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RESEARCH PAPERS

Supplemented Biochar Mitigates the Ammonium Toxicity in Basil (*Ocimum basilicum* L.) Plants

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Abstract—Ammonium (NH_4^+) toxicity adversely affects the basil growth ability and productivity. Biochar amendment is an alternative that used to minimize or reverse the disturbances caused by abiotic stresses. Nevertheless, little is known regarding the mitigating effect of biochar on the NH_4^+-stressed basil. Thus, the present study was conducted to investigate whether biochar amendment could alleviate the NH_4^+ toxicity and determine how biochar helps reduce this degree. To this end, the basil plants were cultivated in a controlled environment (24°C/18°C, 14 h/10 h) and subjected to one of three $NH_4^+ : NO_3^-$ ratios (0 : 100, 50 : 50, and 100 : 0) at a constant N of 13 meq/L, corresponding with biochar treatments (3%, w/w) or without biochar. Chlorosis, stunted growth, and even foliage necrosis, were observed when the basil plants treated with 100% NH_4^+ nutrition. Concomitantly, interrupted photosynthesis and oxidative damage were also imposed by high NH_4^+ supply. By contrast, the presence of biochar significantly attenuated the NH_4^+ toxicity and, accordingly the growth was considerably improved for all three treatments. Additionally, biochar-amended basil plants showed significant increased photosynthesis-related parameters (net photosynthesis, the stomatal conductance, transpiration rates, and water use efficiency) and the antioxidative machinery (improved antioxidant enzymes and declined ROS accumulation and lipid peroxidation). Overall, our data enlightened the fertilization importance of biochar amendment, particularly pertaining to the basil plants at risk of NH_4^+ stresses.

Keywords: *Ocimum basilicum*, photosynthesis, antioxidant defense system, ROS accumulation, biochar effects, PCA analysis
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INTRODUCTION

Nitrogen (N) is a particularly paramount and imperative element that determines the agricultural crop productions. All the plants obtained their N source predominately in forms of ammonium (NH_4^+) and nitrate (NO_3^-) [1, 2]. Both can be absorbed by the plant roots, but the assimilation and utilization process regarding the biochemical and energetic aspects were shown in contrasting manners [3]. Theoretically, the NH_4^+ nutrition is preferred by plants as compared to that of NO_3^-, due to the fact that the assimilation of NH_4^+ is more metabolically efficient than NO_3^- [4].

Paradoxically, only a few plant species manifested better growth when NH_4^+ was applied as the exclusive N source or was unintentionally used [3–5]. Indeed, abundant NH_4^+ nutrition presented on the plants could inevitably cause the acidic stress, which has been shown as the primary cause of ammonium toxicity [6]. Consequently, multiple traits regarding the physiology, morphology, and biochemistry process were severely influenced, for example, the interference of the photosynthetic capacity; the disruption with the overproduction of ROS (reactive oxygen species) level damaging the antioxidant defence system; the impaired metabolic regulation system of the intracellular ions, etc. [3, 5, 6]. Furthermore, these integrated stimuli could further elicit the detrimental visual symptoms which was called NH_4^+ toxicity, as characterized by the stunted plant growth, leaf chlorosis even necrosis, and decreased root system [4, 7, 8].

Basil is a transcendent perennial herb that belongs to the *Lamiaceae* family, it is widely grown in tropical and temperate climates [9]. The demand of basil is increasingly growing due to the versatile properties. Pharmaceutically and gastronomically, it contains not only the essential oil, but also was rich in the distinctive volatile compounds and phytochemicals beneficial for the human health [10, 11]. While essential oils could be exploited as the antioxidants for food industry [12]. Ornamentally, basil also has the decorative properties because of the tender flowers and aromatic foliage [13]. However, our previous research identified that basil was extremely sensitive to high NH_4^+ nutrition [14]. Thus, the breeding of basil with the improvements of yield and quality were highly interrupted by the toxic level NH_4^+ input. Thereafter, the optimized fertilization strategy, such as the applied exogenous substances, could be considered as an efficient method for the alleviation of plant stresses.

Biochar (BC) is an organic raw material made from the thermochemical conversion of plant waste under the anoxia conditions [15, 16]. It has attributed an excellent approach for the crop yield improvement, soil fertility promotion, toxic element adsorption, and abiotic stress mitigation [15–17]. More importantly, numerous reports regarding the biochar applications on retaining the nutrient status were available [15, 17, 18]. For instance, the biochar addition in conjunction with other nitrogen fertilization regime notably increased the nitrogen use efficiency (NUE) and improved the crop yield and quality [19]. Biochar accordingly induced the sorption capacity of N and was expected to promote the photosynthesis ability [19, 20]. Additionally, Jia et al. [21] recently demonstrated that biochar may facilitate the antioxidant enzyme activity and therefore diminish the excess reactive oxygen species (ROS), which significantly enhanced the root activity. However, the study regarding the influences of biochar supplementation on the growth, photosynthesis, and antioxidant systems of NH_4^+-spiked basil, in particular of the alleviation of NH_4^+ toxicity in basil, remains incipient to date [22].

Given the above-mentioned information that plenty of benefits were delivered by the biochar application on the plants. Therefore, the main objective of the study conducted herein was to investigate whether the biochar-derived modifications in resource supplementation may mitigate the adverse effects of NH_4^+ toxicity, improve the growth, photosynthesis, and antioxidant systems in basil seedlings.

MATERIALS AND METHODS

Plant material and growth condition. The basil seed ‘Sweet basil’ was selected as the material and ordered from Blues Seeds Company (Shouguang, China). The seeds were sown in the 128-cell plug trays filled with moistured mini-K substrate (Klasmann-Deilmann GmbH company, Germany). The seeds were then germinated in 7 days after sowing (7 DAS). The basil seedlings were allowed to grow for further 25 days (37 DAS) and cultured with a nutrient solution named MNS (multipurpose nutrient solution) [23]. The seedlings were cultured under an alternating diurnal regime (14 h light by white LED [14] at 240 PPFD plus 10 h darkness) in a plant chamber equipped with air-conditioned temperature at 24°C (light condition) and 18°C (darkness condition).

Treatments and experimental procedures. Subsequently, the basil seedlings with uniform, similar morphology, and turned two true leaves were screened and selected and transplanted to new 128-cell plug trays (38 DAS). The transplanted seedlings were subjected to be irrigated by running tap water for 2 more days to incite all the nutrients leaching (40 DAS).

Afterwards, the basil seedling was equally allocated to be treated by one of three treatment solutions ($NH_4^+ : NO_3^-$ 0 : 100, 50 : 50, and 100 : 0) at a constant N (13 meq/L (Table 1), corresponding with or without 3% (W/W) amended biochar application [24]. The biochar used in this experiment was made at 550–600°C by carbonization for 4–6 h from the waste of maize straw (Yuzhongao Agriculture Technology company, China). The biochar had the specific surface area of 9.0 m^2/g, bulk density of 0.19 g/cm^3, pH of 10.24, total carbon at 650 g/kg, available phosphorus and available potassium at 10.2 and 55.65 g/kg, respectively. This trial performed was in a randomized complete design, by adopting a 2 × 3 factorial layout with three biological replicates, each replicate contains 10 basil plants. Thus, a total of 30 young basil plants underpinning one treatment were used in this study.

Destructive sampling and measurement of plant growth parameters. After the treatment solution was applied every two days for three weeks (60 DAS), the basil plants cultured with different treatment solutions showed distinct appearances. Then the plants were demounted out of the plug tray while the medium were washed off (61 DAS). The whole plant fresh weight (after the surface-blotted with absorbent paper; FW) and the dry weight (after being kept in an air-forced oven at 70°C for 72 h; DW) were determined by an electronic balance. A metal ruler was used to measure the leaf length and width, shoot length, and tap root length. The stem diameter was measured at 1 cm point above the root top with a vernier calliper. The three topmost true leaves were individually collected, quickly frozen in the liquid N_2, and stored at –80°C for further experiment.

Table 1. The nutrition compositions (in meq/L) from the treatment solutions

Nutrients sources	0 : 100 $NH_4^+ : NO_3^-$ without biochar	50 : 50 $NH_4^+ : NO_3^-$ without biochar	100 : 0 $NH_4^+ : NO_3^-$ without biochar
$Mg(NO_3)_2 \cdot 6H_2O$	1.3	0.6	–
KNO_3	4.8	–	–
$Ca(NO_3)_2 \cdot 4H_2O$	6.9	5.9	–
$MgSO_4 \cdot 7H_2O$	1.0	1.4	1.7
KH_2PO_4	1.0	–	2.0
$NH_4H_2PO_4$	–	2.0	–
$(NH_4)_2SO_4$	–	4.5	13
$CaCl_2 \cdot 6H_2O$	–	–	4.9
K_2SO_4	–	4.5	1.2

Estimation of the photosynthesis-related parameters. The photosynthesis-related parameters regarding the net photosynthesis (*P*n), the stomatal conductance (g_s), and transpiration rates (*T*r), were determined by using a portable photosynthesis measurement system (TARGAS-1, PP Systems, Amesbury, United States) before harvest. The water use efficiency (WUE) is calculated by *P*n/*T*r. Briefly, the mentioned parameters were measured from 8:30–11:00 a.m. on three fully expanded true leaves, one leaf was measured for three times. The leaf temperature is about 20°C and the other environmental conditions were identical as the previously set when cultivating the young basil.

Determinations of activities of the key antioxidant enzymes in leaf samples. The frozen leaf samples from different treatments were taken out from the –80°C and finely ground in a mortar over an ice bath. Approximately 0.1 g leaf powder was immediately weighted and mixed with the extraction buffer containing 2% polyvinylpyrrolidone, 1 mM EDTA, 0.05% Triton-x, and 50 mM PBS at pH 7.5. The homogenized solution was centrifuged (13000 rpm, 4°C for 20 min) in order to obtain the supernatant, which was further used for the quantifications of total soluble protein level.

The total protein content was determined by using Bradford's reagents [25]. The antioxidant enzyme activities were spectrophotometrically measured according to our previous publication [23] and calculated on basis of the determined protein content [23]. In specific, NBT (nitroblue tetrazolium) reduction reaction was used for determining the superoxide dismutase (SOD) activity. Catalase (CAT) activity was determined on basis of the reaction regarding the decomposition of H_2O_2 to H_2O. A method using the oxidation of ascorbate was utilized for qualifying the ascorbate peroxidase (APX) activity. Guaiacol peroxidase (POD) was estimated following the oxidation of guaiacol. The activity of dehydroascorbate reductases (DHAR) was measured on basis of the reconversion of dehydroascorbic acid (DHA) into ascorbic acid (ASC).

Determinations of MDA, $O_2^{\bullet -}$, and H_2O_2. The lipid peroxidation degree was reflected in terms of the MDA (malondialdehyde) concentration, while the latter was quantified on basis of the thiobarbituric acid (TBA) reaction [26]. The superoxide ($O_2^{\bullet -}$) content was determined following a sensitive procedure by using hydroxylamine oxidization according to Wu and von Tiedemann [27]. The hydrogen peroxide (H_2O_2) level was colorimetrically determined with a method by Mukherjee and Choudhuri [28]. The detailed procedure can be found in report of Li et al. [29].

Statistics and graphs. All the presented data are means ± SE of no less than 4 biological replications ($n \geq 4$). The significant differences among the treatments were determined following Duncan's multiple range test (one-way ANOVA) when $P = 0.05$ by using SAS v8.2 program (SAS Institute Inc, Cary, NC, USA). The bar graphs were plotted with Garphpad Prism software (8.0.2 Ver, GraphPad Software, Boston, United States). The PCA (principal component analysis) graph was created by Origin 2022 (Origin Lab Corp., Northampton, MA, United States) procedure.

RESULTS

Effect of $NH_4^+ : NO_3^-$ *Ratio and Biochar Application on the Shoot-Related Parameters*

The mixed nutrition of NH_4^+ and NO_3^- supplied or solely NO_3^--treated basil plants exhibited significantly better growth performance as compared to that treated with 100% NH_4^+ nutrition (Fig. 1). In particular, the NH_4^+-spiked basil plants developed the NH_4^+ toxicity symptoms as characterized by the severely inhibited shoot growth, stunted roots, and chlorosis (Fig. 1). These were further evidenced by the recorded shoot-related parameters: the shoot length and stem diame-

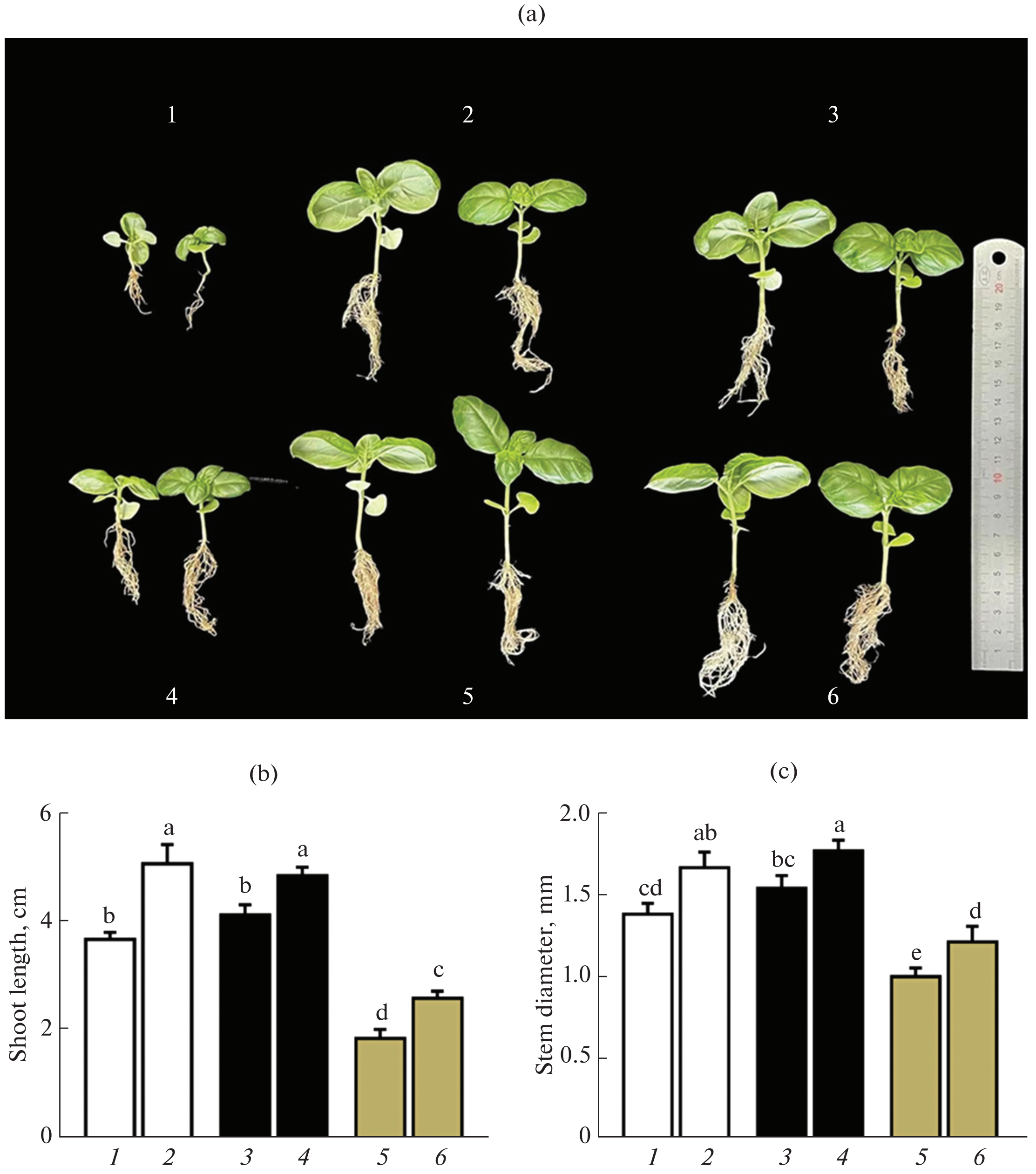

Fig. 1. Effect of three $NH_4^+ : NO_3^-$ ratios and biochar application on the (a) whole view of basil growth regarding (b) shoot length and (c) stem diameter. The significant differences among different treatments were determined following one-way ANOVA according to the Duncan's multiple range test at $P = 0.05$ and denoted by different lower-case letters over bars. Numbers indicate (a) 1: 100 : 0 $NH_4^+ : NO_3^-$ without biochar; 2: 50 : 50 $NH_4^+ : NO_3^-$ without biochar; 3: 0 : 100 $NH_4^+ : NO_3^-$ without biochar; 4: 100 : 0 $NH_4^+ : NO_3^-$ supplemented biochar; 5, 50 : 50 $NH_4^+ : NO_3^-$ supplemented biochar; 6: 0 : 100 $NH_4^+ : NO_3^-$ supplemented biochar; (b) and (c), (*1*) 0 : 100 $NH_4^+ : NO_3^-$ biochar (–), (*2*) 0 : 100 $NH_4^+ : NO_3^-$ biochar (+), (*3*) 50 : 50 $NH_4^+ : NO_3^-$ biochar (–), (*4*) 50 : 50 $NH_4^+ : NO_3^-$ biochar (+), (*5*) 100 :0 $NH_4^+ : NO_3^-$ biochar (–), and (*6*) 100 : 0 $NH_4^+ : NO_3^-$ biochar (+).

ter were markedly diminished by 54.5 and 35.3%, respectively, compared with that of the plants grown in 50 : 50 $NH_4^+ : NO_3^-$ regime (Fig. 1).

However, the supplementation of biochar individually to three $NH_4^+ : NO_3^-$ ratios significantly improved the shoot length and stem diameters, regardless of the $NH_4^+ : NO_3^-$ ratios (Fig. 1). Concomitantly, more important is, the addition of biochar significantly ameliorated the NH_4^+ toxicity degree as was apparent in the Fig. 1. In specific, it is notable that the nourishment of biochar on the NH_4^+-stressed basil plants con-

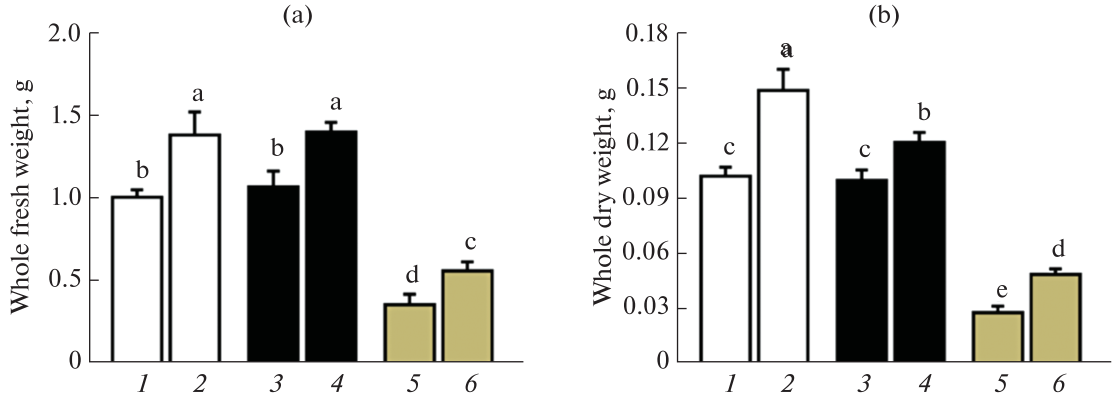

Fig. 2. Effect of three $NH_4^+ : NO_3^-$ ratios and biochar application on the basil plant biomass regarding (a) whole fresh weight and (b) whole dry weight. The significant differences among different treatments were determined by one-way ANOVA according to the Duncan's multiple range test at $P = 0.05$ and denoted by different lower-case letters over bars. Numbers indicate (*1*) 0 : 100 $NH_4^+ : NO_3^-$ biochar (–), (*2*) 0 : 100 $NH_4^+ : NO_3^-$ biochar (+), (*3*) 50 : 50 $NH_4^+ : NO_3^-$ biochar (–), (*4*) 50 : 50 $NH_4^+ : NO_3^-$ biochar (+), (*5*) 100 : 0 $NH_4^+ : NO_3^-$ biochar (–), and (*6*) 100 : 0 $NH_4^+ : NO_3^-$ biochar (+).

siderably increased the shoot length and stem length by 39.4 and 21.8%, respectively (Fig. 1).

Effect of $NH_4^+ : NO_3^-$ *Ratio and Biochar Application on the Plant Biomass*

The basil plant biomass herein in terms of the whole fresh weight and dry weight were also dramatically affected in response to the $NH_4^+ : NO_3^-$ supply with or without biochar supplementations (Fig. 2). Consistent with the findings mentioned above, the 100% NH_4^+-fed basil plants exhibited notable decreases of the whole plant biomass, irrespective of the biochar use considered (Fig. 2). As compared with the basil plants grown in 50 : 50 $NH_4^+ : NO_3^-$ regime, the solely NH_4^+ cultured basil plants significantly decreased by 66.3 and 81.8% of whole fresh weight and dry weight, respectively (Fig. 2).

More importantly, the individual addition of biochar to three $NH_4^+ : NO_3^-$ treatments significantly improved the whole biomass. For instance, the added biochar to the 100 : 0 $NH_4^+ : NO_3^-$ treated plants significantly increased the fresh weight and dry weight by 56.8% and 1.5-fold, respectively (Fig. 2).

Effect of $NH_4^+ : NO_3^-$ *Ratio and Biochar Application on the Leaf- and Root-related Parameters*

The leaf parameters, including the leaf length and width, and the tap root length were all greatly altered when treated with different $NH_4^+ : NO_3^-$ ratios and biochar (Fig. 3).

Similar results with the above were conferred when cultured with different $NH_4^+ : NO_3^-$ treatments. Notably, the leaf length and width together with the tap root length of 100% NH_4^+-fed plants were significantly inhibited (Fig. 3). Compared with the plants cultivated in 50 : 50 $NH_4^+ : NO_3^-$ regime, the leaf length and width along with the tap root length of 100% NH_4^+-fed plants were drastically declined by 55.8, 56.6, and 34.9%, respectively (Fig. 3). It is worthy to note that the NH_4^+ stressed basil plants significantly ameliorated these corresponding three parameters by 57.9, 55.7, and 32.6%, respectively, when biochar was added for the cultivation (Fig. 3).

Photosynthesis-Related Parameters as Affected by the $NH_4^+ : NO_3^-$ *Ratio and Biochar Application*

The photosynthesis traits, such as the net photosynthesis (*P*n), the stomatal conductance (g_s), the transpiration rates (*T*r), and the water use efficiency (WUE) were all greatly altered in response to different $NH_4^+ : NO_3^-$ treatments and biochar applications.

As is apparent in Fig. 4, these four photosynthesis related parameters were severely disturbed when the basil plants were treated with 100% NH_4^+ nutrition. In addition, supplementing the basil plants with biochar significantly improved these four parameters, regardless of the $NH_4^+ : NO_3^-$ ratios. In particular, net photosynthesis (*P*n) was significantly increased by 89.1% in basil plants treated with NH_4^+ alone, while stomatal conductance (g_s) and transpiration rates (*T*r) were sig-

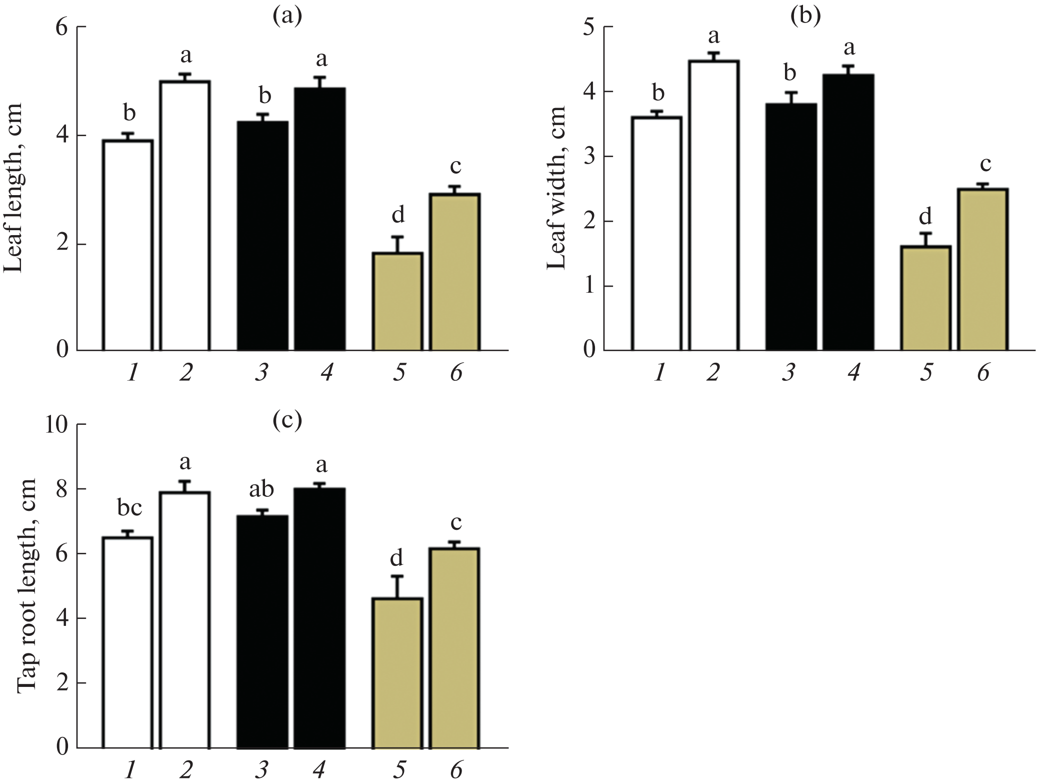

Fig. 3. The basil (a) leaf length, (b) leaf width, and (c) tap root length as affected by three $NH_4^+ : NO_3^-$ ratios and biochar application. The significant differences among different treatments were determined by using one-way ANOVA according to the Duncan's multiple range test at $P = 0.05$ and denoted by different lower-case letters over bars. Numbers indicate (*1*) 0 : 100 $NH_4^+ : NO_3^-$ biochar (–), (*2*) 0 : 100 $NH_4^+ : NO_3^-$ biochar (+), (*3*) 50 : 50 $NH_4^+ : NO_3^-$ biochar (–), (*4*) 50 : 50 $NH_4^+ : NO_3^-$ biochar (+), (*5*) 100 : 0 $NH_4^+ : NO_3^-$ biochar (–), and (*6*) 100 : 0 $NH_4^+ : NO_3^-$ biochar (+).

nificantly improved by 65.6 and 67.6%, respectively (Fig. 4).

Interestingly, the basil plants grown in 50 : 50 $NH_4^+ : NO_3^-$ regime plus biochar exhibited considerably greater net photosynthesis value and higher water use efficiency (WUE) when compared with that grown in 100% NO_3^- nutrition supply (Fig. 4).

The Antioxidant Enzyme Activities as Affected by the $NH_4^+ : NO_3^-$ Ratio and Biochar Application

When the plants suffered the stresses, the oxidative damages would be incited and the plants triggered the antioxidant defense system regarding the antioxidant enzymes. We therefore investigated the antioxidant enzyme productions in response to the $NH_4^+ : NO_3^-$ ratios and biochar applications.

In the absence of biochar, it could be found that 100% NH_4^+ nutrition-treated basil plants notably reinforced the productions of APX, POD, and DHAR by 66.7, 80, and 53.3%, respectively, as compared to that grown in 50 : 50 $NH_4^+ : NO_3^-$ ratio supply (Fig. 5). Nevertheless, it could be noticed that the basil plants cultivated with 100% NH_4^+ nutrition significantly declined the SOD concentration as compared to that grown in either 100% NO_3^- or 50 : 50 $NH_4^+ : NO_3^-$ (Fig. 5). By contrast, the presence of biochar herein for the culture of basil dramatically increased certain antioxidant enzyme activities, especially for the CAT, APX, and DHAR. Take the CAT activity as an example, 0 : 100 $NH_4^+ : NO_3^-$ B (+), 50 : 50 $NH_4^+ : NO_3^-$ B (+), and 100 : 0 $NH_4^+ : NO_3^-$ B (+) significantly improved this level by 46.1, 43.3, and 60%, respectively (Fig. 5).

The Oxidative Stresses (ROS Concentration) as Affected by the $NH_4^+ : NO_3^-$ Ratio and Biochar Application

Thereafter, the oxidative stress degree in terms of the ROS content ($O_2^{\bullet-}$ and H_2O_2) was also determined. It was observed that the basil plants subjected to high NH_4^+ supply pronounced excessive ROS and large amounts of lipid peroxidation (LPO). Herein, the basil plants cultivated in 100 : 0 $NH_4^+ : NO_3^-$ without biochar application significantly increased the $O_2^{\bullet-}$ and

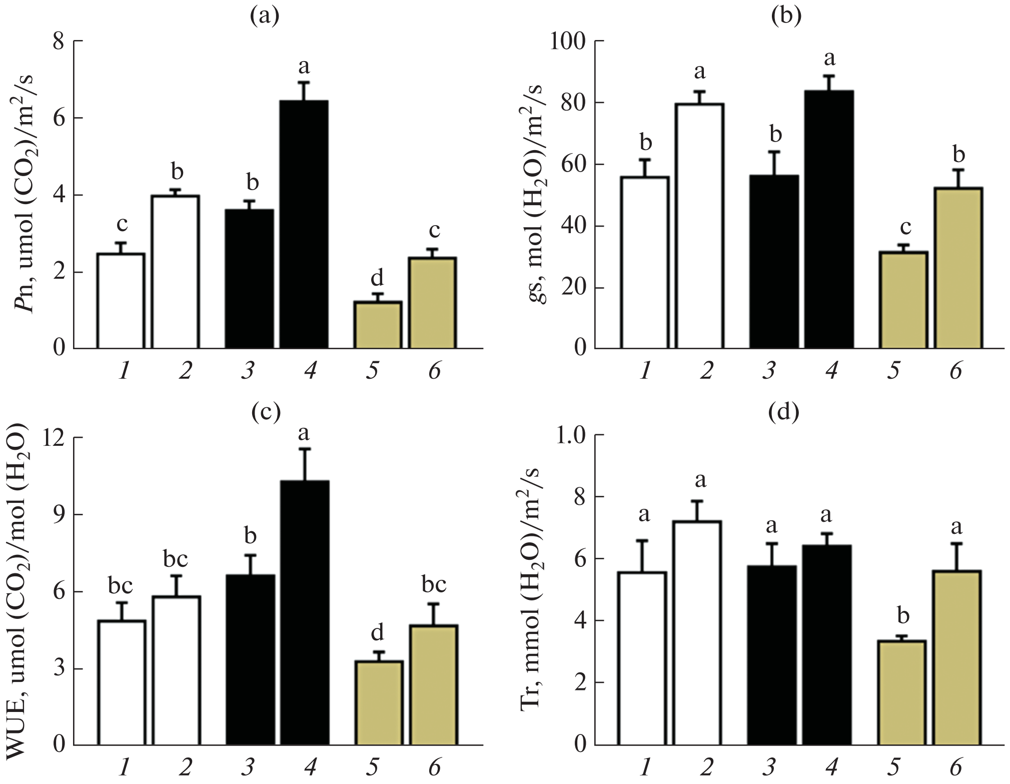

Fig. 4. The basil photosynthesis-related parameters regarding (a) the net photosynthetic rate (*P*n), (b) the stomatal conductance (*g*s), (c) water use efficiency (WUE), and (d) the transpiration rate (*Tr*) in response to three $NH_4^+ : NO_3^-$ ratios and biochar application. The significant differences among different treatments were determined according to one-way ANOVA by the Duncan's multiple range test at $P = 0.05$ and denoted by different lower-case letters over bars. Numbers indicate (*1*) 0 : 100 $NH_4^+ : NO_3^-$ biochar (–), (*2*) 0 : 100 $NH_4^+ : NO_3^-$ biochar (+), (*3*) 50 : 50 $NH_4^+ : NO_3^-$ biochar (–), (*4*) 50 : 50 $NH_4^+ : NO_3^-$ biochar (+), (*5*) 100 : 0 $NH_4^+ : NO_3^-$ biochar (–), and (*6*) 100 : 0 $NH_4^+ : NO_3^-$ biochar (+).

H_2O_2 level by 65.3 and 47.8%, respectively, as compared with that cultured in 50 : 50 $NH_4^+ : NO_3^-$ regime (Fig. 6). Concomitantly, the exclusive NH_4^+ nutrition supplied on the basil plants also markedly improved the MDA content by 44.2% compared to that treated with 50 : 50 $NH_4^+ : NO_3^-$ (Fig. 6).

However, more importantly, the nourishment of biochar on the basil plants significantly decreased the ROS concentration and MDA level, irrespective of the $NH_4^+ : NO_3^-$ supply. Take the NH_4^+-stressed plants as an example, the addition of biochar to three $NH_4^+ : NO_3^-$ ratio distinctly declined the $O_2^{\bullet -}$, H_2O_2, and MDA concentration by 37.29, 31.45, and 11.3%, respectively (Fig. 6).

The PCA Analysis of the Antioxidant Defense System Response to $NH_4^+ : NO_3^-$ *Ratio and Biochar Application*

In order to visualize the response of effects of $NH_4^+ : NO_3^-$ supply and the biochar application on the antioxidant defense system, as well as the relationships among the treatments or parameters, PCA was conducted based on the recorded antioxidant system data set including the antioxidant enzyme activities and the ROS ($O_2^{\bullet -}$, H_2O_2) accumulation as well as the lipid peroxidation (MDA) (Fig. 7).

The computed mode captured the associated parameters by the first two principal components that explained a total of 77.4% of the total recorded data variability (PC1 = 50.1%; PC2 = 27.3%). As is displayed in Fig. 7, the 100% NH_4^+-treated samples were mainly distributed on the right quadrants of the PC1 scatter direction, while other samples were mainly located on the left part of the PC1 scatter plot (Fig. 7). In addition, the biochar treated samples ('B +') were mainly scattered over the PC2, whereas the samples without biochar mainly spread below the PC2 (Fig. 7), which indicated that a negative relation of the antioxidant defense system was conferred between the biochar supplementation and non-biochar supplementation.

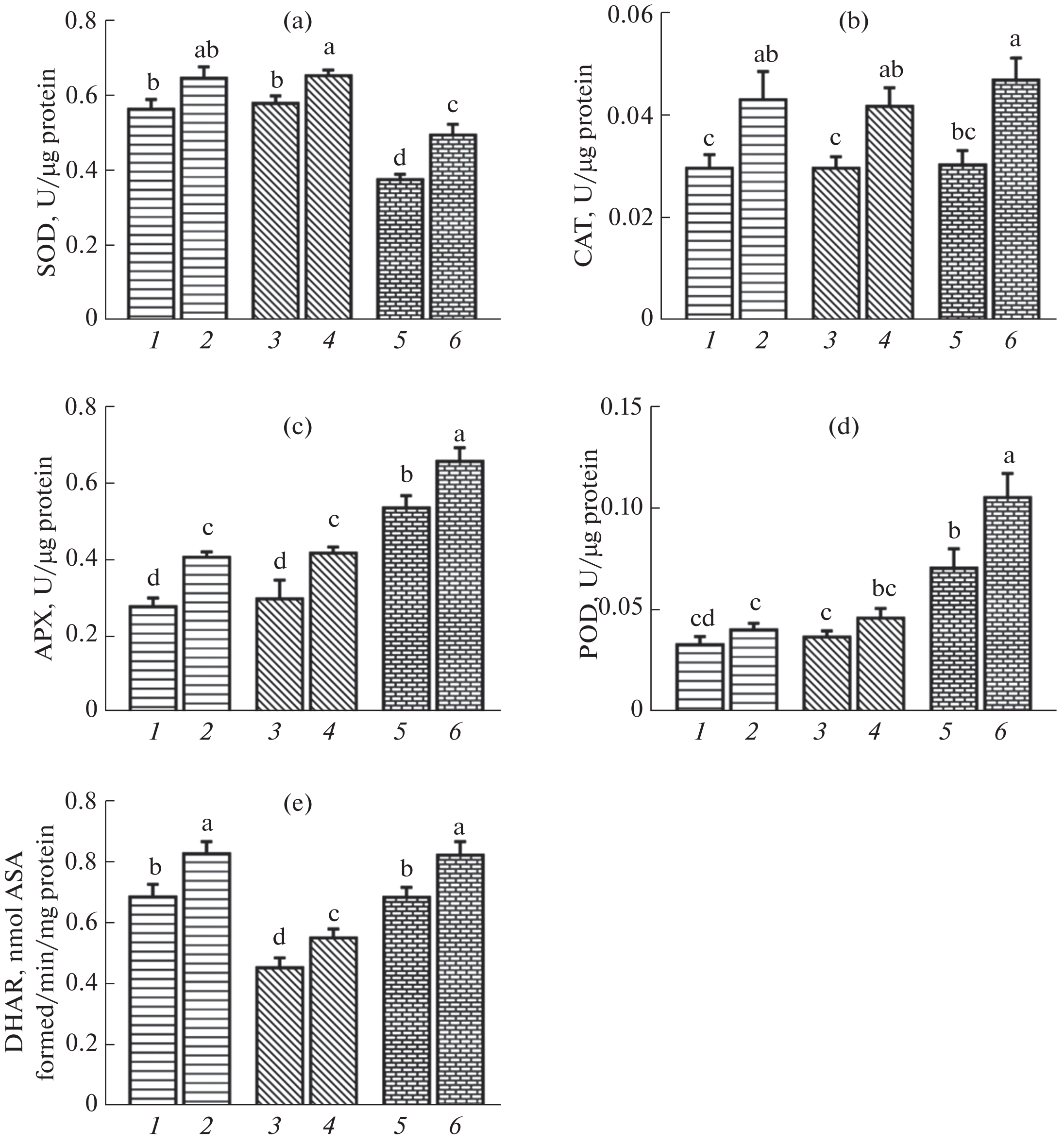

Fig. 5. The antioxidant enzymes level regarding (a) SOD activity, (b) CAT activity, (c) APX activity, (d) POD activity, and (e) DHAR activity in response to three $NH_4^+ : NO_3^-$ ratios and biochar application in basil. The significant differences among different treatments were determined according to one-way ANOVA by the Duncan's multiple range test at $P = 0.05$ and denoted by different lower-case letters over bars. Numbers indicate (*1*) 0 : 100 $NH_4^+ : NO_3^-$ biochar (–), (*2*) 0 : 100 $NH_4^+ : NO_3^-$ biochar (+), (*3*) 50 : 50 $NH_4^+ : NO_3^-$ biochar (–), (*4*) 50 : 50 $NH_4^+ : NO_3^-$ biochar (+), (*5*) 100 : 0 $NH_4^+ : NO_3^-$ biochar (–), and (*6*) 100 : 0 $NH_4^+ : NO_3^-$ biochar (+).

DISCUSSION

The biochar-induced alleviation effects against the NH_4^+ toxicity to date remained speculative even through the biochar benefits have been previously reported in many plant species [15–19]. Therefore, the main objective of this trail carried out herein was to determine whether exogenous biochar will attenuate the damages caused by NH_4^+ toxicity.

Biochar had been shown to improve plenty of desirable plant physiological and morphological aspects, consequently imparting the better growth quality and productions [16]. In this study, the biochar addition to the basil plants significantly improved the growth in terms of the shoot, leaf, and root-related parameters, regardless of the $NH_4^+ : NO_3^-$ ratios (Figs. 1–3). These findings well agreed with multiple previous publications, such as wheat [30] and tomato [31], they all

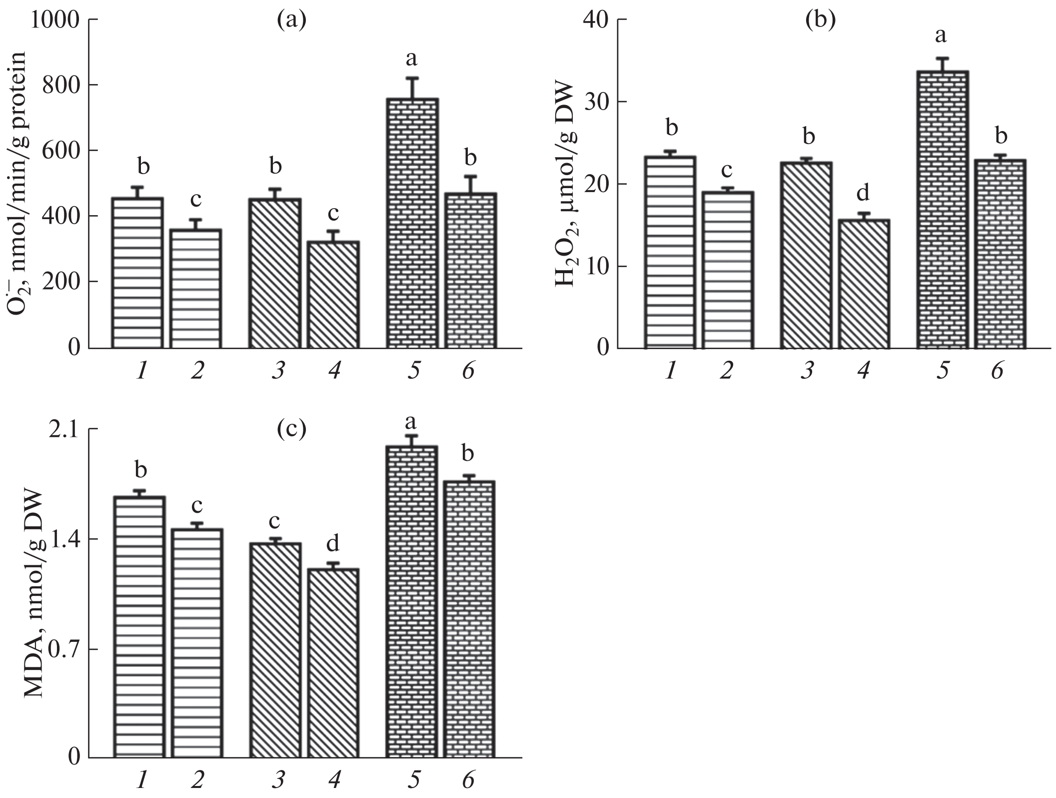

Fig. 6. The ROS accumulations regarding (a) $O_2^{\cdot -}$ content, (b) H_2O_2 content, and lipid peroxidation degree of MDA content in response to three $NH_4^+ : NO_3^-$ ratios and biochar application in basil. The significant differences among different treatments were determined following one-way ANOVA by the Duncan's multiple range test at $P = 0.05$ and denoted by different lower-case letters over bars. Numbers indicate (*1*) 0 : 100 $NH_4^+ : NO_3^-$ biochar (-), (*2*) 0 : 100 $NH_4^+ : NO_3^-$ biochar (+), (*3*) 50 : 50 $NH_4^+ : NO_3^-$ biochar (–), (*4*) 50 : 50 $NH_4^+ : NO_3^-$ biochar (+), (*5*) 100 : 0 $NH_4^+ : NO_3^-$ biochar (–), and (*6*) 100 : 0 $NH_4^+ : NO_3^-$ biochar (+).

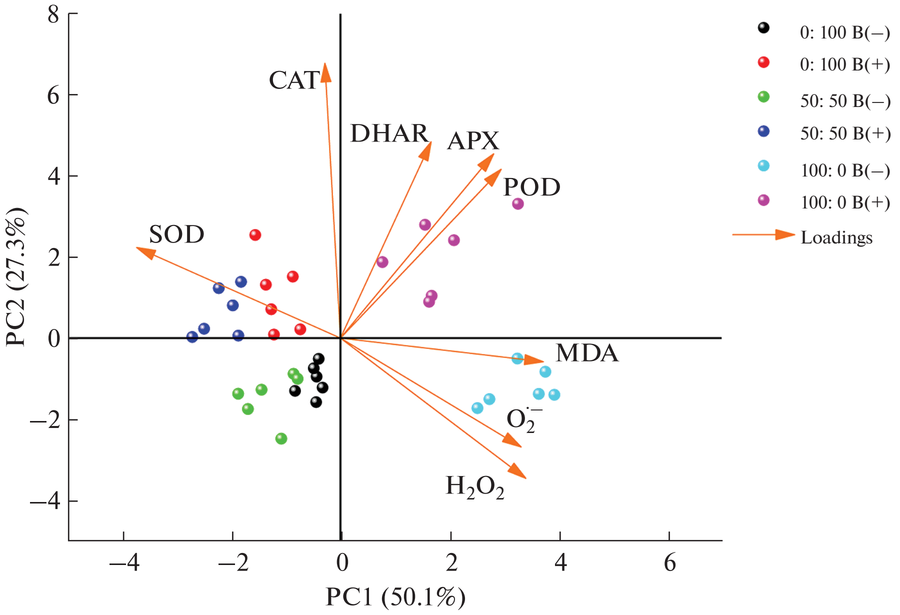

Fig. 7. The principal component analysis (PCA) on the indices underpinning the antioxidant defense and oxidative damage. APX, ascorbate peroxidise; CAT, catalase; DHAR, dehydroascorbate reductase; H_2O_2, hydrogen peroxide; MDA, malondialdehyde; $O_2^{\cdot -}$, superoxide anion; SOD, superoxide dismutase; POD, guaiacol peroxidise.

showed that the plant growth and yield were all augmented after biochar was implemented. Thus, these findings established the beneficial role of biochar involving in the basil growth ability and development, thereby contributing to higher sustainable productions [16, 17, 19].

As previously stated, the 100% NH_4^+ nutrition on basil plants elicited the typical NH_4^+ toxicity phenomenon, as characterized by the stunted growth and chlorosis (Fig. 1). These symptoms were in line with our early reports found that basil was extremely sensitive to high NH_4^+ supply [14].

However, as the most crucial finding, biochar is believed to reinforce the tolerance and consequently mitigate the adverse effects caused by the abiotic stresses, such as the salt stress and drought [16]. In this experiment, the NH_4^+ toxicity degree of basil plants was apparently reduced after the nourishment of biochar (Fig. 1). This phenomenon was mainly ascribed to the fact that biochar has been shown to change the soil physicochemical properties and the plant traits, thereby multiple important plant processes, such as the nutrition absorption and ion regulation, could be ameliorated [16–18]. Therefore, the application of biochar to the basil plants could improve the growth ability and alleviate the NH_4^+ toxicity degree.

The photosynthesis related traits were visibly influenced in response to the $NH_4^+ : NO_3^-$ supply and biochar application (Fig. 4). Indeed, the photosynthetic ability was significantly decreased when 100% NH_4^+ was applied, which was in line with the finding in our previous publication [14]. And the similar photosynthetic damages were found in salvia [23], cabbage [3], and lettuce [3].

The net photosynthesis (*P*n) rate is regarded as an important plant diagnosis technique reflecting the plant physiological status and nutrition assimilations performance [32]. Usually, this value is associated with the stress degree that the plants suffered. It is obvious that the *P*n rate of NH_4^+-derived basil plants was dramatically diminished as comapred to the plants grown in 50: 50 and 0 : 100 $NH_4^+ : NO_3^-$ regime (Fig. 4). It revealed that the 100% NH_4^+-treated plants possessed a disturbed photosynthesis, this was in agreement with the finding in rice [33]. However, the addition of biochar to NH_4^+-stressed basil plants significantly increased the *P*n rate, indicating the damage of NH_4^+ toxicity was reduced by biochar. Similarly, the stomatal conductance (*g*s) is defined as the stomatal opening degree and this measure is related to the carbon uptake concurrently with respect to the water loss [34]. Accordingly, the water use efficiency (WUE) and the transpiration rate (*T*r) were inevitably linked to the stomata opening [35]. In this investigation, the recorded four parameters were all suppressed when the plants under NH_4^+ stresses (Fig. 4). However, these were promoted when biochar was applied. On the one hand, this may be attributed to that biochar addition ameliorated the basil growth ability; on the other hand, biochar diminished the harm to the basil root system, thereby reinforced the photosynthetic ability against the high NH_4^+ nutrition. A previous study also suggested that biochar application improved the *P*n, g_s, WUE, and *T*r [36].

Generally, plants have sophisticated strategies in maintaining the dynamic equilibrium of ROS (reactive oxygen species, mainly by mainly by $O_2^{\bullet -}$ and H_2O_2) regarding the production and detoxification [23]. And this balance was tightly regulated by the antioxidant defence system, such as the stimulated ROS scavenging antioxidant enzymes [36]. However, this balance in plants could be interrupted when suffering severe abiotic stresses [23, 29, 36]. On the one hand, previous studies advocated that high NH_4^+ nutrition-stimulated the osmotic changes could intensify the ROS accumulation, accordingly causing greater lipid peroxidation (MDA) and pigment degradation [3, 7, 8]. In our experiment of biochar-deficiency basil plants, the exclusive NH_4^+ nutrition significantly improved the concentration of APX, POD, and DHAR (Fig. 5). Consistently, as stated above, the $O_2^{\bullet -}$, H_2O_2, and MDA contents of 100% NH_4^+-cultivated plants were shown to be notably higher relative to that grown in other treatments (Fig. 6). On the other hand, the biochar application on the enhancement of antioxidant defense system against the oxidative stresses has been well documented [37]. For instance, the improvements of antioxidant enzymes by exogenous biochar were reported in soybean [37], maize [38], and sugar beet [39]. As the first sensing line of ROS, the SOD could be a prime candidate not only for the transfer of $O_2^{\bullet -}$ to H_2O_2, but also for the efficient H_2O_2 decomposition involving CAT, APX, POD, and DHAR [23, 37, 39]. It has been frequently reported that the exogenous application of biochar increased the antioxidative defense capacity for the reduction of the excessively produced ROS [37–40]. Indeed, as expected, in particular of the 100% NH_4^+ supply, we showed that the investigated antioxidant enzymes herein were significantly improved after biochar nourishment (Fig. 5), illustrating the protective strategy of biochar by basil plants against the NH_4^+ toxicity. Meanwhile, the ROS concentration and the lipid peroxidation degree (MDA level) were also conspicuously declined (Fig. 6), which accordingly suggested that the tight regulation of biochar against the NH_4^+-stressed ROS metabolism [39, 40]. Therefore, biochar is able to increase the antioxidative capacity, as characterized by the reinforced antioxidant enzyme concentration and the declined ROS accumulation together with the decreased lipid peroxidation.

In conclusion, the study undertaken herein firstly showed that the combined nutrition of NH_4^+ and NO_3^- or sole NO_3^- nutrition were more beneficial for basil growth, compared with solely NH_4^+-treated plants. Besides, the basil was identified to be highly sensitive to high NH_4^+ nutrition supply as it exhibited the typical NH_4^+ toxicity symptoms. We also concluded that the biochar addition to the soilless substrate notably ameliorated the basil plant growth through improving the physiological, morphological, and biochemical traits. More importantly, the NH_4^+ toxicity degree by 100% NH_4^+ nutrition-treated basil was significantly attenuated after amended biochar. This alleviating effect of biochar may be attributed to the enhanced photosynthesis, declined lipid peroxidation (MDA), improved antioxidative machinery for scavenging overproduced ROS. Accordingly, NH_4^+ toxicity in basil could be alleviated by exogenous biochar application.

ABBREVIATIONS AND NOTATION

BC	biochar
DAS	days after sowing
NUE	nitrogen use efficiency
ROS	reactive oxygen species
WUE	water use efficiency

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ETHICS APPROVAL AND CONSENT TO PARTICIPATE

This work does not contain any studies involving human and animal subjects.

CONFLICT OF INTEREST

The authors of this work declare that they have no conflicts of interest.

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