Improving soil properties and maize yield under fertilizer reduction using bio-organic matter combined with biochar

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Abstract: The harmless treatment of livestock and poultry reduces the risk of water and soil pollution caused by untreated discard of sick and dead livestock. Chemical fertilizer increases crop yield, while its over-application will lead to serious problems such as agricultural non-point source pollution as well as land acidification and soil compaction. It is of great significance to explore the utilization potential of bio-organic matter originating from harmless treatment of livestock to improve the soil environment and enhance agricultural productivity. This study investigated the effects of different application rates of bio-organic matter (0, 1285, 1928, 2571 kg/hm^2) and biochar addition (0, 10 000 kg/hm^2) on soil properties and crop yield under 20% reduction of chemical nitrogen fertilizer. The results indicated that the application of bio-organic matter combined with biochar improved soil physical structure under fertilizer reduction by decreasing soil bulk density and increasing soil porosity and soil aggregate stability. Compared to that under CK, the soil bulk density was reduced by 1.42%-6.38%, and the soil porosity was increased by 1.17%-7.05%. Compared to conventional fertilization, applying bio-organic matter (1 928 kg/hm^2) ensured sufficient soil nutrients for crop growth under 20% of fertilizer reduction. The soil fertility was further boosted by the addition of biochar. The alkaline nitrogen content peaked under BM3 with 42.08 mg/kg, and the total nitrogen content and soil organic matter content reached their peak values under NM4 treatment, which were 0.97 g/kg and 21.23 g/kg, respectively. The higher the amount of bio-organic matter applied, the higher the grain yield and crop water productivity. The yield gained with bio-organic matter application alone at the rate of 2571 kg/hm^2 under fertilizer reduction (NM4) was 7504 kg/hm^2, which can reach equal yield level with CK, while medium to high addition of bio-organic matter combining biochar (BM3 and BM4 treatments) produced higher grain yield than that under CK. The correlation analysis showed significant positive correlations between total nitrogen and maize yield and between soil organic matter and maize yield. Overall, under 20% fertilizer reduction, applying bio-organic matter at the rate of 1928 kg/hm^2 and combining biochar at the rate of 10 000 kg/hm^2 would be an economical plan to enhance soil physicochemical properties and ensure stable maize yield, and would also supply a scientific way to reuse bio-organic matter originating from harmless treatment of livestock carcasses.

Keywords: fertilizer reduction, harmless treatment of livestock carcasses, soil aggregate, soil improvement, maize
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1 Introduction

Food security is an important guarantee for China's economic development and political construction, and an important basis for ensuring national security. Fertilizer, as the main input factor in agricultural production, rapidly increases crop yields with less labor input. However, the long-term over-application of chemical fertilizers not only increases agricultural production costs, but also leads to serious problems such as agricultural surface pollution and land acidification and sclerosis[1]. In addition, the over-application of chemical fertilizers increases the risk of nutrient leaching from farmland[2], causing harm to the ecological environment and seriously threatening the sustainable development of agriculture in China. Therefore, how to reduce the application of chemical fertilizers and promote the green and sustainable development of agriculture is an important issue of concern for all sectors of the community.

Based on the above problems, many experts and scholars have carried out research in the area of fertilizer reduction and efficiency. They have found that in the process of crop cultivation, fertilizer reduction with the application of bio-organic fertilizers was more effective than conventional fertilization in improving the nutrient content of the soil and soil microorganisms and activity, promoting the growth and development of crops, and ultimately improving yield and planting efficiency[3,4]. Bio-organic fertilizer is a new type of fertilizer made from solid wastes such as livestock and poultry residues and manure, domestic waste, crops, and other solid wastes as raw materials, which are fully fermented by micro-organisms, rapidly deodorized, putrefied, and dehydrated. Hapsoh et al.[5] found that bio-organic fertilizers made from palm oil processing waste (sludge) ensured the normal growth and development of red chilli plants under a 25% reduction in inorganic fertilizers. Bhardwaj et

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al.[6] observed that in a "French Bean-Cauliflower" based cropping system, the combination of 75% regular chemical fertilizer and bio-organic fertilizer made from crop residues saved on chemical fertilizer, while increasing the amount of soil available nitrogen and potassium increased the yield of cauliflower. Zha et al.[7] reported that compared with no bio-organic fertilizer, soil available phosphorus, ammonium nitrogen, sucrase, protease, and urease under sheep manure bio-organic fertilizer treatment were improved, and the growth of strawberry was promoted to some extent. In summary, the application of bio-organic fertilizer is indeed conducive to the supply of soil nutrients, improving the activity of the soil microbial community, and achieving the reduction of chemical fertilizers to ensure both stable and increased crop yields. However, previous studies have focused mainly on bio-organic fertilizers made from materials such as sludge, plant residues, animal manure, and so on, while less research has been conducted on bio-organic fertilizers using the products of harmlessly-treated livestock animal carcasses as raw materials.

Actually, the product of harmlessly-treated livestock animal carcasses is an enormous amount of organic matter that is rich in nutrients, beneficial microbial flora, and a variety of biologically active substances[8]. Zhai et al.[9] chose harmlessly-treated animal carcass manure for oilseed rape planting, and found that it significantly increased the chlorophyll content of oilseed rape, enhancing the photosynthetic efficiency and increasing the accumulation of aboveground dry matter of oilseed rape. It is also reported that the heavy metal content in soil and in the aboveground part of oilseed rape were under a safe level. Therefore, there is a great potential for the bio-organic matter originating from harmless treatment of livestock to be used as a bio-organic fertilizer. This study assumes that using this bio-organic matter in agriculture will improve soil quality and soil fertility as well as promote crop growth and yield, thus reducing the application of chemical fertilizers. However, most of the nitrogen in bio-organic matter exists in organic form and can only be absorbed and utilized by plants through mineralization, which results in a certain gap compared with nitrogen fertilizer.

Biochar, a common soil amendment, is rich in carbon, has a small bulk weight, a large specific surface area, a high adsorption capacity, and a high stability[10,11]. Wang et al.[12] found that biochar application resulted in a lower soil bulk density but higher total organic carbon, effective phosphorus, and total nitrogen, and 15 t/hm^2 biochar promoted the growth and increased the yield of soybean. Yang et al.[13] concluded that 20 t/hm^2 biochar was a reasonable and effective method for increasing the soil organic carbon content, enhancing the stability of the humic acid structure, and improving the rice yield in rice-growing areas in Northeast China. Therefore, the application of biochar in the process of agricultural production effectively improved the physicochemical properties of the soil and increased production. Studies have shown that the combination of biochar and fertilizer can improve nitrogen use efficiency and ensure stable crop yield[14]. Therefore, the applicationofbiochartogetherwithbio-organicmatterwasconsideredin this study.

In summary, this study carried out a field trial of different application rates of bio-organic matter combined with biochar under 20% reduction of nitrogen fertilizer. The aims of this study were: 1) to investigate the impact of bio-organic matter and biochar on soil physical structure and soil fertility, 2) to clarify the influence of bio-organic matter and biochar on corn yield, and 3) to explore the feasibility of applying bio-organic matter alone or combined with biochar under 20% fertilizer reduction, and to discuss their application to ensure equal yield level under traditional fertilization. The study will supply potential utilization methods for harmlessly-treated animal carcass products as bio-organic fertilizer and provide a theoretical basis and practical guidance for chemical fertilizer reduction.

2 Materials and methods

2.1 The experimental area

The experiments were conducted at the Agricultural Water and Hydrology and Aquatic Ecology Experimental Station of Yangzhou University, as shown in Figure 1. The location experiences a subtropical monsoon climate, with an average annual temperature ranging from 15°C to 18°C. The highest temperatures occur in July, while the lowest temperatures are recorded in January. The annual precipitation is approximately 900 mm to 1100 mm, with the majority falling between April and September, accounting for approximately 70% of the total annual rainfall. The field experiments were conducted from March to August 2023; daily

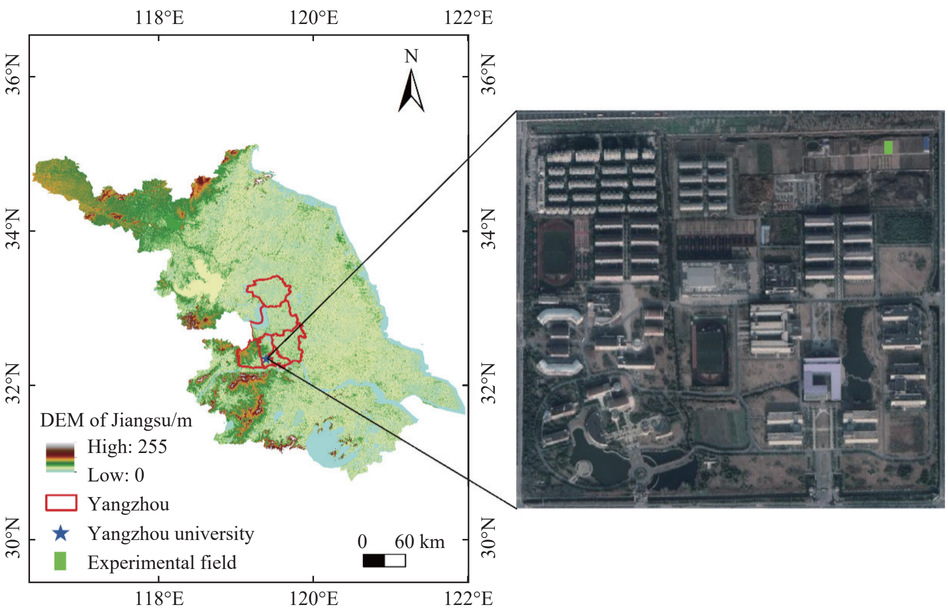


Figure 1 Geographical location of the research site

temperatures and precipitation during the experimental period are shown in Figure 2.

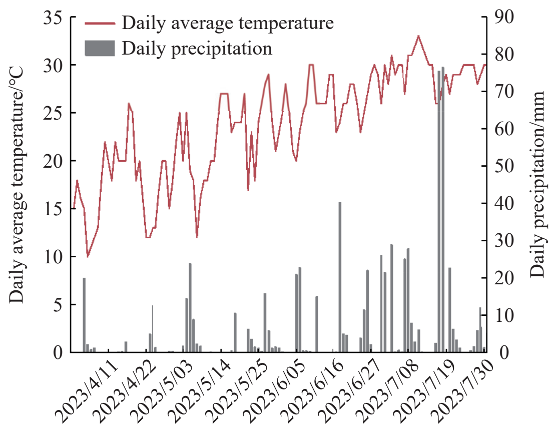


Figure 2 Temperature and effective rainfall conditions during the experimental period

The experimental station was established in the year of 2016, while the land was previously planted with spring maize during 2017-2020 and has been fallow since 2020. The soil properties are listed in Table 1, with the mass fractions of clay, silt, and sand of 4.38%, 32.76%, and 62.86%, respectively. According to international soil texture standards, the soil texture is classified as sandy loam.

Table 1 Soil properties of the experimental area

Soil depth/ cm	Alkaline hydrolyzable nitrogen/ mg·kg^{-1}	Available phosphorus/ mg·kg^{-1}	Available potassium/ mg·kg^{-1}	Total nitrogen/ g·kg^{-1}	Organic matter/ g·kg^{-1}	Bulk density/ g·cm^{-3}	pH
0-20	39.95	15.63	45.02	1.50	8.67	1.42	8.14
20-40	44.42	11.46	42.51	2.22	14.35	1.36	7.79
40-60	10.88	5.64	29.53	1.00	3.62	1.37	7.76

2.2 Experimental materials

The nitrogen fertilizer used in the experiment was urea with a total nitrogen content≥46%; granular phosphate fertilizer with an effective phosphorus content≥12% and water-soluble phosphorus content≥7%; potassium chloride with K_2O content≥60%.

The process of harmless disposal of livestock carcasses in this study was to first put the livestock carcasses into the pre-crushing machine to be crushed into small pieces. When the temperature in the treatment tank was >140°C and the pressure in the tank was ≥ 0.5 MPa, sawdust and straw were added as auxiliary materials and autoclaved for 4 h. When the temperature in the treatment tank was reduced to less than 70°C, composite microbial fungi were added to carry out degradation, and the degradation time was 6-8 h[15]. The bio-organic matter originating from the harmless disposal of livestock carcasses which was used in this study was obtained by composting this product[16]. The bio-organic matter had the following composition: N content (mass fraction) of 7.28%, C content (mass fraction) of 50.91%, H content (mass fraction) of 7.24%, and S content (mass fraction) of 0.63%.

The biochar used in this study was purchased from Henan Jiahe Clean Water Materials Co., Ltd. The biochar originated from maize straw and was produced at a pyrolysis temperature of 500°C with a pyrolysis time of four hours. The biochar had a diameter of 0.15 mm, a pH of 9.0, ash content (mass fraction) of 5.38%, an N content (mass fraction) of 1.55%, and a C content (mass fraction) of 72.31%.

2.3 Experimental design

The experiment employed a completely randomized block design for the addition levels of bio-organic matter (0, 1285, 1928, 2571 kg/hm²) and biochar (0, 10 000 kg/hm²) under 20% nitrogen reduction (180 kg/hm²), a conventional nitrogen application rate (225 kg/hm²) without bio-organic matter or biochar application served as the control (CK), and the detailed treatments are listed in Table 2. Each treatment was replicated three times, totaling 27 plots, each with an area of 96m² (8m×12m). Drainage ditches were manually excavated on the east and west sides of each plot, rectangular in shape, approximately 50 cm wide and 30 cm deep. Before sowing, the appropriate amounts of bio-organic matter and biochar were calculated and applied to each plot, followed by plowing, harrowing, and thorough mixing with the soil of the plow layer. After seeding, fertilization was carried out using trenching methods, with nitrogen fertilizer amounts consistent with those in the experimental treatments, and both phosphorus and potassium fertilizers were applied at 90 kg/m². No further fertilization occurred during the maize growing period, and the other field management practices conformed to local traditional cultivation practices and were the same for all plots.

Table 2 Field experiment treatments

Treatments	Nitrogen reduction ratio/%	Addition amounts/kg·hm^{-2}		
		Nitrogen	Bio-organic matter	Biochar
CK	0	225	0	0
NM0	20	180	0	0
NM2	20	180	1 285	0
NM3	20	180	1 928	0
NM4	20	180	2 571	0
BM0	20	180	0	10 000
BM2	20	180	1 285	10 000
BM3	20	180	1 928	10 000
BM4	20	180	2 571	10 000

The planted maize variety was Su Yu Nuo No. 5, which was sown on April 10, 2023, and harvested on August 1, with a total growth period of approximately 110 d. The maize plants were planted in row-to-row spacing of 80 cm, and the plant-to-plant spacing was 40 cm. The planting density was 60 000 plant/m². The planting depth was 3-5 cm, and the seeds were sown manually with precise spacing.

2.4 Observation and measurement

After the maize was harvested, soil samples from 0-20 cm were collected, avoiding the fertilizer application points and the roots. For each treatment, five samples were collected in an S pattern and mixed into one soil sample. The sample was air-dried and sieved through a 2 mm sieve for the determination of soil physical and chemical properties.

The soil bulk density was measured using the ring knife method after plant harvest, and porosity was calculated as follows:

$$P_t = \left(1 - \frac{p_b}{P_s}\right) \times 100\% \tag{1}$$

where, P_t is the soil porosity, (%); P_s is the soil particle density, which is usually set at 2.65 g/cm³; and p_b is the soil bulk density, g/cm³.

The soil three-phase ratio, consisting of the solid, liquid, and gas phases, was calculated as follows:

$$\text{Soil phase} = (1 - P_t) \times 100 \tag{2}$$

$$\text{Liquid phase} = \text{Soil moisture content} \times p_b \times 100 \tag{3}$$

$$\text{Gas phase} = (1 - P_t - \text{Liquid phase}) \times 100 \tag{4}$$

The deviation of the soil three-phase ratio (R) was calculated as follows:

$$R = \sqrt{(\text{Soil phase} - 50)^2 - (\text{Liquid phase} - 25)^2 - (\text{Gas phase} - 25)^2} \tag{5}$$

The composition of the soil water-stable aggregates was determined by wet sieving. Specifically, 100 g of the sample was placed on a series of sieves with descending mesh sizes of 5 mm, 2 mm, 1 mm, and 0.25 mm from top to bottom. First, the samples were moistened slowly with water, then placed in a bucket and allowed to sit for 5 min. Afterward, the samples were agitated for 3 min by shaking at an amplitude of 3 cm for 50 times, transferred to an aluminum box, and dried at 50°C before weighing. This procedure yields mass measurements for water-stable aggregates of <0.25 mm, 0.25-1 mm, 1-2 mm, 2-5 mm, and >5 mm, respectively. Formulas for calculating the mean weight diameter (MWD), geometric mean diameter (GMD), and the content of aggregates >0.25 mm ($R_{>0.25}$) were as follows:

$$\text{MWD} = \sum_{i=1}^{n} \overline{X_i} \times W_i \tag{6}$$

$$\text{GMD} = \exp\left[\frac{\sum_{i=1}^{n} W_i \ln \overline{X_i}}{\sum_{i=1}^{n} W_i}\right] \tag{7}$$

$$R_{>0.25} = \frac{m_{>0.25}}{m_{\text{T}}} \times 100\% \tag{8}$$

where, $\overline{X_i}$ is the average diameter of each grade of aggregates, mm; W_i is the mass percentage content of each grade of aggregates, (%); $m_{>0.25}$ is the mass of aggregates larger than 0.25 mm, g; m_{T} is the total mass of aggregates of all grades, g.

Soil nutrients were determined by referring to soil agrochemical analysis[17]. Soil available nitrogen (AN) was determined using the alkaline diffusion method, soil total nitrogen (TN) was determined using the Kjeldahl method, and soil organic matter (SOM) was determined using the potassium dichromate-concentrated sulfuric acid external heating method.

At harvest, 12 plants were randomly selected from each plot and brought back to the laboratory for further examination (ear length, ear diameter, number of rows per ear, number of grains per row), where ear length included the bald tip of the maize, and ear diameter was the diameter of the maize at its thickest point. Finally, the 100-grain weight was weighed to calculate the grain yield.

The total water consumption (ET) was calculated as follows, based on the irrigation water:

$$\text{ET} = P + I + \Delta W - R - D \tag{9}$$

where, P is the precipitation; I is the cumulative amount of irrigation water, mm; ΔW is the soil moisture variation, mm; R is the surface runoff; and D is the deep percolation, mm.

There was no irrigation during the whole growth period of maize, and this study assumed that no surface runoff was generated and no leachate was produced, so the equation could be simplified as follows:

$$\text{ET} = P + \Delta W \tag{10}$$

Thus, the water productivity (WP) was calculated as follows:

$$\text{WP} = \frac{Y}{\text{ET}} \tag{11}$$

where, Y is the yield of maize (kg/hm^2).

2.5 Data analysis and statistics

The data were calculated and processed by using Excel 2021. Comparative analyses were conducted to assess the impact of these treatments on soil physicochemical properties and maize yield, and ANOVA variance analysis and Duncan's multiple range tests for significant differences were performed using SPSS 26.0. Spearman's method was selected for correlation analysis. The figures were plotted with Origin 2021.

3 Results

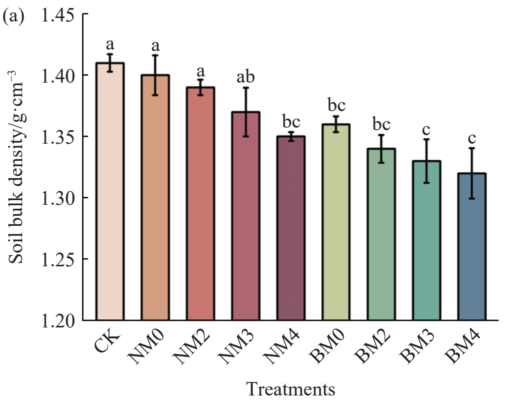
3.1 Soil bulk density and porosity

As shown in Figure 3a, the soil bulk density decreased under treatments with nitrogen reduction, and it continually decreased with bio-organic matter and biochar application. The higher the addition of bio-organic matter was, the lower the soil bulk density was. In the group of bio-organic matter application without biochar (NMs), only the soil bulk density under NM4 was significantly lower than that under CK (p<0.05). In the group of combination of bio-organic matter and biochar addition (BMs), the soil bulk density further decreased, and the values were all significantly lower than those under CK. Notably, biochar addition significantly reduced the soil bulk density under the same bio-organic matter application rate. As shown in Figure 3b, soil porosity was greater under nitrogen reduction than under CK, and it significantly improved under all treatments except NM0. Similarly, the soil porosities under BMs were higher than those under NMs. Overall, bio-organic matter and biochar application under nitrogen reduction can improve soil properties by reducing the bulk density and improving the porosity of the topsoil, thereby improving soil aeration and water permeability.

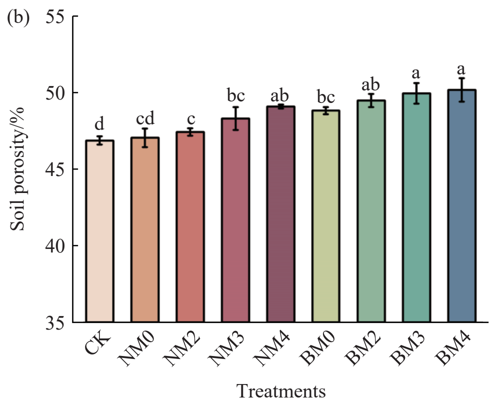
3.2 Soil three-phase ratio

The ideal soil three-phase ratio in agriculture is 50% solid, 25% liquid, and 25% gas; this kind of soil environment is conducive to crop growth. It can be seen from Figure 4 that with bio-organic matter application and its increase under nitrogen reduction, the volume fraction of the solid phase tended to decrease, while the volume fractions of the liquid and gas phases correspondingly increased. When bio-organic matter combined with biochar was applied under nitrogen reduction, the volume fraction of the solid phase decreased by 3.66%-6.21%, while the volume fraction of the gas phase increased by 4.51%-14.20% compared to that of the CK. This indicates that bio-organic matter combined with biochar can obviously promote the soil aeration status. The soil three-phase ratios of NM4 and BM3 were closer to the ideal conditions (50:25:25), which demonstrated that the rational application of bio-organic matter and biochar can optimize the soil physical structure.

The deviation value of the soil three-phase ratio (R), shown in Figure 5, quantifies the spatial distance from the measured soil structure to the ideal ratio of the solid, liquid, and gas phases. According to Figure 5, as the application rate of bio-organic matter increased under nitrogen reduction, the R value continually decreased and reached a minimum value of 1.82 under NM4 in NMs group. The R values under NM4, BM3, and BM4 were significantly lower than those under CK, while the *R* value under BM2 was significantly higher than that under CK. In BMs group, the *R* value initially increased but then decreased as bio-organic matter application increased, which implies that considering the soil three-phase ratio, attention should be paid to the proportion of fertilization. Overall, soil three-phase ratio data suggest that NM4 and BM3 were the most effective treatments for optimizing the soil physical structure towards the ideal three-phase ratio.



a. Soil bulk density at 0-20 cm



b. Soil porosity at 0-20 cm

Note: Different lowercase letters indicate significant differences between treatments ($p<0.05$). Same as below.

Figure 3 Soil bulk density (a) and porosity (b) at 0-20 cm

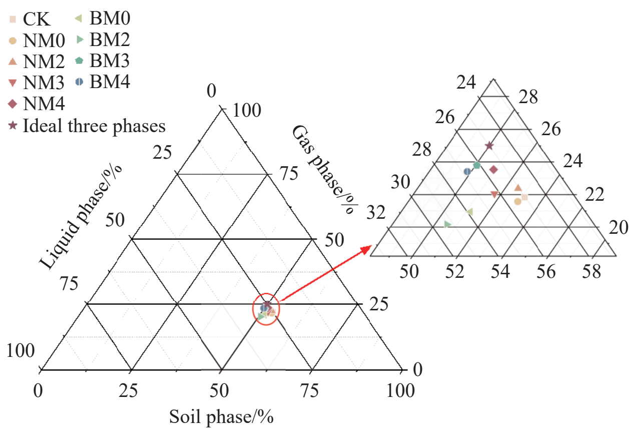


Figure 4 Soil three-phase ratio

Figure 5 shows that the overall trend in the impact of the addition of bio-organic matter on the volume fractions of the three soil phases (solid, liquid, and gas) was as follows: as the amount of bio-organic matter increased, the volume fraction of the solid phase tended to decrease, while the volume fractions of the liquid and gas phases correspondingly increased. When bio-organic matter was applied in combination with biochar, compared to that of the control (CK), the volume fraction of the solid phase decreased by 3.66%-6.21%, the volume fraction of the liquid phase changed by –4.06% to 8.91%, and the volume fraction of the gas phase increased by 4.51%-14.20%. This indicates that the combined application of bio-organic matter and biochar significantly promoted the adjustment of the soil's physical structure, especially in terms of increasing soil porosity. The soil three-phase ratios of NM4 and BM3 were closer to the ideal state (50:25:25), while BM2 had the poorest three-phase ratio (50:21:29), demonstrating that the rational selection of the addition amounts of bio-organic matter and biochar optimized the soil's physical structure.

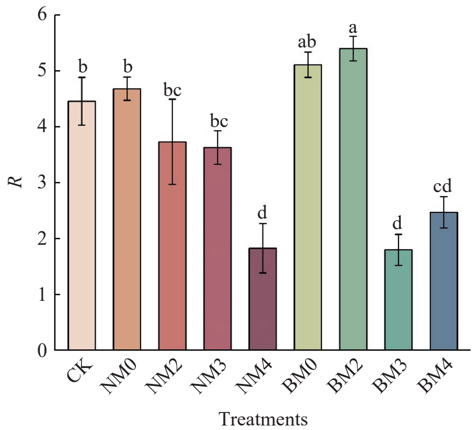


Figure 5 Soil three-phase ratio deviation value *R*

The deviation value *R* of the soil three-phase ratio quantifies the spatial distance from the measured soil structure to the ideal ratio of the solid, liquid, and gas phases. According to Figure 5, as the application rate of bio-organic matter increased after reducing nitrogen levels, *R* progressively decreased, with NM4 decreasing significantly to 1.82%, which was lower than that of the control (CK). When applied in conjunction with biochar, the *R* value initially increased but then decreased as more fertilizer was added, with values of 5.10, 5.39, 1.80, and 2.47, respectively. Notably, BM2 was significantly higher than CK, while BM3 and BM4 were significantly less abundant. These data suggest that treatments NM4 and BM3 were the most effective at optimizing the physical structure of the soil towards the ideal three-phase ratio.

3.3 Soil water-stable aggregates

The application of bio-organic matter and biochar under nitrogen reduction effectively improved the distribution of soil water-stable aggregate components, as shown in Figure 6. Specifically, the proportion of aggregates larger than 5 mm decreased under NM0 compared to that under CK, increased with increasing bio-organic matter application in both the NMs and BMs groups, and reached the maximum of 15.78% under BM3. This indicates that nitrogen reduction decreased the number of large soil aggregates, while the addition of bio-organic matter and biochar increased them. The addition of biochar notably enhanced the quantity of medium aggregates (>1 mm), with the most obvious improvement obtained in treatments combining bio-organic matter and biochar. Compared to the control (CK), the addition of bio-organic matter and biochar significantly increased the quantity of water-stable aggregates larger than 0.25 mm, demonstrating the potential of these amendments to improve soil structure.

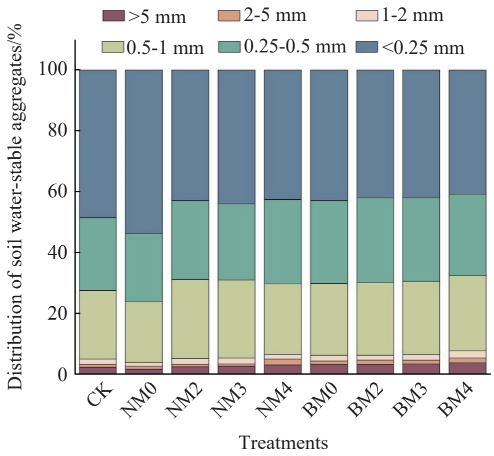


Figure 6 Distribution of soil water-stable aggregates

Soil aggregate mean weight diameter (MWD), geometric mean diameter (GMD), and the proportion of water-stable aggregates over 0.25 mm ($R_{0.25}$) reflected the soil structure. The larger the MWD and GMD, the more stable the soil aggregates. From Table 3, it is observed that nitrogen reduction (NM0) significantly weakened the stability of surface soil aggregates, while the addition of bio-organic matter and biochar improved this phenomenon. Similarly, all MWD, GMD, and $R_{0.25}$ were increased with bio-organic matter application increase in both the NMs and BMs groups. Generally, these three indicators under BMs treatments were higher than those under the corresponding NMs treatments (the same bio-organic matter application rate treatment without biochar), which indicates a strengthening effect of biochar on soil structure. Except for NM0, the MWDs under the other treatments were higher than those under CK; the highest MWD was obtained under BM4, and it was significantly higher than others. The GMD under NM4, BM2, BM3, and BM4 was significantly higher than those under CK and NM0. The highest $R_{0.25}$ was also obtained under BM4, while there was no significant difference among BMs treatments. In general, the soil structure with bio-organic matter application combining biochar was significantly better than that under CK, which implies that biochar improved the soil structure. For a single bio-organic matter, a high application rate would be better to improve soil structure.

Table 3 The stability of soil water-stable aggregates varied among the different treatments

Treatments	MWD/mm	GMD/mm	$R_{0.25}$/%
CK	0.56±0.02^c	0.40±0.02^b	51.42±6.25^c
NM0	0.50±0.03^d	0.37±0.03^c	46.20±5.54^d
NM2	0.58±0.01^c	0.42±0.01^{ab}	55.75±3.52^b
NM3	0.58±0.01^c	0.42±0.01^{ab}	56.01±6.08^b
NM4	0.62±0.01^b	0.43±0.02^a	57.44±4.51^{ab}
BM0	0.61±0.01^b	0.42±0.02^{ab}	57.03±8.49^{ab}
BM2	0.63±0.02^b	0.43±0.01^a	57.97±8.32^{ab}
BM3	0.63±0.02^b	0.43±0.01^a	58.00±3.67^{ab}
BM4	0.67±0.02^a	0.44±0.01^a	59.15±7.12^a

Note: Different lowercase letters behind the same column data indicate significant differences between treatments (p<0.05). Same as below.

3.4 Soil nutrients

The soil alkaline nitrogen (AN), total nitrogen (TN), and soil organic matter (SOM) contents in the topsoil (0-20 cm) at harvest are shown in Figure 7. The soil alkaline nitrogen content is an important indicator used to assess the amount of nitrogen available for plant absorption in the soil. As shown in Figure 7a, the AN content under NM0 was significantly lower than the others. The addition of bio-organic matter and biochar effectively increased the AN content. For single bio-organic matter application, the AN content gradually increased with increasing bio-organic matter rate, and those under NM3 and NM4 were significantly higher than that under control (CK). Furthermore, the AN peaked under BM3 with 42.08 mg/kg. It is interesting that the AN under BM4 significantly decreased to 36.24 mg/kg, which was even lower than that under NM4 with 38.55 mg/kg.

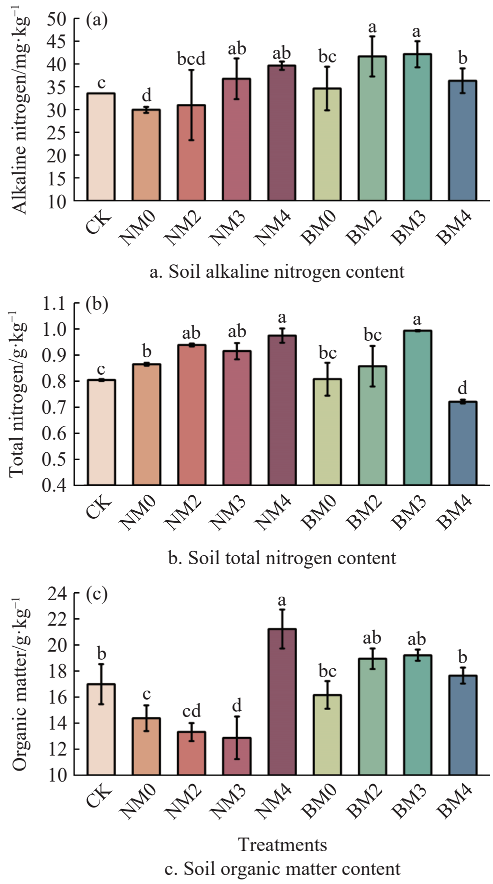


Figure 7 Soil alkaline nitrogen (a), total nitrogen (b), and organic matter (c) content

Total nitrogen content reflects the soil fertility, as shown in Figure 7b. Under reduced nitrogen conditions, the application of bio-organic matter significantly increased the TN content compared to that under CK. Overall, the TN content in the soil increased with the increasing bio-organic matter application rate in the NMs group, and reached a peak of 0.97 g/kg under NM4. In the BMs group, the total nitrogen content first increased and then decreased, and a peak of 0.99 g/kg was obtained under BM3, while the total nitrogen content in BM4 was significantly lower than the others.

Soil organic matter (SOM) is mainly composed of decomposed and synthesized plant residues, animal remains, microorganisms, and their metabolic products. It is the basis for enhancing soil fertility, promoting nutrient cycling, supporting healthy crop growth, and maintaining ecological balance. From Figure 7c, it is evident that the addition of bio-organic matter led to a trend of initially decreasing and then increasing SOM content as the amount of bio-organic matter increased. In the NMs group, the SOM was significantly higher than the others, while the SOM content under NM0, NM2, and NM3 was significantly lower than that under CK. In BMs, the SOM content first decreased with increasing bio-organic matter application, but there was no significant difference with that under CK. The SOM under BM2 and BM3 were significantly higher than those under corresponding treatments of NM2 and NM3, respectively, which indicates that biochar addition stimulates the SOM content when combined with a medium application of bio-organic matter, while for a high rate of bio-fertilizer, it is better to apply it alone.

3.5 Maize yield and water productivity

The maize yield and water productivity (WP) among the different treatments at harvest are listed in Table 4. The number of grains in rows, 100-grain weight, and yield under NM0 were significantly lower than under the traditional fertilization (CK) under 20% nitrogen reduction, and the number of grains per row and length of ears were smaller than under CK, but did not reach significant levels. This indicates that a 20% reduction in nitrogen had no effect on the length of ears and number of grains per row, although it reduced maize yields. Compared with NM0, the addition of only biochar (BM0) under nitrogen reduction conditions increased the number of rows, number of grains per row, ear length, 100-grain weight, and yield of maize, but the other indices except yield did not reach statistical significance. In the NMs and BMs groups, the number of rows, number of grains per row, and ear length increased with increasing bio-fertilizer application, but the differences between the treatments did not reach a significant level. The 100-grain weight and yield also increased with increasing bio-fertilizer application in the two groups, except that the 100-grain weight and yield of BM4 were slightly decreased in comparison with those of BM3. The 100-grain weights of NM2 and BM2 were significantly lower than that of CK, and there was no significant difference with NM0, but the 100-grain weight of NM3 was significantly lower than that of the control, and there was no significant difference with NM0. The 100-grain weights of NM3, NM4, BM3, and BM4 were significantly higher than that of NM0. This indicates that the amount of bio-organic matter needed to reach a certain amount under nitrogen reduction, whether or not biochar was applied. The yield of maize decreased significantly under nitrogen reduction, while the application of bio-fertilizer or biochar, alone or in combination, significantly increased the yield. There was no difference between the yields of BM4 and CK under the condition of single application of bio-organic matter. However, under the condition of bio-organic matter and biochar, the yields of BM3 and BM4 were significantly higher than that of CK, which indicates that the amount of bio-fertilizer applied alone needed to reach 2571 kg/hm², and that the amount of bio-fertilizer applied together with the application of biochar had to reach 1928 kg/hm², in order to ensure that the maize yield did not suffer under nitrogen reduction.

Water productivity (WP) reflects the agricultural output achieved per unit of water resources consumed. As noted in Table 4, WP of NM0 was significantly lower than that of the traditional fertilization (CK) under 20% nitrogen reduction, but this difference was mitigated by the addition of bio-organic matter and biochar. Higher additions resulted in higher WP when bio-organic matter was applied alone, and there was no significant difference between those under NM4 and CK. When bio-organic matter and biochar were applied in combination, the higher the bio-organic matter addition, the higher the WP with BM3. In comparison, WP was slightly lower with BM4. However, WP under BM3 and BM4 both were slightly higher than that under CK. Excessive addition rates were likely to have diminished WP, a result that was already demonstrated by BM4.

Table 4 Maize yield and water productivity among different treatments

Treatment	Number of ears per row	The number of grains per row	Ear length/cm	100-grain weight/g	Grain yield/kg·hm^{-2}	WP/kg·m^{-3}
CK	15.17±1.01^{a}	35.32±3.61^{ab}	15.98±0.23^{ab}	20.80±2.07^{a}	7579±651^{ab}	111.77±9.61^{ab}
NM0	12.23±1.53^{ab}	29.33±1.73^{b}	11.99±1.60^{b}	14.90±1.42^{c}	5904±163^{e}	87.10±2.42^{d}
NM2	13.00±2.02^{b}	31.25±3.79^{ab}	12.68±0.59^{b}	15.57±1.65^{c}	6355±368^{cd}	93.69±5.43^{cd}
NM3	13.69±1.53^{ab}	31.78±1.00^{ab}	13.09±1.56^{b}	19.43±1.15^{ab}	6856±295^{c}	101.12±4.36^{bc}
NM4	13.77±1.01^{ab}	34.30±4.93^{ab}	13.74±1.72^{ab}	21.73±3.84^{a}	7504±360^{b}	110.63±5.32^{ab}
BM0	13.12±0.58^{ab}	32.65±2.31^{ab}	13.13±1.23^{b}	14.57±0.55^{c}	6050±246^{d}	89.18±3.63^{d}
BM2	13.54±1.73^{ab}	32.98±4.04^{ab}	14.46±0.44^{ab}	16.73±0.95^{bc}	6568±202^{cd}	96.82±2.98^{cd}
BM3	15.69±1.91^{a}	36.33±2.20^{a}	17.63±1.03^{a}	22.23±0.76^{a}	7791±318^{a}	114.83±10.59^{a}
BM4	14.36±1.02^{ab}	37.74±1.53^{a}	13.99±1.01^{ab}	21.80±2.17^{a}	7759±229^{a}	114.01±3.37^{a}

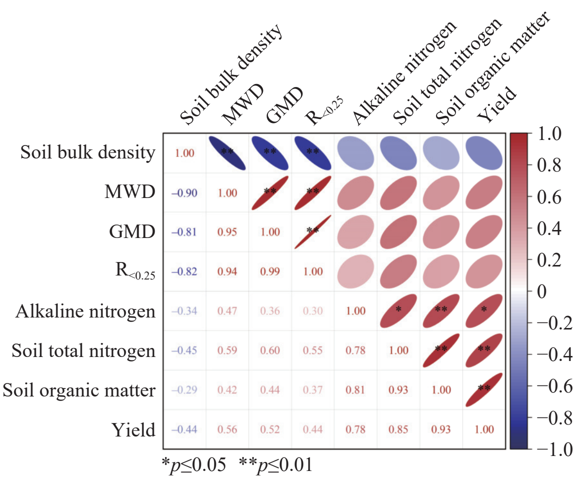


Figure 8 Correlation between soil physicochemical properties and maize yield

3.6 Correlation analysis of soil physicochemical properties and maize yield

The correlation between soil physicochemical properties and maize yield is shown in Figure 8. Soil bulk density was significantly negatively correlated with MWD, GMD, and $R_{<0.25}$ (p<0.01), which indicates that higher soil bulk density may negatively affect soil structure. MWD was significantly positively correlated with GMD and $R_{<0.25}$ (p<0.01). There were extremely significant correlations (p<0.01) between AN, TN, and SOM, and a significant positive correlation (r=0.78) between soil alkaline nitrogen and soil total nitrogen. A significant positive correlation (r=0.78) between AN and maize yield shows the importance of increasing soil nitrogen content for crop growth and yield. Similarly, significant positive correlations between TN and maize yield (r=0.85) and between SOM and maize yield (r=0.93) further emphasize the critical role of soil nutrient management in agricultural production.

4 Discussion

Soil structure plays a crucial role in regulating soil water, nutrients, heat, and atmosphere, serving as the foundation for increasing crop yield and quality. Research has indicated that there is a positive correlation between soil structure suitability and both soil fertility and crop yield[18]. In this study, conventional nitrogen fertilizer application showed high soil bulk density and low soil porosity, leading to soil compaction and hardening. An increase in the bio-organic matter application rate combined with biochar gradually decreased the soil bulk density and improved the soil porosity, which is consistent with the findings of Zhang et al.[19] and Wei et al.[20] Studies have also indicated that the exclusive use of chemical fertilizers affects soil aggregate stability, and as the accumulated years of chemical fertilization increased, the soil MWD, GMD, and $R_{0.25}$ decreased[21]. It is also evidenced that the addition of exogenous organic materials improved the composition and morphological characteristics of the soil aggregates[22]. The results of this study indicated that applying a sufficient amount of bio-organic matter alone increased the number of macro-aggregates in the soil and improved the distribution of soil water-stable aggregates. This suggests that bio-organic matter could promote the aggregation of small soil aggregates into larger ones, enhancing soil structural stability. This can be explained by the fact that bio-organic matter contains abundant organic matter and humic acids, which promote the aggregation of soil particles. Additionally, the diverse nutrients in bio-organic matter provide basic substrates for soil microbes and enzymes[23], thereby further increasing the number of soil aggregates[24]. In this experiment, the application of biochar in addition to bio-organic matter further enhanced the water stability of the soil aggregates. This was because the physical properties of biochar itself could adsorb a large number of fine soil particles, thereby promoting the transformation of small aggregates into larger aggregates[25]. At the same time, biochar is rich in carbon, which enhances the fixation of organic carbon and increases microbial populations and activity, thereby generating more secretions to promote colloid aggregation in the soil[26,27].

Adding a substantial amount of bio-organic fertilizer improved the soil fertility and nutrient-holding capacity[28]. Zeng et al.[29] found that soil total nitrogen content increased with the nitrogen application rate and was mainly concentrated in the 0-40 cm soil layer. This study reached a similar conclusion. Compared to conventional nitrogen fertilization, reducing nitrogen application and supplementing bio-organic matter at a rate of 2571 kg/hm^2 increased the soil total nitrogen content under 20% nitrogen reduction. This was likely due to the rich organic nitrogen sources in the bio-organic matter. When the organic nitrogen was added to a certain level, it was balanced with the nitrogen sources in conventional nitrogen fertilization, thus improving the soil nitrogen supply. In this experiment, reducing nitrogen while applying medium to high rates of bio-organic matter significantly increased the contents of available nitrogen, total nitrogen, and organic matter in the topsoil. This might be due to the nutrient input from the bio-organic matter itself into the soil.

Additionally, the bio-organic matter, which is rich in various bioactive substances, can decompose and transform complex organic materials in the soil, thus rapidly activating nutrients. Biochar itself contains a large amount of minerals, and its ash carries significant quantities of nitrogen, phosphorus, and potassium[30]. However, the sole application of biochar did not yield satisfactory results in enhancing soil surface nutrients, which is consistent with the findings of Ruan et al.[31]. This might have occurred because the biochar addition was relatively low and, due to its physical properties, it adsorbs certain nutrients from the soil. The nutrient-rich bio-organic matter compensated for the nutrient deficiencies of the biochar; the physical properties of the biochar complemented and synergized with the nutrient slow-release function of the bio-organic matter.

In this study, the combined application of high amounts of bio-organic matter and biochar did not effectively improve soil organic matter or total nitrogen content. This might be explained by the fact that the bio-organic matter had a high carbon-to-nitrogen ratio (C/N), which increased the C/N of soil and then decelerated the decomposition of organic matter[32]. The micro-pore structure of biochar adsorbed organic matter, resulting in a reduction of organic matter content in the soil. Although biochar stabilizes organic matter, some organic matter may be permanently adsorbed onto the surface of biochar and not easily decomposed. Reducing nitrogen combined with the application of 2571 kg/hm^2 bio-organic matter increased soil nutrient content more effectively compared to conventional nitrogen application. However, adding biochar on this basis resulted in a decrease. This may be because there was a certain limit to the land's carrying capacity[33], and excessive application of bio-organic matter inhibited soil enzyme activity, reducing the conversion of available nutrients.

Chemical fertilizers combined with an appropriate amount of biochar increased crop yields, but excessive addition in soils with suitable fertility inhibited crop growth and even reduced yields[34]. Reducing chemical fertilizer application and combining it with bio-organic matter in appropriate amounts ensured stable and increasing maize yields, and the improvement effect on soil became more pronounced over the years[35]. These studies indicate that the response mechanism of crop yield to reduced nitrogen and the addition of exogenous organic materials is influenced by factors such as soil fertility and fertilization patterns. This study found that the yield components of maize under reduced nitrogen application combined with appropriate amounts of bio-organic matter and biochar were not significantly different from conventional nitrogen application, and the yield was slightly higher than that under full nitrogen application. The main reason is that the nutrients contained in the appropriate amounts of bio-organic matter compensated for the reduction of nitrogen by 20%, and the biochar acted as a nutrient slow-release agent, extending the availability of soil fertilizers, absorbing and retaining soil nutrients, thereby promoting the formation of maize yield components and increasing yield. This was similar to the findings of Lv[36], who studied the application of chemical fertilizers with organic fertilizers and biochar to foxtail millet. Combining biochar with medium to high rates of bio-organic matter increased water productivity of maize, indicating that water consumption per unit mass increased maize yield. This may be because bio-organic matter and biochar effectively reduced the soil bulk density, causing the dispersed small particle clusters in the soil to coalesce into large clusters, which increased the porosity, reduced the capillary force for soil water to rise, and facilitated water infiltration. This indicates that under the soil conditions of this experiment (with relatively low basic fertility), applying 2571 kg/hm^2 of bio-organic matter under nitrogen reduction could basically ensure a sustained and stable maize yield, and applying 1928 kg/hm^2 or 2571 kg/hm^2 of bio-organic matter with 10 000 kg/hm^2 of biochar under nitrogen reduction could even increase maize yield.

5 Conclusions

1) The application of bio-organic matter originating from harmless disposal of livestock carcasses combined with biochar improved soil physical structure under fertilizer reduction by decreasing soil bulk density and increasing soil porosity and soil aggregate stability of surface soil. Generally, the improvement effect was promoted with an increasing bio-organic matter application rate within the scope of this experiment.

2) The soil alkaline nitrogen and soil organic matter content significantly declined under fertilizer reduction. The soil fertility recovered under low rate bio-organic matter application or biochar addition, and it increased with an increasing bio-organic matter application rate and was boosted by biochar addition. Compared to CK, significant soil fertility improvement was observed under the highest bio-organic matter application alone (NM4) and medium bio-organic matter in combination with biochar (BM3).

3) The grain yield significantly declined with fertilizer reduction, as that under NM0 was significantly lower than that of CK. The yield was enhanced with an increasing bio-organic matter amount, either by applying bio-organic matter alone or combined with biochar. The yield obtained under bio-organic matter application alone at the rate of 2571 kg/hm^2 with nitrogen reduction (NM4) can reach the level of the yield under no nitrogen reduction (CK). The yields under BM3 and BM4 were higher than those under conventional fertilization. Overall, under 20% fertilizer reduction, applying bio-organic matter at the rate of 1928 kg/hm^2 combined with biochar application at the rate of 10 000 kg/hm^2 would be an economical plan to ensure stable maize yield.

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