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Effects of Combined Application of Biochar and Different Types of Nitrogen Fertilizers on Rapeseed Root Growth and Properties of Purple Soil in Southwest China

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Abstract: To demonstrate the effects of combined application of biochar and different types of nitrogen fertilizers on the growth of plant roots and on purple soil properties such as soil nutrients, soil carbon content and soil respiration, a 206-day greenhouse pot experiment with rapeseed was conducted. Three types of nitrogen fertilizer were used: urea (UR), controlled-release urea (RU), a mixture of 60% urea and 40% controlled-release urea (40% RU), and biochar was added at mass fractions of 0% (C0), 2% (C1) and 4% (C2), with a control treatment (CK) without nitrogen fertilizer and biochar. The results showed that biochar significantly improved soil nutrient status, with the best effect observed when 40%RU was co-applied with biochar. The addition of biochar significantly increased soil total organic carbon (TOC) and particulate organic carbon (POC). Soil respiration increased with increasing biochar application, and the combination of 2% biochar and 40% RU showed a reduction in carbon emissions compared to the UR and RU treatments. The number of rapeseed root tips (NT), forks (NF) and crossings (NC) increased significantly with the addition of biochar, and the combination of biochar and 40% RU was more beneficial for root growth and development than RU and UR. Considering the improvement in soil nutrition, increased soil organic carbon content, reduced carbon emissions, and enhanced rapeseed growth and development, the co-application of 2% biochar and 40% RU is recommended for large-scale application in rapeseed cultivation in the hilly purple soil of southwest China.

Keywords: biochar; controlled-release urea; soil nutrients; soil organic carbon; soil respiration

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1. Introduction

Purple soils, which are classified as Regosols in FAO Taxonomy, developed from the fast physical weathering of sedimentary rocks of the Trias–Cretaceous system and are characterized by coarse structure, high permeability, and well-developed interflow [1,2]. They are rich in mineral nutrients and have high soil productivity, but also have shallow soil depth, high erosion, poor drought resistance, and severe degradation [3]. Purple soil is the main soil type in the agricultural areas of southwest China [4]. Due to the humid climate, complex terrain, increased acid rain, and intensified human activities in recent years, soil erosion and soil degradation have become severe in the region. This has led to problems such as thin soil layers, exposed rocks, acidification, and soil nutrient depletion, which have a significant impact on the further development of agriculture and the sustainable use of resources in the area [5]. It is crucial to find agricultural measures that can effectively alleviate soil degradation, improve the ecological environment of farmland soils, and enhance the potential for sustainable development and use of purple soils. In recent years, carbon emissions from agricultural soils and the resulting issue of climate warming have also become a hot topic for domestic and international scholars [6].

Biochar is a highly carbon-rich and stable organic material produced from crop residues (straw and husks), wood chips, and animal manure via pyrolysis under anaerobic or low-oxygen conditions. It is typically in the form of fine particles [7,8]. Due to its porous nature, high cation exchange capacity, and low bulk density, biochar plays an important role in improving soil physicochemical properties, reducing nutrient loss, and enhancing microbial diversity. It has been recognized for its contribution to soil improvement, increased crop productivity, and climate change mitigation [9–14]. A study by Azeem et al. [15] showed that the application of biochar increased soil organic carbon, TA, and soil bulk density, and significantly increased crop yield with or without the use of chemical fertilizers. Liu et al. [16], in their research on purple soils, also showed that the appropriate application of biochar improved soil acidity, increased soil nutrient content, altered microbial populations, and improved crop growth conditions. Studies by Bruun et al. [17], Abiven S et al. [18], Feng L et al. [19], and others have revealed that biochar can promote root growth, increase root density and biomass, and improve root structure. Yang et al. [20] found that the addition of 20 t·hm^{-2} and 40 t·hm^{-2} of biochar not only reduced CH_4 and N_2O emissions but also increased rice yield and soil fertility in their study of rice fields under controlled irrigation. In recent years, using biochar as a slow-release carrier for fertilizers has also attracted considerable attention [21].

Controlled-release urea (RU) is based on ordinary urea and is subjected to physical or chemical treatments that slow the release or decomposition of urea into ammonia without altering the chemical structure of the urea itself. This ensures that the rate of ammonia production matches the rate of plant uptake, improving the efficiency of urea utilization by crops [22]. Compared with conventional urea, RU can significantly reduce nutrient loss, decrease the environmental pollution caused by fertilization, increase nitrogen fertilizer utilization efficiency, improve crop growth and development, and significantly increase yield [23–25]. However, due to the slow nutrient release rate of RU, its sole application can lead to nutrient deficiencies in the early stages of crop growth, which can affect yield [26]. At present, the combined application of RU and conventional urea in appropriate proportions has attracted considerable attention from national and international scholars. Studies by Zhao et al. [27] and Zheng et al. [28] have shown that the co-application of RU and ordinary urea improves crop biomass accumulation, nitrogen accumulation, nitrogen uptake efficiency, and yield. Previous research on the application of RU and conventional urea to crops has mainly focused on aspects such as crop yield, nitrogen fertilizer utilization efficiency, crop physiological and metabolic characteristics, and changes in soil nutrients. However, there is still limited research comparing the effects of biochar combined with different types of nitrogen fertilizer on soil carbon components, soil respiration, and crop root growth. A greenhouse experiment was conducted to investigate the effects of the combined application of biochar and different types of nitrogen fertilizers on rapeseed root growth and purple soil properties in the southwestern hilly region by measuring root characteristics, soil nutrients, soil carbon content and soil respiration.

2. Materials and Methods

2.1. Experimental Materials

The biochar used in this study was provided by Nanjing Qinfeng Straw Co., Ltd. (Nanjing, China). It was produced via the pyrolysis of rice straw at a high temperature of 500 °C under anaerobic conditions. The biochar had a total nitrogen (TA) content of 0.61 g·kg^{-1}, a total phosphorus (TP) content of 1.99 g·kg^{-1}, a total potassium (TK) content of 27.15 g·kg^{-1}, a total organic carbon (TOC) content of 537.97 g·kg^{-1}, and a pH value of 8.7. The RU (N 44.5%) was produced by Jinzhengda Ecological Engineering Group Co., Ltd. (Linyi, China), and had a release period of approximately 120 days. Urea (N 46%), calcium superphosphate (P_2O_5 12%), and potassium chloride (K_2O 60%) were purchased from Sichuan Meifeng Chemical Co., Ltd. (Deyang, China). The rapeseed variety tested was Sanxia You 5, bred by the Chongqing Sanxia Academy of Agricultural Sciences, with a total growth period of approximately 206 days. The tested soil was collected from the 0–20 cm soil layer at the experimental farm of Southwest University, China (29°49′ N, 106°25′ E). The soil was classified as a typical purple soil (medium loam), and its physicochemical properties were as follows: soil bulk density, 1.28 g·cm^{-3}; pH, 6.91; soil organic matter (SOM), 7.22 g·kg^{-1}; TA, 0.62 g·kg^{-1}; alkaline hydrolysis nitrogen (AN), 36.75 mg·kg^{-1}; available phosphorus (AP), 9.46 mg·kg^{-1}; and available potassium (AK), 80.00 mg·kg^{-1}. Plastic pots with a height of 35 cm and a diameter of 25 cm were used for the experiments.

2.2. Experimental Design

The experiment was conducted from October 2017 to May 2018 in a sunlight greenhouse at Southwest University. The pot experiment was carried out with three nitrogen fertilizer treatments (single urea application, single controlled-release urea application, and 60% urea + 40% controlled-release urea, denoted as UR, RU, and 40%RU, respectively) and three biochar gradients (0%, 2%, and 4% biochar-to-soil mass ratio, denoted as C0, C1, and C2, respectively). A control treatment without nitrogen fertilizer and biochar (CK) was included. There were 10 treatments in total, namely CK, URC0, URC1, URC2, RUC0, RUC1, RUC2, 40% RUC0, 40% RUC1, and 40% RUC2 (see Table 1), each with six replicates. N, P, and K were applied at rates of 0.20 g N, 0.15 g P_2O_5, and 0.15 g K_2O per kg of soil (equal to 1 g N·pot^{-1}, 0.75 g P_2O_5·pot^{-1}, and 0.75 g K_2O·pot^{-1}) as a basal fertilizer for rapeseed (as shown in Table 2). Each pot was filled with 5.0 kg of soil (previously air-dried, crushed, and sieved through a 5 mm mesh) mixed thoroughly with the respective fertilizers and biochar according to the experimental design. Five to six rapeseed seeds were sown in each pot, and one seedling was retained at the five-leaf stage. Daily management practices were based on the growth characteristics of rapeseed. The other management practices applied were in accordance with those used by local farmers.

Table 1. Treatment combinations of biochar and nitrogen fertilizer.

Treatment	UR	RU	60% UR + 40% RU (40% RU)
Biochar 0%(C0)	URC0	RUC0	40%RUC0
Biochar 2%(C1)	URC1	RUC1	40%RUC1
Biochar 4%(C2)	URC2	RUC2	40%RUC2

Note: Control treatment (CK) without nitrogen fertilizer and biochar. UR, urea; RU, controlled-release urea.

Table 2. Treatment combinations of biochar and nitrogen fertilizer in each pot.

Treatment	UR (g)	RU (g)	P_2O_5 (g)	K_2O (g)	Biochar (g)	Soil (g)	Total Applied N (g)	Total Applied C (g)
CK	0	0	0.75	0.75	0	5000	0	0
URC0	1	0	0.75	0.75	0	5000	1	0.429
URC1	1	0	0.75	0.75	100	5000	1.061	54.226
URC2	1	0	0.75	0.75	200	5000	1.122	108.023
RUC0	0	1	0.75	0.75	0	5000	1	0.436
RUC1	0	1	0.75	0.75	100	5000	1.061	54.233
RUC2	0	1	0.75	0.75	200	5000	1.122	108.030
40% RUC0	0.6	0.4	0.75	0.75	0	5000	1	0.432
40% RUC1	0.6	0.4	0.75	0.75	100	5000	1.061	54.229
40% RUC2	0.6	0.4	0.75	0.75	200	5000	1.122	108.026

Note: CK, no nitrogen fertilizer and biochar; URC0, single urea application; URC1, urea + 2% biochar; URC2, urea + 4% biochar; RUC0, single controlled-release urea application; RUC1, controlled-release urea + 2% biochar; RUC2, controlled-release urea + 4% biochar; 40% RUC0, 60% urea + 40% controlled-release urea; 40% RUC1, 60% urea + 40% controlled-release urea + 2% biochar; 40% RUC2, 60% urea + 40% controlled-release urea + 4% biochar.

2.3. Sampling and Analyses

During the maturation stage of rapeseed, the plants were carefully removed from the soil while maintaining their overall integrity. The attached soil particles were lightly shaken off, and the roots were rinsed with distilled water and then gently dried with absorbent paper to measure the root characteristics. Soil samples were taken from 0 to 20 cm depth at the rapeseed maturity stage and after harvest. Each soil sample was brought to the laboratory, where it was thoroughly mixed after the removal of soil animals, plant roots, residues, and litter [29]. After natural air drying, the samples were ground and passed through 2 mm and 0.25 mm sieves for the determination of organic carbon components and soil nutrient content.

For the determination of soil basic physicochemical properties, the soil samples obtained after crop harvest were used for analysis. SOM was measured using the potassium dichromate wet combustion procedure in an externally heated oil bath (180 °C, boiling for 5 min) [30]. Total nitrogen (TN) was determined using the semi-micro-Kjeldahl method (digestion with 5 mL concentrated H_2SO_4), and AN was determined using the alkaline hydrolysis diffusion method (1.00 g of a dried soil sample was treated in a diffusion dish with 10 mL of 1.8 mol/L NaOH solution. After diffusion, the sample was absorbed using 3 mL of boric acid and titrated with 0.01 mol/L of hydrochloric acid solution) [31]. TP was measured using the $HClO_4$-H_2SO_4 digestion–molybdenum antimony colorimetric method, and AP was measured using the 0.5 mol L^{-1} $NaHCO_3$ extraction–molybdenum antimony colorimetric method [30]. TK was determined using the sodium hydroxide fusion flame photometric method, and AK was determined using the ammonium acetate extraction flame photometric method [32].

For the determination of soil carbon content, the soil samples collected during the rapeseed maturation stage were used for analysis. TOC was determined using the Shimadzu TOC-VCSH high-sensitivity combustion TOC analyzer (Shimadzu Corporation, Kyoto, Japan) with a dry combustion method [33]. Readily oxidable carbon (ROC) was determined using the potassium permanganate oxidation method [34]. Particulate organic carbon (POC) was determined using the sodium hexametaphosphate dispersion method [35]. Water-soluble organic carbon (WSOC) in the soil was determined by taking 2.5 g of air-dried soil passed through a 2 mm sieve, adding 25 ml of distilled water, shaking at 90 r·min^{-1} for 30 min at 20 °C, centrifuging at 3500 r·min^{-1} for 20 min, filtering through a 0.45 µm microporous membrane, and analyzing the filtrate using a Shimadzu TOC analyzer (TOC-L SSM-5000A and ASI-L, Shimadzu Corporation, Kyoto, Japan) [36].

For soil respiration [37], measurements were taken during the seedling, bud, blooming, and pod stages of rapeseed, with a uniform measurement time of 09:00–11:00 in the morning. The instrument used for measurement was the LI6400 portable photosynthesis system connected to a 6400-09 respiration chamber (Li6400-09, LI-COR Inc., Lincoln, OR, USA (see Figure 1). One measurement point was taken per rapeseed pot, and a self-made PVC ring was placed around each point the day before the measurement to reduce disturbance to the soil. Each PVC ring was measured once, with three cycles and three repetitions per treatment, resulting in a total of nine data points. The average value was calculated as the soil respiration rate (RR).

For the root characteristics of crop plants, at the maturity stage of rapeseed, the diameter at the hypocotyl node was measured using a caliper gauge as an indicator of root collar diameter (root diameter, RD). The roots of rapeseed plants were then severed at the base and thoroughly rinsed with water. The entire root system was then carefully scanned with a root scanner (EPSON V750) and stored as digital images on a computer. The Win RHIZO root analysis system software (Regent Instrument Inc., Quebec, ON, Canada) was used to quantitatively analyze the total root length (RL), the average root diameter (ARD), the number of root tips (NT), the number of root forks (NF), and the number of root crossings (NC) [29].

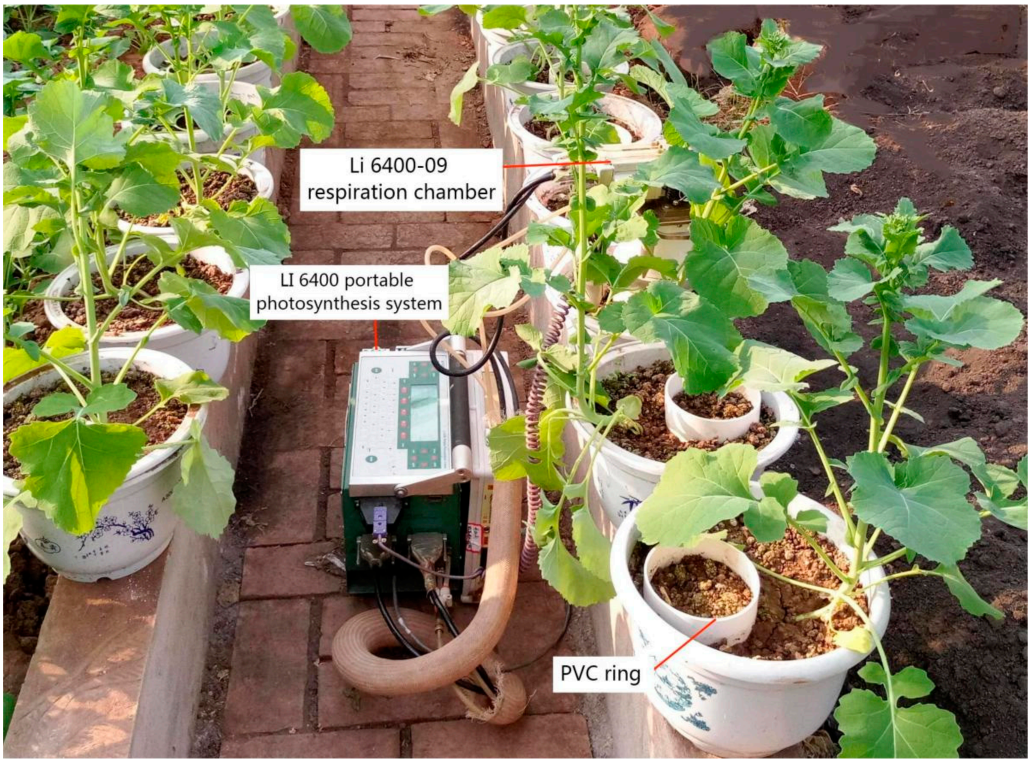

Figure 1. Soil respiration instrument.

2.4. Statistical Analysis

Data were organized and analyzed using Microsoft Excel 2010 and SPSS 17.0. Two-way ANOVA was used to determine differences between treatments. The means were compared using Duncan's test at a 0.05 probability level. The graphs were generated using SigmaPlot 14.0 and Origin 9.0.

3. Results

3.1. Soil Nutrients

As shown in Table 3, the type of nitrogen fertilizer, the gradient of biochar, and their interaction had significant ($p < 0.05$) or highly significant ($p < 0.01$) effects on SOM, TN, TP, TK, AN, AP, and AK (except for the insignificant effect of nitrogen fertilizer type on AP). With increasing biochar content, as shown in Figure 2, SOM and TA content increased significantly ($p < 0.05$) for different types of nitrogen fertilizers (except for the insignificant differences between RUC0 and RUC1, RUC1 and RUC2). The addition of 2% and 4% biochar significantly increased the content of TP, AN, AP, and AK in the 40% RU treatment, and the RU and UR treatments showed significant increases in TP, AK, AP, and AN only when 4% biochar was added ($p < 0.05$).

Table 3. The significance test of the interaction of biochar and nitrogen fertilizer on soil nutrients (p-value was measured after plant removal).

Factors	SOM ($g \cdot kg^{-1}$)	TN ($g \cdot kg^{-1}$)	TP ($g \cdot kg^{-1}$)	TK ($g \cdot kg^{-1}$)	AN ($mg \cdot kg^{-1}$)	AP ($mg \cdot kg^{-1}$)	AK ($mg \cdot kg^{-1}$)
N	**	**	**	**	*	ns	**
B	**	**	**	**	**	**	**
N*B	**	**	**	*	**	**	*

Note: * and ** indicate significant differences at p = 0.05 and p = 0.01, respectively, and ns indicates no significant difference; N: types of nitrogen fertilizer; B: biochar gradient; N*B: types of nitrogen fertilizer and biochar gradient.

From Figure 2, it can also be seen that, without biochar application, the differences among the soil nutrient indicators (except for SOM and TK) between the different types of nitrogen fertilizer were not significant ($p > 0.05$). With the addition of 2% and 4% biochar, there were significant differences in SOM content among different types of nitrogen fertilizers ($p < 0.05$). In these two treatments with different levels of biochar, the SOM content in the 40% RU, UR, and RU treatments was significantly increased by 95.17%, 77.17%, and 26.34% compared with the CK treatment. When 2% biochar was applied, there were no significant differences in the TA content among the different types of nitrogen fertilizers, but all were significantly higher than in the CK treatment. However, when 4% biochar was applied, there were significant differences in the TA content among the different types of nitrogen fertilizers ($p < 0.05$), and the 40% RU treatment had significantly higher TA content than the UR and RU treatments ($p < 0.05$). When 2% biochar was used, the 40% RU treatment had significantly higher TP content than the CK, UR, and RU treatments ($p < 0.05$), and when 4% biochar was used, the RU treatment had significantly higher TP content than the CK, UR, and 40% RU treatments ($p < 0.05$). When 2% biochar was applied, the 40% RU and RU treatments had significantly higher TK content than the CK and UR treatments ($p < 0.05$). When 4% biochar was applied, the RU treatment had significantly higher TK content than the UR and 40% RU treatments ($p < 0.05$), with increases of 37.62%, 14.36%, and 20.86% compared with the CK treatment, respectively. When 2% biochar was applied, there were no significant differences in AN, AP, and AK among the different types of nitrogen fertilizers, and there were no significant differences compared with the CK treatment ($p > 0.05$). When 4% biochar was applied, the 40% RU treatment had significantly higher AN and AK content than the UR and RU treatments, and the 40% RUC2 treatment had significantly increased AN and AK content by 9.84% and 13.32% compared with the CK treatment. The 40% RUC2 and RUC2 treatments had significantly higher AP content than the other treatments ($p < 0.05$), with increases of 138.41% and 103.04% compared with the CK treatment, respectively. This indicates that, with the application of 2% biochar, 40% RU is more conducive to improving soil nutrient status than RU and UR, while with the application of 4% biochar, 40% RU has a better effect on improving soil available nutrients.

3.2. Soil Organic Carbon Components

As shown in Table 4, the type of nitrogen fertilizer, biochar gradient, and their interaction significantly influenced soil carbon indicators (except for the type of nitrogen fertilizer's effect on TOC and POC and the interaction of biochar and nitrogen fertilizer on ROC in the blooming stage, as well as the impact of the type of nitrogen fertilizer in the maturity stage). This indicates that biochar has the main effect on TOC and POC, while WSOC and ROC are greatly influenced by both biochar and the type of nitrogen fertilizer.

According to Table 4, the addition of biochar in combination with all three types of nitrogen fertilizer significantly increased the content of TOC in the soil during the blooming and maturity stages of rapeseed. With an increase in biochar, the content of TOC in the soil showed an increasing trend (except for the RUC2 treatment at the blooming stage), and the 40% RUC2 treatment had the highest TOC content, averaging 13.26 $g \cdot kg^{-1}$. The addition of biochar in combination with all three types of nitrogen fertilizer significantly increased soil POC at all growth stages (except at maturity between 40% RUC0 and 40% RUC1). With an increase in biochar, the content of POC in the soil showed an increasing trend (except for the URC2 treatment during the blooming stage), and the C2 treatment showed significantly higher values than the C1 treatment (except for the differences between URC1 and URC2 during the blooming stage and between 40% RUC1 and 40% RUC2, which were not significant). The 40% RUC2 treatment had the highest content of POC during both the blooming and maturity stages, at 4.93 and 6.05 $g \cdot kg^{-1}$, respectively. This indicates that the addition of 4% biochar is more beneficial for increasing the content of POC in the soil, and the combination of higher biochar dosage (4%) with 40% RU shows better results.

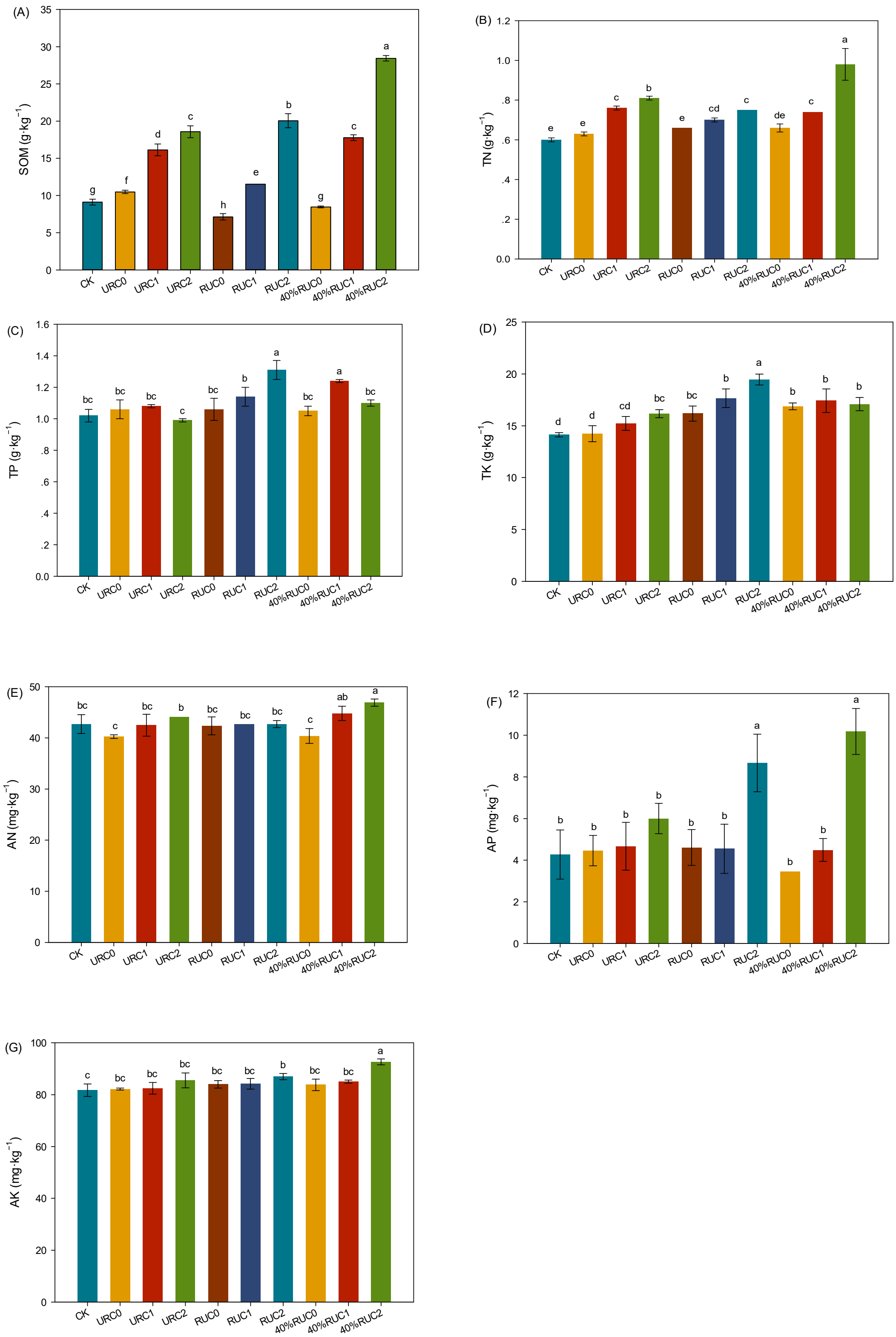

Figure 2. Effects of the combined application of biochar and nitrogen fertilizer on soil nutrients (**A**–**G**). The same lowercase letter represents no significant difference ($p > 0.05$) between treatments. CK, no nitrogen fertilizer and biochar; URC0, single urea application; URC1, urea + 2% biochar; URC2, urea + 4% biochar; RUC0, single controlled-release urea application; RUC1, controlled-release urea + 2% biochar; RUC2, controlled-release urea + 4% biochar; 40% RUC0, 60% urea + 40% controlled-release urea; 40% RUC1, 60% urea + 40% controlled-release urea + 2% biochar; 40% RUC2, 60% urea + 40% controlled-release urea + 4% biochar.

Table 4. The significance test of the interaction of biochar and nitrogen fertilizer on soil nutrients (*p*-value).

Treatment	Blooming Stage				Maturity Stage			
	TOC ($g \cdot kg^{-1}$)	**POC ($g \cdot kg^{-1}$)**	**WSOC ($g \cdot kg^{-1}$)**	**ROC ($g \cdot kg^{-1}$))**	**TOC ($g \cdot kg^{-1}$)**	**POC ($g \cdot kg^{-1}$)**	**WSOC ($g \cdot kg^{-1}$)**	**ROC ($g \cdot kg^{-1}$)**
URC0	6.60 + 0.46 de	3.15 + 0.02 c	0.53 + 0.01 c	9.59 + 0.35 b	6.08 + 0.13 f	3.00 + 0.03 c	0.81 + 0.01 c	3.92 + 0.78 bc
URC1	8.00 + 0.26 bc	4.70 + 0.02 a	0.54 + 0.02 c	12.32 + 0.91 b	9.36 + 0.47 d	4.55 + 0.83 b	1.08 + 0.05 a	6.67 + 0.86 a
URC2	9.69 + 0.20 a	4.63 + 0.00 a	0.61 + 0.06 c	10.31 + 1.02 b	10.78 + 0.47 c	5.34 + 0.41 b	0.97 + 0.01 b	6.39 + 0.17 a
RUC0	6.44 + 0.34 de	3.09 + 0.15 c	0.38 + 0.07 d	8.42 + 0.69 b	4.14 + 0.25 h	3.10 + 0.10 c	0.73 + 0.03 d	6.49 + 0.63 a
RUC1	9.13 + 0.77 a	4.10 + 0.09 b	0.37 + 0.06 d	12.84 + 0.33 a	6.68 + 0.11 e	4.28 + 0.06 b	0.90 + 0.02 b	6.03 + 0.56 a
RUC2	8.84 + 0.64 ab	4.61 + 0.42 a	0.73 + 0.01 b	8.61 + 1.67 b	11.64 + 0.55 b	5.84 + 0.06 a	0.75 + 0.01 cd	5.14 + 0.09 ab
40% RUC0	6.16 + 0.35 e	2.70 + 0.15 d	0.62 + 0.07 c	8.67 + 1.16 b	4.91 + 0.07 g	3.53 + 0.08 c	0.93 + 0.07 b	3.69 + 0.41 c
40% RUC1	7.43 + 0.82 cd	4.45 + 0.32 a	0.60 + 0.02 c	11.94 + 0.89 a	10.31 + 0.23 c	3.62 + 0.15 c	0.92 + 0.03 b	5.14 + 0.54 ab
40% RUC2	10.03 + 0.58 a	4.93 + 0.17 a	0.85 + 0.00 a	7.92 + 0.62 b	16.50 + 0.21 a	6.05 + 0.39 a	0.90 + 0.04 b	5.21 + 0.64 ab
N	ns	ns	**	*	**	ns	**	**
B	**	**	**	**	**	**	**	**
N*B	**	**	**	ns	**	**	**	**

Note: Values with the same letter within a column are not significantly different at $p = 0.05$; * and ** indicate significant differences at $p = 0.05$ and $p = 0.01$, respectively, and ns indicates no significant difference; N: types of nitrogen fertilizer; B: biochar gradient; N*B: types of nitrogen fertilizer and biochar gradient; CK, no nitrogen fertilizer and biochar; URC0, single urea application; URC1, urea + 2% biochar; URC2, urea + 4% biochar; RUC0, single controlled-release urea application; RUC1, controlled-release urea + 2% biochar; RUC2, controlled-release urea + 4% biochar; 40% RUC0, 60% urea + 40% controlled-release urea; 40% RUC1, 60% urea + 40% controlled-release urea + 2% biochar; 40% RUC2, 60% urea + 40% controlled-release urea + 4% biochar.

At the blooming stage, the combination of 2% biochar with all three types of nitrogen fertilizer significantly increased the content of ROC in the soil. During the maturity stage, the combination of biochar with UR and 40% RU significantly increased the ROC content in the soil, except for the RU treatment (as shown in Table 4). With an increase in biochar, the soil ROC content showed an increasing trend in all growth stages (except for the RU treatment in the maturity stage). The combination of 2% biochar with 40% RU showed a more pronounced effect in increasing ROC, while a higher dosage of biochar (4%) had some inhibitory effect. During the blooming stage, only the combination of 4% biochar with RU and 40% RU significantly increased the content of WSOC. During the maturity stage, the combination of biochar with UR significantly increased the WSOC content, while RU only significantly increased it with 2% biochar, and biochar had no significant effect in the 40% RU treatment. Furthermore, the overall trend of WSOC showed an initial increase followed by a decrease with an increase in biochar dosage. The 40% RUC2 treatment had the highest WSOC content during the blooming stage, while URC1 had the highest content during the maturity stage, both significantly higher than the other treatments. This indicates that the influence of biochar and nitrogen fertilizer on WSOC varies at different growth stages and that a higher dosage of biochar (4%) is more conducive to increasing WSOC during the blooming stage, while a higher dosage of biochar during the maturity stage has an inhibitory effect on WSOC.

3.3. Soil Respiration

From Table 5, it can be seen that the effects of biochar dosage and nitrogen fertilizer type on soil respiration varied at different growth stages, with the greatest effect at the blooming stage. During the seedling stage, both biochar dosage and nitrogen fertilizer type significantly influenced soil respiration, and their interaction also reached a significant level. During the bud and pod stages, only the effect of biochar dosage on soil respiration reached a highly significant level. However, during the blooming stage, both biochar dosage and nitrogen fertilizer type had a highly significant impact on soil respiration. This indicates that biochar dosage is the main factor influencing soil respiration, while the effect of nitrogen fertilizer type on soil respiration is sensitive to crop growth conditions.

Table 5. The significance test of the interaction of biochar and nitrogen fertilizer on soil respiration (*p*-value).

Factors	Seedling Stage	Bud Stage	Blooming Stage	Pod Stage
N	**	**	**	**
B	**	**	**	**
N*B	**	**	**	*

Note: * and ** indicate significant differences at $p = 0.05$ and $p = 0.01$, respectively; N: types of nitrogen fertilizer; B: biochar gradient; N*B: types of nitrogen fertilizer and biochar gradient.

According to Figure 3, the soil respiration rate (RR) in rapeseed exhibited an overall trend of initially decreasing, followed by increasing and subsequently decreasing throughout the entire growth period. The lowest RR was observed during the bud stage, while the highest RR occurred during the blooming stage. In addition, RR showed an increasing trend with the increase in biochar application during rapeseed growth (except between the seedling stage URC1 and URC2). At the seedling stage, the addition of 2% and 4% biochar significantly increased RR in the UR treatment, with significant improvements of 55.20% and 50.46%, respectively, compared with the control (CK). The RU and 40% RU treatments only showed a significant increase in soil respiration with the addition of 4% biochar. At the bud stage, the addition of 4% biochar significantly increased RR in the UR and RU treatments, with significant improvements of 41.80% and 43.85%, respectively, compared with CK ($p < 0.05$). However, the addition of biochar had no significant effect on RR in the 40% RU treatment ($p > 0.05$). During the blooming stage, the addition of 2% and 4% biochar significantly increased RR in the UR and 40% RU treatments. Only the RU treatment showed a significant increase in soil respiration with the addition of 4% biochar, with a significant increase of 72.75% compared with CK ($p < 0.05$). During the pod stage, only the addition of 4% biochar significantly increased RR in the RU treatment ($p < 0.05$), while the addition of biochar had no significant effect on RR in the UR and 40% RU treatments ($p > 0.05$). Regardless of the addition of biochar, except during the blooming stage where RU was significantly higher than UR, 40% RU, and URC1 during the seedling stage ($p < 0.05$), there were no significant differences in soil respiration among the different nitrogen fertilizer types during each growth stage ($p > 0.05$). However, the average soil respiration in the 40% RU treatment was the lowest among all growth stages. Therefore, it can be concluded that the addition of 4% biochar significantly increased soil respiration, while the effect of the addition of 2% biochar on soil respiration was generally not significant. In addition, the 40% RU treatment had the lowest average soil respiration rate across all growth stages. This suggests that the combination of 2% biochar and 40% RU can effectively reduce carbon emissions.

3.4. Crop Root Traits

According to Table 6, both the biochar gradient and nitrogen fertilizer type had significant or highly significant effects on RD, ARD, RL, NT, NF, and NC (except for the insignificant effect of nitrogen fertilizer type on NT). Additionally, the interaction effects between biochar gradient and nitrogen fertilizer type also reached a significant level for RD, RL, ARD, and NF. As shown in Figure 4, with an increase in biochar application, the NT, NF, and NC of different nitrogen fertilizer types showed an upward trend. With the increase in biochar, RD, RL, and ARD in the UR and 40% RU treatments showed an initial increase followed by a decrease, while the RU treatment showed a decreasing trend with the increase in biochar, although all treatments were higher than the control (CK). Specifically, the addition of biochar significantly reduced RD in the RU treatment. Moreover, 4% biochar significantly reduced RD in the UR and 40% RU treatments, while 2% biochar significantly increased RD in the 40% RU treatment. Furthermore, 2% biochar significantly increased RL in the UR treatment, while 4% biochar significantly decreased RL in the UR treatment. In addition, 4% biochar significantly reduced ARD in the UR treatment. The addition of biochar significantly increased NT in the 40% RU treatment, and only 4% biochar significantly increased NT in the UR and RU treatments. The addition of biochar significantly increased the NF and NC of different nitrogen fertilizer types ($p < 0.05$). These findings indicate that biochar can promote the root growth and root development of rapeseed in UR and 40% RU treatments. However, excessive biochar application is not conducive to improving the morphological characteristics of rapeseed roots.

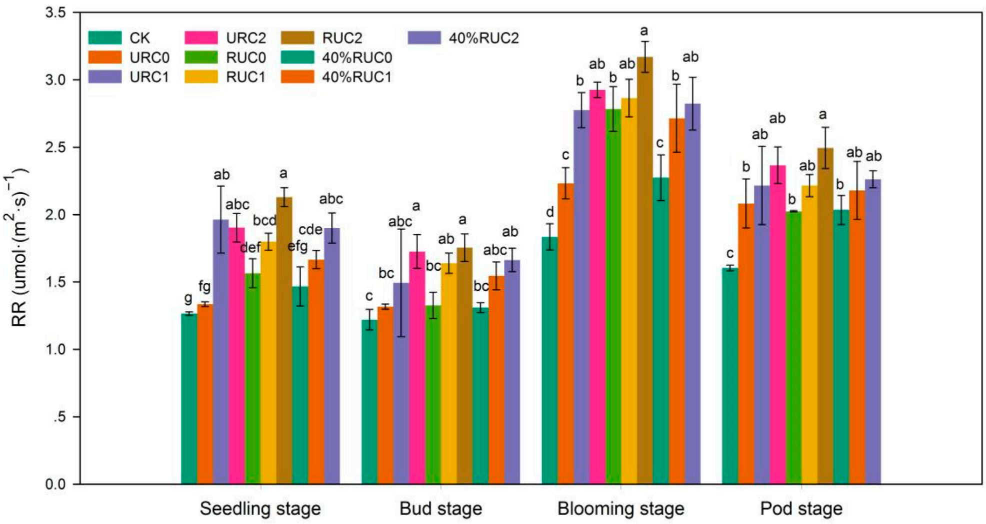

Figure 3. The effects of the combined application of biochar and nitrogen fertilizer on soil respiration. The same lowercase letter represents no significant difference ($p > 0.05$) between treatments. CK, no nitrogen fertilizer and biochar; URC0, single urea application; URC1, urea + 2% biochar; URC2, urea + 4% biochar; RUC0, single controlled-release urea application; RUC1, controlled-release urea + 2% biochar; RUC2, controlled-release urea + 4% biochar; 40% RUC0, 60% urea + 40% controlled-release urea; 40% RUC1, 60% urea + 40% controlled-release urea + 2% biochar; 40% RUC2, 60% urea + 40% controlled-release urea + 4% biochar.

Table 6. The significance test of the interaction of biochar and nitrogen fertilizer on soil respiration (p-value).

Factors	RD (mm)	ARD (mm)	RL (cm)	NT	NF	NC
N	**	**	**	ns	**	*
B	**	**	**	**	**	**
N*B	**	**	**	ns	**	ns

Note: * and ** indicate significant differences at $p = 0.05$ and $p = 0.01$, respectively, and ns indicates no significant difference; N: types of nitrogen fertilizer; B: biochar gradient; N*B: types of nitrogen fertilizer and biochar gradient.

When no biochar was applied, there were no significant differences in RD, RL, ARD, NT, NF, and NC between the different nitrogen fertilizer types ($p > 0.05$), except for significantly higher RL in the UR treatment than in the 40% RU and UR + 40% RU treatments and significantly higher NF in the UR and UR + 40% RU treatments than in the RU treatment ($p < 0.05$). When 2% biochar was added, the 40% RU treatment exhibited a significantly higher RD than the UR and RU treatments; the UR treatment showed significantly higher RL and NF than the 40% RU and RU treatments; and both 40% RU and UR treatments had a significantly higher ARD than the RU treatment ($p < 0.05$). When 4% biochar was added, the 40% RU treatment had a significantly higher ARD than the UR treatment, and the RU treatment showed significantly higher RL and NC than the UR and 40% RU treatments ($p < 0.05$). Overall, the combination of biochar and 40% RU application was more favorable for improving the morphological characteristics of rapeseed roots and promoting root development.

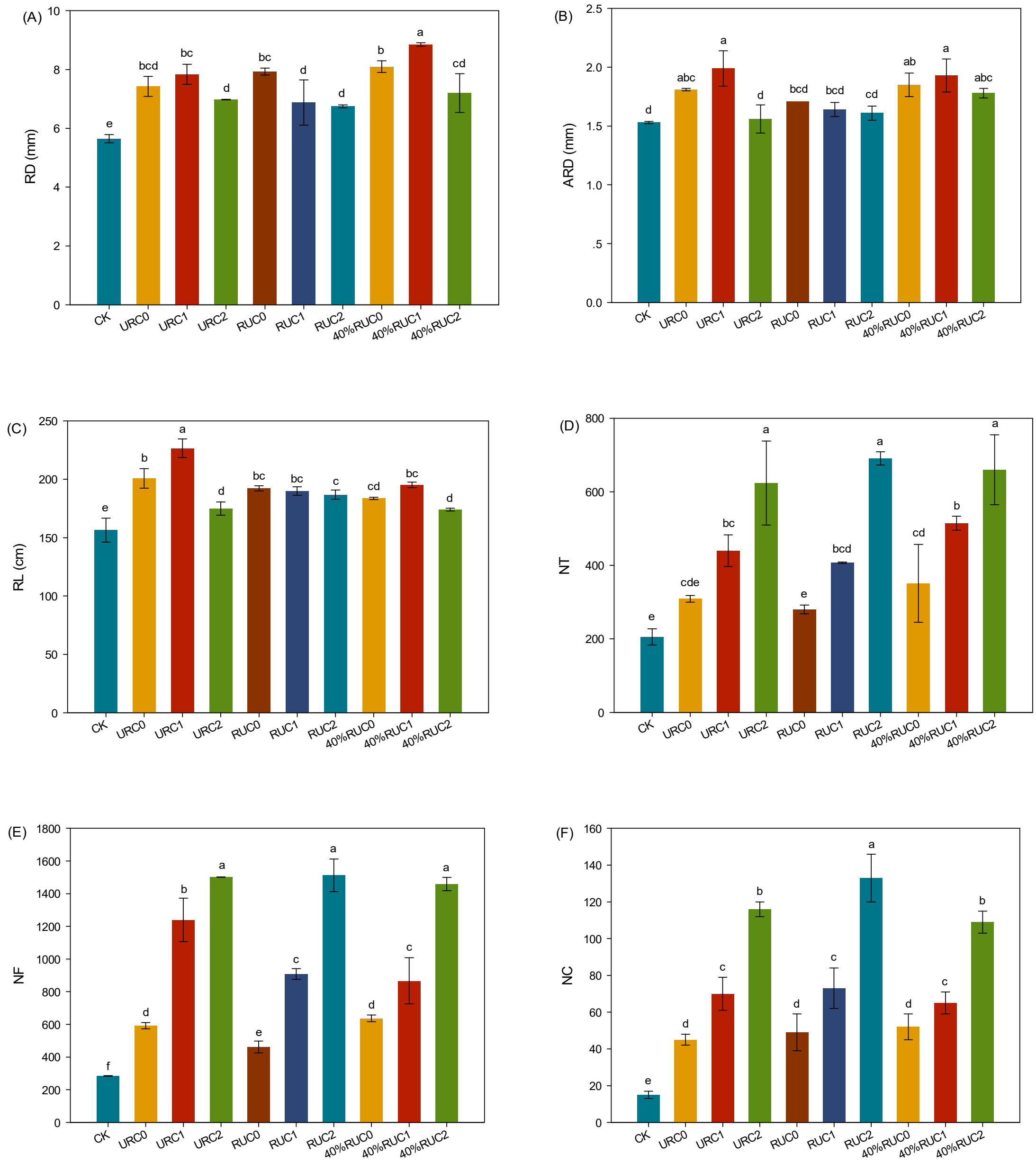

Figure 4. The effects of the combined application of biochar and nitrogen fertilizer on rapeseed root morphology (**A**–**F**). The same lowercase letter represents no significant difference ($p > 0.05$) between treatments. CK, no nitrogen fertilizer and biochar; URC0, single urea application; URC1, urea + 2% biochar; URC2, urea + 4% biochar; RUC0, single controlled-release urea application; RUC1, controlled-release urea + 2% biochar; RUC2, controlled-release urea + 4% biochar; 40% RUC0, 60% urea + 40% controlled-release urea; 40% RUC1, 60% urea + 40% controlled-release urea + 2% biochar; 40% RUC2, 60% urea + 40% controlled-release urea + 4% biochar.

3.5. Correlations between Soil Nutrients, Organic Carbon Components, Respiration, and Crop Root Traits

Correlation analysis was conducted between soil nutrients and mature soil organic carbon components (at the maturity stage), root traits, soil respiration rate (at the pod stage), and C/N ratio, as shown in Figure 5. Among the soil nutrients, SOM showed significant or highly significant positive correlations with TA, AN, AP, and AK. TA showed significant or highly significant positive correlations with SOM, TK, AN, AP, and AK. AN demonstrated significant or highly significant positive correlations with AP, AK, TK, TA, and SOM. The C/N ratio showed significant or highly significant positive correlations with soil SOM, TA, TP, TK, AN, AP, and AK. Among the root traits, NT, NF, and NC showed significant or highly significant positive correlations with SOM, TA, TK, AN, AP, AK, and C/N ratio. NT and NC also exhibited significant correlations with TP, while RD, ARD, and RL showed no significant relationships with soil nutrients. In terms of organic carbon components, TOC and POC showed significant or highly significant positive correlations with SOM, TA, TK, AN, AP, AK, and C/N ratio. WSOC exhibited a significant positive correlation with TA, while ROC showed positive correlations with soil SOM, TA, and AN but did not reach the significance level. TOC and POC were highly significantly and positively correlated with NT and NF, while POC showed a highly significant positive correlation with NC. RD, ARD, and RL showed no significant correlations with soil nutrients, C/N ratio, and soil carbon components (except for a highly significant positive correlation with WSOC) in root traits. RR exhibited significant or highly significant positive correlations with SOM, TA, TK, TP, AP, AK, NT, NF, NC, TOC, POC, and WSOC. These findings indicate that the combined application of biochar and nitrogen fertilizer primarily increases SOM, TA, TK, AN, AP, AK, C/N ratio, TOC, POC, and WSOC, thereby enhancing NT, NF, and NC, ultimately improving RR.

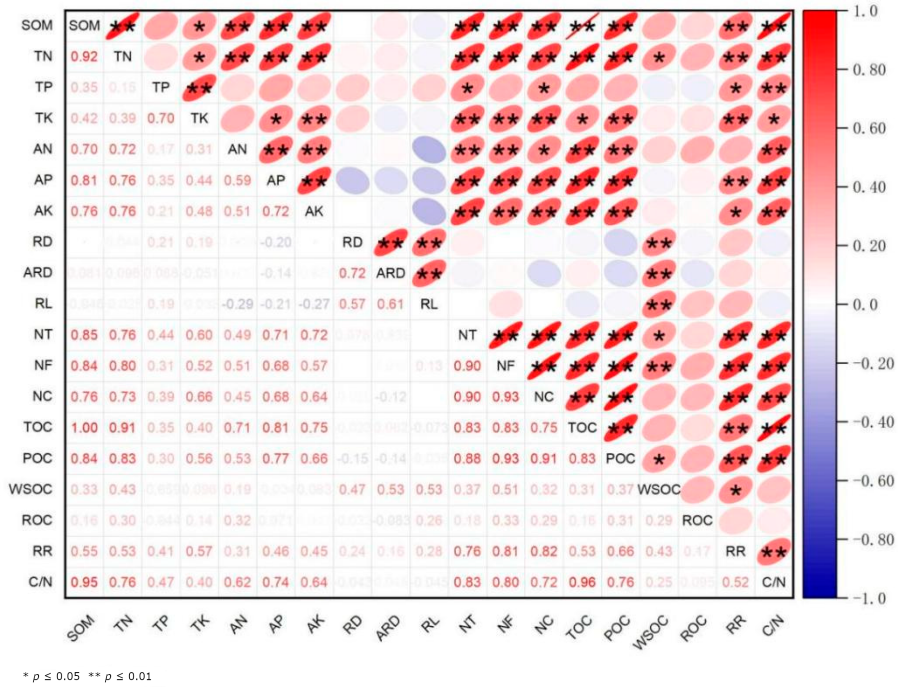

Figure 5. The correlation analysis between soil nutrients, soil organic carbon components, respiration rate, and crop root morphology; * and ** indicate significant differences at p = 0.05 and p = 0.01, respectively.

4. Discussion

4.1. Effects of Biochar and Different Types of Nitrogen Fertilizers on Soil Nutrients

This study shows that as biochar application rates increase, SOM and TA contents increase significantly. There is also a varying degree of increase in TP, TK, AN, AP, and AK. These findings are consistent with previous research on the effects of biochar on other soil types [38], as well as studies by Yao et al. [39], Kizito, S. et al. [40], and Qin Yao et al. [41] on the effects of biochar addition on soil nutrients. On the one hand, biochar itself is rich in mineral nutrients and can increase the content of phosphorus, potassium, calcium, magnesium, and nitrogen in the soil [42]. On the other hand, biochar has a strong adsorption capacity and can effectively bind with nutrients in the soil, reducing nutrient loss and improving the effectiveness of soil nutrients, thereby enhancing soil fertility [43]. However, the effect of adding biochar varies among the different types of nitrogen fertilizers. The reason for this difference may be attributed to the varying rates of nitrogen release or decomposition into ammonia among the three types of nitrogen fertilizers, which affect the content of available nitrogen in the soil. In addition, there is a highly significant positive correlation between available nitrogen and soil TOC, POC, TA, TK, AP, and AK contents (see Figure 5), which indirectly contributes to the differences in SOM and phosphorus–potassium content. Different types of nitrogen fertilizers have different effects on the growth of rapeseed, and the nutrient requirements of rapeseed vary according to its growth stage. Therefore, the impact of the combined application of biochar and different types of nitrogen fertilizers on soil nutrient content also varies. Further research is needed to explore the specific underlying mechanisms.

4.2. Effects of Biochar and Different Types of Nitrogen Fertilizers on Soil Carbon Content

Biochar itself is a carbon-rich material, and its application to soil is equivalent to the direct addition of exogenous organic carbon. This exogenous organic carbon, together with the unique structure of biochar, not only promotes the formation of soil aggregates [44] and the long-term sequestration of soil carbon in the form of POC but also provides suitable habitats and abundant carbon sources for nitrogen-fixing bacteria, stimulating their activity and thereby affecting the TA content of the soil [45]. WSOC and ROC are strongly influenced by plants and microorganisms, making them highly responsive to agricultural practices, including the application of biochar and different nitrogen fertilizer types. This study indicates that biochar is a major factor influencing TOC and POC, while WSOC and ROC are significantly influenced by both biochar and nitrogen fertilizer types, similar to the findings of Linlin Dong et al. [46] and Zhang et al. [47].

It was also found that, with the increase in biochar application, both TOC and POC in the soil showed an upward trend, consistent with previous research results [48]. ROC initially increased and then decreased with the addition of biochar; specifically, 2% biochar promoted an increase in ROC, while 4% biochar had an inhibitory effect. This is inconsistent with the results of other studies [49,50], which reported an increase in ROC content with an increase in biochar application. The reason for this discrepancy may be due to the pot experiment conducted in this study, which is more susceptible to the influence of biochar application rates than field experiments. Furthermore, this study suggests that the 40% RU treatment combined with 4% biochar is more favorable for increasing soil TOC and POC than the UR and RU treatments. This is mainly due to the fact that the 40% RU treatment promotes the synchronous release of available nitrogen and increases the nutrient utilization rate by rapeseed, thereby promoting root growth and increasing root exudates and litter. However, the specific underlying relationship needs further clarification. Additionally, this study also reveals that the effect of biochar on WSOC varies during different growth stages with different nitrogen fertilizer treatments. Specifically, significant effects were observed during the blooming stage for the RU and 40% RU treatments, while significant effects were observed during the maturation stage for the RU and UR treatments. The reasons for this are as follows: (1) Different growth stages show variations in the available nitrogen content in the soil, with the UR treatment having a nitrogen release period of 45–60 days, leading to nitrogen deficiency in the later stages of crop growth. The RU treatment has a nitrogen release period of 120 days, providing limited nutrients for early-stage crop growth, while the 40% RU treatment releases a portion of available nitrogen at both earlier and later stages, which undoubtedly affects plant growth and soil microenvironment at different times [51]. (2) The complex structure of biochar itself and the uncertainty of its action time may also contribute to variations in the microenvironment during the different growth stages. Overall, higher doses of biochar (4%) have a certain inhibitory effect on WSOC during the later stages of crop growth. The reasons for this may be attributed to various factors such as experimental methods, biochar types, crop species, and environmental factors [52]. Further in-depth research is needed to understand the specific temporal effects of biochar application rates and nitrogen fertilizer types on WSOC.

4.3. Effects of Biochar and Different Types of Nitrogen Fertilizers on Soil Respiration

Biochar is considered a permanent carbon sequestration mechanism in soils, as it can persist in the soil for a long time [53]. Many studies have shown that biochar can adsorb enzymes and organic substances in the soil, increase TOC content, and reduce soil CO_2 emissions [54,55]. However, there are also studies that have yielded different results, suggesting that biochar application to soil can enhance organic matter decomposition and increase soil CO_2 emissions [56–58]. The results of this experiment indicate that soil respiration increased with the addition of biochar, and the addition of 4% biochar significantly increased soil respiration, while the effect of 2% biochar on soil respiration was not significant. This suggests that biochar does not promote soil respiration [59] but rather reaches a certain threshold where it significantly increases with an increase in biochar application, thereby promoting soil respiration during the rapeseed growth season and potentially hindering carbon sequestration and emission reduction in agricultural ecosystems. The reasons for this difference may be related to experimental conditions, biochar materials, biochar application rates, soil types, crop species, and the duration of the experiment [60], which need further analysis based on specific circumstances.

The results of this experiment indicate that there was no significant difference in soil respiration during the different growth stages of rapeseed among the different types of nitrogen fertilizers, regardless of the presence of biochar. This may be because soil microbial activity is the primary source of soil respiration, and soil microbial activity is primarily carbon-limited, with nitrogen not being the main factor affecting microbial activity [61]. The lowest average soil respiration was observed in the 40% RU treatment, which may be related to the speed of nitrogen release or conversion to ammonia in different types of nitrogen fertilizers. The specific underlying mechanisms require further research for clarification.

4.4. Effects of Biochar and Different Types of Nitrogen Fertilizers on Root Traits of Rapeseed

Roots are not only important organs for crop water and nutrient uptake but also serve as synthesis organs for biopolymers such as hormones, amino acids, and organic acids. The morphology and physiological functions of roots directly influence the growth, development, yield, and quality of the aerial parts of the plant [62]. Due to the diversity of crops, biochar materials, and soil types, the effects of biochar on crop root growth are inconsistent, with many studies reporting varying degrees of promotion of crop root growth with biochar application [18,63], while other studies have reached contrasting conclusions. This indicates that the positive effects of biochar on crops are influenced by multiple factors, such as soil and crop types, biochar materials and application rates, and climatic conditions [64]. Most studies of the effects of biochar on crop root growth are short-term experiments, and the results are mixed [63]. The positive effects of biochar are mainly attributed to its mineral nutrient content, which can be directly utilized by crops, as well as its large surface area and strong adsorption capacity, enabling good nutrient binding and providing a larger living space for microbial communities, thereby improving soil nutrient status and soil enzyme activity, which is more favorable for crop root growth [65,66]. However, the negative effects of biochar may be due to the improper use of biochar, resulting in excessively high C/N ratios in the soil, which affects the uptake of nitrogen by crops [67]. Additionally, increasing soil pH can inhibit the availability of certain nutrients, thereby suppressing the growth of crop roots when biochar is added [68]. Further in-depth research is therefore needed to fully understand the effects of biochar on crop growth.

5. Conclusions

(1) Biochar significantly improves soil nutrient content. The combination of 40% RU at C1 level is more effective in improving soil nutrient status compared to RU and UR, while at the C2 level, it is more conducive to enhancing soil available nutrients.

(2) Addition of biochar significantly increases TOC and POC content, WSOC is strongly influenced by the type of nitrogen fertilizer that is used. Compared to the combination of UR, RU and biochar, the combination of 40% RU with 4% biochar is more effective in increasing TOC and POC content. The combination of 40%RU with 2% biochar shows a more pronounced effect in increasing ROC. The effect of biochar combined with different nitrogen fertilizer types on WSOC varies with the crop growth stage, but a higher dosage of biochar (4%) has some inhibitory effect on WSOC in the later stages of crop growth.
(3) Overall, the application of 4% biochar significantly increases RR, while the application of 2% biochar and different types of nitrogen fertilizers have no significant impact on soil respiration. Furthermore, the combination of 2% biochar with 40% RU has a carbon emission reduction effect compared to the UR and RU treatments.
(4) NT, NF and NC significantly increase with the addition of biochar, while other root traits show different responses to biochar among different types of nitrogen fertilizers. The combination of biochar with 40% RU is more beneficial for rapeseed root growth and development compared to RU and UR treatments.

In conclusion, the combination of 2% biochar with 40% RU effectively improves soil nutrient status, reduces soil carbon emissions, and promotes rapeseed root growth. Therefore, it can be widely applied in the cultivation of rapeseed in purple soil in the hilly drylands of Southwest China.

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