**ORIGINAL PAPER**

# A Strategy for Reducing Nitrogen Fertilizer Application Based on Application of Biochar: A Case in Northeast China Black Soil Region (Mollisols)

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**Abstract**

Evaluate the effectiveness of using biochar to regulate the erosion and degradation of soil, and establish a quantitative relationship between crop leaf information and soil inorganic nitrogen through functional equations, to guide topdressing and reduce nitrogen fertilizer input. Using field pot experiments, we explored the similarities and differences in soybean growth indicators, yield indicators, and inorganic nitrogen changes under different application ratios of biochar (0, 3, and 6 kg·m$^{-2}$) and nitrogen fertilizer (0, 75, and 150 kg·ha$^{-1}$). The application of biochar to Mollisols significantly increased soil organic carbon (SOC) at the seedling stage, and B2N0 (biochar application rate at 6 kg·m$^{-2}$, nitrogen application rate at 0 kg·ha$^{-1}$) increased SOC by 5.871% compared with the control. Biochar promoted the conversion of soil ammonium nitrogen (NH$_4^+$-N) to soil nitrate nitrogen (NO$_3^-$-N). The NO$_3^-$-N losses of B1N2 (biochar application rate at 3 kg·m$^{-2}$, nitrogen application rate at 150 kg·ha$^{-1}$) and B2N2 (biochar application rate at 6 kg·m$^{-2}$, nitrogen application rate at 150 kg·ha$^{-1}$) were reduced to 42% and 33%, respectively. This difference can be explained by the fact that biochar can prevent the leaching of NO$_3^-$-N with soil water by changing the soil water retention and hydraulic conductivity. Our work demonstrates that the 100-seed-weight of B1N1 (biochar application rate at 3 kg·m$^{-2}$, nitrogen application rate at 75 kg·ha$^{-1}$) increased by 6.55% compared with that of the control. Meanwhile, we have confirmed that the leaf nitrogen contents can accurately predict the changes in NO$_3^-$-N in soil at a depth of 10–20 cm ($R^2$=0.5592–0.7022). Applying a certain amount of biochar before cultivation and applying appropriate topdressing based on leaf information can reduce nitrogen application while maintaining the sustainability of Mollisols.

**Keywords** Biochar · Mollisols · Soybean · Soil organic carbon · Soil inorganic nitrogen

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## 1 Introduction

The black soil (Mollisols) in Northeast China is one of the four black soil belts in the world suitable for farming. Mollisols have more humus, and high fertility, and are suitable for farming. It can provide 1/4 of China's grain output every year (Xu et al. 2023). However, with long-term and high-intensity utilization and the impact of erosion, the organic matter (SOM) content of the cultivated layer of Mollisols in Chinese farmland has decreased from 80 g·kg$^{-1}$ to 38.9 g·kg$^{-1}$ since reclamation (Zhang et al. 2013). The deterioration of the physical and chemical properties of Mollisols weakens the soil's water and fertilizer retention performance, resulting in a gradual reduction in soil aeration and permeability and an increase in bulk density. This results in a continuous decline in soil fertility (Wang et al. 2021). In the context of soil degradation, to maintain crop yield and

increase SOC content, the amount of nitrogen fertilizer input has to be maintained at a high level, but this measure has also led to more nitrogen leaching (Feng and Zhu 2017). The sustainability of the agricultural ecological environment is greatly threatened (Food and Agriculture Organization of the United Nations. 2011).

SOM is the core of soil fertility. According to its composition, source, characteristics, and other properties, it can be divided into soil particulate organic matter and soil mineral-bound organic matter (Lavallee et al. 2020). Soil particulate organic matter is more sensitive to changes in land use patterns or management measures and is also the main source of more active carbon components in soil (Kravchenko et al. 2015). Guo et al. (2019) found that different types of fertilizers and fertilization schemes can have a significant impact on the accumulation and decomposition of soil particulate organic matter; that is, different field management methods can affect the proportion of different components of organic carbon (Bongiorno et al. 2019), thereby affecting soil nutrient cycling, aggregate formation, and other processes (Peng et al. 2015).

In recent years, biochar has become well-known as a carbon-rich soil amendment (Ali et al. 2017; Sohi et al. 2010). In addition, biochar has a relatively large specific surface area compared to other materials and large porosity (Aller et al. 2017; Madari et al. 2017). Its adsorption function can effectively reduce the decomposition of natural soil organic matter (Ventura et al. 2019), regulate the migration and transformation of soil nutrients at the plant-root soil interface (Lehmann et al. 2015), optimize the soil structure and enhance the water-holding capacity of farmland soil (Ali et al. 2017; Fu et al. 2019a, 2019b). However, due to the great differences in the raw materials and soil types applied, the application effects of biochar are different. Yan et al. (2022) applied different rates of biochar in two different ways to moderately degraded Mollisols without fertilization. They found that when biochar was applied at a lower application level (10 t·ha$^{-1}$), there was a phenomenon of crop yield reduction compared to the blank control. Wei et al. (2019) conducted a four-year study to enhance soil fertility on black soil farmland through the annual application of biochar and fertilizer. The findings indicate that plots treated with biochar demonstrated a 33.3% increase in soybean yield compared to those without biochar. He et al. (2020) used red loam soil as the research object and applied nitrogen fertilizer in combination with biochar. The results showed that applying biochar significantly increased the SOC content and could maintain soil fertility for a long time, promoting crop yield. The above results indicate that the application benefits of biochar vary with soil type and land management measures (Laghari et al. 2016). The combined application of biochar and fertilizer has a better effect on maintaining soil fertility. However, the mechanism of

how biochar maintains soil fertility and reduces soil nitrogen leaching is still unclear. In addition, it is also unclear how to reasonably implement reduced fertilization measures after applying biochar.

Based on the above research content, to achieve the goal of "reducing nitrogen" in farmland and rebuilding a fertile cultivation layer, this study takes typical Mollisols in Northeast China as the research object, regulates SOC content by applying different rates of biochar to the soil, and applies different rates fertilization to biochar modified soil. Information on soil leaves at different growth stages under different treatments (leaf nitrogen content, SPAD) and the changes in soil inorganic nitrogen at different soil depths were recorded. Through our research, we propose the following hypotheses: (1) the capacity of biochar to sequester carbon in soil is directly correlated with the quantity of biochar that is implemented; (2) the combined application of biochar and traditional fertilizers can impact the nutrient cycling process of soil, effectively increasing the utilization rate of fertilizers in the soil and subsequently reducing nitrogen loss; and (3) there is a correlation between plant leaf information and soil nutrients. Regulating and applying fertilizer to farmland soil by applying leaf nutrition information and biochar can maximize stable production, improve soil carbon and nitrogen content, and enhance the overall sustainability of farmland soil.

## 2 Materials and Methods

### 2.1 Test Materials

This study was conducted by a pot experiment in the field. The test station was set up in a field test observation station (126°43′7″ E, 45°44′24″ N) in Harbin, Heilongjiang Province, Northeast China. According to the American soil diagnostic classification system, the soil in this area is defined as Mollisols. The soil used for the test was collected in situ from the test site.

In this study, different biochar application rates and fertilizer application rates were established. Biochar was used to adjust the soil organic matter content, and nitrogen fertilizer urea was used to adjust the soil inorganic nitrogen content. The biochar application rates were recorded as B0, B1, and B2, corresponding to the application rates of 0 kg·m$^{-2}$, 3 kg·m$^{-2}$, and 6 kg·m$^{-2}$, respectively. The fertilizer application rates were recorded as N0, N1, and N2, which correspond to application rates of 0 kg·ha$^{-1}$, 75 kg·ha$^{-1}$, and 150 kg·ha$^{-1}$, respectively. In general, a total of 9 treatments were set, and each treatment had 3 repetitions. Soybean was selected as the crop for the experiment, and the selected soybean variety was Dongnong 69, which was provided by the Soybean Research Institute of Northeast Agricultural University. The

planting density was 3 plants per pot. In the above test materials, the biochar used was corn straw biochar, which was fired under a 500 °C high-temperature anaerobic environment. Considering the amount of biochar and the homogeneity of its properties, the biochar used in this study was purchased from Liaoning Jinhefu Agricultural Development Co., Ltd., China. The physicochemical properties of biochar and test soil are shown in Table 1.

In addition, a cylindrical barrel made of PVC material was used as the container for cultivation. The height of the barrel was 38.5 cm, its upper diameter was 32.5 cm, and its lower diameter was 27.5 cm. With reference to the mixing depth of biochar and soil in the field, the mixing depth of biochar and Mollisols was also set to 25 cm. Urea was applied as a basal fertilizer at one time, no top dressing was required in the later growth period, and routine management was carried out in the field. At the same time, to make the soil environment in the pot closer to the field conditions, we reserved 12 round holes with a diameter of 1 cm in the bottom of the bucket. When there was more rainfall, it would not affect the infiltration of water in the soil. A soil pit of the same size as the test barrel was excavated at the original location of the soil, and the test barrel was buried at the same height as the ground to ensure that the temperature and moisture conditions in the barrel were consistent with the field conditions. The amount of fertilizer and the amount of biochar applied in each treatment are shown in Table 2.

## 2.2 Test Content and Measurement Method

In this study, a soybean plant was randomly selected from each pot to mark, the soybean plant height was measured with a ruler, and the soybean stem diameter was recorded with a Vernier caliper. At the same time, a nutrient rapid tester (TYS-3N, Topu, China) was used to record the changes in the nitrogen content and relative chlorophyll value (SPAD) of the middle leaves of soybean plants. To avoid damage to the plant seedlings, after the marked plant grew to more than 20 cm, it was measured every 15 days until the plant height and stem diameter had no obvious changes and the leaves were withered. At the maturity stage, the number of pods, the number of main stem sections, and the hundred-grain weight of the plants were counted. According to the calculation method of Peng et al. (2002), the partial productivity of nitrogen fertilizer (PFP) and the agronomic utilization rate of nitrogen fertilizer (AE) were obtained.

Meanwhile, during the emergence stage (May 23), the seedling stage (June 18), the flower bud differentiation stage (July 20), the flowering and pod-bearing stage (August 2), the bulging stage (August 21), and the maturity stage (September 21), we carried out soil sampling. Soil sampling was conducted with the soil drilling method, and soil cores of the 0–10 cm, 10–20 cm, and 20–30 cm layers were extracted manually by using soil augers. After the soil samples were processed, the SOC content was measured using a Vario TOC cube (Elementar, Germany), and the soil inorganic nitrogen content ($NO_3^-$-N and $NH_4^+$-N) was measured using a flow analyzer (AA3, SEAL Analytical, Germany).

## 2.3 Data Analysis

In this study, SPSS 22.0 and OriginPro 8.5 software were used to process, graph, and tabulate the experimental data. Data results were expressed as the mean $\pm$ standard deviation (S.D.). One-way ANOVAs and multiple-way ANOVAs were used to test the difference between different treatments in soybean agronomic traits and soil nutrient elements, and the least significant difference (LSD) test was used to test the significance of the different treatments ($p < 0.05$).

**Table 1** Physicochemical properties of soil and biochar

| Biochar | | Soil | |
|---|---|---|---|
| pH | 9.14±0.01 | pH | 6.48±0.24 |
| Particle size range (mm) | 1.5~2.0 | Dry density (g•cm$^3$) | 1.40±0.013 |
| C mass fraction (%) | 68.60±3.12 | Sand (%) | 49.84 |
| N mass fraction (%) | 1.28±0.13 | Slit (%) | 35.89 |
| H mass fraction (%) | 2.13±0.18 | Clay (%) | 14.27 |
| S mass fraction (%) | 0.67±0.05 | Natural moisture content (%) | 23 |
| | | Field capacity (%) | 33 |
| | | Porosity (cm$^3$•cm$^{-3}$) | 0.47±0.005 |

**Table 2** The amount of biochar and nitrogen fertilizer applied in each test treatment

| Treatment | CK | B0N1 | B0N2 | B1N0 | B1N1 | B1N2 | B2N0 | B2N1 | B2N2 |
|---|---|---|---|---|---|---|---|---|---|
| Carbamide amount (kg·ha$^{-1}$) | 0 | 75 | 150 | 0 | 75 | 150 | 0 | 75 | 150 |
| Biochar amount (kg·m$^{-2}$) | 0 | 0 | 0 | 3 | 3 | 3 | 6 | 6 | 6 |

# 3 Results

## 3.1 Agronomic Traits of Soybean

### 3.1.1 Stem Diameter

The variation in soybean stem diameter with growth period is shown in Fig. 1. The soybean stem diameter showed a trend of gradually increasing first to a slight decrease in the maturity stage with the growth stages. However, only the treatments with 3 kg·m$^{-2}$ biochar application showed significant differences in the later stage of soybean growth. On August 2, the stem diameter of B1N2 was 1.26 times that of B1N0. In addition, the stem thickness of B0N1 was also 1.21 times that of CK, but within the groups ($p > 0.05$). On August 21, the stem

diameter of B1N2 was 1.19 times that of B1N0. In addition, the stem diameter of B0N2 was 1.1 times that of CK, but within the groups ($p > 0.05$). Based on the above results, a single application of low nitrogen fertilizer had no effect on the growth of soybean stem diameter during the whole growth period of the soybean under the treatment of no adjustment of soil organic matter content or higher soil organic matter content.

### 3.1.2 Plant Height

Soybean plant height increased significantly from the flower bud differentiation stage (July 4) to the flowering and pod-bearing period (August 2). Among them, the CK treatment had the smallest increase in height, which was 2.14 times that of the flower bud differentiation stage,
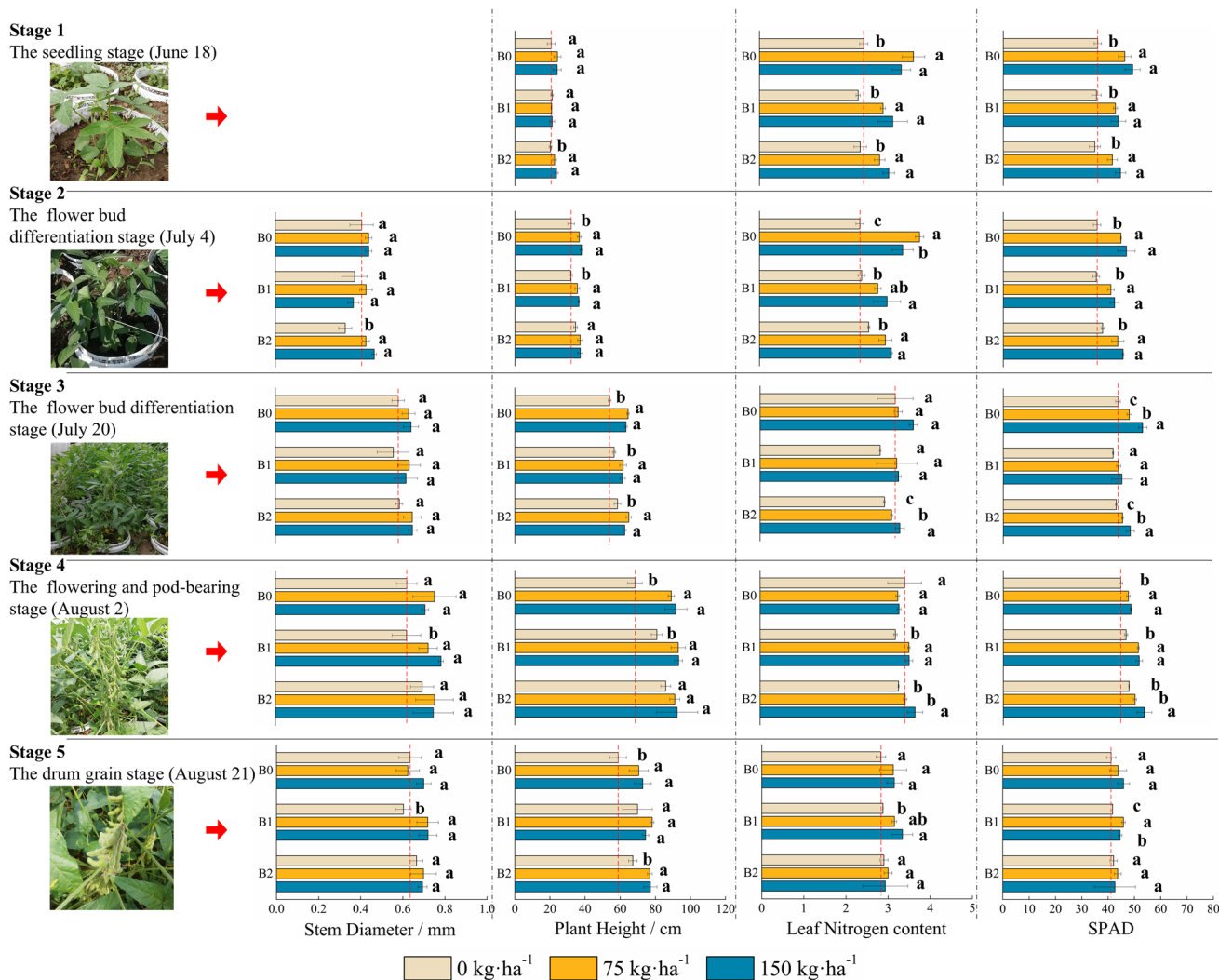


**Fig. 1** Changes in soybean agronomic characteristics under different treatments. The results are presented as the mean ± S.D. Lowercase letters indicate significant differences in the parameters of different treatments (one-way ANOVAs test, $p < 0.05$). B0, B1, and B2 respectively denote the application rates of biochar at 0 kg·m$^{-2}$, 3 kg·m$^{-2}$, and 6 kg·m$^{-2}$, respectively

while the B1N1 treatment had the largest increase, which was 2.62 times that of the flower bud differentiation stage. At the drum grain stage, the crop gradually matured, the top leaves of the soybean plant senesced and fell off, and the plant height decreased slightly (Fig. 1). When comparing the fertilization treatments, it was not difficult to observe that the plant heights of most treatments were significantly different from those of the control, but the difference in plant heights under the two fertilizer application rates was not significant. When no measures were taken to regulate the content of soil organic matter, the difference between the maximum and minimum plant heights on July 20 and August 2 was 19% and 34%, respectively. At the 3 kg·m$^{-2}$ biochar application rate, the difference was reduced to 9% and 15%. At the 6 kg·m$^{-2}$ biochar application rate, the difference dropped to 11% and 7%. In addition, comparing the average plant heights of the three biochar application rates, the performance was in the order of B2 (89.88 cm)> B1 (89.11 cm)> B0 (83.13 cm) on July 20, and the performance was in the order of B1 (74.17 cm)> B2 (73.74 cm)> B0 (67.42 cm) on August 2. In conclusion, soybean plant height could also be increased by regulating the soil organic matter content alone, but the increase value was small. The combination of organic matter content and nitrogen fertilizer supplementation in the soil could increase plant height on the basis of reducing fertilization. In this case, B1N1 was a better treatment.

### 3.1.3 Leaf Nitrogen Content

Under the same amount of biochar application, with the application of urea, the leaf nitrogen content significantly increased. Taking the leaf nitrogen content on June 18 as an example, under the 0 kg·m$^{-2}$ biochar application rate, the leaf nitrogen content in B0N1 was 1.48 times that in CK. Under the 3 kg·m$^{-2}$ biochar application rate, the leaf nitrogen content in B1N2 was 1.35 times that in B1N0. Under the 6 kg·m$^{-2}$ biochar application rate, the leaf nitrogen content in B2N2 was 1.28 times that in B2N0. In the next growth stage, the differences between treatments gradually decreased, and even the differences in leaf nitrogen content values between some nitrogen fertilizer treatments became nonsignificant. For example, in the B0 and B1 treatments on July 20, when the plants entered the maturity stage, as the leaves gradually senesced, the difference in nitrogen content between the groups decreased. Based on the above results, under the B1N2 treatment, the maximum leaf nitrogen content was achieved during the whole soybean growth period (Fig. 1).

### 3.1.4 SPAD

Due to the presence of chloroplasts in plants, plant leaves can undergo photosynthesis to provide energy for plant physiological activities. Chlorophyll is an important component of chloroplasts. When leaf cells divide and grow in the plant, the nutrients they produce can supply the growth and development of the plant. When the plant enters the maturity stage, the chlorophyll value of the leaves decreases, and the lutein value increases, causing the leaves to wither and fall off. In this study, SPAD is the relative value of leaf chlorophyll, and its value can also represent the physiological state of leaves. Figure 1 shows that there were differences in the SPAD value change law between different treatments. The SPAD values of the B0N1 and B0N2 treatments under the 0 kg·m$^{-2}$ biochar application rate reached their maximums on July 20, at 47.84 and 52.93, respectively. The remaining treatments all reached the maximum on August 2. Moreover, under the treatments with different soil organic matter contents, the average SPAD value of the leaves during the whole growth period between the treatment with nitrogen fertilizer and the treatment without fertilization ranged from 3.75 to 8.46 and reached a significant level during the key growth period of soybean ($p<0.05$). In conclusion, it could be concluded that the soil organic matter content could affect the SPAD value of soybean leaves; in addition, the more nitrogen fertilizer was applied, the larger the SPAD value of the leaves.

### 3.1.5 ANOVAs of Soybean Agronomic Characteristics Under Different Treatments

The significance level of soybean agronomic traits under different treatments is shown in Table 3 ($p<0.05$). The results of the variance analysis showed that in different growth periods, applying biochar or nitrogen fertilizer can have a significant impact on soybean agronomic trait indicators ($p<0.05$). In addition, the interaction between biochar and nitrogen fertilizer also had a significant effect on the above-mentioned crop information ($p<0.05$). Biochar and nitrogen fertilizer can interact in the soil to promote plant growth and development.

## 3.2 Effects of Different Degraded Soils on Soil Nutrients

### 3.2.1 SOC

The changes in SOC are shown in Fig. 2. At the initial stage of soybean growth, there was a significant difference between the SOC of the treatment with biochar and that of the treatment without biochar, which was consistent with the

**Table 3** The results of ANOVAs of soybean agronomic traits

| Source | Plant height | | | Stem diameter | | | Leaf nitrogen content | | | SPAD | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Mean square | F-value | Sig. | Mean square | F-value | Sig. | Mean square | F-value | Sig. | Mean square | F-value | Sig. |
| Growth period | 19117.847 | 631.911 | 0.000 | 0.485 | 136.771 | 0.000 | 1.147 | 9.355 | 0.000 | 281.822 | 18.500 | 0.000 |
| Growth period* biochar rates | 12777.048 | 437.042 | 0.000 | 0.292 | 82.300 | 0.000 | 0.914 | 7.784 | 0.000 | 195.658 | 12.951 | 0.000 |
| Growth period* fertilizer rates | 13003.431 | 698.223 | 0.000 | 0.314 | 126.923 | 0.000 | 1.758 | 22.553 | 0.000 | 359.176 | 48.262 | 0.000 |
| Growth period* biochar rates * fertilizer rates | 9776.436 | 561.731 | 0.000 | 0.225 | 91.615 | 0.000 | 1.431 | 19.861 | 0.000 | 275.215 | 38.277 | 0.000 |

*SPAD* relative value of leaf chlorophyll
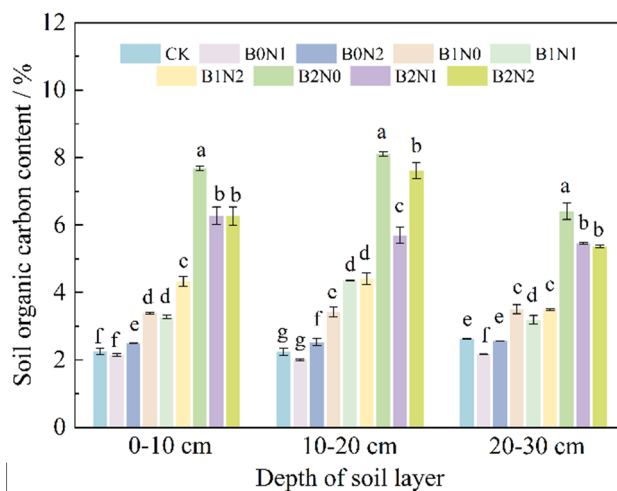


**Fig. 2** Changes in soil organic carbon (SOC) in each soil layer under different treatments. The results are presented as the mean $\pm$ S.D. Lowercase letters indicate significant differences in the parameters of different biochar application treatments (one-way ANOVAs test, $p$ <0.05). The CK group serves as the control, indicating the absence of both fertilizer and biochar application; B0, B1, and B2 respectively denote the application rates of biochar at 0 kg·m$^{-2}$, 3 kg·m$^{-2}$, and 6 kg·m$^{-2}$, while N0, N1, and N2 represent the nitrogen application rates of 0 kg·ha$^{-1}$, 75 kg·ha$^{-1}$, and 150 kg·ha$^{-1}$, respectively

assumption that biochar could regulate the content of soil organic matter. In the 0–10 cm and 10–20 cm soil layers, the SOC content in groups treated with biochar significantly differed from that in groups without biochar ($p$<0.05). Among them, the difference in SOC content between the B2N0 treatment and CK group in the 10-20 cm soil layer at the seedling stage of the crop was up to 5.871%.

### 3.2.2 Soil NH$_4^+$-N

Figure 3 shows the changes in NH$_4^+$-N content in the 0–10 cm, 10–20 cm, and 20–30 cm soil layers and the cumulative consumption of NH$_4^+$-N in the three soil layers under different treatments (cumulative consumption represented the cumulative value of soil NH$_4^+$-N loss in each soil layer until the next growth stage). The NH$_4^+$-N of various treatments showed a gradual decrease from the seedling stage to the maturity stage, indicating that the conventional fertilizer urea was fully released in a short period, and the value range was 6.837~15.785 mg·kg$^{-1}$. In the soybean seedling stage (May 23), compared with the treatment without fertilization, the NH$_4^+$-N content in each treatment had the most obvious difference between the 10 and 20 cm soil layers. For example, the NH$_4^+$-N content in the B2N2 treatment group was 3.92 mg·kg$^{-1}$ more than that in the B2N0 treatment group. However, in the 0–10 cm soil layer, the NH$_4^+$-N content in B2N2 was only 2.102 mg·kg$^{-1}$ more than that in

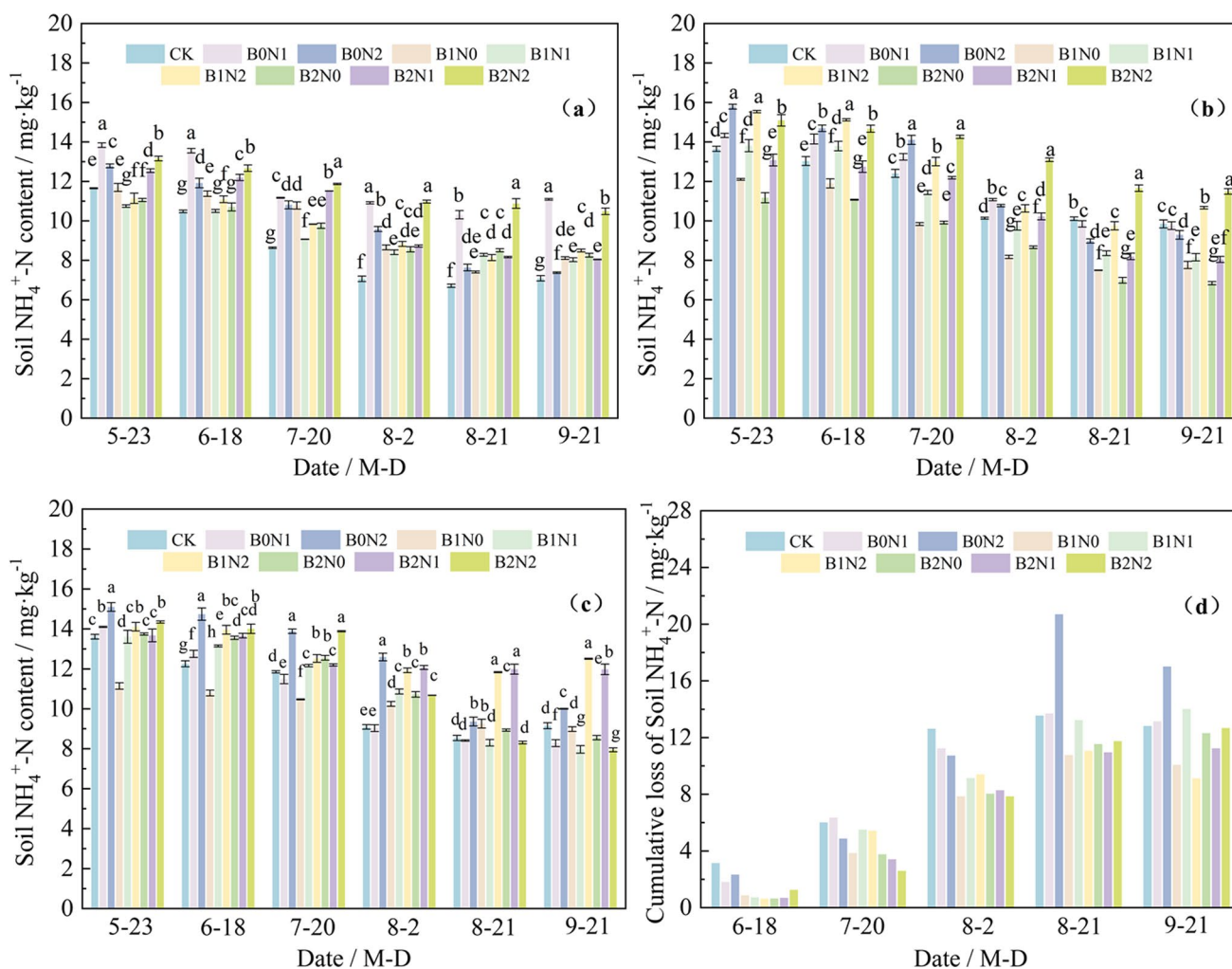**Fig. 3** Changes in soil ammonium nitrogen (NH$_4^+$-N) content in each soil layer under different treatments. **a** 0–10 cm soil layer; **b** 10–20 cm soil layer; **c** 20–30 cm soil layer; **d** cumulative loss of soil NH$_4^+$-N. The results are presented as the mean ± S.D. Lowercase letters indicate significant differences in the parameters of different biochar application treatments (one-way ANOVAs test, $p$ <0.05).

The CK group serves as the control, indicating the absence of both fertilizer and biochar application; B0, B1, and B2 respectively denote the application rates of biochar at 0 kg·m$^{-2}$, 3 kg·m$^{-2}$, and 6 kg·m$^{-2}$; while N0, N1, and N2 represent the nitrogen application rates of 0 kg·ha$^{-1}$, 75 kg·ha$^{-1}$, and 150 kg·ha$^{-1}$, respectively

B2N0, indicating that during the nitrogen release process, NH$_4^+$-N migrated little, and the main process was downward leaching. At the soybean drum grain stage (August 21), in the treatment with co-application of biochar and nitrogen fertilizer, the 20–30 cm soil layer accumulated more ammonium nitrogen than the other layers; among the treatment groups, the NH$_4^+$-N content in B1N2 was 11.831 mg·kg$^{-1}$, and that in B2N1 was 11.984 mg·kg$^{-1}$, both of which were much larger than the 8.538 mg·kg$^{-1}$ in CK.

From the seedling stage to the drum grain stage, the cumulative consumption of NH$_4^+$-N in the soil under the B0N2 treatment was the largest, which was 20.694 mg·kg$^{-1}$. Under the same amount of fertilization, the cumulative consumption of NH$_4^+$-N by B1N2 and B2N2 was 11.063 mg·kg$^{-1}$ and 11.747 mg·kg$^{-1}$, respectively. The above results indicated that increasing the soil organic matter content by adding biochar could effectively reduce the cumulative consumption of soil NH$_4^+$-N.

### 3.2.3 Soil NO$_3^-$N

The soil NO$_3^-$-N content varied from 7.193 to 41.060 mg·kg$^{-1}$ (Fig. 4). Similar to the change in soil NH$_4^+$-N content, the content of soil NO$_3^-$-N in each soil layer also showed a gradual decreasing trend, and the difference between different fertilization treatments and no fertilization was significant ($p$<0.05). In the 0–10 cm soil layer at the seedling stage, the highest NO$_3^-$-N content in B0N2 was 35.817 mg·kg$^{-1}$, while the lowest NO$_3^-$-N content in
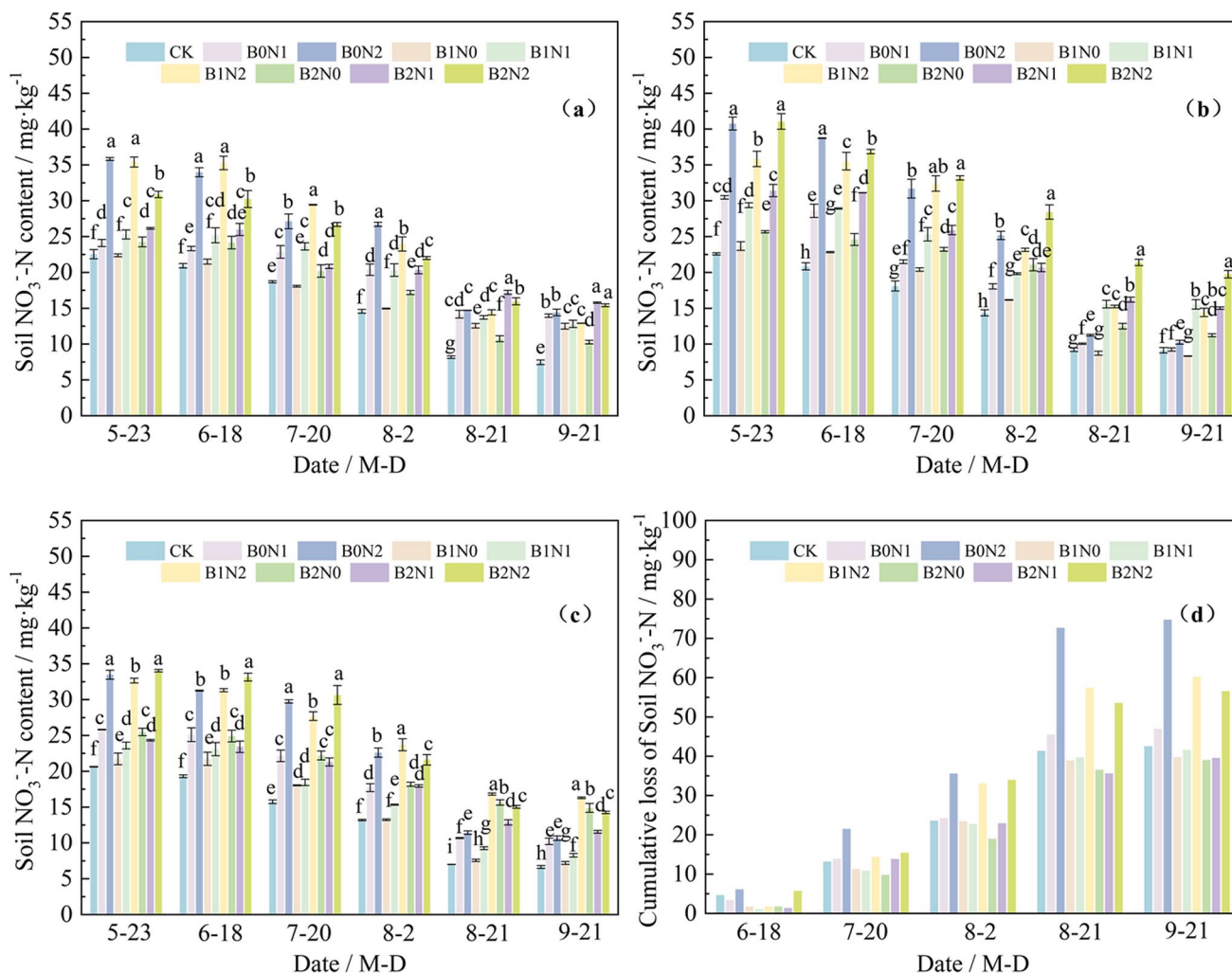
**Fig. 4** Changes in soil nitrate nitrogen ($NO_3^-$-N) content in each soil layer under different treatments. **a** 0–10 cm soil layer; **b** 10–20 cm soil layer; **c** 20–30 cm soil layer; **d** cumulative loss of soil $NO_3^-$-N. The results are presented as the mean ± S.D. Lowercase letters indicate significant differences in the parameters of different biochar application treatments (One-way ANOVAs test, $p < 0.05$). The

CK group serves as the control, indicating the absence of both fertilizer and biochar application; B0, B1, and B2 respectively denote the application rates of biochar at 0 kg·m$^{-2}$, 3 kg·m$^{-2}$, and 6 kg·m$^{-2}$, while N0, N1, and N2 represent the nitrogen application rates of 0 kg·ha$^{-1}$, 75 kg·ha$^{-1}$, and 150 kg·ha$^{-1}$, respectively

CK was 22.533 mg·kg$^{-1}$. At the maturity stage, the highest $NO_3^-$-N content in B1N2 was 15.773 mg·kg$^{-1}$, and the lowest $NO_3^-$-N content in CK was 7.443 mg·kg$^{-1}$. In the 10–20 cm soil layer at the seedling stage, the $NO_3^-$-N content in B2N2 was the highest, which was 18.487 mg·kg$^{-1}$ more than that in CK. With changing soybean growth period, the difference in the $NO_3^-$-N content under different treatments gradually decreased. At the maturity stage, the $NO_3^-$-N content in B2N2 was 10.625 mg·kg$^{-1}$ higher than that in CK. In the 20–30 cm soil layer, the differences between the maximum and minimum soil $NO_3^-$-N in the different treatment groups during each growth period were 13.407,

13.839, 14.887, 10.506, 9.813, and 9.649. This content also gradually decreased after the plants reached the flowering and pod-bearing stages.

In contrast to the change in the cumulative consumption of $NH_4^+$-N in the soil, when the fertilization amount was 150 kg·ha$^{-1}$, the cumulative consumption of soil $NO_3^-$-N at each stage was relatively large. The difference between the maximum and minimum consumption between the treatments during the drum grain stage and the maturity stage was 16.644, 37.065, and 35.734. Under the same fertilization rate, as the amount of biochar used increased, the cumulative consumption of $NO_3^-$-N decreased gradually (Fig. 4d).

## 3.3 The Relationship Between Soil and Plants

### 3.3.1 Correlation of Soil Indexes with Crop Agronomic Character Indexes

Figure 1 shows that when soybeans grew and developed to the drum grain stage, the soybean agronomic trait indicators reached maximum values. Therefore, the initial soybean agronomic trait and SOC and inorganic nitrogen data were selected, and Pearson's correlation analysis was used to evaluate the relationship between different soil nutrient indicators and crop agronomic traits (Fig. 5). The results showed that the soybean plant height and the soil $NO_3^-$-N content for all soil layers were significant, and the $NO_3^-$-N$_{0-10\,cm}$ value reached an extremely significant value, with a correlation coefficient of 0.67. The maximum correlation coefficient between stem diameter and $NO_3^-$-N$_{20-30\,cm}$ was 0.52. Leaf nitrogen and SPAD were mainly affected by $SOC_{20-30\,cm}$, and the correlation coefficients were 0.57 and 0.79, respectively.

Meanwhile, the stepwise regression analysis was used to obtain fitting models of soybean agronomic traits and soil carbon and nitrogen indicators. All models had good fitting effects, and all had significance. $NO_3^-$-N$_{0-10cm}$ and $SOC_{10-20cm}$ explained 52.8% of the change in plant height, $NO_3^-$-N$_{20-30cm}$ explained 24% of the change in stem diameter, $SOC_{10-20cm}$ and $SOC_{20-30cm}$ explained 39.3% of the change in leaf nitrogen content, and $SOC_{20-30cm}$ and $NO_3^-$-N$_{0-10cm}$ explained 76.5% of the change in SPAD (Table 4). These results also explained the relationship between soil nutrient indicators and plant agronomic trait indicators.
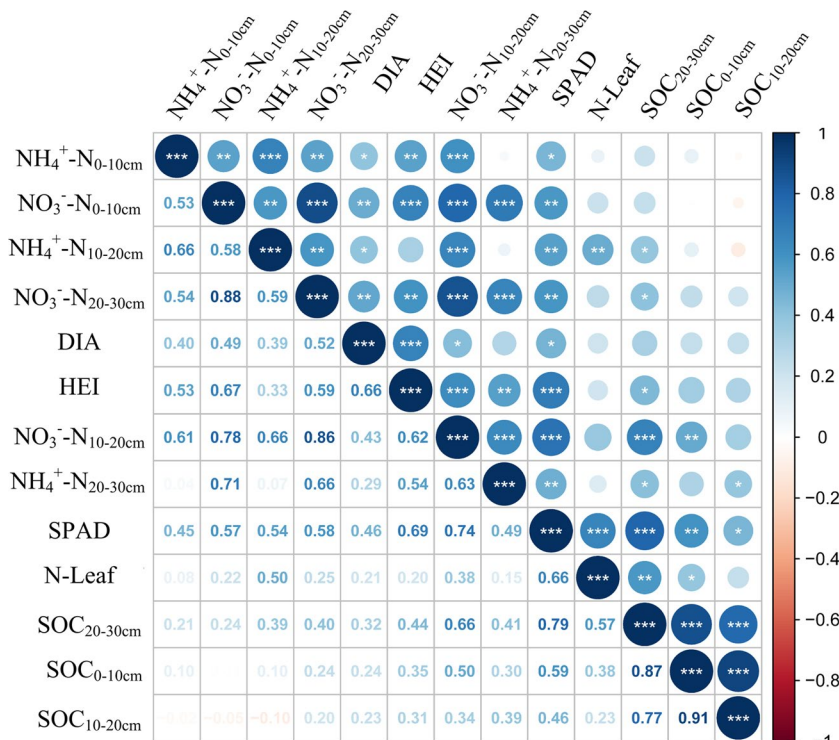
**Table 4** Stepwise regression analysis to identify driving factors explaining soybean height (HEI), soybean diameter (DIA), leaf nitrogen content (N-leaf), relative value of leaf chlorophyll (SPAD) using soil organic carbon content ($SOC_{0-10cm}$, $SOC_{10-20cm}$, $SOC_{20-30cm}$), soil nitrate nitrogen content ($NO_3^-$-N$_{0-10cm}$, $NO_3^-$-N$_{10-20cm}$, and $NO_3^-$-N$_{20-30cm}$), and soil ammonium nitrogen content ($NH_4^+$-N$_{0-10cm}$, $NH_4^+$-N$_{10-20cm}$, and $NH_4^+$-N$_{20-30cm}$) as independent parameters

| Stepwise multiple regression model | $R^2$ | $F$ | $p$ |
|---|---|---|---|
| HEI= 0.686 $NO_3^-$-N$_{0-10cm}$ + 0.343 $SOC_{10-20cm}$ | 0.528 | 15.524 | < 0.001 |
| DIA= 0.519 $NO_3^-$-N$_{20-30cm}$ | 0.240 | 9.197 | 0.006 |
| N-leaf= 0.976 $SOC_{20-30cm}$ − 0.522 $SOC_{10-20cm}$ | 0.393 | 9.422 | 0.001 |
| SPAD= 0.694 $SOC_{20-30cm}$ + 0.409 $NO_3^-$-N$_{0-10cm}$ | 0.765 | 43.403 | < 0.001 |

### 3.3.2 Response Relationship Between Leaf Nitrogen Content and Soil Nitrogen

Scharf et al. (2006) mentioned that leaf chlorophyll content (SPAD) can predict the availability of soil nitrogen. To clarify whether the SPAD value can accurately simulate the nitrogen content level of soil under the condition of mixed application of biochar and nitrogen fertilizer, this study constructed the response function of SPAD value and soil nitrate nitrogen content. The specific results are shown in Fig. 6.

In each period of soybean growth and development, the leaf SPAD value and soil $NO_3^-$-N content showed a synergistic upward trend, and there was a significant linear
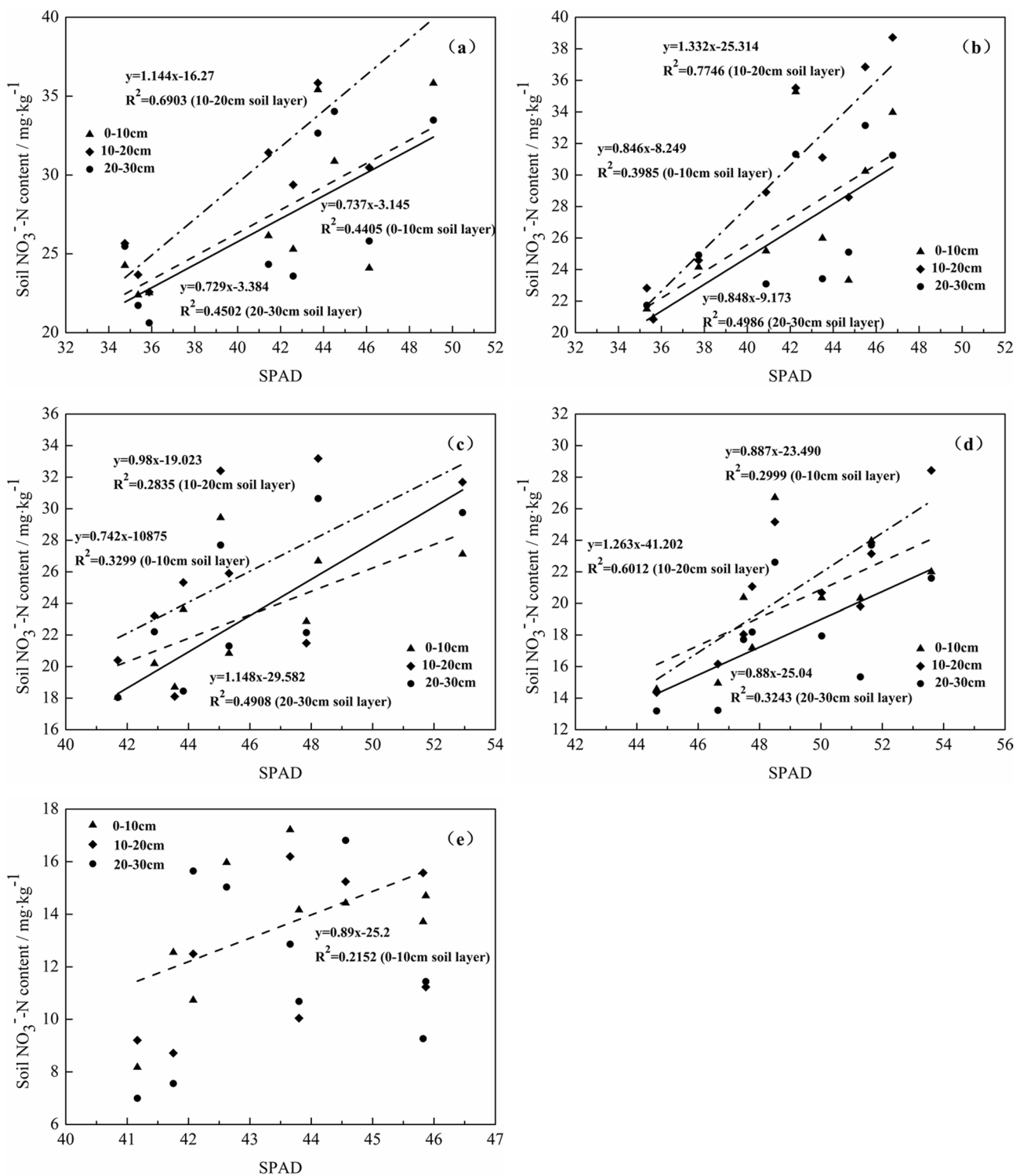
**Fig. 5** Correlation matrix of different soybean agronomic traits and soil nutrient indexes. The figure displays correlation coefficient between the two indicators, while the symbol * indicates statistical significance with $p$<0.05. Soybean height (HEI), soybean diameter (DIA), leaf nitrogen content (N-leaf), relative value of leaf chlorophyll (SPAD) using soil organic carbon content ($SOC_{0-10cm}$, $SOC_{10-20cm}$, $SOC_{20-30cm}$), soil nitrate nitrogen content ($NO_3^-$-N$_{0-10cm}$, $NO_3^-$-N$_{10-20cm}$, $NO_3^-$-N$_{20-30cm}$), and soil ammonium nitrogen content ($NH_4^+$-N$_{0-10cm}$, $NH_4^+$-N$_{10-20cm}$, $NH_4^+$-N$_{20-30cm}$) as independent parameters

**Fig. 6** Response relationship between SPAD (relative value of leaf chlorophyll) and $NO_3^-$-N (soil nitrate nitrogen content) at different growth stages. **a** June 18; **b** July 4; **c** July 20; **d** August 2; **e** August 21. The fitting method was linear fitting

correlation between the two. Comparing different soil layers, the fitting effect of the nitrate nitrogen content in the 0–10 cm soil layer was poor, and the coefficients of determination

($R^2$) were 0.4405, 0.3985, 0.3299, 0.2999, and 0.2152, respectively. Until August 21, SPAD had a functional relationship with only the $NO_3^-$-N content in the 0–10 cm soil
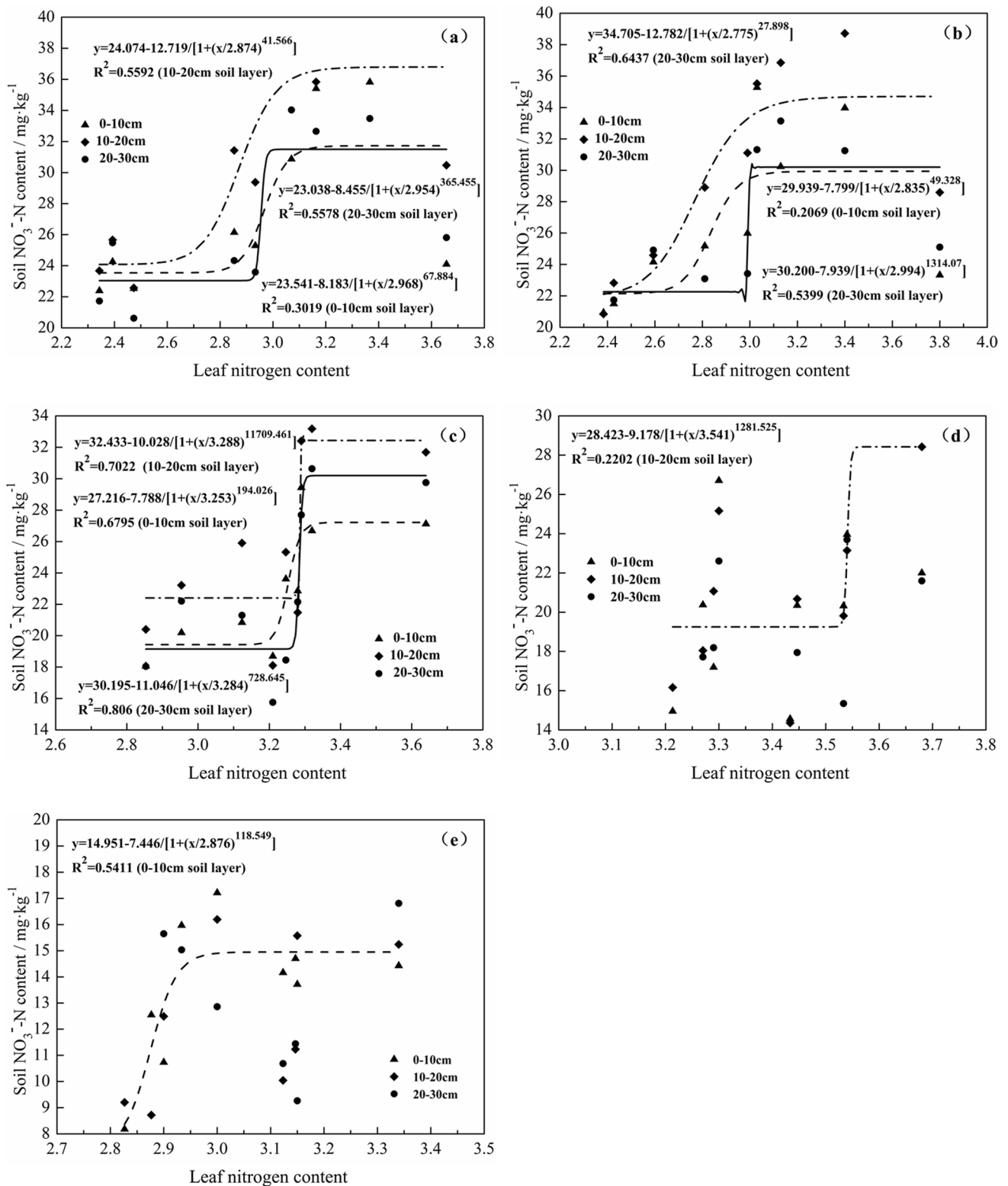
**Fig. 7** Response relationship between leaf nitrogen content and $NO_3^-$-N (soil nitrate nitrogen content) at different growth stages. **a** June 18; **b** July 4; **c** July 20; **d** August 2; **e** August 21. The fitting method was logistic curve fitting

layer. Therefore, in this study, the response functions of leaf nitrogen content and $NO_3^--N$ content were constructed at the same time. The result is shown in Fig. 7.

During the soybean seedling stage and flower bud differentiation stage, the leaf nitrogen content and soil $NO_3^--N$ content showed a synergistic upward trend, and there was a significant logistic curve relationship between them. Comparing different soil layers, the fitting effect of the $NO_3^--N$ content in the 0–10 cm soil layer was poor. The coefficients of determination ($R^2$) on June 18 and July 4 were only 0.3019 and 0.2069, respectively, and the simulation accuracy under other soil layers was higher. In the flowering and pod-bearing stage, only the leaf nitrogen content in the 10–20 cm soil layer had a functional relationship with the soil $NO_3^--N$ content, but the coefficient of determination ($R^2$) was only 0.2202. At the soybean drum grain stage, only the leaf nitrogen content in the 0–10 cm soil layer had a functional relationship with the soil $NO_3^--N$ content.

### 3.4 Yield Index of Soybean Under Different Treatments

Table 5 shows the soybean 100-seed weight, the number of main stem sections, the number of pods per plant, the partial productivity of nitrogen fertilizer (PFP), and the agronomic utilization of nitrogen (PE) under different treatments. Except for those in the B1N0, B2N0, and B2N1 groups, the 100-seed weights of soybeans in the treatment groups were greater than those in the CK group, and the 100-seed weights of the B1N1 and B2N2 treatment groups were 1.38 g and 1.26 g more than those in the CK group, respectively. However, the number of main stem sections for different treatments ranged from 12 to 13.4, and the number of pods ranged from 21 to 31.82. Considering the numerical values of the three parameters, B1N1 and B1N2 were relatively high-yielding. At the same time, Table 5 shows that the smaller amount of fertilizer had a higher PFP. The application of biochar improved AE,

among which the B1N1 treatment had the most obvious effect, with a value of 2.63.

At the same time, to further explore the relationship between crop agronomic traits and the abovementioned yield indicators, the method of path analysis was used to calculate the abovementioned indicator data. The results showed that soybean plant height had an effect on the number of main stem segments of soybean. The normalized path value was 0.880>0, and this path showed a significance level of 0.01 ($z=3.420$, $p=0.001<0.01$). The standardized path coefficient value of soybean stem diameter to pod number was 0.479>0, and this path showed a significance level of 0.05 ($z=2.354$, $p=0.019<0.05$). The standardized path coefficient value of soybean stalk diameter to crop grain weight was 0.739>0, and this path showed a significance level of 0.01 ($z=4.454$, $p=0.000<0.01$). All three had a positive relationship. However, the soil nutrient indicators in the critical growth period of crops did not show a direct influence on the yield indicators of crops ($p>0.05$).

## 4 Discussion

The organic carbon contained in biochar was divided into two categories. One type of organic carbon contained relatively stable aromatic carbon, which directly contributed to soil organic matter, and the other type of organic carbon was easily degradable with weak resistance to mineralization, which could be converted into soluble organic carbon to supplement the soil carbon pool (Cen et al. 2021; Plaza et al. 2016; Zimmerman 2010). In our research, the SOC content increased sharply with the increase in the rate of biochar application. Taking the 10–20 cm soil layer as an example, the application of biochar increased the SOC content 1.528~3.623 times during the whole soybean growth period, which was similar to the test result obtained by (Dong et al. 2018).

**Table 5** Soybean 100-seed weight, number of main stem sections, and number of pods per plant under different treatments

| Treatment | CK | B0N1 | B0N2 | B1N0 | B1N1 | B1N2 | B2N0 | B2N1 | B2N2 |
|---|---|---|---|---|---|---|---|---|---|
| 100-grain weight (g) | 21.07±0.23 c | 21.85±0.01 b | 21.14±0.06 c | 19.75±0.14 e | 22.45±0.10 a | 21.53±0.48 bc | 21.06±0.09 c | 20.56±0.58 d | 22.33±0.15 a |
| Number of main stem sections | 12.00±0.23 c | 13.40±0.70 a | 13.08±1.08 ab | 12.82±1.17abc | 13.05±0.85 a | 13.36±0.67 ab | 12.73±1.01abc | 13.17±0.83 bc | 12.42±2.02 bc |
| Number of pods | 21.00±4.33 | 29.70±8.25 | 29.33±5.76 | 26.09±8.90 | 30.70±5.12 | 31.82±4.69 | 28.82±7.49 | 27.75±5.46 | 26.75±7.52 |
| PFP (g grain·$g^{-1}$ N) | – | 21.10 | 11.12 | – | 22.44 | 11.96 | – | 22.71 | 11.82 |
| AE (g grain·$g^{-1}$ N) | – | 1.29 | 1.22 | – | 2.63 | 2.05 | – | 2.90 | 1.92 |

The results are presented as the mean ± S.D. There were significant differences only in "100-grain weight" and "Number of main stem sections" among different treatments. Lowercase letters indicate significant differences in the parameters of different biochar application treatments (one-way ANOVAs test, $p<0.05$). The CK group serves as the control, indicating the absence of both fertilizer and biochar application; B0, B1, and B2 respectively denote the application rates of biochar at 0 kg·$m^{-2}$, 3 kg·$m^{-2}$, and 6 kg·$m^{-2}$, while N0, N1, and N2 represent the nitrogen application rates of 0 kg·$ha^{-1}$, 75 kg·$ha^{-1}$, and 150 kg·$ha^{-1}$, respectively. The PFP refers to the partial productivity of nitrogen fertilizer, and the AE refers to the agronomic utilization rate of nitrogen fertilizer

However, the research results of Yan et al. (2022) showed that compared to the treatment without biochar, the application of 10 t·ha$^{-1}$ biochar resulted in a decrease in crop yield. This seems to contradict our hypothesis that by inputting carbon sources into the soil, arable land actually decreases. When the application amount of biochar was greater than 10 t·ha$^{-1}$, crop yield could be improved. We believe that this phenomenon is caused by the following reasons: When biochar was applied to the soil, it was evenly mixed in the cultivation layer. Solid sample testing methods were used to pretreat the samples. The "biochar-soil" complex was mixed with each other, and the organic carbon in biochar was higher, significantly increasing the common organic carbon content of the "biochar-soil" complex and causing a sharp increase in the organic carbon content in the cultivation layer soil. There are significant variances in the properties of biochar that arise due to the preparation of raw materials and methods utilized (Major et al. 2010). Biochar prepared under low-temperature and rapid pyrolysis conditions contains a substantial amount of easily degradable constituents, with the biochar itself forming organic carbon during preparation (Chen et al. 2018). The application of biochar to soil significantly alters the composition and network structure of fungal and bacterial communities, while also promoting antagonistic or competitive interactions between different bacterial communities and substrates (Chen et al. 2019). On the one hand, the mixing of biochar with soil propels the mineralization of soil organic carbon and the mineralization of biochar itself, resulting in a positive excitation effect that accelerates the loss of organic carbon and decreases soil organic carbon content (Smith et al. 2010). On the other hand, as unstable components in biochar decompose rapidly and cultivation time prolonged, biochar promotes the physical protection of soil aggregates, significantly inhibiting the decomposition of background soil organic carbon and leading to a negative excitation effect. Interestingly, this effect is positively correlated with an increase in bacterial and fungal diversity (Kerré et al. 2016; Chen et al. 2019). In instances where the quantity of applied biochar is low, the supplemented organic carbon content in the soil is relatively low, ultimately resulting in the consumption of organic carbon in the background soil under the positive excitation effect. The above process reduced the effective carbon components involved in crop growth and nutrient absorption, and altered nutrient cycling in the plow layer, thereby limiting crop growth and development, and leading to crop yield reduction. Therefore, when the application amount of biochar is within a reasonable threshold, it can effectively regulate the SOC, thereby improving the nutrient cycling process within the topsoil and maintaining soil fertility.

$NH_4^+$-N and $NO_3^-$-N are two forms of soil nitrogen ions that are easily absorbed and utilized by plants (Thi Thu Nhan et al. 2017). However, in the soil, these two ionic forms of nitrogen are both mobile and are not easily adsorbed by soil colloids (Li 2008). When nitrogen in the soil is leached, it may deplete soil fertility (Laird et al. 2010). In this study, the contents of $NH_4^+$-N and $NO_3^-$-N in each soil layer were at the maximum during the seedling stage of soybean cultivation. The overall nitrogen content of the 10-20 cm soil layer was higher than that of the other soil layers. Considering that the fertilization depth in this study was 10 cm, the inorganic nitrogen in the farmland soil had a downward migration phenomenon. In this soil layer, the maximum and minimum $NH_4^+$-N extreme difference between the contents of $NH_4^+$-N and $NO_3^-$-N was quite different. The above results indicated that after urea fertilizer was applied, it was converted into ammonium bicarbonate in a short period and then converted into nitrate by nitrification (Datta 1987). Although our pot experiment was set up in the field, and the soil height in the pot was consistent with its surface, it was found in the preliminary experiment that the temperature of the soil in the pot was still slightly higher than that of the field soil; the above process would also accelerate the volatilization of nitrogen fertilizer (Cameron et al. 2013). At this stage, the threshold for temperature change and water storage capacity in the field satisfies the basic prerequisites for nitrification (Sahrawat 2008). With the application of biochar, the pH value and carbon content of the soil are significantly enriched, while the large pores obtained between the biochar and soil enhance internal oxygen circulation within the soil. These factors are advantageous for fostering soil nitrification. At the same time, under the 10-20 cm soil layer in the seedling stage, the $NH_4^+$-N content of the CK was 13.647 mg·kg$^{-1}$. The $NH_4^+$-N contents of the B1N0 treatment and B2N0 treatment groups were 12.099 mg·kg$^{-1}$ and 11.171 mg·kg$^{-1}$, respectively. The $NO_3^-$-N content was 22.573 mg·kg$^{-1}$, 23.500 mg·kg$^{-1}$ and 25.665 mg·kg$^{-1}$ in the CK, B1N0 and B2N0 groups, respectively. The correlation coefficient between $NH_4^+$-N and $NO_3^-$-N contents in the 10-20 cm soil layer was extremely significant, with a value of 0.66 (Fig. 6). The aforementioned collected data also substantiate that with an augmented application of biochar, the soil's $NH_4^+$-N concentration declines, in contrast to a rise in $NO_3^-$-N content. As a result, it can be inferred that biochar fosters the transformation of $NH_4^+$-N to $NO_3^-$-N in soil. This conclusion was also similar to the results of Nelissen et al. (2012). Figure 3(d) and Fig. 4(d) show the total consumption of $NH_4^+$-N and $NO_3^-$-N, respectively, in each soil layer. After a long growth period, the $NO_3^-$-N content in the 0-30 cm soil layer of the crop from the seedling stage to the maturity stage showed stable consumption, and the $NH_4^+$-N content showed a trend of cumulative consumption to a slight increase in the maturity stage. Taking the

change in $NO_3^-$-N content as an example, although fertilizer was applied, especially when the fertilizer amount reached 150 kg·ha$^{-1}$, the B0N2 treatment increased the nitrogen loss by approximately 75% compared with the CK group. This proved that high fertilization levels trigger more nitrogen leaching. However, it is worth noting that the nitrogen loss of the B1N2 and B2N2 groups was reduced to 42% and 33%, respectively. This may be attributed to the high adsorption capacity of biochar. On the one hand, the application of biochar reduced the downward leaching of soil moisture (Li et al. 2022; Li et al. 2020), slowing the downward migration of soil solutes. On the other hand, biochar adsorbed smaller soil particles, changing the particle size of existing aggregates in the soil and resulting in new changes in the water retention and hydraulic conductivity of the soil (Fu et al. 2019a). In addition, biochar has many positively charged functional groups, which can adsorb limited nitrate (Li et al. 2018), effectively intercept nutrients, and reduce nitrogen leaching. At the same time, according to the information provided in Fig. 6, only the SOC in the 20–30 cm soil layer strongly correlated with the $NO_3^-$-N in the 10–20 cm soil layer, indicating that a single increase in SOC content had a limited impact on the leaching of soil nitrogen.

The majority of the nitrogen that plants can absorb and utilize is nitrogen in the nitrate structure (Leghari et al. 2016); leaf nitrogen is closely related to the nitrogen content of root soil. Because farmers do not have independent laboratories, it is difficult for them to monitor soil nitrogen dynamics by sampling soil in different growth periods, and it is impossible for them to judge the dynamic changes in soil nitrogen over time. Therefore, farmers tend to apply a large amount of fertilizer to the field. However, too much fertilizer is likely to lead to agricultural nonpoint source pollution and endanger farmland soil health. At the end of the 20th century, some scientists proposed using the SPAD analyzer to estimate the nitrogen dynamics of farmland soil and optimize the soil nutrient structure by topdressing when sensing that the soil nitrogen is insufficient (Peng et al. 1996; Vos 1998); this method has been widely used in rice, wheat, and maize (Argenta et al. 2004; Lopez-Bellido et al. 2004; Tremblay et al. 2010). Referring to the above research results, this study used a portable leaf measuring instrument to apply the same principle for measuring the SPAD value and leaf nitrogen content of soybean leaves treated under different soil organic matter contents and different fertilization levels. The results showed that, for the soil in the 0–10 cm soil layer, the coefficient of determination of the response function was low whether using leaf nitrogen fitting or SPAD value fitting (Figs. 6 and 7). This may have been caused by the higher surface soil temperature in the potted container, which encouraged the hydrolysis of urea in the soil to volatilize in the form of ammonia gas, resulting in unstable soil nitrogen content (Cameron et al. 2013). For the 10–20 cm soil layer, the determination coefficient of the fitting equation obtained from the nitrogen content of soybean leaves reached

0.5592 or more ($p<0.05$), indicating that during this period, portable chlorophyll meters can be used to predict the actual nutrient changes in the soil (Fig. 7). Biochar and fertilizer were mixed in Mollisols, and it was observed that the soybean plant height and soybean stem diameter were significantly improved (Fig. 1). The results of the analysis of variance showed that there were significant differences in soybean agronomic traits under the interaction of different rates of biochar and nitrogen fertilizer (Table 3). Meanwhile, the mixed application of biochar and fertilizer can promote the accumulation of nitrogen and increase the SPAD value of soybean leaves. However, in our previous study, it was found that when only biochar was applied, changing the application method and application rates of biochar, the leaf nitrogen content, and SPAD content did not exhibit significant differences (Li et al. 2020). The results of the path analysis in this paper show that only plant height and stem diameter can affect the yield index of crops. The above results also prove that the leaf information of crops can reflect the changes in nutrients in the root zone of crops. Based on the above research results, this study determines that the effect of the mixed application of biochar and fertilizer on crop yield follows the following relationship: when nitrogen fertilizer is applied to the soil, the contents of two kinds of soil inorganic nitrogen, $NH_4^+$-N and $NO_3^-$-N, which can be directly absorbed and utilized by plants in the soil, are increased (Figs. 3 and 4). Due to the application of biochar, the conversion of soil $NH_4^+$-N to $NO_3^-$-N is promoted. At the same time, considering that $NO_3^-$-N is more easily absorbed and utilized by crops (Raven et al. 1992), compared with CK, the plant height, and stem diameter of the plants treated with the mixed application of biochar and fertilizer increased (Table 5). Plants with enhanced plant height and stem diameter obtain more main stem nodes, pod numbers, and grain weight, thereby achieving higher yields. Compared with each treatment in this study, B1N1 had the best effect in terms of yield and soybean growth. In the process of agricultural production, farmers' management decisions on cultivated land are still based on economic benefits (Luo et al. 2017). Therefore, we recommend the application of a biochar amendment at a rate of 3 kg·m$^{-2}$ to the soil tillage layer, along with a reduction in the nitrogen fertilizer application before cultivation. After the flowering and pod setting stage, foliar fertilizer should be applied differently for top dressing based on the fertility status reflected by the nitrogen content of the crop leaves. This measure can not only reduce fertilizer input but also effectively prevent nonpoint source pollution caused by excessive fertilizer.

## 5 Conclusion

The following conclusions can be drawn from the application of the nine different biochar and fertilizer application strategies to evaluate soil fertility and crop

growth. When the biochar application rate exceeds 3 kg m$^{-2}$, the organic carbon content within the biochar and the organic carbon generated by the positive excitation effect within the soil formation outstrip the organic carbon consumed by the negative excitation effect of the soil, consequently promoting soil organic carbon storage. Biochar-mediated storage of soil organic carbon supports the survival of soil microorganisms and improves soil pH, moisture retention, and aeration, thereby facilitating soil nitrification and the transformation of ammonium into nitrate nitrogen, ultimately boosting the total content of soil nitrate nitrogen. Additionally, biochar application can curb soil inorganic nitrogen leaching owing to its strong nitrogen adsorption capacity, water retention ability, and attraction of nitrogen groups that enhance nitrate nitrogen retention. This research demonstrates that nitrate nitrogen leaching can be decreased by up to 42% under B1N2 treatment. The study also confirms that reducing fertilizer input while applying biochar can result in higher crop yields. During the flower bud differentiation and flowering and podding stages, there was a logistic curve correlation between leaf nitrogen content and soil nitrate nitrogen content, and the relationship was accurately defined. Therefore, one can accurately estimate the nitrate nitrogen content from the leaf nitrogen content before flowering and podding in the soil of the soybean root zone. Based on the above research results, it is believed that applying biochar at a minimum rate of 3 kg m$^{-2}$ rate to the field before crop cultivation, coupled with a limited quantity of base fertilizer and topdressing based on soybean leaf information before the flowering and pod setting stage, can minimize nitrogen input into the farmland.

## Declarations

**Competing Interests** The authors declare no competing interests.

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