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Research Article

Mechanisms of Stress Alleviation after Lime and Biochar Applications for *Brassica napus* L. in Cadmium-Contaminated Soil

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Lime and biochar amendments are widely used to immobilize cadmium (Cd) in agricultural soils and to ensure food security. However, the effects of these two soil amendments on the mechanisms of Cd stress alleviation in crops are unclear. Therefore, the effects of lime and biochar applications on Cd uptake, transport, subcellular distribution, antioxidant system, N metabolism, and related factors were examined in a soil-*Brassica napus* L. (*B. napus*) system. We found that lime application significantly increased the root Cd content by 41.5% but decreased Cd TF and shoot Cd by 81.0% and 74.3%, respectively, whereas biochar amendment decreased root and shoot Cd contents by 67.6% and 34.3%, respectively, but increased Cd TF by 104.1%. Lime treatment immobilized Cd in the cell wall of the root to reduce Cd transport, but biochar treatment increased the soluble fraction of Cd in root cells to improve the migration capacity of Cd. The significant negative relationship between the soil exchangeable Cd and Ca and the positive relationships between Cd and Ca both in shoot and root indicated that the Ca mediated Cd transport from soil to *B. napus* after lime and biochar applications. Additionally, lime amendment increased Cd proportion in the root cell walls to immobilize Cd, but biochar amendment increased Cd proportion in the soluble fraction to enhance Cd migration. Furthermore, biochar application significantly increased SOD, CAT, and POD by 17.5%, 95.4%, and 26.6%, whereas lime amendment only significantly enhanced CAT by 51.0%. Besides, both of biochar and lime applications increased shoot N content and GDH activity, but only the shoot NO_3^- content and nitrate reductase under biochar treatment were significantly altered. Overall, these findings suggested that lime is more efficient in reducing the transport of Cd from underground to aboveground and in improving Cd tolerance, whereas biochar tends to improve the antioxidant capacity and facilitate N metabolism. These results will provide significant strategies for selecting appropriate amendments to ensure the crops safety.

1. Introduction

Cadmium (Cd) levels in agricultural soils are continuously increasing in China [1, 2], which is usually due to human activities, such as sewage irrigation, atmospheric deposition, and application of chemical fertilizers and pesticides [3–5]. Cd is a nonessential element for plants but can disrupt physiological processes, accumulate in crops, and even enter the food chain to threaten human health since it has a relatively high solubility in soil and high translocation in plants [1, 6, 7]. It is of great significance to remediate Cd-contaminated agricultural soil.

Application of soil amendments is commonly used to remediate Cd-contaminated agricultural soils, which can decrease the Cd contents in crops by immobilizing Cd through complexation, adsorption, or ion exchange [8]. Lime and biochar are typical soil amendments because of their low cost, availability, and high efficiency [8–11]. Lime can effectively decrease the ability/bioavailability of Cd in soils by increasing soil pH, which thereby restricts crop Cd uptake [11, 12]. The large amount calcium (Ca^{2+}) derived from liming materials not only has a significant positive effect on soil aggregation and structural stability, but also mediate the growth and stress alleviation of plant because it was the essential macroelement [13, 14]. However, little is known about the effect of Ca on plant stress alleviation after lime application in Cd-contaminated soils. Biochar has larger surface areas, higher cation exchange capacity, and higher porosity compared to other amendments [2, 4]. Numerous studies have shown that biochar can immobilize Cd and act as a slow-acting fertilizer [4, 15, 16], whereas the underlying mechanisms of stress alleviation in plants after biochar amendment are still unclear.

Crops have developed various mechanisms to cope with Cd stress, such as cell wall fixation, vacuolar compartmentalization, and antioxidant systems [6]. Cell wall components, namely, pectin, cellulose, and hemicellulose, can fix Cd by negatively charged groups such as $-COO^-$, -OH, and -SH [17, 18]. Ca^{2+} is an essential macroelement and also cross-linked with negatively charged $-COO^-$ to contribute to the cell wall structure and extensibility [13]. Larger Ca^{2+} contents in roots may stimulate the cell wall to produce more $-COO^-$, which may promote the fixation of more Cd^{2+} because of the same divalent cations and share transport channels and binding sites [19]. Both of lime and biochar amendments could change the soil exchangeable Ca^{2+}; however, whether these amendments mediated Cd transformation and tolerance in plants by Ca dynamics in the soil-plant system is still unclear. In addition, vacuoles can compartmentalize Cd by forming complexes with phytochelatins, S_2^-, organic acid anions, and amino acids [1]. These two tolerance mechanisms tend to fix Cd belowground and thus reduce its transport to the aerial plant parts. Although many studies have reported that lime and biochar can significantly reduce the available Cd contents in soils and decrease Cd concentrations in plants [11, 12, 20], whether the subcellular reallocation, cell wall composition, and the underlying physiological mechanisms are different after lime and biochar amendment remains unclear.

Plants can also stimulate the antioxidant enzyme system to alleviate oxidative stress (i.e., membrane permeability and subsequent generation of reactive oxygen species (ROS)) caused by Cd, such as by synthesizing superoxide (SOD), catalase (CAT), and peroxidase (POD) [21]. Some studies showed that biochar amendments enhanced SOD, CAT, and POD activities and reduced the ROS content [15, 22, 23]. Lime application also improved the activities of antioxidant enzymes by decreasing Cd stress [24]. The activity of plant antioxidant enzymes also increased with soil nutrient status [25–27]. But biochar application increased nutrient supply in Cd-contaminated soil, and the mechanism of its influence on antioxidant enzyme activity needs further study.

Nitrogen (N) is an important component of various structural, functional, and genetic molecules in plant cells and plays a vital role in defending various stresses [27–29]. Soil amendments not only change Cd migration and plant resistance but may also affect N metabolism. Previous studies have found that applications of lime [30] and biochar [31] could increase N availability. Furthermore, lime enhanced the activity of nitrate reductase (NR) and N use efficiency of maize [32]. Biochar also enhanced the N use efficiency by improving the activities of N metabolic enzymes (e.g., NR, glutamine synthetase (GS), and glutamate dehydrogenase (GDH), which accelerated nitrate (NO_3^-) assimilation in crabapple and cabbage [31, 33]. However, these studies were all conducted in clean soil that were not contaminated by heavy metals. The effect of lime or biochar on plant N metabolism in Cd-contaminated agricultural systems needs to be further explored.

Brassica napus L. (*B. napus*) is a widely cultivated oil crop in southern China, but its growth is constrained by Cd stress and low N use efficiency [17, 34]. Previous studies focused on the stabilization mechanism of soil Cd under the applications of lime and biochar, but little attention was paid to the tolerance mechanism of Cd in plant after lime and biochar treatments. To better understand the comprehensive effects of commonly used soil amendments on Cd-contaminated soil-*B. napus* systems, we conducted *B. napus* pot experiments with lime or biochar application. Specifically, we hypothesized that (1) the Cd migration in soil-*B. napus* systems varies between lime and biochar treatments, (2) the biochar treatment had stronger impacts on *B. napus* antioxidant system, and (3) the N metabolism for *B. napus*.

2. Materials and Methods

2.1. Soil Collection, Plant Materials, and Growth Conditions. Cd-contaminated soil (0–15 cm of tillage layer) was collected from an oilseed rape-rice rotation system in Zhuzhou (26.82°N, 113.53°E), China. The total Cd content was 1.66 mg/kg, and the pH was 5.58 in the soil, which exceeded the risk intervention value (0.3 mg/kg) but was lower than the risk control value (2 mg/kg) when compared to the standard values for paddy soil (GB 15618–2018). It is essential to remediate these moderately Cd-contaminated agricultural soils and maintain their quality and health for the sustainability of ecosystem services and food security because of the limited cultivated area in China [1]. The soil was air-dried and ground to pass through a 2-mm nylon sieve, and then, it was thoroughly mixed for the pot experiment. This study involved three treatments (four replicates each): no amendment (CK), 0.3% lime (the common application rate in this contaminated region), and 1.0% biochar (produced from rice straw at 250°C for 2.5 h; the common amendment rate from [8, 22, 23]). The characterization method and information in the biochar are presented in the Supporting Information (Table S1, Figure S1). Each pot contained 1.0 kg of soil and base fertilizers (e.g., 0.25 g/kg urea, 0.41 g/kg $Ca(H_2PO_4)_2{\cdot}H_2O$, 0.13 g/kg KCl, and 0.05 g/kg H_3BO_3). After stabilization for one week, two plump *B. napus* (Westar 10) were transplanted into black plastic pots with different treatments. These pots were placed in a greenhouse at a temperature of 20~25°C and 55~60% relative humidity for 40 days and were irrigated with ultrapure water.

At the end of cultivation, samples from soils, roots, and shoots were collected and thoroughly mixed and then divided into multiple subsamples according to the requirements of the property to be analyzed.

2.2. Determination of Soil and Plant Elements. The soil samples were ground and passed through sieves (2 mm and 0.149 mm) after air-drying. The soil pH levels were measured at a v/w ratio of 2.5 with a pH meter (Seven Compact S220, Mettler Toledo). Ammonium acetate (NH_4OAc, 1 M) was used to extract the exchangeable Ca (EX-Ca) in the soil [35]. The soil was digested in an acid mixture of $HNO_3/HCl/HClO_4$ [36] by an electronic heat digestion furnace (DigiBlock EHD36, Lab Tech) for Cd and Ca analysis. The soil exchangeable Cd (EX-Cd) was extracted with 1.0 M $MgCl_2$ solution (v/w ratio, 10 : 1) [37]. In addition, the roots and shoots were ground into fine powder and subsequently digested in an acid mixture of $HNO_3/HClO_4$ (4 : 1, v/v) at 200°C for 2 h [38].

The obtained elements were quantified with inductively coupled plasma mass spectrometry (ICP-MS, NexION™ 350X; PerkinElmer, MA, USA). EX-Ca was quantified with a flame atomic absorption spectrophotometer (AA-6880, Shimadzu, Japan). The Chinese national standard reference materials (e.g., GSS-6, GSS-8, and CSV-2) were used to determine the reliability of the results. The recovery rate for soil and plant Cd was 88–98% and 95–102%, respectively.

2.3. Cd Determinations in Subcellular Structures and Xylem Sap. Fresh roots and shoots were used to determine the Cd contents at the subcellular level by using a method described by Wu et al. [38]. Fresh samples were ground with a mixed solution (e.g., sucrose, dithiothreitol, and Tris-HCl buffer solution) and separated into cell walls, organelles, and soluble parts by different centrifugal forces. Xylem sap was collected based on the method of Lu et al. [14]. Finally, the method of element determination described in the previous section was used to determine the Cd contents in the subcellular structures and xylem sap.

2.4. Determination of Cell Wall Components. The cell wall material of the shoots was separated according to Hu and Brown [39]. The levels of the different cell wall components (e.g., ionic soluble pectin (ISP), covalent soluble pectin (CSP), lignin, cellulose, and hemicellulose) were determined using the corresponding kits (Suzhou Comin Biotechnology Co., Ltd, China).

2.5. Determination of ROS, MDA, and Antioxidant Enzymes in Shoots. A malondialdehyde (MDA) supernatant was obtained by grinding fresh shoots in 0.1% (w/v) cold trichloroacetic acid and centrifuging at 4°C for 30 min (6,000×*g*) [21]. To determine the H_2O_2 and $O_2^-\cdot$ content and POD, SOD, and CAT activities, fresh shoots were ground in 0.05 M phosphate buffer and were then centrifuged at 12,000*g* for 10 min at 4°C [23]. Finally, the levels or activities of MDA, H_2O_2, O_2^-, SOD, CAT, and POD in the supernatants were measured according to the kit instructions (Suzhou Comin Biotechnology Co., Ltd, China).

2.6. Determination of N Metabolism Traits in Shoots. The N concentration in shoots was determined with the Kjeldahl method. The shoot NO_3^- concentration was determined spectrophotometrically using the salicylic acid nitration method according to Xiong et al. [40]. The activities of NR, GS, and GDH were measured by using a modified method based on Ma et al. [29] and Zhang et al. [41].

2.7. Bioconcentration Factor (BCF) and Translocation Factor (TF). The bioconcentration factor (BCF) was defined as the ratio of the Cd concentration in the roots to the Cd concentration in the soil; the translocation factor (TF) was defined as the ratio of the Cd concentration in the roots to that in the shoots. These calculation formulas are based on Mujeeb et al. [42].

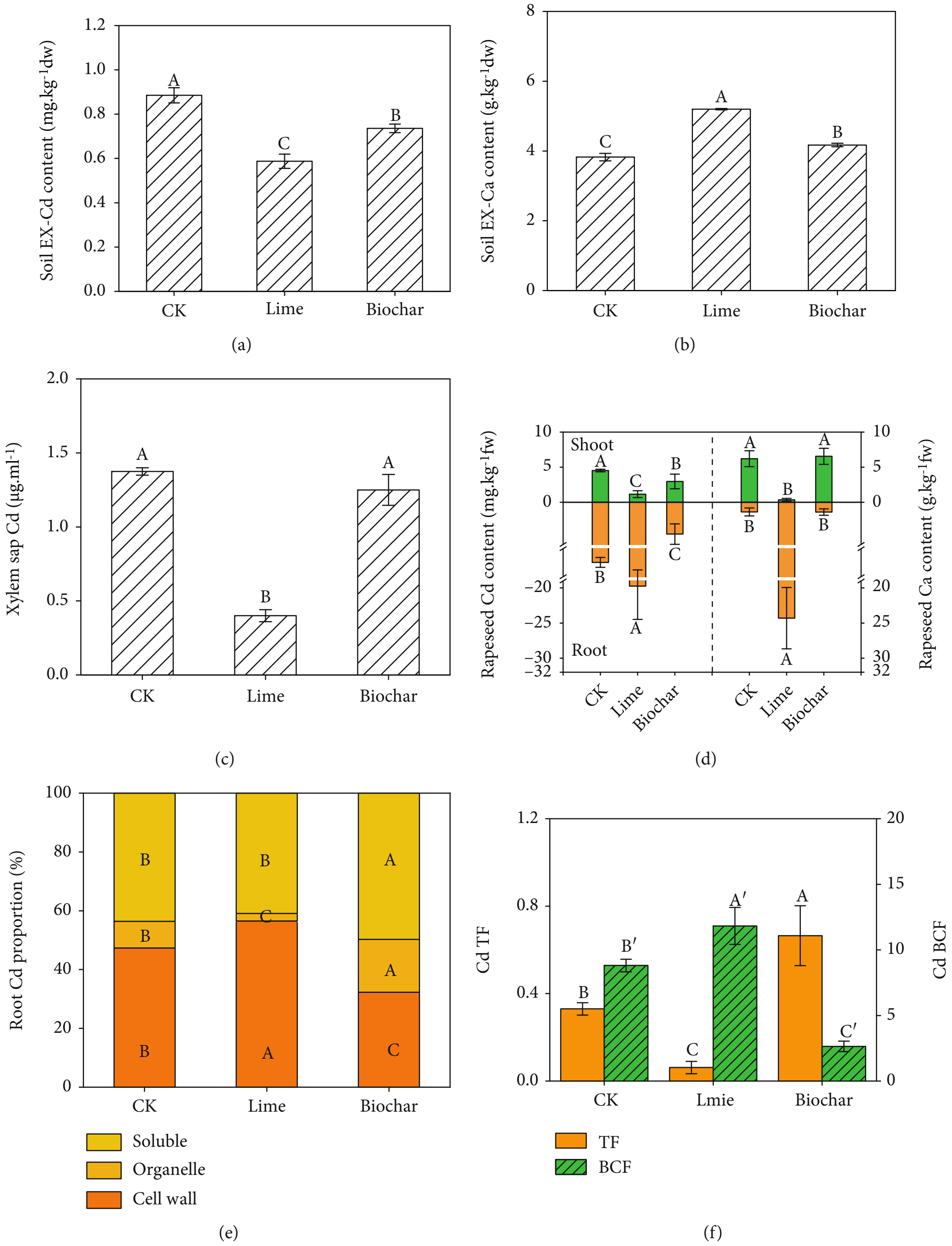
2.8. Statistical Analysis. One-way analysis of variance (ANOVA) and least significant difference (LSD) were conducted to analyze the differences among treatments. Linear regression analyses were used to determine the correlations among variables ($P < 0.05$ and $P < 0.01$). Principal component analysis (PCA) was used to analyze the relationships among the *B. napus* traits and identify a few traits that explain most of the variance observed in a large of manifest traits. Statistical analyses were conducted using SPSS ver. 25.0 (SPSS Inc.) and R ver.4.0.2.

3. Results

3.1. Cd Transfer in the Soil-B. napus System. The soil EX-Cd content reduced by 33.6% and 16.9% after applications of lime and biochar (Figure 1(a), $P < 0.05$), respectively, while the pH increased by 1.58 and 0.77 units, respectively. In addition, the EX-Ca content increased by 35.8% and 9.0% under the lime and biochar treatments, respectively (Figure 1(b), $P < 0.05$). Furthermore, neither lime nor biochar significantly reduced the total Cd contents in the soil (Figure S2a). The EX-Ca content and pH were significantly negatively related to the EX-Cd values (Figure 1(h), $P < 0.05$).

The Cd accumulation patterns were different for *B. napus* under the lime and biochar amendments (Figure 1(d), $P < 0.05$). For the lime treatment, the root Cd content increased by 41.5%, and the shoot Cd content decreased by 74.3%. However, the root and shoot Cd level were reduced by 67.6% and 34.3%, respectively, under the biochar treatment. The BCF decreased (70.0%) after biochar amendment, but no significant changes were found for the lime application (Figure 1(f)). Furthermore, the Cd content in xylem sap decreased by 71.0% after lime application, but those after biochar amendment did not change significantly (Figure 1(c)). The TF values decreased significantly by 81.0% after lime application, while that for the biochar treatments increased by 104.1% (Figure 1(f), $P < 0.05$). Additionally, the Cd concentration was positively correlated with the Ca content ($P < 0.05$) in both the roots and shoots (Figure 1(g)).

3.2. Subcellular Distribution of Cd and Cell Wall Fractions. The Cd redistribution in the roots was markedly different at the subcellular level after the lime and biochar applications (Figure 1(e), $P < 0.05$). The Cd proportion in the cell walls increased by 19.4%, while that in the organelle fraction decreased by 7.18% and that in the soluble fraction did not



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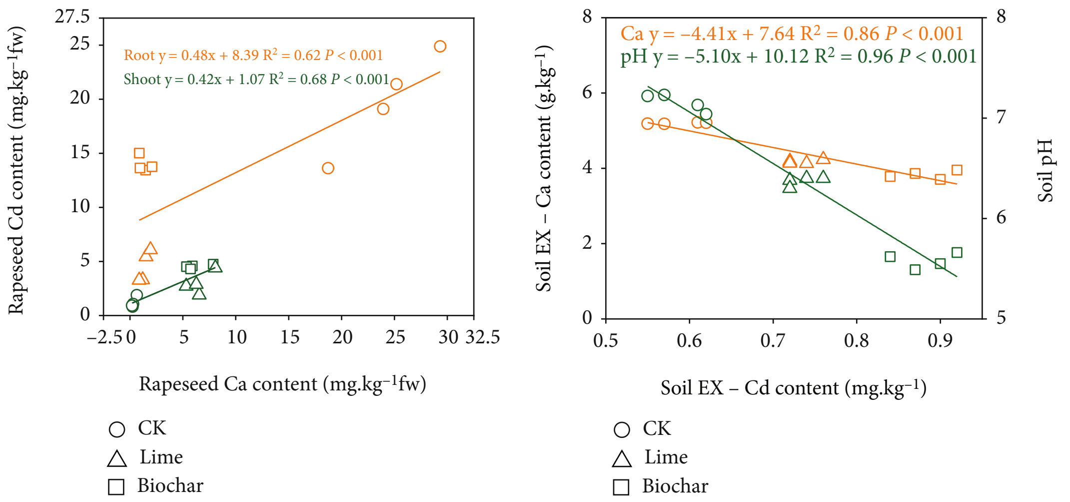


(g)

(h)

FIGURE 1: Cd accumulations and related factors in soil-*B. napus* system under three treatments. *B. napus*: *Brassica napus L.*; EX-Cd: exchangeable cadmium; EX-Ca: exchangeable calcium; TF: translocation factor; and BCF: bioconcentration factor. Means ± SD ($n = 4$). Different lowercase letters indicate significant differences ($P < 0.05$) between the three treatments.

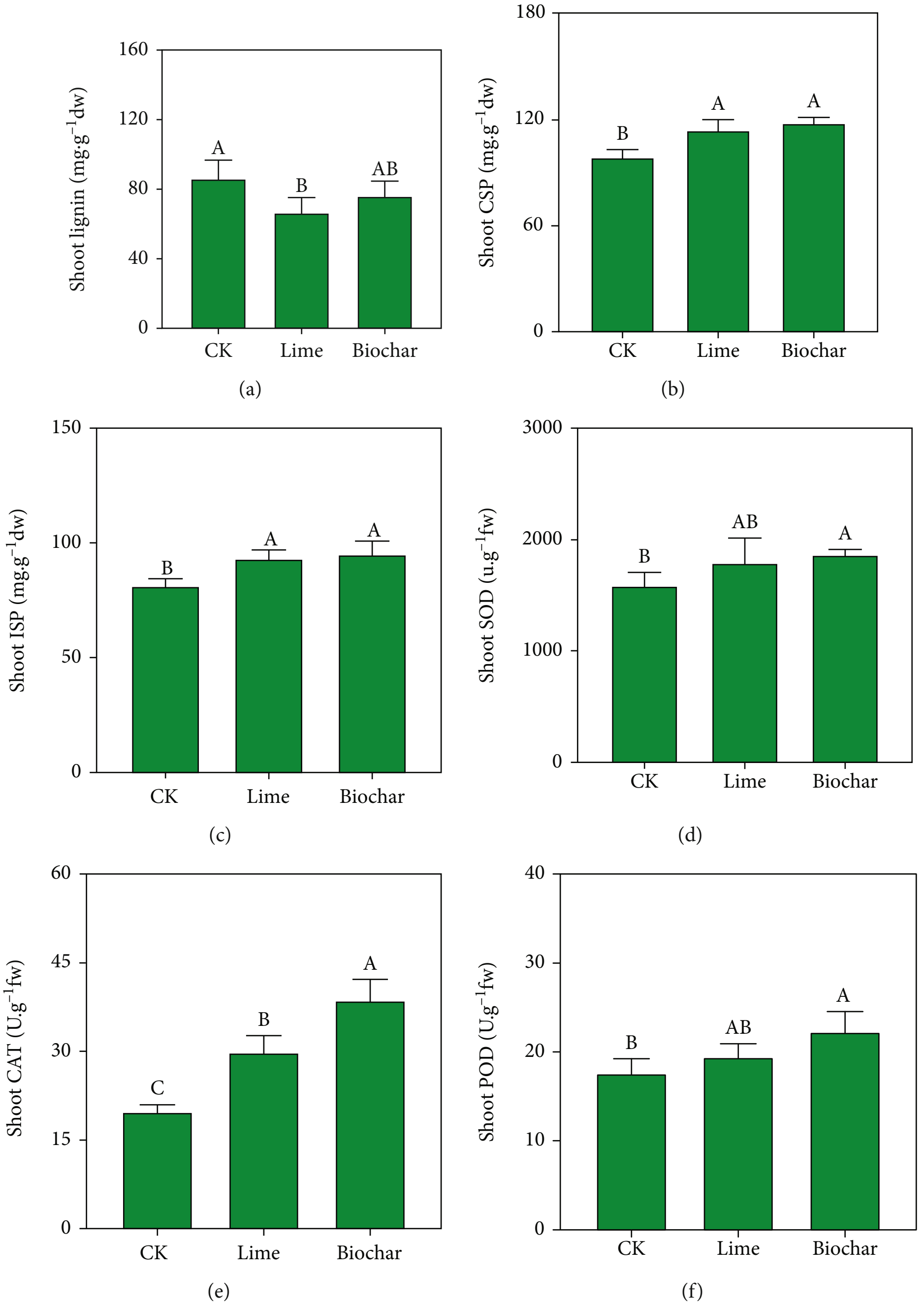
change after lime treatment. The Cd proportions in the soluble and organelle fractions increased by 98.8% and 14.0%, respectively, whereas that in the cell wall fraction decreased by 31.9% after biochar amendment. However, the Cd redistributions in the shoots among the three treatments were not significantly different (Figure S2e, $P > 0.05$). However, the Cd redistributions in the shoots among the three treatments were not significantly different (Figure S2e, $P > 0.05$).

Regarding the cell wall fractions in the shoots, the lignin content reduced by 22.8% and 11.6% after the applications of lime and biochar, respectively (Figure 2(a)). The CSP and ISP levels increased by 15.5% and 14.6%, respectively, for the lime treatment, while those for the biochar amendment increased by 19.7% and 16.8%, respectively (Figures 2(b) and 2(c), $P < 0.05$). No significant differences were found in the hemicellulose and cellulose levels among all treatments. (Figure S2b–S2c, $P > 0.05$). In addition, the lignin content was positively correlated with the Cd content in shoots (Figure 3(a), $P < 0.05$).

3.3. The Antioxidant System in the Shoots. For the biochar treatment, the SOD, CAT, and POD activities increased significantly increased by 17.5%, 95.4%, and 26.6%, respectively (Figures 2(d)–2(f), $P < 0.05$). However, only the CAT activity was significantly enhanced (by 51.0%) after lime application (Figure 2(e), $P < 0.05$). In addition, the MDA and H_2O_2 levels were significantly reduced by both lime and biochar amendments (by 20.2% and 29.0%, respectively, and by 16.0% and 16.6%, respectively, Figures 2(g) and 2(i), $P < 0.05$). The $O_2^{-}\cdot$ concentrations were markedly reduced after the lime application, while no significant changes were observed after biochar application (Figure 2(h)). Furthermore, the MDA, $O_2^{-}\cdot$, and H_2O_2 levels were positively correlated with Cd contents (Figures 3(b)–3(d), $P < 0.05$), but no correlations were found among the antioxidant enzymes and the Cd content (Figure S3a–c, $P > 0.05$).

3.4. N Metabolism in B. napus. The N content in roots increased significantly after both the lime and biochar applications, while the increment for the latter was 2.3 times greater than that of CK (Figure 2(j), $P < 0.05$). The NO_3^- content decreased by 19.7% and the NR activity improved by 18.2% in the shoots under biochar application ($P < 0.05$), while no significant changes were found for the lime amendment (Figures 2(l) and 2(m), $P > 0.05$). The GDH activity improved by 49.3% and 38.0% (Figure 2(n), $P < 0.05$) after the applications of lime and biochar, respectively. No significant differences in the GS activity were observed among all treatments (Figure S2d, $P > 0.05$). Furthermore, negative correlations were found for the Cd content and the N content in the roots and shoots (Figures 3(e) and 3(f), $P < 0.05$) and for the Cd content with the GDH activity in the shoots (Figure 3(g), $P < 0.05$). In addition, no relationships were found between the Cd content and NO_3^- and NR (Figure S3d–e, $P > 0.05$).

3.5. Principal Component Analysis. PCA was used to explore the patterns of the traits associated with stress alleviation in *B. napus* by combining the data across the three treatments (Figure 4). PCA axis 1 showed strong positive loadings for root Cd and shoot N but negative loadings for MDA, ROS (H_2O_2 and $O_2^{-}\cdot$), lignin, shoot Ca, Cd TF, and Cd in the shoot, xylem sap, and root organelle fractions. PCA axis 2 had strong positive loadings for shoot NO_3^-, root Cd and cell wall Cd fractions but negative loadings for antioxidant enzymes (e.g., CAT, POD, and SOD), N metabolism (e.g., root N, NR, and GDH), pectin (CSP and ISP), and Cd in the root soluble fraction. Additionally, the detoxification traits (e.g., root Ca content and cell wall Cd fractions), which



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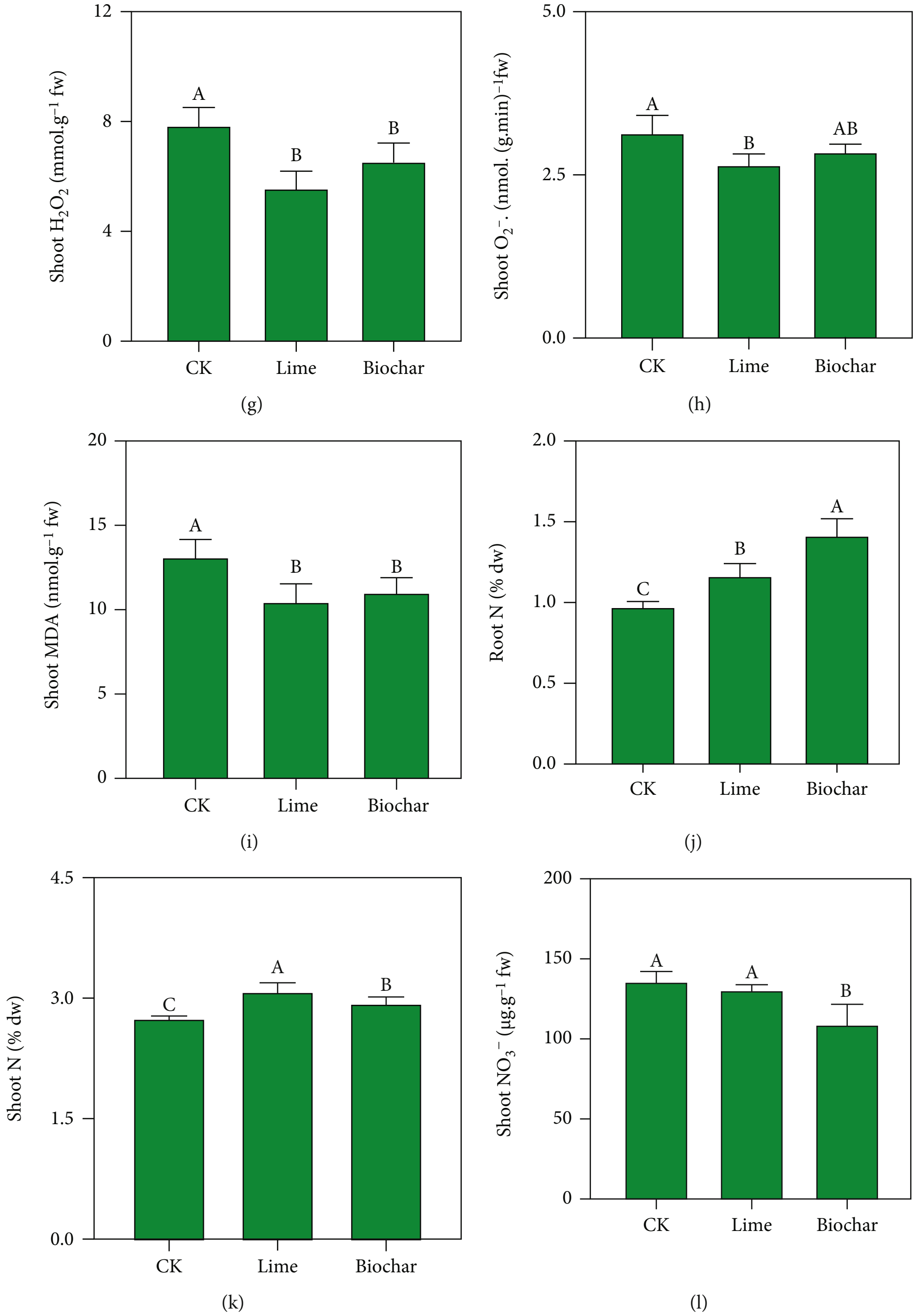

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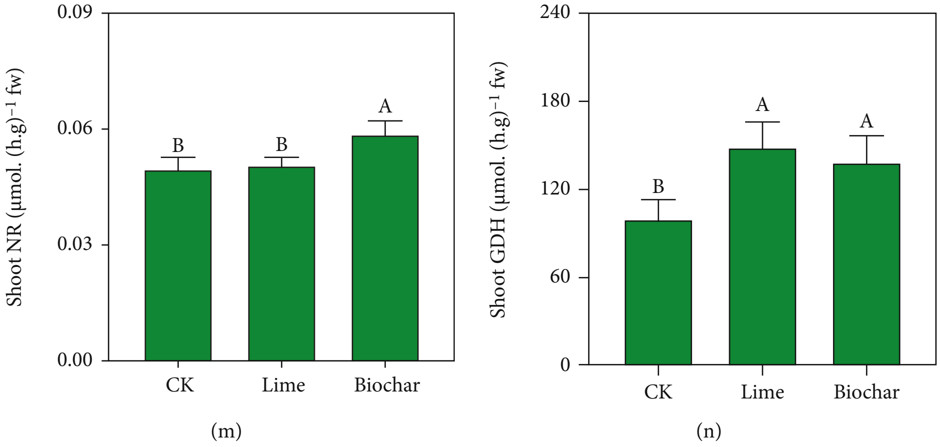


(m)

(n)

Figure 2: Responses of *B. napus* physiological characteristics under three treatments. *B. napus*: *Brassica napus L.*; ISP: ion-bound pectin; CSP: covalently bound pectin; MDA: malondialdehyde; H_2O_2: hydrogen peroxide; $O_2^{-}\cdot$: superoxide anion; SOD: superoxide dismutase; CAT: catalase; POD: peroxidase; NO_3^{-}: nitrate; NR: nitrate reductase; GDH, and glutamate dehydrogenase. Means ± SD ($n = 4$). Different lowercase letters indicate significant differences ($P < 0.05$) between the three treatments.

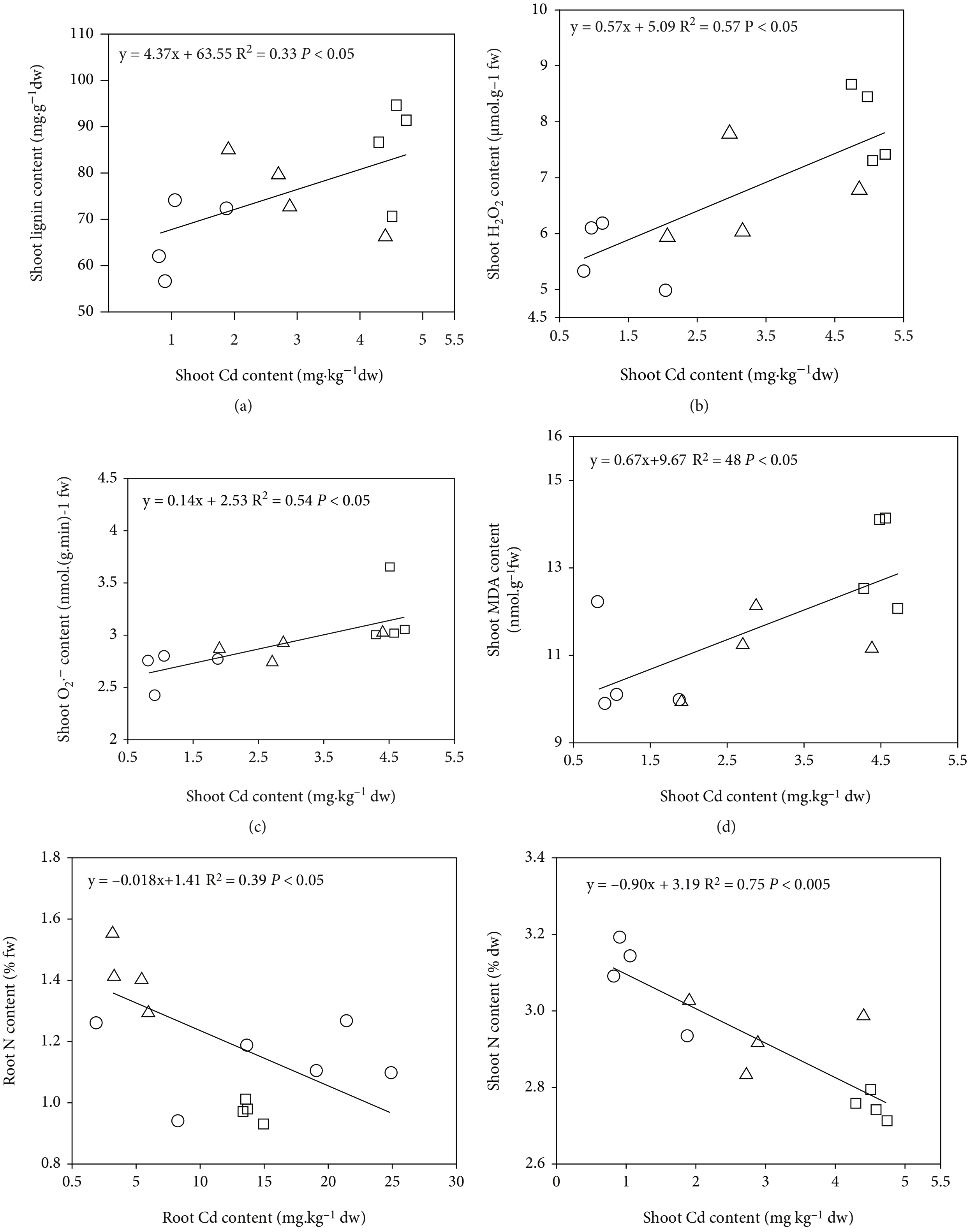
are shown in the upper right part of Figure 4, are associated with lime treatment, while different traits (e.g., antioxidant enzymes and N metabolism), which are shown in the lower part of Figure 4, are associated with biochar treatment.

4. Discussion

4.1. Effects of Lime and Biochar on Cd Translocation in Soil-B. napus System. Partially supporting our first hypothesis, we found that the Cd migration patterns from soil to roots and from roots to shoots between lime and biochar treatments were different, whereas the Cd distribution in the shoots showed similar dynamics. Specifically, the Cd content in the *B. napus* roots increased after lime amendment but decreased after biochar amendment (Figure 1(d)), and these different root Cd levels resulted in differences in the BCF (Figure 1(f)). The divergent effects of lime and biochar on the root Cd content and BCF may result from the influence of Ca since the root Cd content increased with Ca (Figure 1(g)). Ca and Cd are both divalent cations and share many transporters, transport channels, and binding sites [43]. Lu et al. [14] demonstrated that Cd could enter plant guard cells through Ca channels and synergistic interactions and thus accelerate Cd accumulation in plants. Therefore, although the soil Cd was immobilized after lime application (Figure 1(a)), large amounts of Ca may entice the transport channel to promote Cd absorption by the roots. Moreover, biochar application reduced the root Cd content (Figure 1(d)). Numerous studies demonstrated that the application of biochar to soil significantly decreased the phytoavailability of Cd and its uptake by *B. napus* [4, 44, 45]. These results may be attributed to the increased soil pH and formation of an organic matter Cd fraction that prevented Cd uptake by the roots [8, 46].

The patterns of subcellular localization (e.g., cell wall, organelles, and soluble fraction) can help determine Cd accumulation, translocation, and detoxification mechanisms in plants [17]. First, the cell wall Cd fractions in the roots increased significantly after lime application, but not after biochar amendment (Figure 1(e)). Recent studies have indicated that the cell wall is the most important defense mechanism because of the negative charges present on it (i.e., -COO^{-}, -OH, and -SH), which can bind Cd [17–19]. Ca^{2+}, an essential component that is cross-linked with negatively charged -COO^{-}, contributes to the cell wall structure and extensibility [13]. Cd^{2+} can replace Ca^{2+} because of its stronger ion binding and replacement capabilities [19]. Therefore, a larger Ca^{2+} uptake by roots after lime application may stimulate the cell wall to produce more -COO^{-}, which then promotes the fixation of more Cd^{2+} [19]. In addition, the Cd content in the xylem sap was significantly reduced after lime application, but not after biochar amendment, indicating that the amount of Cd that migrated from the underground to aboveground plant parts was greatly reduced. These differences in root Cd content, cell wall Cd fractions in the roots, and Cd contents in the xylem sap further result in a lower TF in lime treatment but a higher one in the biochar treatment. Second, the soluble Cd fraction in the roots was not significantly altered after lime amendment but increased by biochar application (Figure 1(e)), which is consistent with the results of Li et al. [16]. The mobility of the soluble fraction of Cd is stronger than those of the cell wall and organelle components [17, 38]. The increased soluble Cd fraction could further enhance Cd translocation from roots to shoots and then lead to a higher TF level after biochar amendment (Figure 1(f)) [16].

The cell wall Cd fractions in the shoots were not significantly changed (Figure S2e), while some of the cell wall components for the shoots were significantly altered and showed similar changes after lime and biochar amendments (Figures 2(a)–2(c)). We found that the lignin amounts in the shoots decreased significantly and were positively correlated with the Cd contents (Figure 3(a), $P < 0.05$), which was consistent with other studies [47, 48]. Moura et al. [49] indicated that lignin deposition may result from Cd stress in plant cells. Furthermore, the lignification of cell



(a)

(b)

(c)

(d)

(e)

(f)

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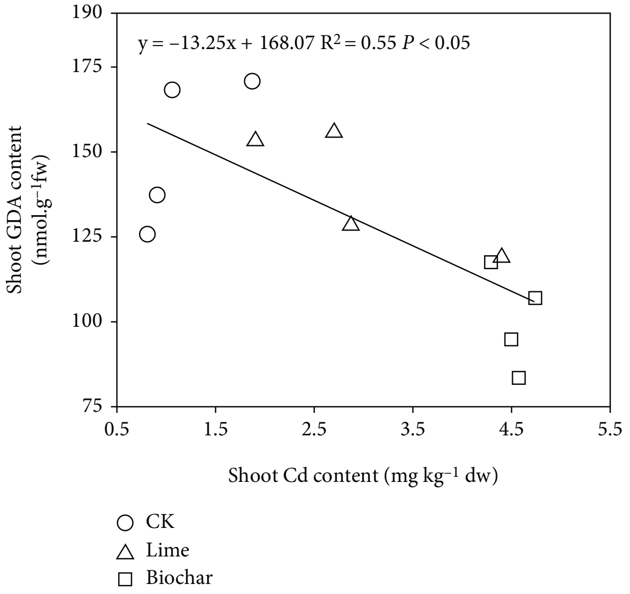

(g)

FIGURE 3: Relationships between Cd accumulation and related physiological traits. MDA: malondialdehyde; H_2O_2: hydrogen peroxide; $O_2^-\cdot$: superoxide anion; and GDH: glutamate dehydrogenase.

walls can also inhibit plant growth [18]. Therefore, the lignin content decreased in shoots indicated that applications of lime and biochar reduced Cd stress on shoot cells. Moreover, the pectin (e.g., CSP and ISP) levels increased after the lime and biochar applications, which indicated the alleviation of Cd toxicity to shoot cells [38].

4.2. Responses of the Antioxidant System of B. napus after Lime and Biochar Applications. ROS (O_2^- and H_2O_2) and MDA accumulate in plant cells under Cd stress, which can result in organelle damage [21]. This study found that the ROS and MDA levels decreased after both lime and biochar applications (Figures 2(g)–2(i)), which was consistent with the studies of Kamran et al. [45] and Huang et al. [24]. Additionally, the ROS and MDA levels in the shoots exhibited a positive relationship with the Cd content (Figures 3(b)–3(d)). These results implied that the decreased Cd contents in the shoots after lime and biochar applications could directly alleviate oxidative stress [24, 45]. Furthermore, the antioxidant system is an important defense mechanism for plants to cope with Cd stress. For example, SOD can convert $O_2^-\cdot$ to H_2O_2, and CAT and POD can degrade H_2O_2 to H_2O, thus reducing the MDA content in cells [21, 47]. Supporting our second hypothesis, biochar amendment had relatively greater impacts on antioxidant enzyme activities. Specifically, the SOD, CAT, and POD activities were enhanced significantly after biochar application, while only the CAT activity improved after lime application (Figures 2(d)–2(f)). Previous studies found that biochar treatment could upregulate pathways and genes associated with plant defense, thereby enhancing enzyme activities [50, 51]. Numerous studies also proved that biochar application improved the activities of SOD, CAT, and POD and reduced the MDA and ROS contents, which decreased oxidative stress [15, 22, 23, 45]. Therefore, compared with lime application, biochar amendment tends to exert stronger effects on antioxidant enzymes to avoid oxidative damage.

4.3. N Metabolism Responses in B. napus after Lime and Biochar Applications. B. napus production is often N-limited because of the low N use efficiency [34]. In the present study, the N content increased in both roots and shoots under lime and biochar applications (Figures 2(j) and 2(k)). Other studies have also found that the applications of lime and biochar increased the N contents in crops [30–32, 51]. Additionally, the N content was negatively correlated with the Cd content in both roots and shoots (Figures 3(e) and 3(f)), implying that decreased Cd contents may improve the N uptake of *B. napus*. NO_3^- and N metabolic enzymes (such as NR, GS, and GDH) are important limiting factors for the N metabolism process in plants [28, 29, 52]. Yang et al. [27] found that the plant N content and metabolic enzyme activities decreased under Cd stress. Our study found that the NO_3^- content decreased and the NR activity was improved in the shoots after biochar application, while no significant changes were observed after lime amendment (Figures 2(l) and 2(m)), supporting our third hypothesis. This result implied that biochar application accelerated the process of N assimilation, i.e., incentivized NR activity to improve the conversion of NO_3^- to NO_2-. Some studies also found that biochar application improved the activities of metabolic enzymes (e.g., NR and GDH) in crops [31, 53]. Therefore, in comparison with lime application, biochar amendment was not only an efficient way to reduce Cd stress but was also an appropriate method to enhance N use efficiency for N-limited crops.

The PCA results showed that higher Cd levels increased oxidative stress since the higher ROS and lignin contents in

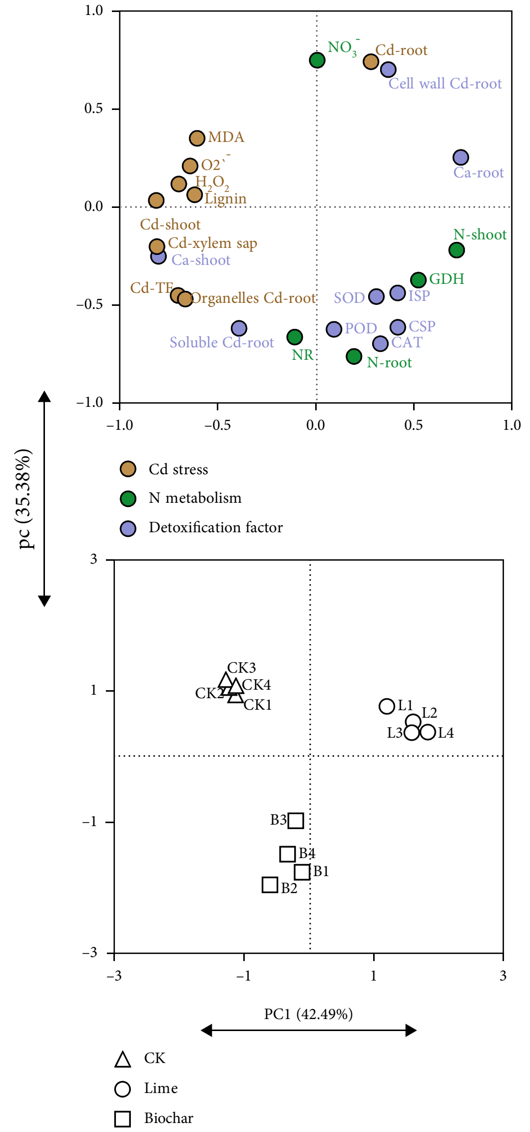

Figure 4: Principal component analysis. Principal component analysis for (a) the 23 traits and (b) the 3 treatments for the first two axes. ISP: ion-bound pectin; CSP: covalently bound pectin; MDA: malondialdehyde; H_2O_2: hydrogen peroxide; $O_2^{\cdot-}$: superoxide anion; SOD: superoxide dismutase; CAT: catalase; POD: peroxidase; NO_3^-: nitrate; NR: nitrate reductase; GDH: glutamate dehydrogenase; and TF: translocation factor.

shoots were accompanied by higher Cd levels (Figure 4(a)). Additionally, the PCA results further confirmed the different mechanisms that alleviate Cd stress in *B. napus* after lime and biochar applications. First, the root Ca level was negatively correlated with the Cd TF and Cd level in the shoots and xylem sap, which further demonstrated that an increase in root Ca could prevent Cd translocation to reduce shoot damage after lime application. Additionally, the antioxidant enzymes (e.g., SOD, CAT, and POD) and N metabolism (e.g., root N content, NO_3^-, NR, and GDH activity) on the lower sides were associated with biochar treatments, and the N metabolism traits were closely related to the antioxidant enzyme activities, which indicated that higher N metabolism might facilitate antioxidant enzyme synthesis to enhance Cd tolerance after biochar application. Yao et.al [25] reported that supplemental N increased activity of antioxidant enzymes (POD, SOD, and CAT) and reduced the ROS content. Additionally, the N deficiency reduced the antioxidant enzyme activities in plants [26].

5. Conclusion

B. napus growth in southern China is usually restricted by Cd stress and low N use efficiency. Large amounts of soil Ca promoted the uptake of Cd by the roots but blocked Cd on the cell wall and reduced Cd translocation from roots to shoots after lime application. In addition, lime amendment also enhanced CAT activity to Cd stress in the shoot. In contrast, biochar application immobilized soil Cd to decrease the uptake of Cd by *B. napus*. Biochar application also improved SOD, CAT, and POD activities to alleviate oxidative stress. Furthermore, the N metabolic processes were accelerated only after biochar application. Therefore, lime is more efficient in reducing the migration of Cd from belowground to crops and improving Cd tolerance, while biochar tends to facilitate N metabolism and antioxidant capacity. These studies will provide significant information for the appropriate selection of remediation approaches. These findings will improve information for the remediation mechanism of soil amendments in agricultural ecosystems and provide strategies for selecting appropriate amendments that both decrease Cd accumulation and enhance resistance in crops.

Data Availability

The datasets used or analyzed during the current study are available from the corresponding author on reasonable request.

Conflicts of Interest

We declare that we have no financial and personal relationships with other people or organizations that can inappropriately influence our work.

Authors' Contributions

Ming Lei designed this study. Zhuoqing Li and Xinqi Wang analyzed the results and wrote the original draft. Zhuoqing Li, Xinqi Wang, and Beibei Zhang performed experiments. Boqing Tie, Tehreem Ayaz, and Xia Lu reviewed and edited this paper.

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Supplementary Materials

Supplementary data to this article can be found online at https://doi.org/10.1155/2022/4195119. *(Supplementary materials)*

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