earthworm activity at different soil moisture levels (High, Medium, Low) and soil depths (0–5 cm, 5–10 cm, 10–15 cm). Bars show mean ± SE ($n$ = 4).

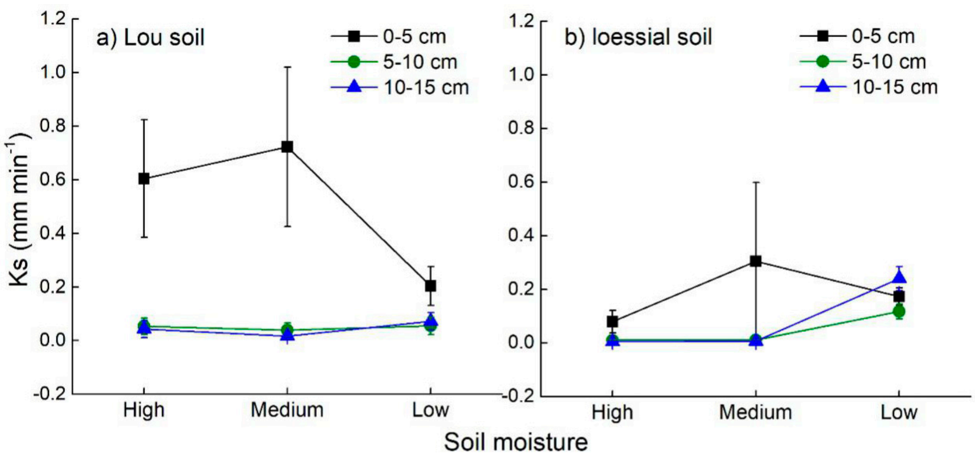


**Figure 3.** Saturated hydraulic conductivity (Ks) following earthworm activity at different soil moisture levels (High, Medium, Low) and soil depths (0–5 cm, 5–10 cm, 10–15 cm). Bars show mean ± SE ($n$ = 4).

### 3.4. *Effect of Earthworm Activity on Soil Aggregation and Hydraulic Characteristics*

$AG_L$ content, Ks, and field capacity were significantly positively correlated with earthworm activity in terms of burrow area in Lou soil ($p < 0.05$, Figure 5a,c,e). As for loessial soil, burrow area showed no significant correlation with $AG_L$ content, Ks, and field capacity (Figure 5b,d,f).

**Table 2.** ANOVA of the effects of soil moisture, soil type, and soil depth on earthworm burrowing activities (number of burrows, area of burrows), soil aggregates ($Ag_L$ and $Ag_S$), soil hydraulic characteristics (soil hydraulic conductivity (Ks) and field capacity); *p*–values for significant effects and interactions are shown in bold. $AG_L$ indicates large soil aggregates (AG > 2 mm); $AG_S$ indicates small soil aggregates (0.25 mm < AG < 2 mm).

| | **Number of Burrows** | | **Area of Burrows (%)** | | **Area of Macropores (%)** | | **$AG_L$ (%)** | | **$AG_S$ (%)** | | **Ks (mm min$^{-1}$)** | | **Field Capacity (%)** | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | ***F* Values** | ***p* Values** | ***F* Values** | ***p* Values** | ***F* Values** | ***p* Values** | ***F* Values** | ***p* Values** | ***F* Values** | ***p* Values** | ***F* Values** | ***p* Values** | ***F* Values** | ***p* Values** |
| Soil moisture | 8.98 | **0.002** | 23.51 | **<0.001** | 10.16 | **<0.001** | 7.72 | **0.001** | 0.32 | 0.73 | 0.29 | 0.75 | 33.76 | **<0.001** |
| Soil type | 0.02 | 0.90 | 27.08 | **<0.001** | 0.56 | 0.46 | 0.86 | 0.36 | 11.01 | **0.002** | 1.74 | 0.19 | 3.518 | 0.07 |
| Soil depth | — | — | 90.91 | **<0.001** | 10.56 | **<0.001** | 83.16 | **<0.001** | 0.06 | 0.94 | 9.52 | **<0.001** | 32.57 | **<0.001** |
| Soil moisture × soil type | 0.15 | 0.86 | 1.76 | 0.18 | 0.23 | 0.80 | 4.05 | **0.02** | 4.45 | **0.02** | 1.70 | 0.20 | 3.90 | **0.03** |
| Soil moisture × soil depth | — | — | 5.25 | **0.001** | 3.07 | **0.02** | 9.80 | **<0.001** | 0.41 | 0.80 | 1.57 | 0.20 | 1.88 | 0.13 |
| Soil type × soil depth | — | — | 3.33 | **0.04** | 0.34 | 0.71 | 7.61 | **<0.001** | 0.54 | 0.59 | 3.42 | **0.04** | 7.51 | **0.001** |
| Soil moisture × soil type × soil depth | — | — | 1.26 | 0.30 | 0.44 | 0.78 | 1.00 | 0.42 | 0.23 | 0.92 | 0.25 | 0.91 | 1.86 | 0.13 |

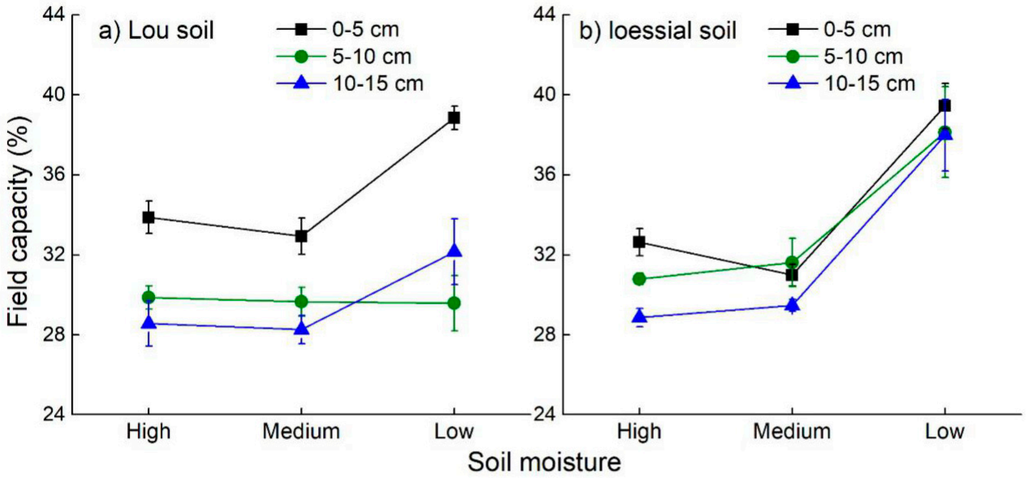


**Figure 4.** Field capacity following earthworm activity at different soil moisture levels (High, Medium, Low) and soil depths (0–5 cm, 5–10 cm, 10–15 cm). Bars show mean ± SE ($n$ = 4).

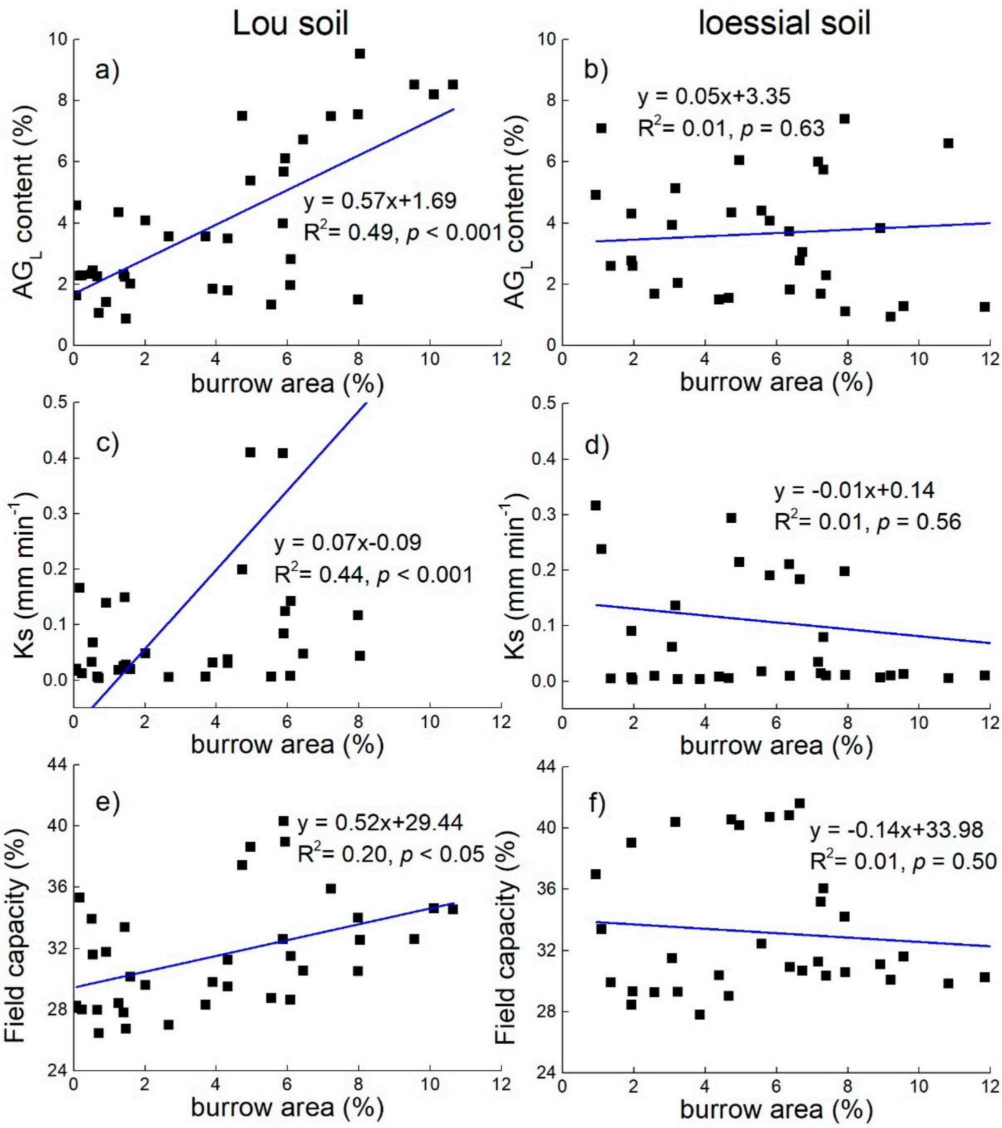


**Figure 5.** Relationship between burrow area of earthworms and $AG_L$ content (**a**,**b**), Ks (**c**,**d**) and field capacity (**e**,**f**) at 0–15 cm soil depth in two soil types. Bars show mean ± SE ($n$ = 12).

## 4. Discussion

Several studies have described the characteristics of earthworm burrowing activity and its impacts on soil properties and processes, such as the aggregate formation and hydrological processes, under different soil moisture conditions [12,24,27]. In general, a wet environment is more conducive to earthworm presence and activity, and the burrowing was consequently greater in moister condition. A previous study conducted under laboratory conditions found that the burrowing activity of *E. fetida* was significantly higher at 80% than at 60% moisture content in peat moss [10], which was consistent with our findings that burrowing activity was greater at High and Medium than at Low moisture contents. However, only a few studies document the relationship between soil moisture and the burrowing activity of *E. fetida* and other epigeic species. Studies on endogeic and anecic species have observed that a decreasing water potential or low water content in the soil could limit burrowing activity and cause the aestivation of earthworms [6,9,11].

The influence of soil moisture on earthworm burrowing activity varied with soil type and was greater in loessial soil than Lou soil under same moisture conditions, which may due to the differences in physical and chemical properties of the two soils. By comparing the burrowing rates of E. *fetida*, Kwak et al. [39] highlighted the important impacts of soil properties on earthworm activity. Soil organic matter is identified as the most important environmental factor controlling the growth and activity of earthworms, followed by soil moisture [6]. As epigeic earthworms are known to live on organic matter in the top layer of soil [6,40], the higher SOC in loessial soil compared to Lou soil (Table S1) could be one of the reasons for the larger burrowing area in loessial soil. Epigeic earthworms are also known to burrow within the upper 20 cm of the soil [41], likely explaining the decreasing burrowing activity of *E. fetida* with increasing soil depth for both the soils in our study. Our results further imply the greater influence of *E. fetida* on surface soil processes in contrast to subsurface soil. While giant earthworms such as anecic species can produce large burrows, epigeic and endogeic species nonetheless play a dominant role in burrow formation on account of the huge number of these species [41]. Thus, greater focus is needed on burrowing activities of epigeic species.

Aggregate formation is one of the typical functions of earthworms that influence soil hydrological processes. Earthworm casting activity is well–known for its great influences on soil aggregate formation [21,42]. The increase in $AG_L$ content with increased burrowing area confirmed the positive effects of earthworm activity on soil aggregation. Numerous studies found that earthworm aggregate formation decreased when the soil changed from wet to dry along with reduced burrowing and casting activities [9,20–22,43]. Part of our findings was consistent with those studies, in that $AG_L$ content was highest in High moisture for 0–5 cm, but at 5–10 cm and 10–15 cm depths, it was highest in Low moisture treatment. The higher $AG_L$ content in the drier soil could be related to the burrowing of earthworms. Though earthworms compact the soil matrix when they go through the soil and improve the aggregation potential, the intense burrowing activity could destroy aggregates into small particles [44]. Particularly, aggregates formed in humid environments are less shaped [45]; water saturation may weaken the bonding agents inside these aggregates [46], decreasing the stability of aggregates more easily dispersed by external factors, e.g., rainfall, burrowing of macrofauna, animals trampling [47,48]. Therefore, less disturbance of earthworm burrowing and more stable aggregates in drier soil may result in more water–stable $AG_L$ contents in the low moisture treatment at 5–10 cm and 10–15 cm in this study.

The effects of burrowing activity on soil porosity and hydraulic properties have been well–documented [25,26]. Ks is a function of the macroporosity and macropore morphology [26], which is closely related to the burrowing activity of earthworms [24]. Increases in Ks with earthworm introduction were reported in several microcosm studies [24,26]. This is confirmed by our findings for Lou soil, i.e., Ks was significantly positively correlated with earthworm burrow area (Figure 5c). However, no significant differences in Ks between different moisture treatments were recorded ($p > 0.05$), despite the significantly higher burrowing activity in wetter soil (Table 1). There was little correlation between burrow area and Ks in loessial soil (Figure 5d). Indeed, the movement of water through earthworm burrows depends on the structure and morphological characteristics of the burrows. Several studies have stated that increasing inner diameter, connectivity, and tortuosity of burrows can effectively improve soil water conductivity and infiltration [49,50], while burrows with many branches may reduce water conductivity. Although *E. fetida* formed many more burrows and macropores in soils with higher moisture content, the burrows were found to be generally horizontal and highly branched (Table 1, Figure 1), limiting vertical hydraulic conductivity [28]. Thus, while the introduction of *E. fetida* contributed to increasing water conductivity, no significant differences in Ks were found in relation to moisture content. The variation of Ks with soil depth could be related to the distinct burrowing patterns of *E. fetida* at different soil depths. Bastardie et al. [24] found that Ks decreased rapidly at 0–10 cm and was almost constant at a deeper depth, caused by fewer functional and continuous burrows of *Lumbricus rubellus* (an epgeic species) with the increasing soil depth. Similarly, Ks in our study was highest at 0–5 cm, decreased rapidly at 5–10 cm, and remained constant at 10–15 cm, due to the decreasing burrowing accompanied by the reduction in the macropores at a deeper depth.

Earthworm activities also play an important role on soil water storage, with some studies finding that decompacting endogeic species decrease soil water holding capacity [51,52]. Similarly, field capacity in our study was decreased by the burrowing activity of earthworms and was significantly higher in soils with fewer burrows at Low treatments for each soil layer. Earthworm burrows are the important pathways of preferential flow and could facilitate water discharge. Ernst et al. [5] found that soil water storage decreased as earthworm burrows improved aeration and then promoted soil evaporation. The lower water discharge capacity and evaporation due to lower macroporosity and burrowing activities in drier soil could drive a significantly higher field capacity in lower moisture soils. In addition, the beneficial influences of soil aggregates on water holding capacity have been widely verified [53,54], and Hallam and Hodson [29] noted a consistent relationship between increases in soil aggregates and water holding capacity in soils with earthworms. This could explain the increase in field capacity with stronger earthworm activity in Lou soil. The effects and feedback within the soil, however, are highly correlated with the characteristics of earthworm burrows; thus, further research about the interaction between earthworm burrow features, soil properties, and the influence on soil hydraulic characterization is critically needed.

## 5. Conclusions

*E. fetida* can generate large number of burrows both in Lou and loessial soils and strongly influences soil aggregation and hydraulic properties. The burrows, macropores, and branches of burrows produced by burrowing activity of *E. fetida* increased with soil moisture; burrowing activity was higher in loessial soil than in Lou soil. *E. fetida* can influence soil hydraulic properties variously by changing soil porosity and aggregates at different soil moisture condition. This study provided baseline information required for further research on the role of earthworms on ecosystem processes on the Loess Plateau.

**Supplementary Materials:** The following are available online at http://www.mdpi.com/2071-1050/12/21/9303/s1, Table S1: Soil characteristics: soil organic carbon (SOC), pH, particle size distribution (clay, silt, and sand) of Lou and loessial soil from the Loess Plateau.

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