

Article

Study on the Improved Black Soil Structure Under Biological Tillage on *Brassica chinensis* L. Yield

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Abstract

The degradation of soil structure in black soils has become a key factor limiting the productivity of farmland ecosystems. However, systematic studies on restoring soil physical properties and improving crop yields through biological tillage remain scarce. In this study, *Eisenia fetida* was employed as a biological tillage agent to create soil macropores. An orthogonal experiment with three factors was conducted to investigate the mechanisms by which different gradients of soil moisture, decomposed straw, and soil compaction affect soil pore structure and the yield of *Brassica chinensis* L. X-ray-computed tomography (CT) was used to quantitatively characterize the macropore network mediated by earthworms. The results indicated that the critical threshold conditions for optimized biological tillage were 50 g of decomposed straw, a compaction of 50–150 kPa, and a soil moisture content of 30–37%. Under these conditions, earthworm activity significantly enhanced the leaf dry weight of *Brassica chinensis* L. by approximately 55.29%, while root dry weight increased by 96.60%. Compared with treatments of low soil moisture combined with 50 g of decomposed straw, higher moisture levels further increased total biomass by 75.46%. Compared with the control, earthworm-induced macropores had 27 times more pore throats than abiotic pores, and network models showed significantly improved connectivity, indicating enhanced soil structure. This study revealed a synergistic threshold of water–food–physical resistance regulation for soil structural improvement under biological tillage and innovatively proposed a biological tillage evaluation system based on CT-quantified pore networks and root structure–function relationships. These findings provide a theoretical basis for the ecological restoration of degraded black soils.

Keywords: black soil conservation; soil pore structure; earthworms; crop biomass; X-ray computed tomography (CT)



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1. Introduction

Black soils, characterized by their high organic matter content and well-developed aggregate structure, are regarded as strategic resources for sustaining global food security [1]. It is estimated that crop yield losses due to degradation in global black soil regions reach as high as 12% annually, resulting in direct economic losses exceeding USD 27 billion [2].

However, long-term improper tillage practices, the insufficient application of organic fertilizers, and a low rate of straw return have caused the continuous degradation of black soils. In the black soil region of northeast China, soil water-holding capacity has declined to its original state, while bulk density has increased, severely restricting root growth and nutrient uptake [3,4]. Such degradation is not only a consequence of physical structure destruction but is also closely related to a decline in soil biota [5]. Studies have shown that earthworm density in degraded black soils is much lower than under natural conditions, resulting in insufficient biogenic pores and further aggravating soil compaction [6,7]. Although the application of organic amendments can temporarily alleviate soil compaction, their restorative effect is considerably weaker than that of biogenic pore formation [8]. Currently, the combined use of earthworms, straw, and *Bacillus subtilis* has become one of the important strategies for soil structure restoration [9,10].

Biological tillage is an environmentally friendly and cost-effective approach to soil restoration [11], with earthworms often referred to as “ecosystem engineers” [12]. Through their movement, earthworms create intersecting burrows that form macropores, effectively increasing soil porosity [13,14]. These burrows are typically cylindrical in shape, with high regularity and roundness, which enhances their continuity and permeability. Moreover, the macropore networks created by earthworms are highly complex, significantly enhancing soil structural connectivity and further optimizing the transport efficiency of water and air within the soil [15]. Therefore, earthworms play a crucial role in regulating soil pore structure, pore size distribution, and connectivity. Previous studies have reported that earthworms markedly improve soil water infiltration and retention capacity while effectively mitigating soil degradation [16,17]. Research by Ma et al. (2021) [18] further demonstrated that the hydraulic conductivity of earthworm burrows is influenced by complex interactions between soil physicochemical properties and earthworm density. The diameter, tortuosity, and connectivity of burrows are key determinants of water movement in soils [19]. In addition, earthworm burrowing behavior exhibits considerable variability, which is often related to environmental conditions. Factors such as soil organic matter content, available food resources, soil moisture, soil compaction, and seasonal changes can directly or indirectly affect earthworm activity [20].

Previous research, using food-attraction experiments and computed tomography (CT) imaging, has demonstrated that decomposed straw significantly promotes earthworm activity. Moreover, combining straw with *Bacillus subtilis* provides a viable food source for earthworms, greatly improving their survival rate in structurally degraded black soils. Nevertheless, the mechanistic role of earthworms in biological tillage remains unclear, and current studies are largely theoretical, with limited translation to field-scale applications [21]. Although the beneficial effects of earthworms on soil structural improvement have been established, three major limitations remain unresolved. First, the influence of moisture stress gradients on the stability of earthworm burrows has not been quantitatively characterized. Most existing studies have focused on the effect of soil moisture on vermicomposting [22], while the critical soil moisture range in which earthworms effectively improve soil structure is still unknown. Second, the availability threshold of food resources (e.g., decomposed straw) has not been determined. Severe organic matter loss in degraded soils often fails to meet the food requirements for earthworm activity [23]. Third, the mechanical resistance of soil compaction to earthworm bioturbation has only been qualitatively described, with limited studies employing CT-based three-dimensional reconstruction to visualize burrow networks [24]. These research gaps have greatly hindered the field application of earthworm-based biological tillage. Therefore, identifying viable food sources for earthworms under field conditions, as well as elucidating the effects

of soil moisture and compaction on earthworm activity, are key issues for advancing the practical application of biological tillage technology.

On one hand, this study focuses on plant growth status. By comparing the biomass and root morphology of *Brassica chinensis* L., it screens out the factors that have significant impacts on plant growth and further determines the optimal implementation strategies for earthworms to improve soil structure and increase crop yield. Second, soil micropore characteristics were quantified using CT imaging combined with AVIZO 9.2 software to systematically analyze changes in pore morphology, channel structure, and connectivity induced by decomposed straw. Three-dimensional reconstruction was applied to visualize the spatial distribution and morphological features of pores, enabling the direct comparisons of soil microstructures under different treatments and clarifying the key factors influencing soil structure [25]. Collectively, this study further explored the effects of biological tillage-induced soil structural improvement on *Brassica chinensis* L., growth, providing theoretical evidence for differences in plant performance and offering specific, practical strategies for applying biological tillage to improve soil microstructure, enhance crop yields, and promote field-scale applications. The primary hypothesis of this study is as follows: Utilizing the attraction of the *Eisenia fetida* to decomposed straw as a biological tillage technique, by regulating the synergistic thresholds of soil moisture, decomposed straw application rate, and soil compaction, the pore structure of degraded black soil can be improved, thereby promoting the growth and yield enhancement of *Brassica chinensis* L. The expected results are as follows: Under optimized conditions of 50 g decomposed straw, 50–150 kPa soil compaction, and 30–37% soil moisture content, the biological tillage method may significantly increase three-dimensional porosity. This could substantially elevate *Brassica chinensis* L. leaf dry weight, root dry weight, and total biomass, with soil moisture potentially serving as the core factor driving biomass growth.

2. Materials and Methods

2.1. Experimental Materials and Soil Preparation

Brassica chinensis L. seeds with a short growth cycle were purchased from Degao Seed Industry Co., Ltd. (Dezhou, China). At sowing, the seeds were planted in a mixture of soil and decomposed straw with *Eisenia fetida*. The earthworms used in this study were *Eisenia fetida* (Commercial name: “Taiping No. 2”), obtained from Junlong Ecological Technology Co., Ltd. (Shenzhen, China). This species is an epigeic–endogeic earthworm with a body length of 35–130 mm, body width of 3–5 mm, and 80–110 segments, mainly active in the humus layer. *Eisenia fetida* is characterized by a high reproduction rate and strong stress tolerance and is most commonly found in organic soils at depths of approximately 20 cm [26]. After transport to the laboratory, earthworms were rinsed and pre-incubated with the test black soil in separate boxes for 7 days to acclimate them to the experimental soil environment. After acclimation, vigorous individuals were selected for the formal experiments. The selection of *Eisenia fetida* was based on our previous findings that its activity, coupled with maize straw addition and *Bacillus subtilis* inoculation, could generate a reticulated macropore structure that facilitates the improvement of black soil structure [27].

The soil used in the experiment was typical black soil collected from a maize field at the Agricultural Expo Garden in Changchun, Jilin Province, Northeast China. Samples were collected at a depth of 0–20 cm using PVC ring cutters (100 mm inner diameter, 200 mm height) to obtain intact soil cores, which corresponded to the depth most suitable for earthworm survival. According to particle size distribution analysis performed with a BT-9300ST analyzer (Bettersize Instruments, Dandong, China), the soil was classified as sandy loam. Its detailed physicochemical properties are shown in Table 1. During soil pretreatment,

visible stones and plant residues were removed, and the soil was sieved through a 1 mm mesh to maintain the stability of native aggregates and ensure soil homogeneity [28].

Table 1. The soil properties of the experiment.

Soil Moisture Content (%)	Temperature (°C)	PH	Soil Bulk Density (g·cm ³)	Clay (g·kg ^{−1})	Sand (g·kg ^{−1})	Silt (g·kg ^{−1})
19.6–21.4	23.2–24.7	6.8–7.9	1.6–2.1	107.1 ± 3	823 ± 7	69.9 ± 6
Soil aggregate fractionation	>2 mm	1–2 mm	0.5–1 mm	0.25–0.5 mm	<0.25 mm	
1	4.33	10.69	9.91	7.06	68.02	
2	65.58	2.47	3.09	0.30	28.56	
3	12.26	6.77	6.78	7.05	67.15	
Total Nitrogen (TN) g/kg	Total Phosphorus (TP) g/kg	Total Potassium (TK) g/kg	Alkaline Hydrolyzable Nitrogen (AHN) mg/kg	Available Phosphorus (AP) mg/kg	Available Potassium (AK) mg/kg	Total Organic Carbon (TOC)%
9.456	2.711	21.018	638.37	152.60	1426.95	8.55
9.736	2.565	21.297	658.43	145.25	1414.20	9.53
10.016	2.744	19.563	650.75	151.15	1462.50	8.11

2.2. Pot Experiment Design and Treatments

The pot experiment was conducted in a greenhouse at the Agricultural Expo Garden, Changchun, Jilin Province, China, from 28 November 2024 to 4 January 2025. Plastic pots with a height of 30 cm and a width of 20 cm were used. The experimental soil was sieved to 1 mm and then packed into the pots. Each pot contained soil, earthworms, and decomposed straw inoculated with *Bacillus subtilis*. Five individual earthworms were introduced into each pot, with the density determined according to the equivalent field-scale distribution per square meter. Earthworms were initially placed at the bottom of the pots and were attracted to the decomposed straw, thereby facilitating the formation of soil macropores [10].

Two levels of decomposed straw were applied: 50 g and 100 g per pot. These amounts were derived from the minimum and maximum rates of straw return commonly applied in field conditions [29]. Soil moisture content classifications are derived from the moisture characteristic curves of reference subjects and crop growth requirements, simulating moist and dry conditions [30]. The soil compaction level is set based on field conditions in the study area and crop growth requirements, simulating both loose and compacted soil states. After soil column preparation, three *Brassica chinensis* L. seeds were sown in each pot. The greenhouse temperature was maintained at 20–25 °C. Soil moisture was monitored daily using a soil moisture–temperature–salinity meter (YP-WSYP, Youyunpu Optoelectronic Technology Co., Ltd., Weifang, China). The required irrigation volume was calculated from soil bulk volume and daily measured soil moisture content, and water was manually supplied to maintain the preset soil moisture gradients.

At the end of the growth cycle, plants were harvested, oven-dried, and weighed to determine key biomass indicators. Each treatment was replicated three times, with an additional control group (CK). The control group (CK) consisted of no earthworms and decomposed straw, comprising a total of 51 pots (Figure 1). The experimental factors and treatment levels are summarized in Tables 2 and 3.



Figure 1. Comparison of *Brassica chinensis* L. at germination, growth, and maturity phase in the pot experiment.

Table 2. Orthogonal design of experimental factors and levels.

Level	Factor A Composted Straw	Factor B Soil Moisture Content	Factor C Soil Firmness
1	50 g	9–16%	50–150 kPa
2	100 g	16–23%	150–250 kPa
3		23–30%	250–350 kPa
4		30–37%	350–450 kPa

Table 3. Orthogonal experimental conditions ($L_{16} (2^1 \times 4^2)$).

Case	Factor A	Factor B	Factor C	Experimental Parameter Composted Straw (g)	Soil Moisture Content (%)	Soil Firmness (kPa)
1	1	1	1	50 g	9–16%	50–150 kPa
2	1	1	3	50 g	9–16%	250–350 kPa
3	1	2	2	50 g	16–23%	150–250 kPa
4	1	2	4	50 g	16–23%	350–450 kPa
5	1	3	2	50 g	23–30%	150–250 kPa
6	1	3	4	50 g	23–30%	350–450 kPa
7	1	4	1	50 g	30–37%	50–150 kPa
8	1	4	3	50 g	30–37%	250–350 kPa
9	2	1	2	100 g	9–16%	150–250 kPa
10	2	1	4	100 g	9–16%	350–450 kPa
11	2	2	1	100 g	16–23%	50–150 kPa
12	2	2	3	100 g	16–23%	250–350 kPa
13	2	3	1	100 g	23–30%	50–150 kPa
14	2	3	3	100 g	23–30%	250–350 kPa
15	2	4	2	100 g	30–37%	150–250 kPa
16	2	4	4	100 g	30–37%	350–450 kPa

2.3. CT Image Acquisition Andprocessing

Five representative samples (T7, T1, T16, T6, and CK) were selected for pore structure measurement. These treatments represented the optimal groups from the orthogonal experiment and were also used to compare soil structure under different conditions of soil moisture, soil compaction, and decomposed straw application. X-ray computed tomography (CT) scanning was employed to obtain the pore structure (Sunshine32 XCT, Dachang, Shenyang, China) [31]. Samples were placed horizontally on the scanning platform and

rotated 180°. The scanning time was 1.50 s with the following parameters: collimation 8×0.55 , peak voltage 140 kV, tube current 140 mA, slice thickness 0.55 mm, voxel resolution $512 \times 512 \times 261 \mu\text{m}^3$, and field of view $512 \times 512 \text{ mm}^2$. Three-dimensional reconstruction and the analysis of image data were performed using Avizo software (Thermo Fisher Scientific, Waltham, MA, USA). A multi-threshold segmentation approach was applied to accurately extract pore features, which effectively resolved the problem of unclear boundary segmentation associated with a single-threshold method [32]. To obtain the actual three-dimensional skeletal structure of the sample, this study proceeded as follows: (1) Scanning yielded cross-sectional images with a resolution of 0.55 mm per pixel. Through this scanning technique, we successfully acquired detailed cross-sectional images of the sample. (2) The acquired cross-sectional images underwent a series of digital image processing steps, including noise reduction filtering, grayscale enhancement, OTSU thresholding, and morphological opening operations. These steps enabled the extraction of aggregate images within the sample. Subsequently, three-dimensional reconstruction was performed on the aggregate image sequence. A three-dimensional watershed algorithm was applied to segment the reconstructed three-dimensional aggregate boundaries. (3) Virtual aggregate screening: Aggregate volume was calculated based on voxel counts, volume, and equivalent diameters of segmented aggregate particles. A 26-connectome voxel connection method was employed to partition aggregates within the 3D skeleton structure using labeling techniques. Assigning distinct colors to each aggregate particle highlighted the accuracy of adherent aggregate segmentation through this color differentiation approach [33]. Key parameters, including the three-dimensional porosity and planar porosity of four models, were analyzed to evaluate the geometric characteristics of earthworm-mediated pore structures and the connectivity of pore throats and their effects on *Brassica chinensis* L. growth.

2.4. Data Analysis

An orthogonal range analysis combined with one-way analysis of variance (ANOVA) was used to assess the effects of different treatments on soil physical properties under biological tillage. The variances prior to analysis of variance satisfy the assumptions of normality and homogeneity, with no outliers or missing data. *Brassica chinensis* L. growth parameters under different treatments were also tested using one-way ANOVA. Prior to conducting the aforementioned statistical analyses, all datasets were tested to determine whether they met the normality and homogeneity of variance requirements for the test assumptions. Statistical significance was set at $p < 0.05$, and pairwise comparisons were conducted using the least significant difference (LSD) method. All statistical analyses were performed with SPSS 20.0 (IBM SPSS Inc., Chicago, IL, USA).

3. Results and Discussion

3.1. Effects of Biological Tillage on *Brassica Chinensis* L. Growth Parameters Under Different Soil Structures

In the pot experiment, the effects of biological tillage (earthworm and decomposed straw treatment) on *Brassica chinensis* L. growth indices were evaluated (Figure 2). Compared with the treatment without earthworms and decomposed straw, the biological tillage treatment markedly increased shoot biomass: fresh weight increased by approximately 36.45% and dry weight by 55.29%. Root biomass also showed significant improvement, with fresh weight increasing by 78.26% and dry weight by 96.60%. These results indicate that biological tillage significantly promoted biomass accumulation and enhanced plant growth [34].

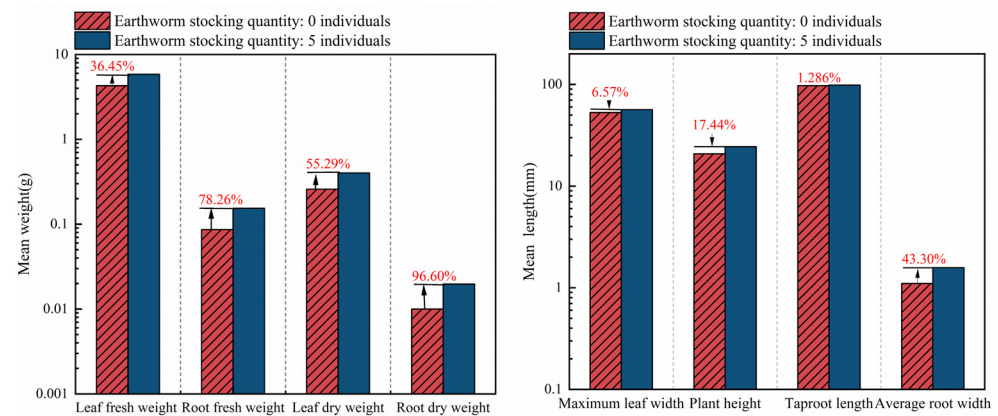


Figure 2. Average weight chart of growth and morphological indices of *Brassica chinensis* L. with and without biological tillage treatment.

Regarding morphological traits, *Brassica chinensis* L. grown in biological tillage-treated soil exhibited increases in maximum leaf width (6.57%) and plant height (17.44%). Taproot length did not change significantly, likely due to the vertical growth limitation imposed by the pot height. However, average root diameter increased by 43.30%, suggesting that biological tillage may facilitate lateral root expansion and enhance nutrient uptake. Overall, these findings demonstrate that biological tillage not only significantly improves biomass production in *Brassica chinensis* L. but also optimizes root morphology to improve nutrient acquisition, confirming its role as a key growth-promoting factor in cultivation systems [35].

3.2. Effects of Biological Tillage Under Different Food Sources, Soil Moisture, and Soil Compaction Conditions on Soil Water and Temperature

Soil water plays multiple essential roles: it is indispensable for plant growth and physiology, it regulates soil temperature, and it directly influences the effectiveness of earthworm-mediated soil structural improvement. Thus, soil moisture and temperature were considered critical environmental factors in this study [36]. Soil moisture in all treatments was maintained at preset levels through manual regulation, and the focus was placed on analyzing the dynamic variations in temperature and moisture within the earthworm activity layer.

Soil temperature dynamics at the 20 cm depth during the entire growth period are shown in Figure 3. Regardless of food source, soil moisture, or compaction conditions, soil temperature in the earthworm activity layer followed a consistent three-phase trend. The first stage (days 1–13, corresponding to sowing and seedling emergence) showed a rapid accumulation of heat, with an increase of 7.7–7.9 °C, accompanied by a decline of 3.1–3.4% in soil water content. This cumulative warming provided favorable conditions for seed germination. The second stage (days 13–28, vegetative growth) was characterized by a gradual decline in soil temperature, ultimately reaching a minimum of 14.7 °C; the cooling rate remained below 7 °C. The third stage (days 28–41, maturation) showed the stabilization of soil temperature around 19.2 °C, coinciding with the main nutrient accumulation phase of the crop.

Soil moisture dynamics under different treatments are also presented in Figure 3. Changes in soil water content appeared closely related to earthworm activity. While most treatments showed only minor differences between initial and final values, significant fluctuations were observed in treatments T6, T7, T11, T14, and T15 during the early monitoring period, likely due to increased macroporosity caused by earthworm burrowing. Substantial variations in soil water content were observed only during the first 20 days; once biogenic macropores were formed, their complex and interlaced structure effectively

reduced water loss, suggesting a water-retention effect of earthworm-induced macropores during this stage [14].

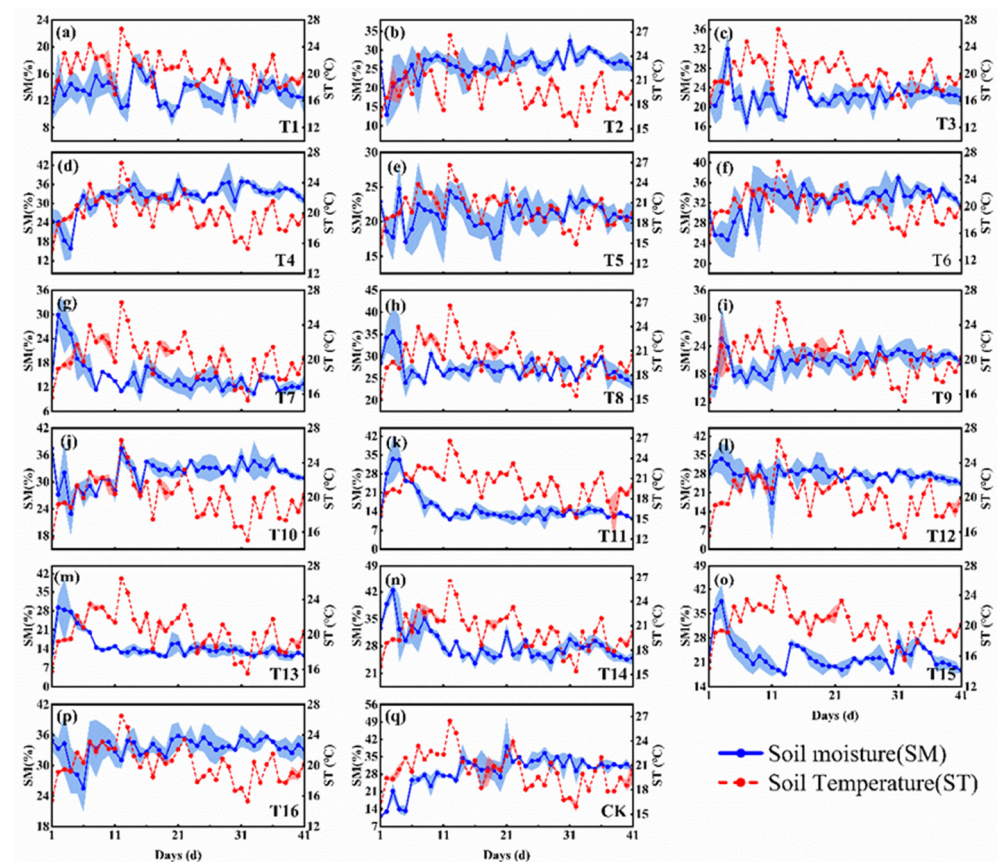


Figure 3. Changes in soil moisture and temperature in the earthworm activity layer, (a–q) show the changes in soil moisture and soil temperature of T1–CK. The red line represents soil temperature, the blue line represents soil moisture, and the expanded parts (shaded areas) in red and blue indicate the error values.

At the macro level, the results highlight the dynamic changes in soil water and temperature in the earthworm activity layer. Plant roots absorb water directly from the soil to support physiological processes, and higher levels of available soil water generally promote growth. However, it should be noted that the effects of earthworm-induced macropores on soil water are dynamic and interact with both plant growth and earthworm activity, warranting further study.

Differences in soil water content were observed under different food sources, moisture levels, and soil compaction conditions (Figure 3). For instance, Zhang et al. [37] reported that straw return significantly affected soil moisture and temperature, thereby influencing maize emergence rate, particularly under low application rates. This is consistent with the present study, where decomposed straw served as both a food source for earthworms and a moisture-retaining substrate. Similarly, Hafez et al. [38] demonstrated that vermicompost application enhances available soil water, and our results also suggest that earthworm activity increased soil water availability. Collectively, these findings indicate that variations in soil water content were jointly driven by earthworm burrowing and the application rate of decomposed straw. Nevertheless, in field production systems, the influence of earthworms on soil moisture should not be overstated, as they serve primarily as an auxiliary factor altering water distribution in the tillage layer.

3.3. Effects of Biological Tillage on Biological Characteristics, Root Systems, and Yield of *Brassica Chinensis* L. Under Different Conditions of Earthworm Food, Soil Moisture, and Soil Compaction

In this study, range analysis was employed, and this is depicted in Table 4 below.

Table 4. Orthogonal design and experimental results.

	A	B	C	A	B	C	A	B	C
	Aboveground Biomass			Root biomass			Total Biomass		
k1	102.7416667	97.72333333	111.9233333	0.020875	0.01525	0.02325	0.471833333	0.30975	0.502416667
k2	94.46291667	97.14916667	98.88666667	0.018291667	0.01775	0.0175	0.364	0.37575	0.409916667
k3		86.8075	94.42083333		0.0165	0.020666667		0.394333333	0.391
k4		112.7291667	89.17833333		0.028833333	0.016916667		0.591833333	0.368333333
R	8.27875	25.92166667	22.745	0.002583333	0.013583333	0.006333333	0.107833333	0.282083333	0.134083333
Influence Factors (from Large to Small)		B > C > A			B > C > A			B > C > A	
Optimal Combination		A1B4C1			A1B4C1			A1B4C1	
	Root shoot ratio			Mean root diameter			Taproot length		
k1	0.045960974	0.05532323	0.047462601	1.754166667	1.256666667	1.795	102.7416667	97.72333333	111.9233333
k2	0.05640854	0.050656619	0.045596167	1.398333333	1.4725	1.565833333	94.46291667	97.14916667	98.88666667
k3		0.045216981	0.060541836		1.5225	1.47		86.8075	94.42083333
k4		0.053541105	0.051138425		2.053333333	1.474166667		112.7291667	89.17833333
R	0.010447567	0.010107343	0.014945669	0.355833333	0.796666667	0.325	8.27875	25.92166667	22.745
Influence Factors (from Large to Small)		C > A > B			B > A > C			B > C > A	
Optimal Combination		A2B1C3			A1B4C1			A1B4C1	

3.3.1. Effects of Biological Tillage on Biomass and Root–Shoot Ratio of *Brassica Chinensis* L.

From the range analysis, it can be seen that the optimal group for aboveground biomass is A1B4C1, and the optimal group for root–shoot ratio is A2B1C3. Additionally, soil moisture content ranging from 9% to 23% is defined as low moisture content, while a range from 23% to 37% is defined as high moisture content. The aboveground biomass of *Brassica chinensis* L. is shown in Figure 4a,b. Aboveground biomass can reflect vegetation growth status, productivity, and carbon cycling processes. In addition, under different treatment conditions, there are significant differences in the response of the aboveground biomass of *Brassica chinensis* L. to soil moisture content, soil compaction, and composted straw. Compared with the treatment of low soil moisture content and 50 g composted straw, the treatment of low soil moisture content and 100 g composted straw decreased the aboveground biomass by 10.08% ($F = 0.235$, $p = 0.645$). Meanwhile, the treatment of high soil moisture content and 50 g composted straw significantly increased the aboveground biomass by 74.81% ($F = 10.719$, $p = 0.017$). Furthermore, under high soil moisture content, the aboveground biomass in the 100 g composted straw treatment was significantly lower than that in the 50 g composted straw treatment ($F = 2.667$, $p = 0.154$). This result indicates that high soil moisture content and 50 g of composted straw synergistically promote the aboveground growth of *Brassica chinensis* L. Its mechanism may be to indirectly improve plant nutrient absorption by enhancing earthworm activity and metabolic efficiency, as well as soil water retention capacity. Soil compaction has a significant restrictive effect on aboveground biomass. Specifically, under the same amount of earthworm food, the difference in aboveground biomass between the loose soil compaction range (50–250 kPa) and the compact soil compaction range (250–450 kPa) is relatively small, and it shows no significant difference. Earthworm biological tillage can improve soil structure through peristalsis; even under higher soil compaction, earthworm peristalsis can still improve soil pore structure. However, compared with loose soil, the aboveground biomass in compact soil decreased by 17.25% ($F = 0.536$, $p = 0.476$). This indicates that earthworm food and soil moisture content are the key factors restricting the earthworm improvement effect.

These two factors can promote earthworm peristalsis and improve soil structure, thereby promoting the increase in aboveground biomass.

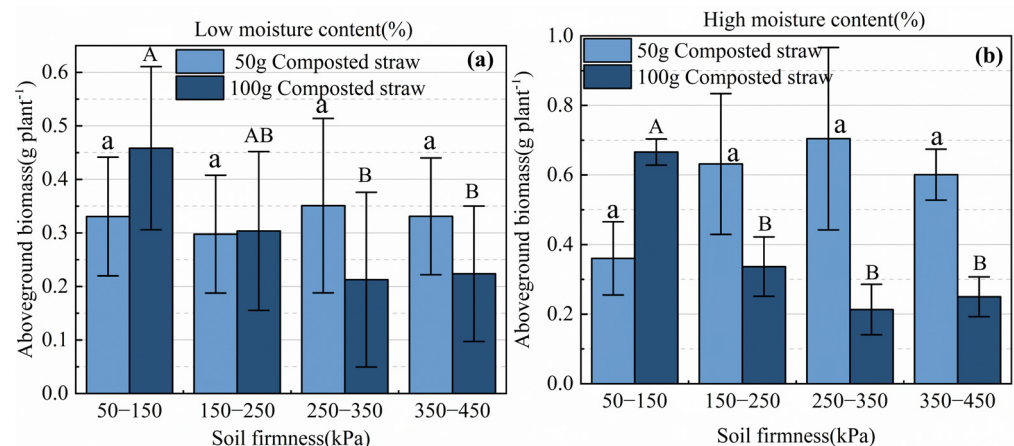


Figure 4. Effects of earthworm biological tillage on aboveground biomass of *Brassica chinensis* L. (a,b) Aboveground biomass under different soil compaction levels with high and low soil moisture contents. The data are presented as means $\pm$ SE. Treatment groups labeled with the same lowercase letters for 50 g of decomposed straw showed no significant differences according to Tukey's multiple comparison test; different letters indicate significant differences. Treatment groups labeled with the same uppercase letters for 100 g of decomposed straw showed no significant differences according to Tukey's multiple comparison test; different letters indicate significant differences ($p < 0.05$).

The root biomass of *Brassica chinensis* L. is shown in Figure 5a,b. The change trend of root biomass and the optimal group obtained through range analysis are consistent with those of aboveground biomass. However, there is no significant difference in the response of root biomass to each treatment factor, and it is inferred that this may be due to pot cultivation restricting root growth. Under the conditions of low soil moisture content and soil compaction of 50–250 kPa, the root biomass in the 100 g composted straw treatment was higher than that in the 50 g composted straw treatment ($F = 1.683$, $p = 0.324$). Under high soil moisture content conditions, the 100 g composted straw treatment still maintained relatively high root biomass in the high soil compaction range (250–450 kPa). Compared with the low soil moisture content treatment under the same soil compaction, it showed no significant difference ($F = 0.002$, $p = 0.966$). Earthworm biological tillage can significantly increase root biomass. However, between loose soil (with soil compaction of 50–250 kPa) and compact soil, the difference in root biomass is relatively small ($F = 0.131$, $p = 0.723$). Specifically, under the treatment of high soil moisture content and 50 g composted straw, the root biomass in relatively compact soil (250–350 kPa) reached the maximum value of $0.03733 \text{ g plant}^{-1}$, which was significantly higher by 43.58% than that in relatively compact soil (350–450 kPa). Under high soil moisture content conditions, earthworm peristalsis can promote soil aggregate formation and pore connectivity, enhance the root osmotic pressure regulation capacity, loosen compact soil, and thereby reduce the variation range of crop root biomass. Under loose soil conditions, the root biomass in the treatment of high soil moisture content and 100 g composted straw was $0.04733 \text{ g plant}^{-1}$, which was 75.30% higher than that in compact soil under the same treatment. However, although earthworms in compact soil can locally improve soil structure through peristalsis, the overall root expansion may still be restricted by pot cultivation. This results in little difference in root biomass between compact soil (with soil pores improved via earthworm peristalsis) and loose soil. This phenomenon indicates that earthworm biological tillage has a good improvement effect on root growth and can narrow the difference in root biomass between compact soil and loose soil.

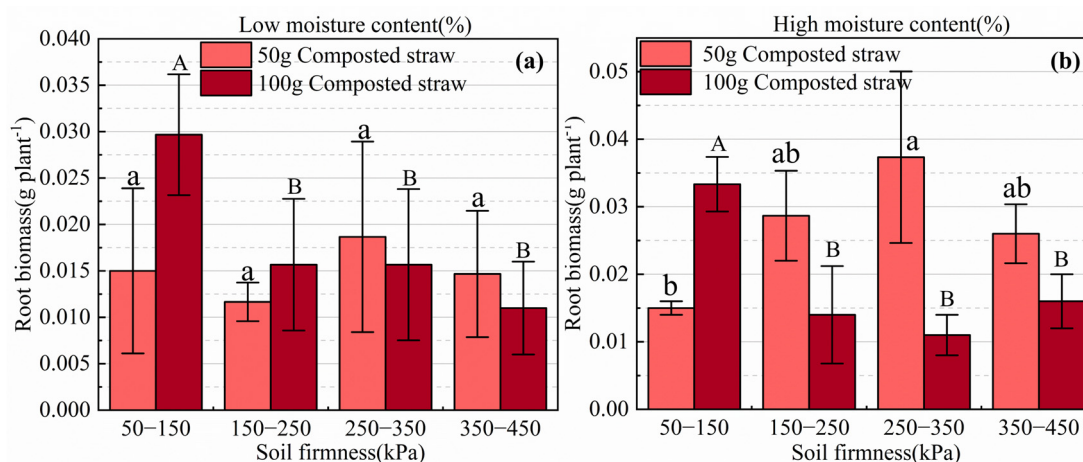


Figure 5. Effects of earthworm biological tillage on root biomass of *Brassica chinensis* L. (a,b) Root biomass under different soil compaction levels with high and low soil moisture contents. The data are presented as means $\pm$ SE. Treatment groups labeled with the same lowercase letters for 50 g of decomposed straw showed no significant differences according to Tukey's multiple comparison test; different letters indicate significant differences. Treatment groups labeled with the same uppercase letters for 100 g of decomposed straw showed no significant differences according to Tukey's multiple comparison test; different letters indicate significant differences ($p < 0.05$).

The total biomass of *Brassica chinensis* L. is shown in Figure 6a,b. The variation characteristics and trends of total biomass can reflect the overall resource allocation strategy of plants. Specifically, under the treatment of high soil moisture content and 50 g composted straw, the total biomass reached the maximum value of $2.40433 \text{ g plant}^{-1}$. It was significantly 75.46% higher than that in the treatment of low soil moisture content and 50 g composted straw ($F = 10.445$, $p = 0.018$), and also significantly 75.28% higher than that in the treatment of low soil moisture content and 100 g composted straw ($F = 4.967$, $p = 0.067$). The promotion effect of earthworm biological tillage on total biomass is particularly prominent under the treatment of high soil moisture content and 50 g composted straw. However, soil compaction has a relatively small impact on total biomass; moreover, in compact soil, total biomass shows a marginal decreasing trend as the amount of earthworm food increases. The inferred reason is that earthworms in compact soil face increased peristalsis resistance and can only locally increase soil pores, failing to fully improve soil structure, which in turn restricts the further increase in total biomass.

The root–shoot ratios of *Brassica chinensis* L. are shown in Figure 7a,b. The root–shoot ratio is the ratio of root biomass to aboveground biomass, and its variation can reveal the resource allocation strategy of *Brassica chinensis* L. under different treatments: under drought conditions, the roots prioritize growth to absorb water, leading to an increase in the root–shoot ratio; when nitrogen fertilizer is sufficient, the growth of the aboveground part accelerates, resulting in a decrease in the root–shoot ratio. Compared with the 50 g composted straw treatment, the 100 g composted straw treatment significantly increased the root–shoot ratio by 22.73% ($F = 4.821$, $p = 0.045$), indicating that sufficient earthworm food can promote earthworm peristalsis while also enhancing the growth allocation to the roots. Compared with the high soil moisture content treatment, the low soil moisture content treatment increased the root–shoot ratio by 7.31% ($F = 0.442$, $p = 0.517$). Notably, the difference in root–shoot ratio between different soil moisture content treatments is not significant, indicating that the regulatory effect of earthworm biological tillage on the root–shoot allocation of *Brassica chinensis* L. exhibits consistency under different moisture conditions.

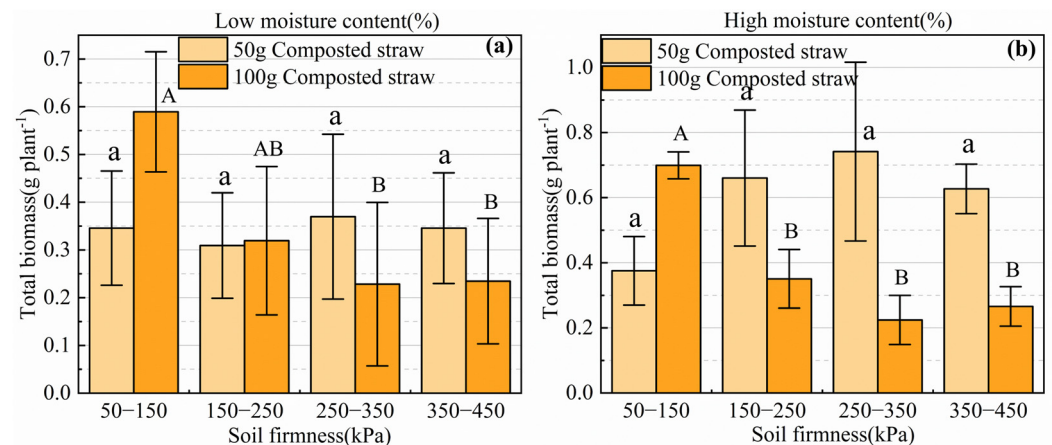


Figure 6. Effects of earthworm biological tillage on total biomass of *Brassica chinensis* L. (a,b) Total biomass under different soil compaction levels with high and low soil moisture contents; The data are presented as means $\pm$ SE. Treatment groups labeled with the same lowercase letters for 50 g of decomposed straw showed no significant differences according to Tukey's multiple comparison test; different letters indicate significant differences. Treatment groups labeled with the same uppercase letters for 100 g of decomposed straw showed no significant differences according to Tukey's multiple comparison test; different letters indicate significant differences ($p < 0.05$).

To summarize, range analysis shows that the optimal treatment for *Brassica chinensis* L. biomass is A1B4C1, while the optimal treatment for the root–shoot ratio is A2B1C3. This result is consistent with that of the variance analysis. These findings indicate that the regulation of root–shoot allocation by earthworm biological tillage exhibits consistency under different moisture conditions. Furthermore, the study reveals that high soil moisture content and an appropriate amount of composted straw are key factors promoting the growth of *Brassica chinensis* L. earthworm biological tillage synergistically regulates biomass allocation and resource utilization by improving soil structure. This is highly similar to the effects of soil moisture content on crops observed in Wang's study of winter wheat root growth dynamics under water stress [39].

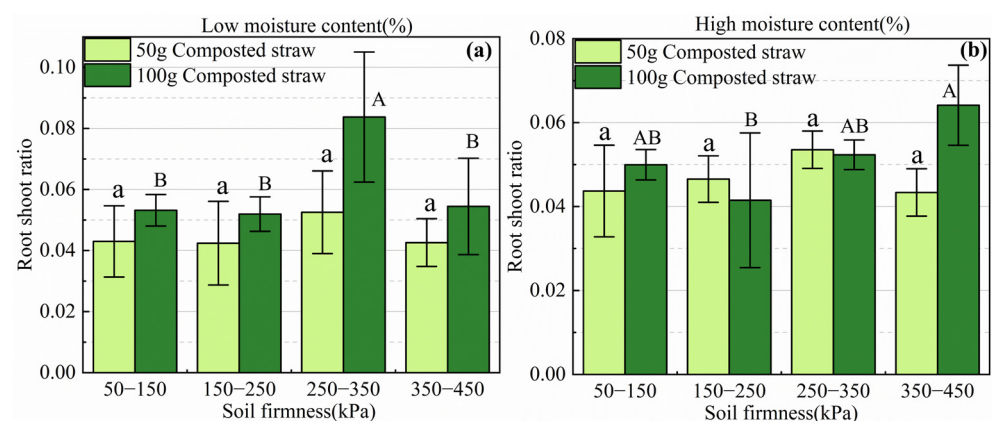


Figure 7. Effects of earthworm biological tillage on root–shoot ratio of *Brassica chinensis* L. (a,b) Root–shoot ratios under different soil compaction levels with high and low soil moisture contents; The data are presented as means $\pm$ SE. Treatment groups labeled with the same lowercase letters for 50 g of decomposed straw showed no significant differences according to Tukey's multiple comparison test; different letters indicate significant differences. Treatment groups labeled with the same uppercase letters for 100 g of decomposed straw showed no significant differences according to Tukey's multiple comparison test; different letters indicate significant differences ($p < 0.05$).

3.3.2. Effects of Biological Tillage on the Root System of *Brassica Chinensis* L.

As shown in Figure 8a,b, the main root length of *Brassica chinensis* L. is presented. Main root length can reflect the root system development status, nutrient absorption capacity of plants, and the supporting basis for the growth of aboveground parts [40]. Compared with the treatment of low soil moisture content and 50 g composted straw, the treatment of low soil moisture content and 100 g composted straw reduced the main root length by 6.13% ($F = 0.398$, $p = 0.552$). Furthermore, variance analysis shows that neither soil moisture content nor the amount of composted straw has a significant effect on the main root length. Soil compaction has a significant restrictive effect on main root length. The main root length under soil compaction in the 50–150 range (loose) is significantly higher than that in the 350–450 range (compact) by 25.5% ($F = 6.305$, $p = 0.046$). Specifically, under the treatment of high soil moisture content and 50 g composted straw, the main root length under soil compaction in the 50–150 range is 39.67% higher than that in the 350–450 range. Earthworm biological tillage can improve soil structure through earthworm peristalsis; however, even with the action of earthworms, relatively high soil compaction still cannot completely break the physical constraints, leading to hindered expansion of the main root. In contrast, in loose soil, earthworm peristalsis is more likely to form effective pores, which significantly enhances the growth potential of the main root. This result indicates that the synergistic effect of soil moisture content and composted straw fails to promote main root growth; however, earthworm peristalsis indirectly improves the soil compaction environment around the roots, thereby stimulating the elongation of the main root.

In Figure 9a,b, the average root diameter of *Brassica chinensis* L. is presented; this indicator reflects the root system development status and nutrient absorption capacity of *Brassica chinensis* L. Compared with the treatment of low soil moisture content and 50 g composted straw, the treatment of high soil moisture content and 50 g composted straw significantly increased the average root diameter by 41.68% ($F = 10.504$, $p = 0.018$). Compared with the treatment of low soil moisture content and 100 g composted straw, the treatment of high soil moisture content and 50 g composted straw significantly increased the average root diameter by 61% ($F = 11.672$, $p = 0.014$). Under low soil moisture content, the treatment with 50 g composted straw has an average value of 5.81 mm for average root diameter, while the treatment with 100 g composted straw has an average value of 5.11 mm; under high soil moisture content, the treatment with 50 g composted straw has an average value of 8.23 mm. This indicates that high soil moisture content and 50 g of composted straw synergistically promote root system development and indirectly improve the environment for root nutrient absorption and expansion. Furthermore, soil compaction has no significant restrictive effect on average root diameter. This suggests that the amount of composted straw and soil moisture content are key factors restricting the earthworm's improvement effect; these two factors can affect root expansion and diameter development by regulating earthworm peristalsis efficiency and soil pore structure. This conclusion aligns with Zhao's research, which demonstrated that the combined application of green manure and crop straw enhances crop productivity and improves soil quality. Compared to controls receiving only chemical fertilizer or no fertilizer, organic amendments (straw, manure, green manure, and the green manure–straw combination) improved topsoil quality. This aligns with the concept explored in this study that earthworms and decomposed straw enhance crop yields [41].

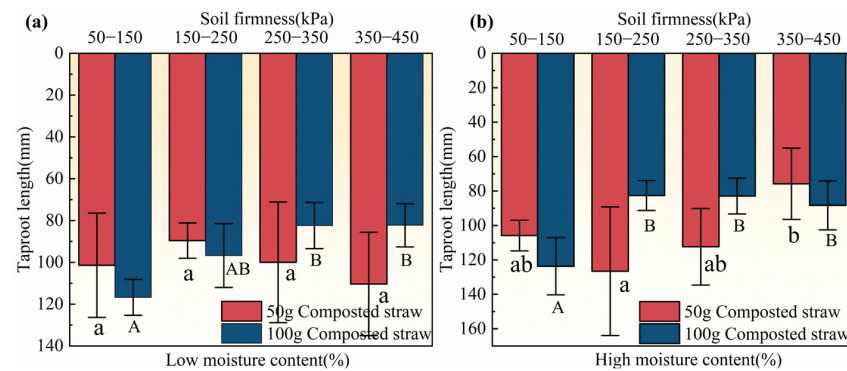


Figure 8. Effects of earthworm biological tillage on main taproot length of *Brassica chinensis* L. (a,b) Taproot length under different soil compaction levels with high and low soil moisture contents. The data are presented as means $\pm$ SE. Treatment groups labeled with the same lowercase letters for 50 g of decomposed straw showed no significant differences according to Tukey's multiple comparison test; different letters indicate significant differences. Treatment groups labeled with the same uppercase letters for 100 g of decomposed straw showed no significant differences according to Tukey's multiple comparison test; different letters indicate significant differences ($p < 0.05$).

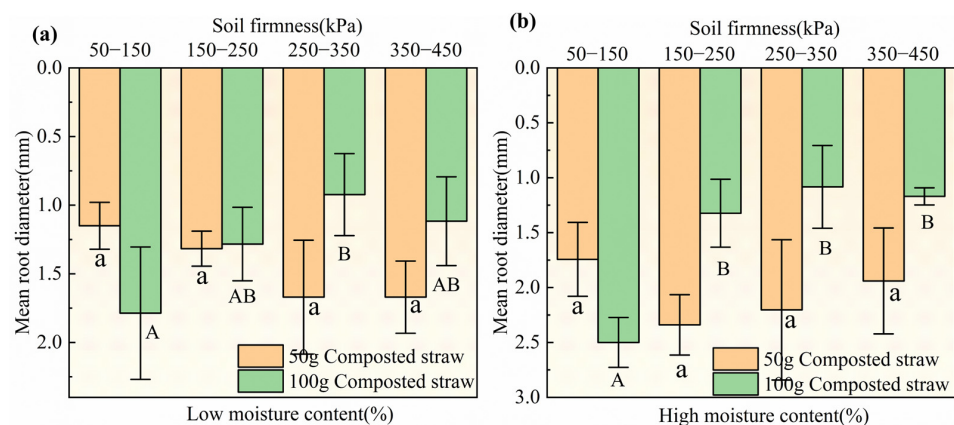


Figure 9. Effects of earthworm biological tillage on main mean root diameter of *Brassica chinensis* L. (a,b) Mean root diameter under different soil compaction levels with high and low soil moisture contents. The data are presented as means $\pm$ SE. Treatment groups labeled with the same lowercase letters for 50 g of decomposed straw showed no significant differences according to Tukey's multiple comparison test; different letters indicate significant differences. Treatment groups labeled with the same uppercase letters for 100 g of decomposed straw showed no significant differences according to Tukey's multiple comparison test; different letters indicate significant differences ($p < 0.05$).

3.3.3. Structural Equation Model (SEM) of *Brassica Chinensis* L. Biomass Under Composted Straw, Soil Moisture Content, and Soil Compaction

Key environmental factors were screened out through correlation analysis, and a structural equation model (SEM) was further established to reveal the comprehensive impact mechanism of composted straw, soil compaction, and soil moisture content on *Brassica chinensis* L. biomass, as shown in Figure 10. SEM analysis shows that soil moisture variation may be the core driving factor promoting the growth of *Brassica chinensis* L. biomass, and it significantly increases biomass through the direct path (path coefficient = 0.95, $p < 0.05$) [42]. Soil compaction, as a key environmental restrictive factor, has no significant direct impact on soil moisture (path coefficient = -0.12 , $p \geq 0.05$), which indicates that the effect of soil compaction on water retention may be masked by other factors. Soil temperature is an important mediating variable that connects environmental factors and biomass [43]. It not only significantly promotes the increase in *Brassica chinensis* L. biomass through a direct path (path coefficient = 0.27, $p < 0.05$), but also indirectly improves the root growth environment by positively regulating soil moisture (path coefficient = 0.26, $p < 0.05$).

The overall fitting indices of the model are good (CFI = 0.91, RMR = 0.04, SRMR = 0.05), which verifies the reliability of the relationships between variables. The results of the structural equation model (SEM) indicate that soil moisture is the key variable affecting *Brassica chinensis* L. yield (path coefficient = 0.95, $p < 0.05$). Composted straw treatment not only directly promotes yield but may also indirectly enhance the crop growth environment by improving soil moisture. Although soil moisture content has no significant direct effect on yield, it exerts an indirect promoting effect by significantly increasing soil moisture (path coefficient = 0.28, $p < 0.05$). By contrast, soil compaction did not exhibit a significant effect in this model.

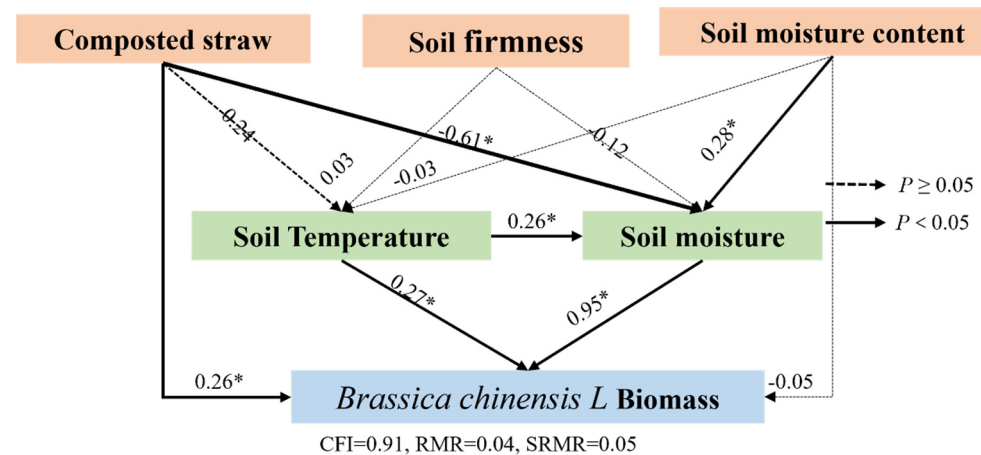


Figure 10. Structural equation model (SEM) of *Brassica chinensis* L. biomass under earthworm biological tillage, * in the figure indicates that the path coefficient is statistically significant.

3.4. Effects of Earthworm Biological Tillage on Soil Structure Under Different Conditions of Earthworm Food, Soil Moisture, and Soil Compaction

Based on the results of the orthogonal experiment and the above-mentioned discussion, Group T7 (A1B4C1), Group T1 (A1B1C1), Group T16 (A2B4C4), Group T6 (A1B3C4), and the Control Group (CK) were selected for CT scanning. Additionally, AVIZO software was used to calculate soil porosity and establish the equivalent pore network model. Porosity reflects the proportion of voids inside a material, i.e., the ratio of pore volume to total volume, while the pore network model (PNM) reflects how pores are distributed and connected within the material, thereby affecting the macroscopic properties of the material [44]. The 3D porosity and surface porosity of earthworm-induced pore structure under earthworm biological tillage are shown in Table 5 and Figure 11. In these depictions, the black areas represent earthworm pores, which are distributed in different regions of the sample space in the form of tubular and conical shapes, while the small white areas represent the soil matrix, showing irregular shapes. Results show that compared with the Control Group (CK), under the conditions of 50 g composted straw, high soil moisture content, and a loose soil compaction threshold of 50–150 kPa, the three-dimensional porosity of Group T7 is 0.2884249 and the three-dimensional porosity of the CK is 0.00194. This significant multiple increase may be attributed to the high level of earthworm activity, which promoted rapid root expansion. In comparison with the 6.9-fold increase from the theoretical research stage, the significant difference in the increase multiple may be due to the presence of planted crops. The analysis of surface porosity, as shown in Figure 11, indicates that Group T7 exhibits a sudden and significant increase in porosity in CT images 51 to 77. The data reveal that under earthworm biological tillage, earthworm activity successfully forms a large number of macropores, with the maximum porosity reaching 0.684. This demonstrates that earthworm biological tillage significantly increases the variability and scale of soil porosity. The surface porosity distribution of Group T7 shows a sudden increase, with

lower porosity in the upper part of the pot and higher porosity in the lower part. This phenomenon may be related to earthworms being food-driven during the experiment, and the composted straw was placed in the lower part of the pot [45]. Compared with Group T7, the porosity distributions of Groups T1, T16, and T6 are relatively similar, all exhibiting a sudden increase in surface porosity at CT image 51. This reflects that the earthworms in these groups were all creeping toward the food source. The porosity of the Control Group (CK) is uniformly distributed overall, indicating that the absence of earthworms in this group has no effect on porosity. Although the curves of the other groups (excluding Group T7) in Figure 11 are not obvious, numerically, the maximum porosity of the Control Group (CK) is only 0.0034, while those of the other groups all reach above 0.1, showing a significant gap. Although earthworm biological pores account for a small proportion of the soil volume, they play an important role in promoting the combination of plant litter. Existing studies have shown that the porosity of earthworm biological pores is significantly higher than the overall porosity of the soil, mainly because the biological pores formed by earthworms' burrowing activities in the soil have higher permeability [46]. By synthesizing these results, it can be inferred that under earthworm biological tillage, earthworms' biological activities have significantly improved soil porosity.

Table 5. Three-dimensional porosity.

Dispose	Volume Fraction	Label Volume (μm^3)	Mask Volume (μm^3)	Label Voxel Count	Mask Voxel Count
T7	0.2884249	2.7×10^{23}	9.4×10^{23}	3,714,177	12,877,500
T1	0.123758	1.3×10^{23}	10.8×10^{23}	1,679,644	13,572,000
T16	0.009291	9.7×10^{21}	10.4×10^{23}	77,880	8,382,010
T6	0.004650	7.5×10^{21}	16.0×10^{23}	84,511	18,173,750
CK	0.00194	2.4×10^{21}	12.2×10^{23}	22,653	11,660,836

The equivalent pore network model (PNM) is widely used for accurately describing pore structures. It can provide detailed data on the size and spatial distribution of pores and throats, which is crucial for understanding the impact of earthworm activities on soil pores [47]. The pore network model (PNM) reflects properties such as connectivity and hydraulic conductivity. At the macroscopic level, the results show that pore structures exhibit significant differences under different driving conditions. In the Group T7 samples, within a favorable threshold range, the pore system formed by biological tillage developed more interlaced and connected structures, thereby significantly increasing soil porosity. After the further processing of the pore space, three pore network models were obtained, as shown in Figure 12. These models include the following: (1) The overall pore network, as shown in Figure 12a–e. (2) Ball-and-stick models that reflect the sizes of pores and throats, as shown in Figure 12f–j. In these models, red represents pores with larger volumes, while blue represents smaller soil pores; the color coding applies similarly to throats. (3) Pore skeleton diagrams that show the size of throats, as shown in Figure 12k–o. The color indicates the width of pore channels: colors closer to red correspond to larger pore channels, while blue corresponds to smaller ones. Statistical results show that the number of throats under earthworm driving is approximately 27 times higher than that without earthworm driving. This change indicates that earthworms significantly enhance soil pore connectivity. In addition, the reconstructed macropores are mainly cylindrical, which is consistent with the typical pore characteristics formed by earthworms [48]. Compared with conventional systems (i.e., systems without earthworm driving), the earthworm-driven pore volume is significantly increased, and this model clearly demonstrates the changes in pore structure [49].

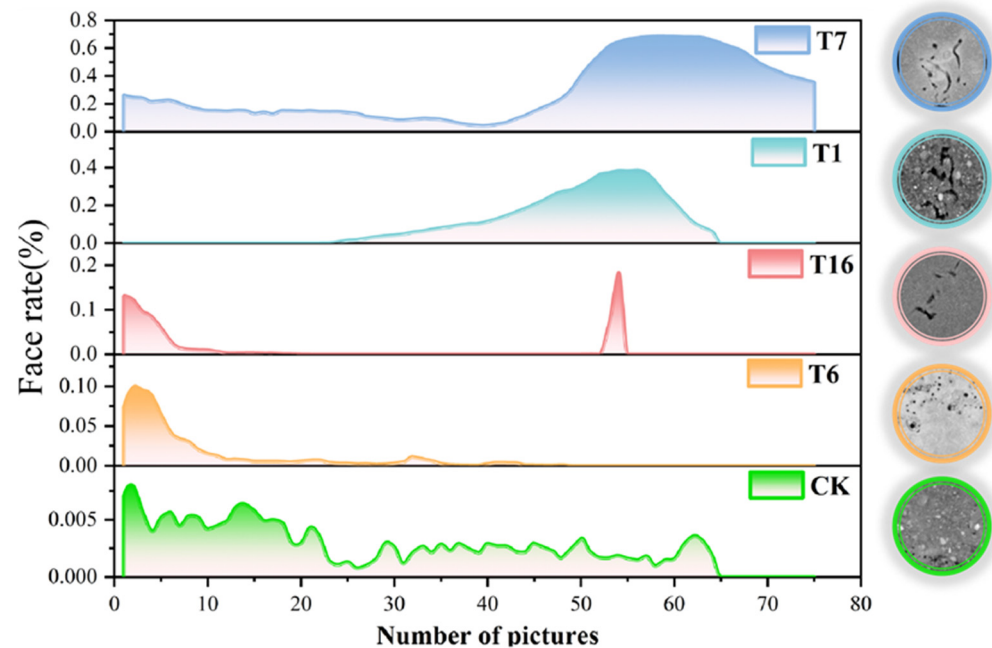


Figure 11. Surface porosity diagram.

To summarize, the groups A1B4C1, A1B1C1, A2B4C4, A1B3C4, and the Control Group (CK) were selected to compare the images of the crops when they were fresh, as shown in Figure 13.

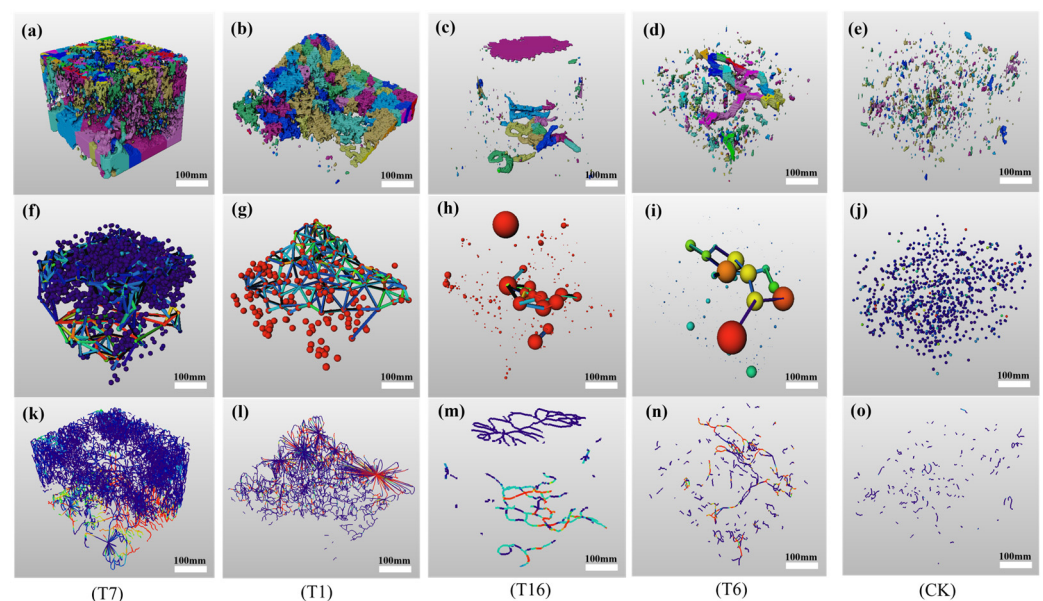


Figure 12. A network model. Color is used to distinguish earthworm pores from conventional pores. In the stick model, red indicates that the pore is larger and blue indicates that the pore is smaller, and the color of the connected pipe is the same as that of the pipe. In the skeleton model, red indicates that the pore throat pipe is larger and purple indicates that it is smaller. Pore network models: (a) PNM of group T7; (b) PNM of group T1; (c) PNM of group T16; (d) PNM of group T6; (e) PNM of control group (CK); (f) ball-and-stick model of group T7; (g) ball-and-stick model of group T1; (h) ball-and-stick model of group T16; (i) ball-and-stick model of group T6; (j) ball-and-stick model of control group (CK); (k) pore skeleton diagram of group T7; (l) pore skeleton diagram of group T1; (m) pore skeleton diagram of group T16; (n) pore skeleton diagram of group T6; (o) pore skeleton diagram of control group (CK).

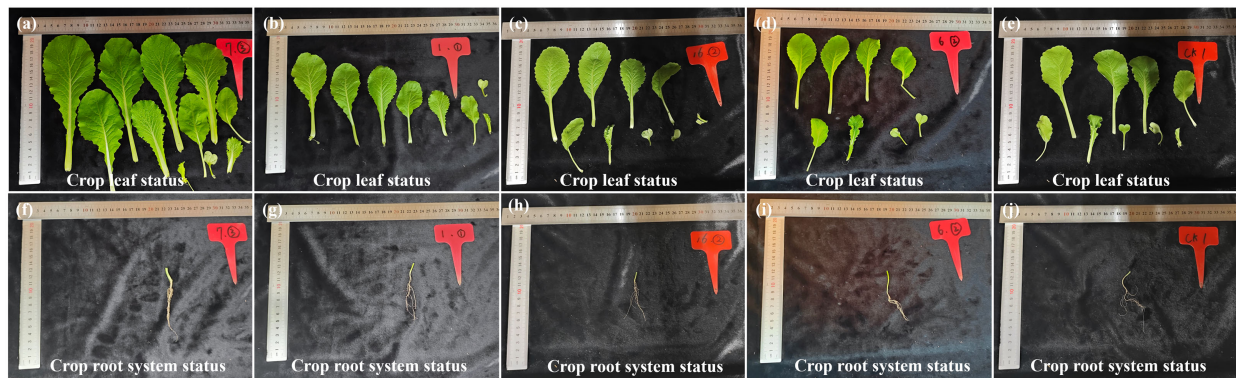


Figure 13. Figure (a–j) show the diagrams of leaves and roots of *Brassica chinensis* L. in groups A1B4C1, A1B1C1, A2B4C4, A1B3C4, and control group (CK). The figure illustrates crop leaf states: (a–e) represent A1B4C1, A1B1C1, A2B4C4, A1B3C4, and the control group (CK), respectively. The images (f–j) show the root conditions of the crop under treatments A1B4C1, A1B1C1, A2B4C4, A1B3C4, and the control group CK, respectively.

4. Conclusions

This study systematically explored the regulatory mechanism of earthworm (*Eisenia fetida*)-mediated biological tillage for the structural improvement of degraded black soil and its effect on the yield of *Brassica chinensis* L. through orthogonal pot experiments and CT scanning technology. The synergistic optimization thresholds were determined as follows: 50 g composted straw, 50–150 kPa soil compaction, and 30–37% field capacity. Under these conditions, earthworm activities significantly enhanced the soil macropore network: the 3D porosity increased by 288 times compared with the Control Group (CK); the equivalent pore network model (PNM) intuitively showed improved pore connectivity, with the formation of penetrating cylindrical pores; and the number of pore throats increased by 27 times under biological tillage. This not only optimized the soil water and gas transmission efficiency but also directly promoted the crop growth response: the leaf dry weight increased by 55.29%, the root dry weight increased by 96.60%, and the total biomass increased by 75.46%. Of the root architecture indicators, the average root diameter increased by 41.68% under high water content, and the taproot length in loose soil was significantly higher than that in compacted soil. These results indicate that earthworms indirectly support root expansion by locally improving the physical environment. The structural equation model (SEM) further verified the interaction mechanism of environmental factors, revealing that soil moisture was the core driving factor for biomass growth, with a direct path coefficient of 0.95 ($p < 0.05$). In conclusion, this study provides a theoretical basis for the application of earthworm biological tillage technology in degraded soil remediation and crop yield improvement. By optimizing the conditions of earthworm biological tillage, soil structure can be effectively improved, plant growth can be promoted, and new ideas and methods can be provided for the development of sustainable agriculture.

This study has certain limitations: the limited volume of the pots restricted earthworm activity space, causing the pores formed by their burrowing to concentrate primarily in the lower regions where straw was placed. This failed to simulate the three-dimensional, uniformly distributed macropore network found at field scale. The height of the pots also limited the vertical growth of the *Brassica chinensis* L. taproots, resulting in no significant change in root length or restriction by pot depth. This caused deviations in root morphology compared to natural field growth conditions. The experimental scale was small, focusing solely on a single crop (*Brassica chinensis* L.) and a single earthworm species (*Eisenia fetida*). The experimental period was short-term, covering only one growth season of *Brassica chinensis* L., making it difficult to reveal the dynamic effects of long-term straw decompo-

sition and continuous earthworm reproduction on pore network stability. Ultimately, we were able to simulate complex field environmental stresses; calibrate key parameters based on field data; adjust technical solutions according to different soil textures and climatic characteristics in the northeast black soil region; and help to achieve the field adaptation and promotion of biological tillage technology.

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Data Availability Statement: The original contributions presented in the study are included in the article, and further inquiries can be directed to the corresponding author.

Conflicts of Interest: The authors declare no conflicts of interest.

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