Long-term biochar application promotes rice productivity by regulating root dynamic development and reducing nitrogen leaching

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Funding information
National Key Research and Development Program, Grant/Award Number: 2017YFD0200107; National Natural Science Foundation of China, Grant/Award Number: 31660597 and 31700382; Central Public-interest Scientific Institution Basal Research Fund for Chinese Academy of Tropical Agricultural Sciences, Grant/Award Number: BSRF201906; Ningxia Key R&D Program, Grant/Award Number: 2019BBF02026

Abstract
Biochar is beneficial for improving soil quality and crop productivity. However, the long-term effects of biochar addition on temporal dynamics of plant shoot and root growth, and the changes in soil properties and nitrogen (N) leaching are still obscure. Here, based on a long-term (7 years) biochar field experiment with rice in northwest China, we investigated the effects of two biochar rates (0 and 9 t ha⁻¹ year⁻¹) and two N fertilizer rates (0 and 300 kg N ha⁻¹ year⁻¹) on shoot and root growth, root morphology, N leaching, and soil physicochemical properties. The results showed that both biochar and N fertilizer significantly promoted rice growth, with their interaction significant only in some cases. Both fertilizers enhanced rice shoot biomass and N accumulation in various growth stages as well as increased grain yield. Nitrogen fertilizer significantly promoted root growth regardless of biochar application. However, biochar application without N fertilizer increased root biomass and length during the whole growth period, except in the booting stage; biochar with N application promoted root growth at tillering, reduced root biomass but maintained root length with low root diameter and high specific root length during the jointing and booting stages, and then delayed root senescence in the grain filling stage. Long-term applications of biochar and N fertilizer reduced 10%–12% bulk density of topsoil compared to the control treatment with no N fertilizer and no biochar. Long-term biochar application also improved soil total organic carbon and concentrations of available N, phosphorus, and potassium. In addition, biochar and N fertilizer applied together significantly reduced nitrate and ammonium concentration in leachate at different soil depths. In conclusion, biochar could regulate root growth, root morphology, soil properties, and N leaching to increase rice N fertilizer-use efficiency.

Keywords
N leaching, nutrient availability, rice yield, root morphology, root turnover, soil physical properties, temporal dynamics
In agricultural systems, widespread use of inorganic nitrogen (N) fertilizers has significantly improved crop yields, but also concomitantly caused environmental problems. Biochar, as a carbonaceous material obtained after thermal treatment of biomass residues (Albuquerque et al., 2013), represents a potential sustainable option for decreasing N fertilizer application without yield penalty (Laird et al., 2010; Lehmann et al., 2011). Biochar application may enhance sustainability of the agricultural systems (Blanco-Canqui, 2017; Jiang et al., 2020) due to: increasing soil porosity and decreasing soil bulk density; high content of recalcitrant carbon favoring soil carbon sequestration; high cation exchange capacity (CEC) that may contribute to decreased N leaching and nitrous oxide (N₂O) emissions; as well as the improvements in soil fertility and crop yields (Dai et al., 2020; Liu et al., 2020; Sohi et al., 2010). Biochar amendments can also enhance the root systems and regulate root morphology, thus promoting nutrient absorption and crop productivity (Olmo & Villar, 2019; Prendergast-Miller et al., 2014; Xiang et al., 2017). However, it is important to understand the mechanisms by which biochar addition influences the dynamics of root and shoot growth and N leaching, as well as the changes in root morphology during the whole growth period.

The effect of biochar application on crop shoot and root growth can vary greatly (Jeffery et al., 2011; Olmo & Villar, 2019; Prendergast-Miller et al., 2014); for example, it was influenced by the soil nutrient content (Clough et al., 2013; van Zwieten et al., 2010). In the fertile soil, there was a clear positive biochar effect that was absent in the low-fertility soil (Noguera et al., 2010). Other studies showed that biochar significantly improved plant growth and doubled grain production with mineral fertilization (Steiner et al., 2007), and increased root length with N fertilization (Prendergast-Miller et al., 2011). However, some studies reported that biochar addition increased crop yield and root growth regardless of nitrogen application rate (Backer et al., 2017).

The effects of biochar on crop growth differed depending on the growth stage. For example, biochar increased root biomass and root development between early vegetative growth and flowering due to increased soil nutrient availability linked to the biochar high CEC, followed by improved grain yield (Backer et al., 2017; Xiang et al., 2017), thus reducing the risk of nutrient leaching through improved nutrient-use efficiency. In maize, studies on the growth dynamics in different growth stages showed that increased root development in the seedling stage effectively increased the aboveground biomass (Li et al., 2016; Zhang et al., 2019), and high root vitality in the booting stage promoted nutrient accumulation and increased grain yield (Li et al., 2019). However, there is still a lack of systematic research on how the interaction between biochar and nutrient input influences plant growth and N leaching, especially regarding potential synchronization of the root and shoot growth in each growth stage.

Application of biochar and/or N fertilizer to the soil is known to influence soil structure and nutrient availability (Jiang et al., 2020; Luo et al., 2019), and therefore may impact root morphology (Olmo & Villar, 2019; Xiang et al., 2017). For example, increased specific root length (SRL; the root length per unit mass; Backer et al., 2017; Olmo & Villar, 2019; Xiang et al., 2017) is the most commonly reported effect of biochar application to soils, but increased or decreased root diameter (RD; Amendola et al., 2017; Sun et al., 2020; Xiang et al., 2017) and increased or decreased root tissue density (RTD; the root mass per unit of root volume; Brennan et al., 2014; Bruun et al., 2014; Olmo et al., 2016) have also been reported. In theory, if the root system is regarded as a cylinder, without considering the variability of the root shape, according to the equation SRL = 4/(π × RD² × RTD), SRL is inversely proportional to RD and RTD (Bergmann et al., 2020), implying that SRL increases with decreasing RD and/or RTD. These contradictory findings suggest that the effect of biochar application on root morphological traits may be complex and variable depending on the plant species and soil conditions.

In nutrient-poor soil environments, plant roots could increase the absorption area and improve nutrient acquisition efficiency with lower root construction cost by changing root traits, such as increasing root:shoot ratio and SRL as well as decreasing RTD (Lambers et al., 2006; White et al., 2013). In contrast, in nutrient-rich soil environments, plant species generally increase RD and decrease SRL (Wang et al., 2018), and also reduce fine root turnover and prolong root life span (Burton et al., 2000). In addition, nitrogen fertilizer application can increase rice root biomass and rhizodeposition, thus stabilizing soil aggregates (Luo et al., 2019). Given both biochar and fertilizer application have a significant impact on root morphology, revealing the effects of long-term application of biochar and N fertilizer on root morphological traits may improve our understanding of the mechanisms governing efficient utilization of soil nutrients by crops.

Rice is the staple food for nearly half of the world’s population; rice production worldwide is expected to be 161 million metric tons per year on 503 million hectares in 2021–2022 (Chauhan et al., 2017). China, as the largest producer of rice, supplied about one third of the world’s rice on about one fifth of the world’s rice paddy land (Frolking et al., 2002). To achieve such high grain yields, high rates of N fertilizers were applied in rice paddies (Peng et al., 2009), exacerbating a risk of N leaching (Liu et al., 2012). The rice roots respond to variable soil N supply (Ju et al., 2015; Xu et al., 2018), but there is a paucity of knowledge about whether long-term application of biochar together with N fertilizer may influence the growth
and morphological traits of rice roots in different growth stages to synergistically decrease soil N leaching and increase aboveground growth.

To address these issues, we characterized the effects of long-term application of biochar and N fertilizer on rice shoot and root growth, root morphology, soil N leaching and soil properties, as well as grain yield by monitoring growth dynamics throughout the rice growth period in northwest China. We aimed to answer the following questions: (a) whether shoot and root growth in different growth stages is regulated synergistically by biochar and N fertilizer applications, (b) how root morphological traits respond to different biochar and N supplies, and (c) whether long-term application of biochar and N fertilizer can reduce N leaching and improve rice productivity.

## 2 | MATERIALS AND METHODS

### 2.1 | Study site and experimental design

This experiment was conducted in the experimental field of Zhengxinyuan Modern Agriculture Company at Yesheng Town in Qingtongxia City of Ningxia Hui Autonomous Region, China (106°11′35″E, 38°07′26″N). The site belongs to the temperate continental monsoon climatic zone with a mean annual temperature of 8.9°C and mean annual precipitation of 192.9 mm. The soil is classified as anthropogenic alluvium. The topsoil (0–20 cm) total organic carbon (TOC) in 2017 was 12.7 g/kg, and the total nitrogen (N) and total phosphorus (P) were 0.94 and 0.60 g/kg, respectively. Soil available N, P, and potassium (K) were 82, 22, and 122 mg/kg, respectively. Soil pH in water was 8.56, and bulk density was 1.56 g/cm³. The relevant methods are described below.

The long-term field experiment included two biochar rates (B0, no biochar supply; B+, biochar supply at 9 t ha⁻¹ year⁻¹) and two N fertilizer rates (N0, no N supply; N+, N supply at 300 kg N ha⁻¹ year⁻¹) since 2012. These four treatments were combined in a fully factorial manner in a randomized complete block design with four replicates. A total of 16 plots (each measuring 13 m × 5 m) were established. Each plot was separated by plastic film (approximately 1.56 g/cm²). The relevant methods are described below.

The standard N application rate for the local farming area was applied as urea (46% N) to the soil surface; 50% of 300 kg N/ha was applied as basal fertilizer before transplanting (May 25), 30% was applied at tillering (June 9), and the remaining 20% was applied at jointing (stem elongation stage, June 24). Double superphosphate and KCl were also applied as basal fertilizers to the soil surface before transplanting at rates of 90 kg P₂O₅/ha and 90 kg K₂O/ha.

Biochar was produced by pyrolysis of wheat straw at 240–360°C by Shandong New Energy Company. Biochar had C, N, P, and K total contents (w/w) of 66%, 0.49%, 0.1%, and 1.6%, respectively, with a pH (H₂O) of 7.78. Biochar was applied together with basal fertilizers by broadcasting on the soil surface and was incorporated into the soil by plowing to a depth of approximately 15 cm in May. To maintain consistency, plowing was also performed in the plots without biochar treatment.

Rice (cv. Ningjing 43) was sown in a nursery bed on May 1. Rice seedlings were transplanted on May 29 and harvested on September 29. Crop management was consistent across plots and years.

### 2.2 | Soil sampling and analysis

During rice growth in 2017 and 2018, soil water samples used for the leaching calculations were collected from lysimeters, as described by Riley et al. (2001). Four PPR (polypropylene) equilibrium-tension lysimeters (0.19 m²) were installed at the desired depths (20, 60, and 100 cm below the soil surface) in each plot. Soil leachate samples were collected using 100 ml plastic syringes and were transferred to plastic tubes and stored at 4°C before analysis. Samples were taken once in May (1 day after fertilization), seven times in June (1, 4, 7, and 10 days after fertilization), four times in July (every 10 days), and two times in August (every 15 days), resulting in 14 sampling times during the crop growth period. Nitrate and ammonium concentrations of the soil leachate were measured by the continuous flow technique (TRACS 2000 system, Ran and Luebbe).

Five soil samples (0–20 cm) from two diagonal lines through each plot were collected and pooled into one composite sample in 2017. A subsample of soil was frozen at −20°C for measuring soil available N and soil pH, and the remaining soil was air-dried for measuring other soil properties. Soil bulk density was measured using 100 cm³ cylinders.

Soil TOC content was determined using a total carbon analyzer (MULTIN/C 2100). Soil total N content was measured using the Kjeldahl method (Bao, 2000). Soil total P was determined spectrophotometrically after digestion by HClO₄. Soil available N (KCl-extractable ammonium and nitrate) was determined using a Continuous Flow Analyzer (AutoAnalyzer 3; Bran & Luebbe). Soil available P (NaHCO₃-extractable P) was measured by the Olsen-P method (Sims, 2000). Soil available K was determined by atomic absorption spectrophotometry after extraction with ammonium acetate. The soil pH (1:2.5, soil:water) was assayed potentiometrically.
2.3 Rice growth and root trait measurements

The changes in rice shoot growth and N uptake, as well as root growth and morphological traits, were assessed in different growth stages. Rice plants were harvested manually from five 0.25 m² areas diagonally across each plot in the tillering, jointing, booting, and grain filling stages. For the root sampling, a soil block (0.5 m length × 0.5 m width × 0.5 m deep) was excavated; more than 95% of rice root length is concentrated in 0–0.4 m (Kondo et al., 2000). Rice shoot samples were oven-dried to a constant weight at 80°C, weighed, finely ground, sieved, and analyzed for total N content using the same method as for soil total N.

Fresh rice root samples were washed under running water, spread in water with minimal overlap, and then scanned on an Epson Expression 10,000 XL desktop scanner (resolution 300 dpi). Root images were analyzed using WinRHIZO software (Regent Instruments) to obtain the average RD, volume, and total length. Root samples were oven-dried to a constant weight at 80°C and weighed. The SRL was calculated as the root total length divided by its dry mass. The RTD was calculated as the ratio of root dry mass to its volume. Additionally, at rice maturity in 2017, rice grains were manually harvested from five 1 m² areas diagonally across each plot, oven-dried to a constant weight at 80°C, and weighed to determine the rice yield.

2.4 Data analysis

Two-way repeated measures analysis of variance (ANOVA) was used to test the effects of N fertilizer and biochar application and their interactions on shoot parameters (such as shoot biomass and N accumulation) and root growth (such as root biomass and length), as well as root/shoot biomass ratio across the whole growth period in two consecutive seasons using SPSS 23.0 (SPSS Inc.). In a given growth stage, the independent-sample t test was performed to determine significant differences in shoot and root growth between the two biochar treatments at the same N rate in 2017 and 2018. Additionally, the independent-sample t test was performed to determine significant differences in root morphological traits (such as RD, SRL, and RTD) in a given growth stage between the two biochar treatments at the same N rate, as well as between the two N fertilizer treatments at the same biochar rate.

Two-way ANOVA with post-hoc Tukey HSD tests was used to test N fertilizer and biochar supply effects on soil physicochemical properties (0–20 cm); when the interaction was nonsignificant, we just tested the difference between the two levels of each main effect using the independent-sample t test. Furthermore, the independent-sample t test was also performed to examine the differences in leaching of N forms between different rates of biochar supply on a given sampling date. All figures were plotted using R version 3.6.1.

3 RESULTS

3.1 Changes in rice growth and root traits

Both N fertilizer and biochar supply had significant influence on rice above- and belowground biomass during the whole growth period in two consecutive seasons (Table S1). However, there were different changes in rice shoot and root growth during the growth stages, responding to N fertilizer and biochar application (Figure 1). Considering that the growth patterns of shoots and roots in 2017 and 2018 were similar, here we only took the crop growth in 2017 as an example (Figure 1). Shoot growth kept increasing after adding N fertilizer and/or biochar with time (Figure 1a,b), whereas root growth reached the peak in the jointing and booting stages (Figure 1e–h). Although the extent of increase in shoot biomass and shoot N accumulation was greater in the N+ than the N0 treatments, in both situations increases were greater in the treatment with than without biochar application during the whole growth period (Figure 1a–d).

Root biomass and length significantly increased after applying N fertilizer, irrespective of biochar rates, albeit the responses to biochar rates differed at various growth stages (Figure 1f,h). Specifically, root biomass and length both increased after applying biochar without N fertilizer, except in the booting stage (Figure 1e,g). In contrast, root biomass significantly decreased (by 55%) after adding biochar and N fertilizer in the middle stages (i.e., jointing and booting), whereas root length did not change significantly (Figure 1f,h). Biochar addition without N fertilizer significantly increased root/shoot ratio at grain filling (Figure 1i), but when added with N fertilizer significantly decreased root/shoot ratio, except in the grain filling stage (Figure 1j).

Nitrogen fertilizer and biochar supply had differential effects on root morphological traits in different growth stages (Figure 2; Figure S1). For example, in 2017, N fertilizer significantly increased RD and decreased SRL when no biochar was added (Figure S1a,c). With biochar application, SRL significantly decreased at tillering and increased at jointing and booting, whereas RTD increased at tillering and decreased at the other three stages in response to N fertilizer application (Figure S1d,f). Biochar application without N fertilizer significantly increased RD and decreased RTD at tillering and grain filling, and decreased SRL in the jointing and grain filling stages (Figure 2). However, biochar supply with N fertilization significantly decreased RD and increased SRL at jointing and booting (Figure 2b,d).
FIGURE 1  Effects of different biochar application and N fertilizer rates on rice shoot (a–d) and root growth (e–j) during different growth stages in 2017 and 2018. N0_B0 indicates the treatment with no biochar and no N fertilizer, and N0_B+ indicates the treatment with biochar and zero N fertilizer; N+_B0 indicates the treatment with no application of biochar and N fertilizer addition, and N+_B+ indicates the treatment with application of biochar and N fertilizer addition. Asterisks for a growth stage denote significant differences between biochar treatments at the same N fertilizer rate using the independent-sample \( t \) test. ***\( p < .001 \), **\( p < .01 \), *\( p \leq .05 \), ns, nonsignificant at \( \alpha = 0.05 \). Values are mean ± SE (n = 4).
3.2 | Soil properties and N leaching

Long-term N fertilizer and/or biochar application had no significant effect on topsoil (0–20 cm) total N, total P and pH values, but significantly influenced TOC, available nutrients, and soil bulk density (Figure 3; Table S2). For example, biochar supply significantly increased (by 40%) the content of TOC irrespective of N fertilizer addition (Figure 3a). The combined applications of N fertilizer and biochar significantly increased (by 8%) soil available N compared with all the other treatments (Figure 3b). In the treatments with biochar addition, soil available P significantly increased (by 33% and 48% without and with N fertilizer, respectively; Figure 3c). Soil available K significantly increased (by 51%) after adding only biochar (Figure 3d). Long-term N fertilizer and/or biochar supply significantly decreased soil bulk density (by 10%–12%) compared to the non-amended control (Figure 3e).

Clear seasonal and soil depth dynamic changes in nitrate and ammonium concentrations in the leachate were observed in the N fertilization treatments in the two growing seasons (Figure 4; Figure S2). The peaks of N leaching, especially in ammonium concentration, occurred during the tillering and jointing stages. Biochar supply tended to decrease N leaching, particularly at tillering and jointing. Taking the tillering stage in 2017 as an example, the maximum decrease of nitrate concentration in the leachate was more than 65% at 20 and 60 cm depths, declining to 37% at a depth of 100 cm, whereas the maximum decrease
of ammonium concentration in the leachate exceeded 90% in all soil layers (Figure 4). However, averaged over the whole growth period, the leaching intensity of nitrate was similar in all three soil layers, but that of ammonium was stronger in the topsoil than deeper, regardless of biochar addition (Figure 4; Figure S2).

TABLE 1  Effects of N fertilizer and biochar application on rice grain yield (t/ha) in 2017 and 2018. N0 and N+ indicate no application and the application of N fertilizer treatments, respectively. B0 and B+ indicate no biochar control and the application of biochar treatments, respectively. Different lowercase letters in a column denote significant differences among the treatments at \( \alpha = 0.05 \) based on two-way ANOVA. Where the N × B interaction was nonsignificant, only the main effects were presented.

| Treatment | n | 2017       | 2018       | Significance |
|-----------|---|------------|------------|--------------|
| N0_B0     | 4 | 4.25 ± 0.06 c |           |              |
| N0_B+     | 4 | 4.44 ± 0.04 c |           |              |
| N+_B0     | 4 | 8.30 ± 0.10 b |           |              |
| N+_B+     | 4 | 9.04 ± 0.15 a |           |              |
| N0        | 8 | 4.49 ± 0.08 *** |        |              |
| N+        | 8 | 8.92 ± 0.09 ** |          |              |
| B0        | 8 | 6.56 ± 0.98 ** |          |              |
| B+        | 8 | 6.85 ± 1.01    |          |              |

\( p \) value

|           | 2017 | 2018 |
|-----------|------|------|
| N         | <.001| <.001|
| B         | .002 | .007 |
| N × B     | .025 | .385 |

Note: Values are mean ± SE (n = 4 or 8).

***p < .001; **p < .01.
3.3 Rice yield

Both N fertilizer and biochar supply had significant influence on rice grain yield in the two consecutive growing seasons ($p \leq .007$), but the interaction was significant only in 2017 (Table 1). Compared with the non-amended control, the application of biochar and/or N fertilizer tended to increase rice yield. In particular, the rice yield was increased twofold by after adding N fertilizer in both seasons (Table 1).

4 DISCUSSION

4.1 Biochar and N fertilizer modify shoot and root development during the whole growth period

Biochar can increase plant productivity, especially when applied together with fertilizers (Backer et al., 2017; Nan et al., 2020). In the N fertilizer treatments in 2017, biochar application consistently promoted rice shoot biomass and N accumulation during the whole growth period (Figure 1b,d). In contrast, the root biomass and total root length exhibited different dynamics. Biochar application promoted both root biomass and length at tillering and grain filling (Figure 1f,h). Root development during the early stage is a key to acquisition of soil resources (Li et al., 2019; Zhang et al., 2019; Figure 1). Interestingly, root biomass in the treatments with N fertilizer and biochar application was lower than that in the treatment with N application only during the jointing and booting stages (Figure 1f). This finding is inconsistent with previous meta-analysis studies that showed biochar application improved (He et al., 2020; Xiang et al., 2017) or had no influence on root biomass (Biederman & Harpole, 2013). Thus, biochar application might not be the most important proxy describing the belowground processes and responses (Song et al., 2020). The root/shoot ratio was lower in the treatment with both N fertilizer and biochar application compared with the N fertilizer only treatment during the whole growth period, except the grain filling stage (Figure 1j). This finding suggested that crops tended to allocate more resource to aboveground growth when the soil in fertile soil (Poorter et al., 2012).

Compared with root biomass, root length at jointing and booting showed no difference between the two biochar rates in the treatments with N fertilizer (Figure 1h); this is likely to have resulted in small RD and high SRL in these mid-growth stages in the treatment with both biochar and N fertilizer applied (Figure 2b,d). The increased growth of thin roots in the biochar treatment (Sun et al., 2020) also improved root activity to effectively take up N and decrease N leaching (Cao et al., 2019). Additional, long-term biochar application enhanced soil P availability (Figure 3c; Gao et al., 2019) and decreased soil bulk density (Figure 3e; Blanco-Canqui, 2017). These favorable soil conditions would allow thin roots to penetrate soil more easily, thus exploring soil for available P efficiently (with low energy investment). As rice entered the reproductive stage, the root biomass and length in the treatment with N fertilizer only declined significantly, but biochar application decreased the rate of root death and maintained root biomass/length at grain filling (Figure 1e,g). This may be beneficial for nutrient uptake during the reproductive growth stage as well as for the final yield (Table 1; Li et al., 2019).

Under no applying N fertilizer environments, biochar application promoted shoot growth, except at tillering (Figure 1a), and also enhanced root growth (biomass and length) during the whole growth period except at booting (Figure 1e,g). Biochar was found to provide a small amount of nutrient directly and also indirectly alter soil nutrient content to promote root growth (Ding et al., 2016; Prendergast-Miller et al., 2014). In the study presented here, after seven successive years of biochar application, the soil available N, P, and TOC content was significantly higher, and soil bulk density lower, compared with the treatments without biochar application (Figure 3). Thus, rice plants in the zero biochar treatments were exposed to conditions of relatively low nutrient availability and high soil compaction stress (Figure 3). This might have been the reason for rice in the no biochar treatment having significantly smaller RD, but higher RTD, in the tillering stage (Figure 2a,e), which could be save the whole root cost investment and more easily to explore soil nutrient at a distance (Materechera et al., 1991; McCormack et al., 2015).

We found that the effect of biochar on rice shoot and root growth was not always synchronized, mostly depending on the soil nutrient environment and plant growth stages (Figure 1; Biederman & Harpole, 2013). In the present study, a consistent root growth pattern was that biochar (regardless of N fertilizer rates) promoted root growth in the early stage, and delayed root senescence in the late growth stage, suggesting that long-term biochar application may provide the soil conditions conducive to maximizing root function. Although the root morphological traits varied among treatments and growth stages (Figure 2), their adjustments also played an important role in plant adaptation to the complex soil environmental changes caused by long-term biochar and nitrogen applications (Blanco-Canqui, 2017; El-Naggar et al., 2019).

4.2 Biochar addition improved rice productivity and changed soil properties and N leaching

Increased crop production in biochar-treated soils has been reported widely (Ali et al., 2020; Biederman & Harpole, 2013; Jeffery et al., 2011). Similarly, in the current
study, a small but significant increase in rice yield was observed after adding biochar irrespective of N fertilizer (Table 1). A growing body of evidence shows that long-term biochar application can promote crop productivity by changing soil physical conditions, improving soil fertility and decreasing N leaching (Biederman & Harpole, 2013; Blanco-Canqui, 2017). The porous structure of biochar applied to soil would influence soil physical properties (Ali et al., 2020; Lu et al., 2014). In the present study, long-term application of biochar and/or N fertilizer significantly reduced (by 10%–12%) bulk density of the topsoil (0–20 cm) relative to the soil without biochar and N fertilizer addition (Figure 3e). Similar results have been confirmed in previous studies (Głąb et al., 2016; Liu et al., 2016; Omondi et al., 2016). Low soil bulk density is beneficial to root growth and proliferation in soil by reducing mechanical resistance (Bruun et al., 2014).

Addition of biochar can improve soil fertility (Gao et al., 2019; Lehmann et al., 2003; Xiang et al., 2017; Yuan et al., 2019). In this study, we found that biochar application resulted in a significant increase in soil TOC, irrespective of N fertilizer addition (Figure 3b). This finding confirms the role of biochar in contributing to C storage in soil (Lehmann, 2007; Lehmann & Joseph, 2015). In addition, the combined applications of biochar and N fertilizer significantly increased soil available N, biochar application alone increased soil K content, and biochar enhanced soil P availability with and without N fertilizer (Figure 3). These biochar effects may contribute to the nutrient supply to crops. Generally, the increases in soil available nutrients could be related to biochar characteristics. The biochar’s large surface area and the negative surface charge (Bird et al., 2008; Cheng et al., 2008) increased the CEC of the soil and enhanced nutrient retention (El-Naggar et al., 2019). In addition, biochar as a C source stimulated microbial activity to improve nutrient cycling (Gao & DeLuca, 2018; Gomez et al., 2014; Zhang et al., 2018).

Biochar application can increase soil water holding capacity, microbial biomass, ion exchange, and net N mineralization of the soil to reduce nitrate and ammonium (Clough et al., 2013; Ding et al., 2010; Liu et al., 2019; Major et al., 2009; Xu et al., 2016). In the present study, biochar decreased N leaching, but the effect size depended on the growth stage and soil depth (Figure 4; Figure S2). The nitrate concentration in soil leachate at different soil depths was similarly high, but ammonium concentration decreased significantly with soil depth regardless of biochar application, suggesting nitrate leaching was a major problem in this ecosystem. Nitrate is soluble in water and its diffusion coefficient in soil ($10^{-10} \text{m}^2/\text{s}$) is close to the diffusion coefficient in aqueous solution (Tinker & Nye, 2000). In contrast, ammonium can easily be adsorbed onto negatively charged clay minerals in soil. The much higher CEC of biochar than soil might have been the dominant reason for a decrease in ammonium concentration in the leachate (Clough et al., 2013).

The mechanisms of biochar decreasing N leaching by changing soil physical, chemical, and hydraulic properties have been discussed in previous reviews (Clough et al., 2013; Major et al., 2009). Based on our findings, we suggested that the enhanced plant–soil interactions in biochar-amended soils could be important for understanding N leaching. In the present study, the decrease of N leaching after adding biochar was large mainly in the tillering and jointing stages (Figure 4; Figure S2). In these growth stages, biochar application increased root length and root absorption area (tillering; Figure 1d), reduced RD and increased SRL (jointing; Figure 2), suggesting improved root activity and nutrient uptake rate (Hodge, 2006). Indeed, these root changes effectively promoted rice N accumulation and N fertilizer-use efficiency (Figure 1). In addition, root distribution down the profile is important for intercepting and taking up nitrate to decrease its leaching (Lynch, 2019). Although the biochar was applied to the topsoil (0–20 cm), its influence on N leaching could extend to the deep layers (Figure 4; Figure S2). Thus, characterizing the changes in root vertical distribution in the soil profile (e.g. 0–100 cm) and clarifying a role of root–soil–microbe interactions in nutrient leaching under the long-term biochar application may advance our understanding of biochar effects.

5 CONCLUSION

This study showed that biochar application enhanced rice shoot biomass and N accumulation during the whole growth period and increased grain yield, especially when applied together with N fertilizer. With N fertilizer application, biochar promoted root growth in the early stage, reduced root biomass but maintained root length with low RD and high SRL during the middle growth stages, and then delayed root senescence in the late growth stage. When no N fertilizer was applied, biochar also promoted root growth in the early stage, and maintained strong root growth in the late growth stage. Additionally, biochar application decreased topsoil bulk density and improved soil TOC and available P content, regardless of N fertilizer rates. Applied together, N fertilizer and biochar increased soil available N content and decreased N leaching. In summary, biochar application can enhance crop production by improving the dynamics of root development and soil properties.

ACKNOWLEDGEMENTS

This study was supported by Ningxia Key R&D Program (2019BBF02026), National Key Research and Development Program (2017YFD0200107), the National Natural Science Foundation of China (31700382 and 31660597) and Central...
DATA AVAILABILITY STATEMENT
The data that support the findings of this study are available from the corresponding author upon reasonable request.

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REFERENCES
Alburquerque, J. A., Salazar, P., Barrón, V., Torrent, J., del Campillo, M. D. C., Gallardo, A., & Villar, R. (2013). Enhanced wheat yield by biochar addition under different mineral fertilization levels. *Agronomy for Sustainable Development*, 33(3), 475–484. https://doi.org/10.1007/s11393-012-0128-3

Alix, I., He, L., Ullah, S., Quan, Z., Wei, S., Iqbal, M., Munisif, F., Shah, T., Xuan, Y., Luo, Y., Tianyuan, L. I., & Ligeng, J. (2020). Biochar addition coupled with nitrogen fertilization impacts on soil quality, crop productivity, and nitrogen uptake under double-cropping system. *Food and Energy Security*, 9. https://doi.org/10.1002/fes.2308

Amendola, C., Montagnoli, A., Terzaghi, M., Trupiano, D., Oliva, F., Baronti, S., Miglietta, F., Chiariante, D., & Scippa, G. S. (2017). Short-term effects of biochar on grapevine fine root dynamics and arbuscular mycorrhizae production. *Agriculture, Ecosystems and Environment*, 239, 236–245. https://doi.org/10.1016/j.agee.2017.01.025

Backer, R. G. M., Saeed, W., Seguin, P., & Smith, D. L. (2017). Root traits and nitrogen fertilizer recovery efficiency of corn grown in biochar-amended soil under greenhouse conditions. *Plant and Soil*, 415(1–2), 465–477. https://doi.org/10.1007/s11104-017-3180-6

Bao, S. D. (2000). *Soil and agricultural chemistry analysis*. China Agriculture Press.

Bergmann, J., Weigelt, A., van der Plas, F., Laughlin, D. C., Kuyper, T. W., Guerrero-Ramirez, N., Valverde-Barrantes, O. J., Bruehlheide, H., Freschet, G. T., Iversen, C. M., Kattge, J., McCormack, M. L., T. W., Guerrero-Ramirez, N., Meier, I. C., Rigolot, C., Semchenko, M., Sweeney, C., Sarmah, A. K., & Ok, Y. S. (2019). Biochar application to low fertility soils: A review of current status, and future prospects. *Geoderma*, 337, 536–554. https://doi.org/10.1016/j.geoderma.2018.09.034

Biederman, L. A., & Harpole, W. S. (2013). Biochar and its effects on plant productivity and nutrient cycling: A meta-analysis. *GCB Bioenergy*, 5(2), 202–214. https://doi.org/10.1111/gcbb.12037

Bird, M. I., Ascough, P. L., Young, I. M., Wood, C. V., & Scott, A. C. (2008). X-ray microtomographic imaging of charcoal. *Journal of Archaeological Science*, 35(10), 2698–2706. https://doi.org/10.1016/j.jas.2008.04.018

Blanco-Canqui, H. (2017). Biochar and soil physical properties. *Soil Science Society of America Journal*, 81(4), 687–711. https://doi.org/10.2136/ssaj2017.01.0017

Brennan, A., Jiménez, E. M., Puschenreiter, M., Alburquerque, J. A., & Switzer, C. (2014). Effects of biochar amendment on root traits and contaminant availability of maize plants in a copper and arsenic impacted soil. *Plant and Soil*, 379(1–2), 351–360. https://doi.org/10.1007/s11104-014-2074-0

Bruun, E. W., Petersen, C. T., Hansen, E., Holm, J. K., & Haugaard-Nielsen, H. (2014). Biochar amendment to coarse sandy subsoil improves root growth and increases water retention. *Soil Use and Management*, 30(1), 109–118. https://doi.org/10.1111/sum.12102

Burton, A. J., Pregitzer, K. S., & Hendrick, R. L. (2000). Relationships between fine root dynamics and nitrogen availability in Michigan northern hardwood forests. *Oecologia*, 125(3), 389–399. https://doi.org/10.1007/s004420000455

Cao, H., Ning, L., Sun, M. L., Feng, F., Li, P., Yue, S., Song, J., Zhang, W., & Yang, H. (2019). Biochar can increase nitrogen use efficiency of *Malus hapearensis* by modulating nitrate reduction of soil and root. *Applied Soil Ecology*, 135, 25–32. https://doi.org/10.1016/j.apsoil.2018.11.002

Chauhan, B. S., Jabran, K., & Mahajan, G. (2017). *Rice production worldwide*. *Rice production worldwide*. Springer International Publishing. https://doi.org/10.1007/978-3-319-47516-5

Cheng, C. H., Lehmann, J., & Engelhard, M. H. (2008). Natural oxidation of black carbon in soils: Changes in molecular form and surface charge along a climosequence. *Geochimica et Cosmochimica Acta*, 72(6), 1598–1610. https://doi.org/10.1016/j.gca.2008.01.010

Clough, T., Condron, L., Kammann, C., & Müller, C. (2013). A review of biochar and soil nitrogen dynamics. *Agronomy*, 3(2), 275–293. https://doi.org/10.3390/agronomy3020275

Dai, Y., Zheng, H., Jiang, Z., & Xing, B. (2020). Combined effects of biochar properties and soil conditions on plant growth: A meta-analysis. *Science of the Total Environment*, 713, 136635. https://doi.org/10.1016/j.scitotenv.2020.136635

Ding, Y., Liu, Y., Liu, S., Li, Z., Tan, X., Huang, X., Zeng, G., Zhou, L. U., & Zheng, B. (2016). Biochar to improve soil fertility. A review. *Agronomy for Sustainable Development*, 36(2), 36. https://doi.org/10.1007/s11353-016-0372-z

Ding, Y., Liu, Y. X., Wu, W. X., Shi, D. Z., Yang, M., & Zhong, Z. K. (2010). Evaluation of biochar effects on nitrogen retention and leaching in multi-layered soil columns. *Water, Air, and Soil Pollution*, 213(1–4), 47–55. https://doi.org/10.1007/s11270-010-0366-4

El-Naggar, A., Lee, S. S., Rinkleje, J., Farooq, M., Song, H., Sarmah, A. K., & Ok, Y. S. (2019). Biochar application to low fertility soils: A review of current status, and future prospects. *Geoderma*, 337, 536–554. https://doi.org/10.1016/j.geoderma.2018.09.034

Frolking, S., Qui, J., Boles, S., Xiao, X., Liu, J., Zhuang, Y., Li, C., & Qin, X. (2002). Combining remote sensing and ground census data to develop new maps of the distribution of rice agriculture in China. *Global Biogeochemical Cycles*, 16(4), 38–1–38–10. https://doi.org/10.1029/2001gb001425

Gao, S., & DeLuca, T. H. (2018). Wood biochar impacts soil phosphorus dynamics and microbial communities in organically-managed croplands. *Soil Biology and Biochemistry*, 126, 144–150. https://doi.org/10.1016/j.soilbio.2018.09.002

Gao, S., DeLuca, T. H., & Cleveland, C. C. (2019). Biochar additions alter phosphorus and nitrogen availability in agricultural ecosystems: A meta-analysis. *Science of the Total Environment*, 654, 463–472. https://doi.org/10.1016/j.scitotenv.2018.11.124

Głab, T., Palmsowska, J., Zaleski, T., & Gondek, K. (2016). Effect of biochar application on soil hydrological properties and physical quality of sandy soil. *Geoderma*, 281, 11–20. https://doi.org/10.1016/j.geoderma.2016.06.028

Gomez, J. D., Denef, K., Stewart, C. E., Zheng, J., & Cotrufo, M. F. (2014). Biochar addition rate influences soil microbial abundance and activity in temperate soils. *European Journal of Soil Science*, 65(1), 28–39. https://doi.org/10.1111/ejss.12097

He, Y., Yao, Y., Ji, Y., Deng, J., Zhou, G., Liu, R., & Bai, S. H. (2020). Biochar amendment boosts photosynthesis and biomass in C3 but
properties using meta-analysis of literature data. *Geoderma*, 274, 28–34. https://doi.org/10.1016/j.geoderma.2016.03.029

Peng, S., Tang, Q., & Zou, Y. (2009). Current status and challenges of rice production in China. *Plant Production Science*, 12(1), 3–8. https://doi.org/10.1626/pps.12.3

Pooter, H., Niklas, K. J., Reich, P. B., Oleksyn, J., Poot, P., & Mommer, L. (2012). Biomass allocation to leaves, stems and roots: Meta-analyses of interspecific variation and environmental control. *New Phytologist*, 193(1), 30–50. https://doi.org/10.1111/j.1469-8137.2011.03952.x

Prendergast-Miller, M. T., Duvall, M., & Sohi, S. P. (2011). Localisation of nitrate in the rhizosphere of biochar-amended soils. *Soil Biology and Biochemistry*, 43(11), 2243–2246. https://doi.org/10.1016/j.soilbio.2011.07.019

Prendergast-Miller, M. T., Duvall, M., & Sohi, S. P. (2014). Biochar-root interactions are mediated by biochar nutrient content and impacts on soil nutrient availability. *European Journal of Soil Science*, 65(1), 173–185. https://doi.org/10.1111/ejss.12079

Riley, W. J., Ortiz-Monasterio, I., & Matson, P. A. (2001). Nitrogen leaching and soil nitrate, nitrite, and ammonium levels under irrigated wheat in Northern Mexico. *Nutrient Cycling in Agroecosystems*, 61(3), 223–236. https://doi.org/10.1023/A:1013758116346

Sims, J. T. (2000). Soil test phosphorus: Olsen P. In G. M. Pierzynski (Ed.), *Methods of phosphorus analysis for soils, sediments, residuals, and waters*, Southern Cooperative Series Bulletin No. 396 (pp. 20–21). Raleigh, NC: North Carolina State University.

Sohi, S. P., Krull, E., Lopez-Capel, E., & Bol, R. (2010). A review of biochar and its use and function in soil. In *Advances in agronomy* (Vol. 105, pp. 47–82). Academic Press. https://doi.org/10.1016/S0065-2113(10)05002-9

Song, X., Razavi, B. S., Ludwig, B., Zamanian, K., Zang, H., Kuzyakov, Y., Dippold, M. A., & Gunina, A. (2020). Combined biochar and nitrogen application stimulates enzyme activity and root plasticity. *Science of the Total Environment*, 735, 139393. https://doi.org/10.1016/j.scitotenv.2020.139393

Steiner, C., Teixeira, W. G., Lehmann, J., Nehls, T., de Macêdo, J. L. V., Blum, W. E. H., & Zech, W. (2007). Long term effects of manure, charcoal and mineral fertilization on crop production and fertility on a highly weathered Central Amazonian upland soil. *Plant and Soil*, 291(1–2), 275–290. https://doi.org/10.1007/s11104-007-9193-9

Sun, C., Wang, D., Shen, X., Li, C., Liu, J., Lan, T., Wang, W., Xie, H., & Zhang, Y. (2020). Effects of biochar, compost and straw input on root exudation of maize (*Zea mays* L.): From function to morphology. *Agriculture, Ecosystems and Environment*, 297(April), 106952. https://doi.org/10.1016/j.agee.2020.106952

Tinker, P. B., & Nye, P. H. (2000). *Solute movement in the rhizosphere*. Oxford University Press.

Van Zwieten, L., Kimber, S., Morris, S., Chan, K. Y., Downie, A., Rust, J., Joseph, S., & Cowie, A. (2010). Effects of biochar from slow pyrolysis of papermill waste on agronomic performance and soil fertility. *Plant and Soil*, 327(1), 235–246. https://doi.org/10.1007/s11104-009-0050-x

Wang, Y., Zhang, T., Wang, R., & Zhao, Y. (2018). Recent advances in auxin research in rice and their implications for crop improvement. *Journal of Experimental Botany*, 69(2), 255–263. https://doi.org/10.1093/jxb/erx228

White, P. J., George, T. S., Gregory, P. J., Bengough, A. G., Hallett, P. D., & McKenzie, B. M. (2013). Matching roots to their environment. *Annals of Botany*, 112(2), 207–222. https://doi.org/10.1093/aob/mct123

Xiang, Y., Deng, Q., Duan, H., & Guo, Y. (2017). Effects of biochar application on root traits: A meta-analysis. *GCB Bioenergy*, 9(10), 1563–1572. https://doi.org/10.1111/gcbb.12449

Xu, G., Liu, D., Wang, H., & Li, Y. (2018). Morphological and physiological traits of rice roots and their relationships to yield and nitrogen utilization as influenced by irrigation regime and nitrogen rate. *Agricultural Water Management*, 203, 385–394. https://doi.org/10.1016/j.agwat.2018.02.033

Xu, N., Tan, G., Wang, H., & Gai, X. (2016). Effect of biochar additions to soil on nitrogen leaching, microbial biomass and bacterial community structure. *European Journal of Soil Biology*, 74, 1–8. https://doi.org/10.1016/j.ejsobi.2016.02.004

Yuan, P., Wang, J., Pan, Y., Shen, B., & Wu, C. (2019). Review of biochar for the management of contaminated soil: Preparation, application and prospect. *Science of the Total Environment*, 659, 473–490. https://doi.org/10.1016/j.scitotenv.2018.12.400

Zhang, D., Wang, Y., Tang, X., Zhang, A., Li, H., & Rengel, Z. (2019). Early priority effects of occupying a nutrient patch do not influence final maize growth in intensive cropping systems. *Plant and Soil*, 442(1–2), 285–298. https://doi.org/10.1007/s11104-019-04155-1

Zhang, L., Jing, Y., Xiang, Y., Zhang, R., & Lu, H. (2018). Responses of soil microbial community structure changes and activities to biochar addition: A meta-analysis. *Science of the Total Environment*, 643, 926–935. https://doi.org/10.1016/j.scitotenv.2018.06.231

**SUPPORTING INFORMATION**

Additional supporting information may be found online in the Supporting Information section.

**How to cite this article:** Liu, B., Li, H., Li, H., Zhang, A., & Rengel, Z. Long-term biochar application promotes rice productivity by regulating root dynamic development and reducing nitrogen leaching. *GCB Bioenergy*. 2021;13:257–268. https://doi.org/10.1111/gcbb.12766